DESIGN, SYNTHESIS, AND STRUCTURE-ACTIVITY RELATIONSHIP INVESTIGATION OF 3',4'-DISUBSTITUTED PYRANOCHROMONE DERIVATIVES WITH DIVERSE BIOLOGICAL ACTIVITIES

Ting Zhou

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Approved by

Kuo-Hsiung Lee, PhD

(Advisor)

Kenneth F. Bastow, PhD

Jian Liu, PhD

Qian Shi, PhD

Chin-Ho Chen, PhD

ABSTRACT

TING ZHOU: DESIGN, SYNTHESIS, AND STRUCTURE-ACTIVITY RELATIONSHIP INVESTIGATION OF 3',4'-DISUBSTITUTED PYRANOCHROMONE DERIVATIVES WITH DIVERSE BIOLOGICAL ACTIVITIES

(Under the direction of Kenan Professor Kuo-Hsiung Lee)

The overall goals of this research are to design and synthesize novel 3',4'-disubstituted pyranochromone derivatives, to evaluate their anti-HIV activity and chemosensitizing potency, to elucidate structure-activity relationships (SAR), and to discover novel chemical entities.

3'*R*,4'*R*-Di-camphanoyl-khellactone analogs (DCKs) was first identified in our laboratory with high anti-HIV activity against wild-type HIV strain early in 1994. Using SAR-directed strategy, 3'*R*,4'*R*-di-camphanoyl-pyranochromone analogs (DCPs) were synthesized and evaluated in a continuing study, which showed high potency against both wild-type and multi-RT inhibitor-resistant strains. Mechanism study of DCK and its derivatives suggested that the compounds target HIV-RT in a unique way, by inhibiting its DNA-dependent DNA polymerase activity.

A microwave-assisted one-pot reaction was first developed in our study and successfully applied in the preparation of 2',2'-dimethyl-chromone motifs which were

key intermediates in DCK and DCP derivatives. The highly efficient microwave synthesis shrinked the reaction time over 12-fold with much higher yield than traditional heating system previously reported.

Utilizing the optimized synthesis method, novel DCP analogs were designed and synthesized to further explore functionality requirements on the pyranochromone ring and to improve water solubility. SAR studies led us identified 5-alkyl-DCPs (**98**, **102**) with increased efficacy against both wild-type and drug-resistant HIV strains. Simultaneously, novel DCPs (**98, 115**) also presented 5- and 10-time higher water-solubility than 2-EDCP (**69**).

Furthermore, we explored the interaction between the planar ring and the binding pocket by constructing a tri-aryl-conjugated system, pyranoxanthones (DCXs). DCX and its analogs maintained and even increased the π-π stacking interaction with the residues inside the binding pocket. Several DCX analogs (**131**, **135**, **144**) increased anti-HIV potency against both virus strains and six analogs exhibited improved therapeutic index (TI) compared to the control 2-EDCP (**69**).

Finally, we discovered that 3',4'-disubstituted pyranochomone (DSP) analogs showed strong potential to reverse multi-drug resistance (MDR) in cancer cell line. A SAR study were established and three DSP analogs (**194**, **197** and **204**) fully resolved vincristine (VCR) resistance in KB-Vin cancer cells, which are over 2-fold more potent than verapamil, a prototype chemosensitizer. Preliminary mechanism study revealed that the chemosensitizing activity of DSP analogs results from the inhibition of cellular P-glycoprotein (P-gp) function.

DEDICATION

To my husband Kwun Wah for his unlimited love and support throughout my graduate school.

To my parents for their constant encouragement of my study and career decisions.

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AIDS	acquired immunodeficiency syndrome
ABC	ATP-binding cassette
ARV	AIDS related viruses
AZT	zidovudine
BBB	blood-brain barrier
CNS	central nervous system
CCR5	C-C chemokine receptor type 5
CXCR4	C-X-C chemokine receptor type 4
DCK	3'*R*,4'*R*-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone
DCP	3'*R*,4'*R*-dicamphanoyl-2',2'-dimethyldihydropyranochromone
DCX	1*R*,2*R*-3,3-dimethyldihydroxanthone
DDDP	DNA-dependent DNA polymerase
DMAP	4-dimethylaminopyridine
DMDCK	(±)-3'-*O*-4'-O-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone
DMF	*N,N*-dimethylformamide
DSP	3'R,4'R-disubstituted-pyranochromone
Eaton's reagent	phosphorus pentoxide solution
EC_{50}	half maximal effective concentration
2-EDCP	2-ethyl DCP
ER	endoplasmic reticulum
Env glycoprotein	envelope glycoprotein
FDA	food and drug administration

FIs	fusion inhibitors
GI	gastrointestine
Gp41	glycoprotein 41
Gp120	glycoprotein 120
HAART	highly active anti-retroviral therapy
HB	Hydrogen bond
HIV	human immunodeficiency virus
HIV/RTMDR-1	Multi-RT-inhibitor resistant HIV strain
1H NMR	the proton nuclear magnetic resonance
HPLC	High-performance liquid chromatography
HTLV-III	human T-lymphotrophic virus III
IN	integrase
LAV	lymphadenopathy associated virus
MAE	mean absolute errors
MCPBA	meta-chloroperoxybenzoic acid
4-MDCK	4-methyl-DCK
MDR	multi-drug resistance
mp	melting point (°C)
MS	mass spectrum
MW	microwave
NBS	*N*-bromosuccinimide
NF-κB	nuclear factor kappa B
NNRTIs	non-nucleoside reverse transcriptase inhibitors

NRTIs	nucleoside/nucleotide reverse transcriptase inhibitors
PBMC	Peripheral blood mononuclear cells
P-gp	p-glycoprotein
PIs	protease inhibitors
PLS	partial least square
PR	protease
PTLC	preparative thin layer chromatography
QSAR	quantitative structure-activity relationship
mRNA	messenger RNA
THF	tetrahydrofuran
TI	therapeutic Index
R^2	correlation coefficients
RDDP	RNA-dependent DNA polymerase
RNasH	ribonuclease H
RT	reverse transcriptase
SAR	structure-activity relationship
VCR	vincristine
WS	water solubility
µM	micromolar concentration

CHAPTER 1

HIV/AIDS AND TREATMENTS

1.1 Overview of Acquired Immunodeficiency Syndrome (AIDS)

Acquired immunodeficiency syndrome (AIDS) is a set of symptoms and infections resulting from the damage to the human immune system caused by the human immunodeficiency virus (HIV). [1] The first case in the United States (US) was reported in 1981. [2] In 1987, the first antiretroviral drug zidovudine (AZT), a nucleoside analog, was approved by the US Food and Drug Administration (FDA) for the treatment of AIDS patients. Since then, enormous progress has been made in both basic and clinical research. However, neither a cure for this disease nor a vaccine to stop the spread of the pandemic is available.

By 2007, the Joint United Nations Program on HIV/AIDS reported that an estimated 33.4 million people were living with HIV worldwide, including 2.1 million children. [3] In the US, an estimated 1.1 million persons were living with diagnosed or undiagnosed HIV/AIDS at the end of 2006 and 42,655 new cases of HIV/AIDS in adults, adolescents and children were diagnosed in 2007 alone, based on reports by Centers of Disease Control and Prevention. [4]

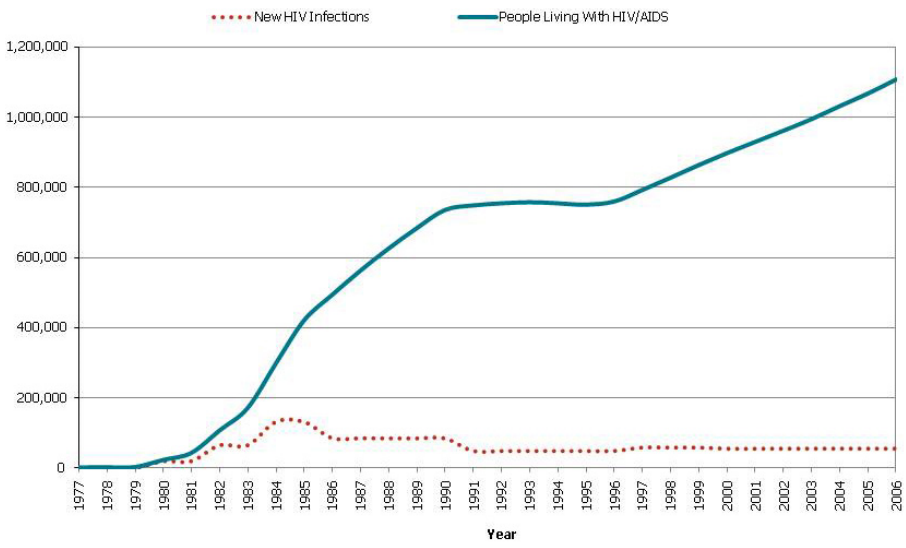


Figure 1-1. HIV incidence and prevalence, United Stetes, 1997-2006. Adapted from CDC. HIV prevalence estimtes- United States 2006

1.2 HIV

HIV is a member of the lentiviruses, a subfamily of the retroviruses. Infection with HIV occurs by the transfer of virus in blood, semen, vaginal fluid, pre-ejaculate, or breast milk. The four major routes for transmission of HIV are unsafe sex, contaminated needles, breast milk, and vertical transmission (transmission from an infected mother to her baby at birth). HIV can infect various immune cells, such as CD4+ T lymphocyte cells, macrophages that express the CD4 molecule on the surface, and dendritic cells.

Two types of HIV are known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered. HIV-1 is more virulent and infective, and it accounts for the vast majority of HIV infections worldwide, with HIV-2 present mainly in Western Africa. [5]

The first isolation of the new human retrovirus, subsequently shown to be the causative agent of AIDS, was reported in 1983 by Montagnier and colleagues.[6] The

virus was isolated from the lymph node lymphocytes of a French homosexual patient with generalized hyperplastic lymphadenopathy. The virus was named lymphadenopathy-associated virus (LAV-I). The association of LAV to AIDS remained obscure until Dr. Robert Gallo reported the isolation and characterization of a retrovirus from patients with or at risk of AIDS. This virus was named human T-lymphotrophic virus III (HTLV-III) in 1984.[7] Subsequent studies have shown LAV and HTLV-III to be virtually identical. In addition, similar viruses known as AIDS-related viruses (ARV) were isolated from homosexuals in San Francisco.[8] All isolates of these related retroviruses are now termed human immunodeficiency virus type I (HIV-I).[9-13]

1.2.1 Structure of HIV-1

The mature HIV-1 particles are spherical with a diameter of about 100-120 nanometers. (Figure 1-2) HIV particles surround themselves with a coat of fatty material known as the viral envelope. The spikes projecting from this envelope are formed from the Env glycoprotein, a heterodimer that contains the surface gp120, which is non-covalently attached to the transmembrane subunit gp41. Each spike contains three gp120 and three gp41 molecules, held together in a triangular symmetry.[14] The

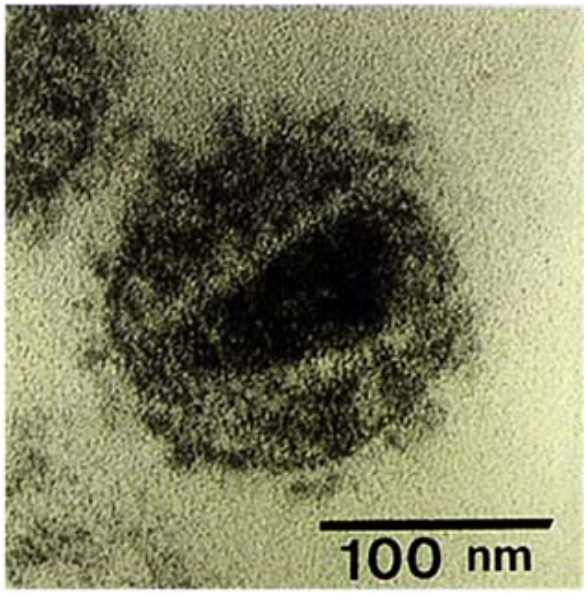


Figure 1-2. HIV at the electron microscope. Adapted from Montagnier et al. [13]

glycoprotein complex plays a significant role to facilitate viral entry, which enables the virus to attach to and fuse with target cells and initiate the infectious cycle. [15, 16,17] Below the viral envelope is a layer called the matrix, which is composed of an association of the viral protein p17.[18] The function of the matrix is to ensure the integrity of the virion particle. The viral capsid is usually bullet-shaped and is made from protein p24. [19] Inside the capsid are three enzymes required for HIV replication called reverse transcriptase (RT), integrase (IN), and protease (PR). Also held within the core is HIV's genetic material, which consists of two copies of positive single-stranded RNA. (Figure 1-3)

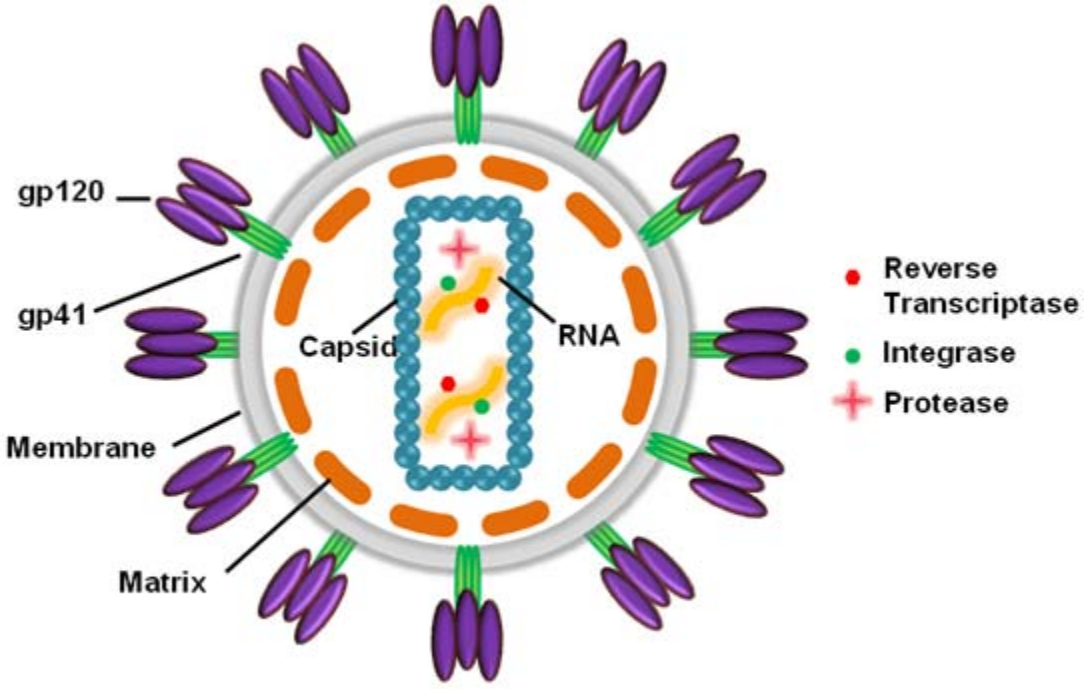


Figure 1-3. Structure of a mature HIV-1 Particle

The RNA genome of HIV-1 encodes the major structural and non-structural proteins common to all replication-competent retroviruses. [20] (Figure 1-4) From its 5' to 3' –end, the genome contains *gag* (for group-specific antigen), *pol* (for

polymerase), and *env* (for envelope glycoprotein) genes, which encode corresponding structural proteins. The *gag* gene encodes a polyprotein precursor, $Pr55^{Gag}$, which is cleaved by the viral PR to release four mature gag proteins, matrix (MA), capsid (CA), nucleocapsid (NC), and p6. The *pol*-encoded enzymes are initially synthesized as part of a large polyprotein precursor, $Pr160^{Gagpol}$. The individual *pol*-encoded enzymes, PR, RT, and IN, are cleaved from $Pr160^{Gagpol}$ by the viral PR. The *env* gene encodes the envelope glycoprotein precursor gp160. The gp160 is processed not by PR but instead by a cellular protease in Golgi apparatus, which generates the surface Env glycoprotein gp120 and the transmembrane glycoprotein gp41. The function of each protein will be described in detail in Chapter 1.2.2.

HIV-1 also encodes a number of regulatory proteins, Tat, Rev, and accessory proteins Vpu, Vif, Vpr, and Nef. Tat is critical for transcription from HIV-1LTR, and Rev plays a major role in the transport of viral RNAs from the nucleus to the cytoplasm.

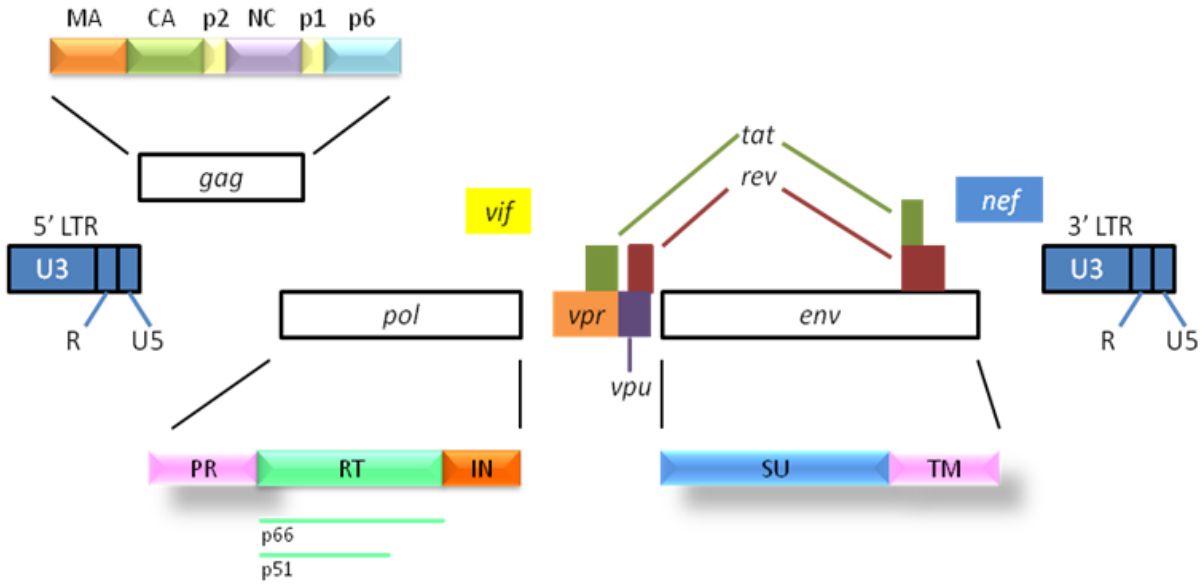


Figure 1-4. Genome structure of HIV-1. The relative locations of the HIV-1 open reading frames *gag, pol, env, vif, vpr, vpu, nef, tat,* and *rev* are indicated. The 5' and 3' LTRs are shown.

1.2.2 Replication Cycle of HIV-1

HIV replication proceeds in a series of events that can be divided into several steps. Based on current understanding, molecular events in the replication cycle of HIV-1 include the following steps (Figure 1-5): virus entry and fusion (1), reverse transcription (2), integration and gene expression (3), and assembly and maturation (4).

(1) Virus entry and fusion

The entry of HIV into the host cell requires the presence of certain receptors (CD4 receptors) and co-receptors (e.g., CCR5 and CXCR4) on the cell surface. [20] These cellular receptors interact with virus protein complexes composed of gp120 and gp41 glycoproteins, which are embedded in the viral envelope. When HIV approaches its target cell, gp120 binds to the CD4 receptors. Binding to CD4 triggers a conformational change in gp120 that exposes a binding site for a

chemokine receptor that acts as a co-receptor.[17] Interaction with the co-receptor promotes the formation of a transient intermediate, in which gp41 spans both the viral and the cellular membrane and facilitates the fusion of the two membranes.[21]

However, a recent study explored the fusion process by using population-based measurements of the viral content delivered into the cytosol and time-resolved imaging of single viruses. The study demonstrated that HIV-1 predominantly uses existing cellular endosomes as transport carriers to gain access to the cytoplasm, rather than using conventional fusion upon interacting with cognate cellular receptors on the plasma membrane.[22]

(2) Reverse transcription

The viral nuclear capsid enters the host cell and releases two RNA strains and three essential replication enzymes: IN, PR and RT. RT begins the reverse transcription of viral RNA. It has two catalytic domains, the ribonuclease H (RNaseH) active site and polymerase active site. Single-stranded viral RNA is transcribed into a RNA-DNA double helix. RNaseH then breaks down the RNA. Finally, the polymerase completes the remaining DNA strand and forms a DNA double helix.

(3) Integration and gene expression

The viral DNA double helix is imported into the nucleus in a process regulated by the viral protein Vpr. Then, the viral integrase goes into action and cleaves a di-nucleotide from each 3' end of the DNA, creating two sticky ends. Integrase then transfers the DNA into the cell nucleus, where it also makes a staggered cleavage in the cellular target DNA and facilitates connection of virus DNA to the ends of

cleaved cellular DNA. The integrated DNA is called the “provirus”.

After integration, the host cell genome contains the genetic information of HIV. Activation of the cell by certain cellular transcription factors, such as NF-κB, induces the transcription of proviral DNA into mRNAs that ultimately encode the full complement of structural, regulatory, and accessory proteins used to direct virus replication. The HIV-1 LTR, composed of three regions, U3, R and U5, serves as the site of transcriptional initiation and harbors *cis*-acting elements required for RNA synthesis.[23] Tat transactivates LTR-driven gene expression.[24] The mRNAs are then translated into proteins using the cell machinery.

(4) Assembly and maturation

Following the synthesis of the full complement of viral proteins, the assembly process begins. The assembly takes place at the plasma membrane of the infected cell, and Gag precursor polyprotein $Pr55^{Gag}$ plays an important role in membrane binding, targeting, and encapsidation.[25,26] The MA domain of Gag is largely responsible for targeting and binding the plasma membrane. The specific encapsidation of retroviral RNAs into virus particles is mediated by interaction between packaging signal and the NC domain of Gag protein. Thus, the NC domain of Gag protein promotes the encapsidation of the viral RNA genome and the association with viral Env glycoproteins, gp41 and gp120 and stimulates budding from the cell.

Maturation occurs during or shortly after virus release from the plasma membrane of the host cell. During maturation, HIV PR cleaves the polyprotein precursors into individual mature HIV proteins and enzymes.[27] The mature virus is

then able to infect another cell.

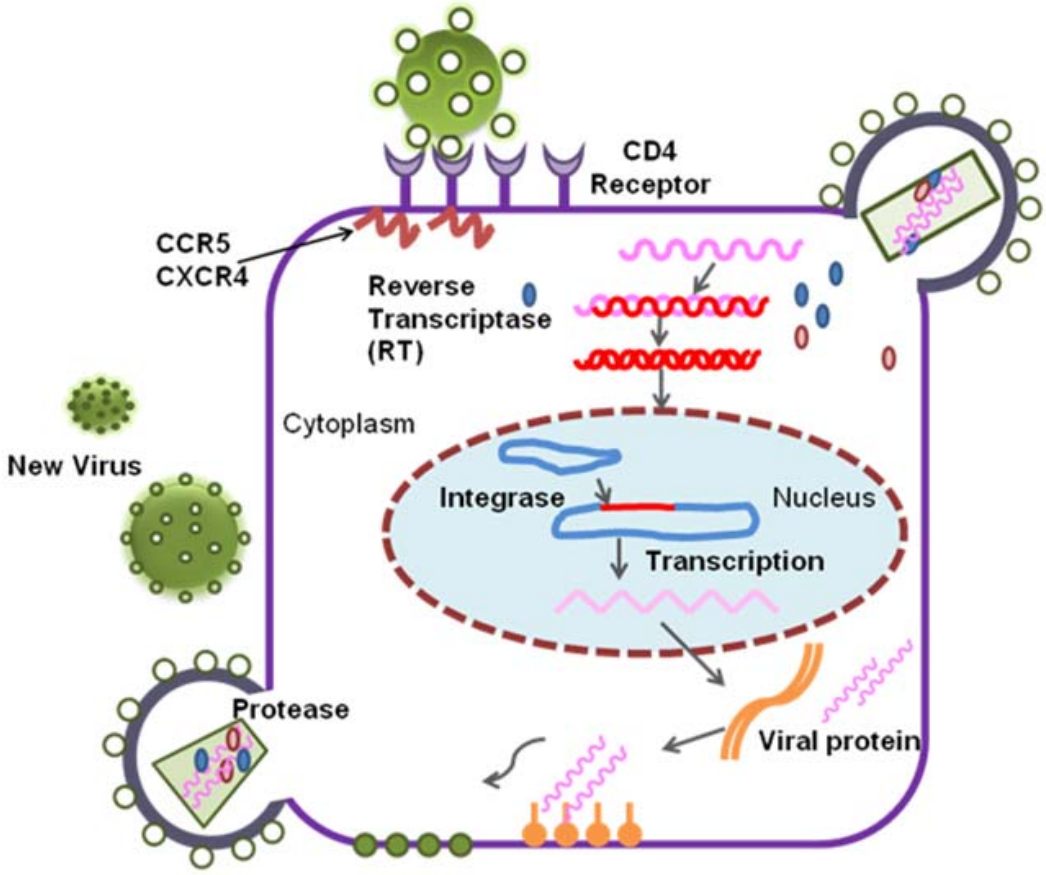


Figure 1-5. Replication of HIV-1

1.3 Current Anti-retroviral Drugs

Currently, no vaccine or cure for HIV or AIDS is publically available. However, the introduction of antiretroviral drugs has significantly improved the prognosis of infected individuals who have access to treatment. Current anti-retroviral drugs/agents fall into six categories based on their different mechanisms: (A) a fusion inhibitor (FI); (B) entry inhibitor (CCR5 co-receptor antagonist), (C) nucleoside/nucleotide viral RT inhibitors (NRTIs), (D) non-nucleoside RT inhibitors

(NNRTIs), (E) HIV integrase strand transfer inhibitors, and (F) protease inhibitors (PIs).

(A) Fusion inhibitors

Fusion inhibitors prevent HIV from entering target cells. [28] Enfuvirtide (T-20) is the first and only fusion inhibitor approved by FDA for use in treatment-experienced patients.(Figure 1-6) [29-33] Enfuvirtide is a synthetic 36-amino acid peptide that mimics the residues 127-162 of the extracellular portion of gp41 and prevents the conformational change of gp41 required for virus-cell fusion.[34] Enfuvirtide is considered to be a drug with a low genetic barrier to resistance development.[28,35] Other limitations associated with enfuvirtide treatment include the high cost of the peptide manufacturing process and its mode of delivery. The drug must be administered by subcutaneous injection because oral administration leads to rapid degradation in the gastrointestinal (GI) tract.[36]

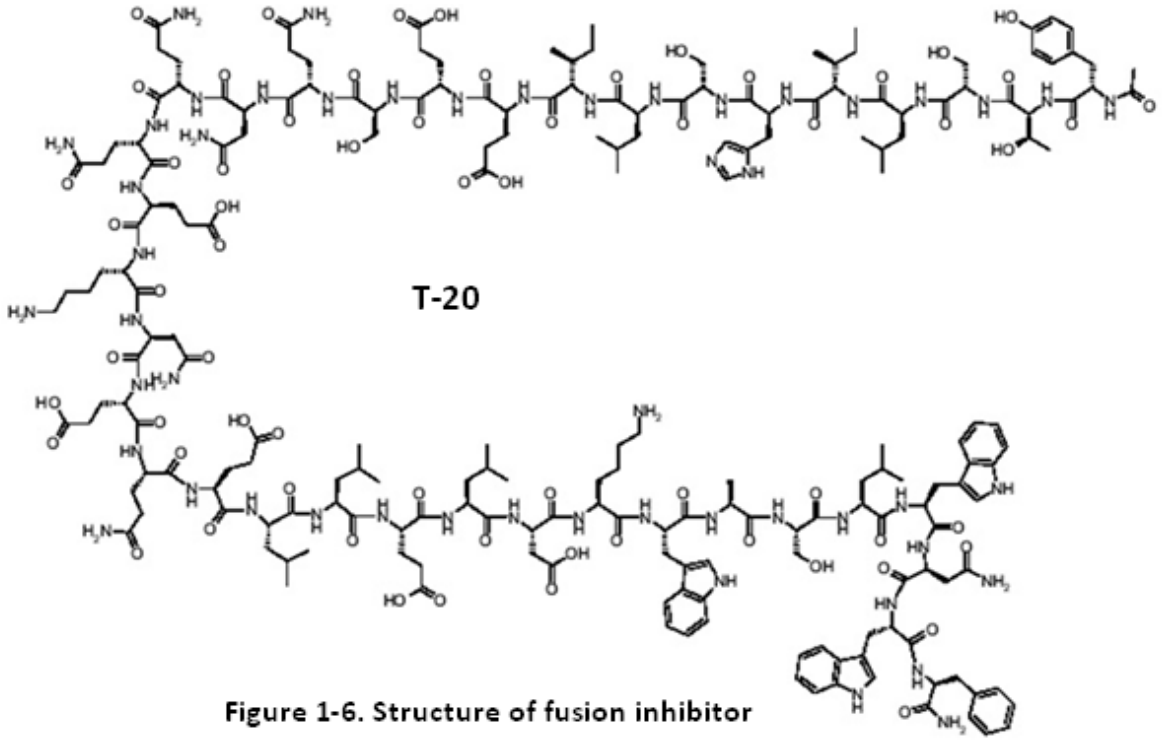
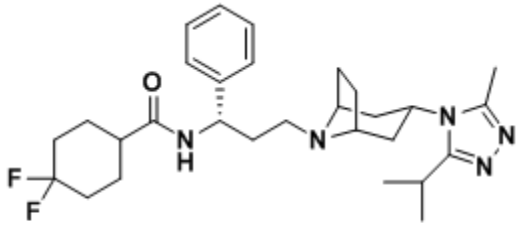


Figure 1-6. Structure of fusion inhibitor

(B) CCR5 antagonist

The CCR5 antagonists are antiretroviral agents with an extracellular, host-targeted mechanism of action against HIV. Maraviroc was the first approved oral receptor-binding inhibitor. (Figure 1-7)[37] The binding of the medication with CCR5 causes a conformational change that blocks gp41 mediated fusion of viral and cellular membranes.[38,39] However, maraviroc has no efficacy against CXCR4 tropic virus and minimal effect on mixed/dual-tropic virus.[40] Hence, FDA approval of maraviroc stipulates that it is used as therapy for treatment-experienced adult patients in which only R5-tropic HIV-1 is detectable.[37]



Maraviroc

Figure 1-7. Structure of entry inhibitor

(C) Nucleoside/nucleotide RT inhibitors (NRTIs)

NRTIs are nucleoside/nucleotide analogs that replace natural nucleotides in the viral DNA nucleic acid sequence. [41,42] NRTIs were also the first drugs to be used clinically as antiretroviral agents and they remain in the mainstream of therapy against HIV infections. FDA approved NRTIs include AZT, d4T, 3TC, ddC, ddI, ABC, and FTC. (Figure 1-8) [43-45] All analogs mimic endogenous nucleosides and are metabolically activated by host cellular kinases to their corresponding triphosphate forms, which are then incorporated into DNA by HIV-1 RT and therefore terminate the DNA chain elongation. [46] Major limitations include mitochondrial toxicity,[47,48] lack of activity in some cell types, and susceptibility to viral resistance.[49]

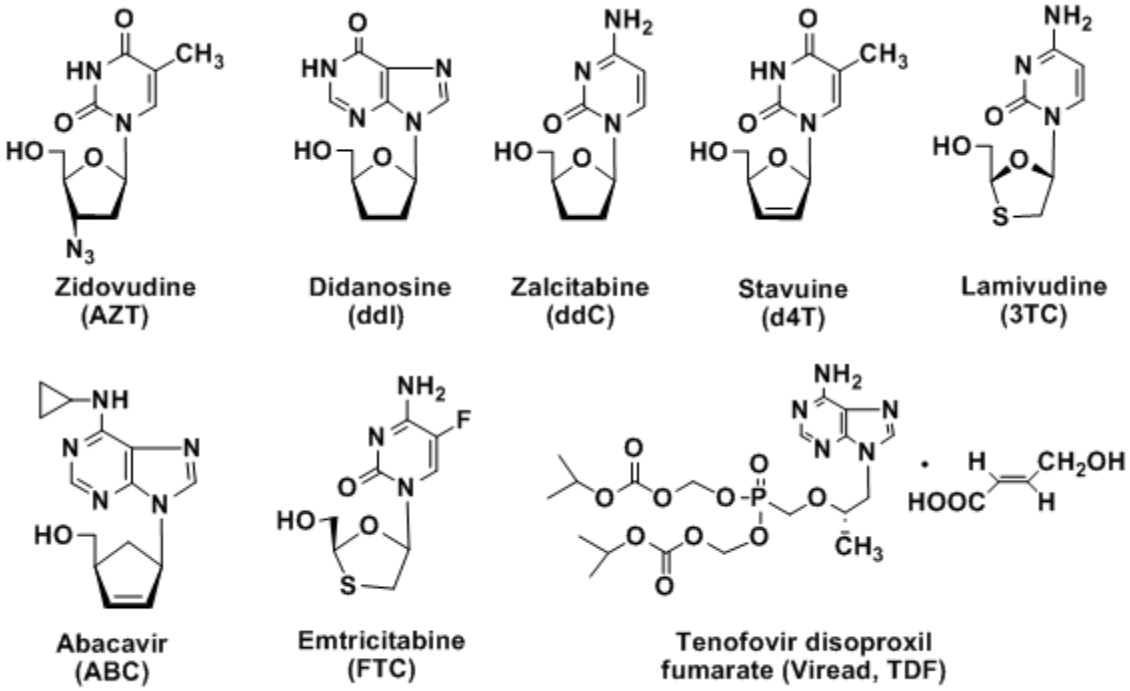


Figure 1-8. Structures of NRTIs

(D) Non-nucleoside RT inhibitors (NNRTIs)

Compared with NRTIs, NNRTIs are small allosteric inhibitors. They non-competitively inhibit DNA polymerization, and bind to a non-active site pocket in RT.[50,51] NNRTIs are structurally and chemically diverse, with more than 30 structurally different classes of compounds being identified.[52] The FDA has approved four NNRTIs: nevirapine, delavirdine, efavirenz, and etravirine. (Figure 1-9) The major limitation of NNRTIs is their low genetic barrier to resistance. Single point mutations at the NNRTI binding site can cause high levels of resistance and cross-resistance to other NNRTIs.[53] Etravirine is a newly approved second generation NNRTI. Its structure flexibility allows it to bind efficiently to the binding pocket even in the presence of significant NNRTI resistance-associated mutations. However, etravirine still loses its potency when two or more mutations occur simultaneously.[54]

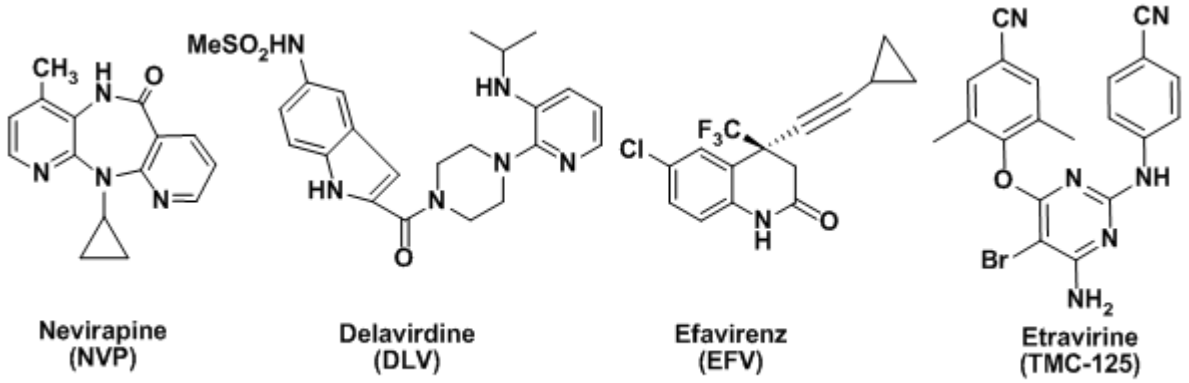
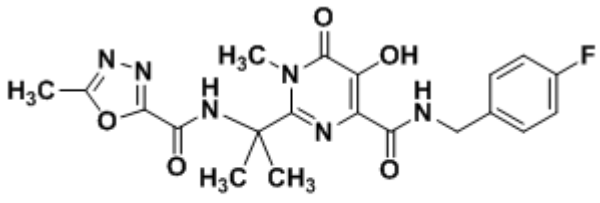


Figure 1-9. Structures of NNRTIs

(E) HIV IN inhibitor

IN is the newest target of AIDS and retroviral therapy.[55] Raltegravir is the first and the only IN inhibitor approved by the FDA (2007) for the treatment of patients who are failing highly active anti-retroviral therapy (HAART). (Figure 1-10)[56] The medication is well-tolerated and highly potent, and has a good pharmacokinetic profile.[56-58] However, as observed for other anti-retrovirials, specific resistance mutations have been identified in patients failing to respond to treatment with raltegravir.[59,60] Varying-degrees of cross-resistance to IN inhibitors have also been reported.[61,62]



Raltegravir

Figure 1-10. Structure of integrase inhibitor

(F) Protease inhibitors (PIs)

PIs are non-hydrolysable transition state peptidomimetics of HIV protease cleavage sites. [63] PIs selectively inhibit the cleavage of HIV gag and gag-pol

polyproteins, thereby preventing viral maturation.[64] Among the 25 available anti-HIV drugs, nine of them, saquinavir, indinavir, ritonavir, nelfinavir, darunavir, lopinavir, fosamprenavir calcium, atazanavir, amprenavir, and tipranavir, target the HIV PR. Due to their peptidomimetic nature, PIs suffer from poor aqueous solubility, low bioavailability, and short plasma half-lives.[65] Toxicities of PIs include insulin resistance and lipodystrophy.[66] PIs are also highly susceptible to viral resistance.[67,68]

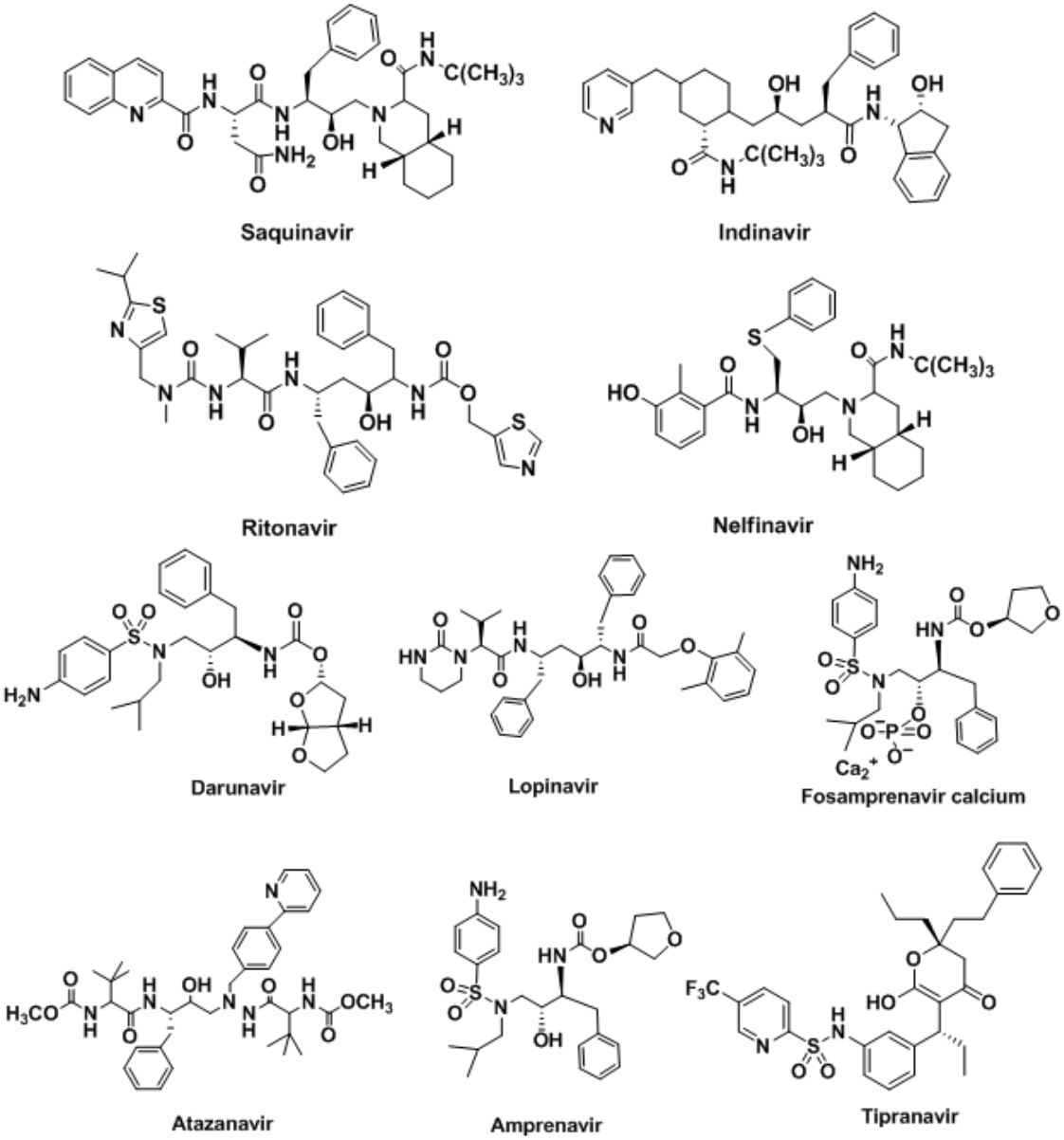


Figure 1-11. Structures of PIs

1.4 Current retroviral Therapy and Its Issues

In the nearly 30 years of research, 25 anti-retroviral drugs have been approved for the treatment of HIV infection in the US. The introduction of antiretroviral drugs has significantly improved the prognosis of infected individuals who have access to treatment. The revolutionary introduction of HAART resulted in dramatically decreased morbidity, improved life expectancy, and cost-effective care for HIV-1 infected individuals. HAART is a combination of at least three drugs, including either a PI or an NNRTI and two NRTIs.[69] HAART slows and prevents the emergence of drug resistant virus. Minor variants with pre-existing drug resistance to one drug can be suppressed by other drugs in a combination. In addition, drug combination can be additive or synergistic, blocking HIV-1 replication more extensively than individual drugs and, thus, preventing the development of new drug resistant mutations.

However, some emerging issues are having increasingly detrimental impacts on the treatment options and disease outcome. While HAART has been successful in prolonging the life-spans of HIV infected patients, it does not allow viral eradication. In addition, as a consequence of the long-term use of anti-retrovirals, a larger dose-increase is often required due to decreased susceptibility. Development of viral resistance is rapid, which makes use of current therapeutic approaches toward AIDS difficult. Furthermore, the treatment itself has serious adverse side effects on the liver and hematophietic organs (e.g., bone marrow suppression and anemia), metabolic effects resulting in increased levels of lipid and sugar in the blood, as well as peripheral neuropathy.[70-72] Another concern is the high cost of lifelong treatment. The expensive cost of HAART also limits access of a broad range of HIV-infected patients to the medications. These limitations have stimulated an emergent need for new anti-HIV agents, especially those with new structures and (or) new action mechanisms to improve convenience, reduce toxicity, and particularly to provide antiretroviral activity against viral strains resistant to the currently available antiretroviral agents.

1.5 References

1. Weiss RA. How does HIV cause AIDS? *Science*, NY, **1993**; 260(5112):1273-1279.
2. CDC. *Pneumocystis pneumonia*---Los Angeles. *MMWR,* **1981**;30:32.
3. Joint United Nations Programme on HIV/AIDS. *2007 AIDS epidemic update: latest developments in the global AIDS epidemic.* Geneva: UNAIDS, **2007**.
4. CDC. HIV/AIDS Surveillance Report. **2007**;19.
5. Gilbert PB, McKeague IW, Eisen G, Mullins C, Gueye NA, Mboup S, Kanki PJ. Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Statistics in Medicine* **2003**; 22(4):573-593.
6. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*, NY **1983**; 220(4599):868-871.
7. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science, NY **1984**;224(4648):497-500.
8. Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM, Oshiro LS. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science*, NY **1984**; 225(4664):840-842.
9. Alizon M, Sonigo P, Barre-Sinoussi F, Chermann JC, Tiollais P, Montagnier L, Wain-Hobson S. Molecular cloning of lymphadenopathy-associated virus. *Nature* **1984**; 312(5996):757-760.
10. Thomson BJ, Dalgleish AG. Human retroviruses. *Blood Rev* **1988**; 2(4):211-221.
11. Wain-Hobson S, Sonigo P, Danos O, Cole S, Alizon M. Nucleotide sequence of the AIDS virus, LAV. *Cell* **1985**; 40(1):9-17.
12. Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, Doran ER, Rafalski JA, Whitehorn EA, Baumeister K, et al. Complete nucleotide sequence of the AIDS virus, HTLV-III. *Nature* **1985**; 313(6000):277-284.
13. Montagnier L. 25 years after HIV discovery: prospects for cure and vaccine (Nobel lecture). *Angew Chem Int Ed Engl* **2009**; 48(32):5815-5826.
14. Sierra S, Kupfer B, Kaiser R. Basics of the virology of HIV-1 and its replication. *J Clin Virol* **2005**; 34(4):233-244.
15. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **1998**; 393(6686):648-659.
16. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* **1998**; 393(6686):705-711.
17. Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, Hendrickson WA, Sodroski J. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science*, NY **1998**; 280(5371):1949-1953.

18. Freed EO. HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology* **1998**; 251(1):1-15.

19. Alcaraz LA, Del Alamo M, Mateu MG, Neira JL. Structural mobility of the monomeric C-terminal domain of the HIV-1 capsid protein. *The FEBS Journal* **2008**; 275(13):3299-3311.

20. Doms RW, Trono D. The plasma membrane as a combat zone in the HIV battlefield. *Genes Dev* **2000**; 14(21):2677-2688.

21. Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. *Annual Review of Biochemistry* **2001**; 70:777-810.

22. Miyauchi K, Kim Y, Latinovic O, Morozov V, Melikyan GB. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell* **2009**; 137(3):433-444.

23. Freed EO, Martin MA, editors. HIVs and Their Replication. 4th Volume; **2001**. 1971-2041 p.

24. Dayton AI, Sodroksi JG, Rosen CA. The *trans*-activator gene of the human T cell lymphotropic virus type III Is required for replication. *Cell* **1986**; 44(941-947).

25. Facke M, Janetzko A, Shoeman RL, Krausslich HG. A large deletion in the matrix domain of the human immunodeficiency virus gag gene redirects virus particle assembly from the plasma membrane to the endoplasmic reticulum. *Journal of Virology* **1993**; 67(8):4972-4980.

26. Freed EO, Orenstein JM, Buckler-White AJ, Martin MA. Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. *Journal of Virology* **1994**; 68(8):5311-5320.

27. Wlodawer A, Erickson JW. Structure-based inhibitors of HIV-1 protease. *Annual Review of Biochemistry* **1993**; 62:543-585.

28. Greenberg M, Cammack N, Salgo M, Smiley L. HIV fusion and its inhibition in antiretroviral therapy. *Reviews in Medical Virology* **2004**;14(5):321-337.

29. Cooper DA, Lange JM. Peptide inhibitors of virus-cell fusion: enfuvirtide as a case study in clinical discovery and development. *The Lancet Infectious Diseases* **2004**; 4(7):426-436.

30. Fletcher CV. Enfuvirtide, a new drug for HIV infection. *Lancet* **2003**; 361(9369):1577-1578.

31. Lazzarin A, Clotet B, Cooper D, Reynes J, Arasteh K, Nelson M, Katlama C, Stellbrink HJ, Delfraissy JF, Lange J, Huson L, DeMasi R, Wat C, Delehanty J, Drobnes C, Salgo M. Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *The New England Journal of Medicine* **2003**; 348(22):2186-2195.

32. Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, Walmsley S, Cohen C, Kuritzkes DR, Eron JJ, Jr., Chung J, DeMasi R, Donatacci L, Drobnes C, Delehanty J, Salgo M. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *The New England Journal of Medicine* **2003**; 348(22):2175-2185.

33. Morse C, Maldarelli F. Enfuvirtide antiviral activity despite rebound viremia and resistance mutations: fitness tampering or a case of persistent braking on entering? *The Journal of Infectious Diseases* **2007**; 195(3):318-321.

34. Wild CT, Shugars DC, Greenwell TK, McDanal CB, Matthews TJ. Peptides corresponding to a predictive alpha-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection. *PNAS* **1994**; 91(21):9770-9774.

35. Greenberg ML, Cammack N. Resistance to enfuvirtide, the first HIV fusion inhibitor. *The Journal of Antimicrobial Chemotherapy* **2004**; 54(2):333-340.

36. LaBonte J, Lebbos J, Kirkpatrick P. Enfuvirtide. *Nature Reviews* **2003**; 2(5):345-346.

37. http://www.fda.gov/bbs/topics/NEWS/2007/NEW01677.html. http://wwwfdagov/bbs/topics/NEWS/2007/NEW01677html.

38. Yang H, Rotstein DM. Novel CCR5 antagonists for the treatment of HIV infection: a review of compounds patented in **2006 - 2008**. *Expert Opinion on Therapeutic Patents*; 20(3):325-354.

39. Huang W, Toma J, Fransen S, Stawiski E, Reeves JD, Whitcomb JM, Parkin N, Petropoulos CJ. Coreceptor tropism can be influenced by amino acid substitutions in the gp41 transmembrane subunit of human immunodeficiency virus type 1 envelope protein. *Journal of Virology,* **2008**; 82(11):5584-5593.

40. Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. Update of the drug resistance mutations in HIV-1. *Top HIV Med,* **2008**; 16(5):138-145.

41. Painter GR, Almond MR, Mao S, Liotta DC. Biochemical and mechanistic basis for the activity of nucleoside analogue inhibitors of HIV reverse transcriptase. *Current Topics in Medicinal Chemistry* **2004**; 4(10):1035-1044.

42. Sharma PL, Nurpeisov V, Hernandez-Santiago B, Beltran T, Schinazi RF. Nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Current Topics in Medicinal Chemistry,* **2004**; 4(9):895-919.

43. Cimons M. U.S. approves sale of AZT to AIDS patients. Los Angeles Times 1987:1.

44. Hirsch MS. Chemotherapy of human immunodeficiency virus infections: current practice and future prospects. *The Journal of Infectious Diseases,* **1990**; 161(5):845-857.

45. Nelson M, Schiavone M. Emtricitabine (FTC) for the treatment of HIV infection. *International Journal of Clinical Practice,* **2004**; 58(5):504-510.

46. Schneider B, Sarfati R, Deville-Bonne D, Veron M. Role of nucleoside diphosphate kinase in the activation of anti-HIV nucleoside analogs. *Journal of Bioenergetics and Biomembranes,* **2000**; 32(3):317-324.

47. Cossarizza A, Moyle G. Antiretroviral nucleoside and nucleotide analogues and mitochondria. *AIDS* (London, England), **2004**; 18(2):137-151.

48. Blanche S, Tardieu M, Rustin P, Slama A, Barret B, Firtion G, Ciraru-Vigneron N, Lacroix C, Rouzioux C, Mandelbrot L, Desguerre I, Rotig A, Mayaux MJ, Delfraissy JF. Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues. *Lancet,* **1999**;354(9184):1084-1089.

49. Gallant JE, Gerondelis PZ, Wainberg MA, Shulman NS, Haubrich RH, St Clair M, Lanier ER, Hellmann NS, Richman DD. Nucleoside and nucleotide analogue reverse transcriptase inhibitors: a clinical review of antiretroviral resistance. *Antiviral Therapy* **2003**; 8(6):489-506.

50. De Clercq E. Perspectives of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Farmaco* **1999**; 54(1-2):26-45.

51. Zhang Z, Hamatake R, Hong Z. Clinical utility of current NNRTIs and perspectives of new agents in this class under development. *Antiviral Chemistry & Chemotherapy* **2004**; 15(3):121-134.

52. De Clercq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antiviral Research* **1998**; 38(3):153-179.

53. Mackie NE, Fidler S, Tamm N, Clarke JR, Back D, Weber JN, Taylor GP. Clinical implications of stopping nevirapine-based antiretroviral therapy: relative pharmacokinetics and avoidance of drug resistance. *HIV Medicine* **2004**; 5(3):180-184.

54. Frater AJ, Beardall A, Ariyoshi K, Churchill D, Galpin S, Clarke JR, Weber JN, McClure MO. Impact of baseline polymorphisms in RT and protease on outcome of highly active antiretroviral therapy in HIV-1-infected African patients. *AIDS* (London, England) **2001**; 15(12):1493-1502.

55. Marchand C, Maddali K, Metifiot M, Pommier Y. HIV-1 IN inhibitors: 2010 update and perspectives. *Current Topics in Medicinal Chemistry,* **2009**; 9(11):1016-1037.

56. Pace P, Rowley M. Integrase inhibitors for the treatment of HIV infection. *Current Opinion in Drug Discovery & Development,* **2008**; 11(4):471-479.

57. Al-Mawsawi LQ, Al-Safi RI, Neamati N. Anti-infectives: clinical progress of HIV-1 integrase inhibitors. *Expert Opinion on Emerging Drugs,* **2008**; 13(2):213-225.

58. Iwamoto M, Wenning LA, Petry AS, Laethem M, De Smet M, Kost JT, Merschman SA, Strohmaier KM, Ramael S, Lasseter KC, Stone JA, Gottesdiener KM, Wagner JA. Safety, tolerability, and pharmacokinetics of raltegravir after single and multiple doses in healthy subjects. *Clinical Pharmacology and Therapeutics,* **2008**; 83(2):293-299.

59. Hu Z, Kuritzkes DR. Effect of Raltegravir Resistance Mutations in HIV-1 Integrase on Viral Fitness. *Journal of Acquired Immune Deficiency Syndromes*, **2010**, July 14.

60. Clavel F. HIV resistance to raltegravir. *European Journal of Medical Research* **2009**; 14 Suppl 3:47-54.

61. Hazuda DJ, Anthony NJ, Gomez RP, Jolly SM, Wai JS, Zhuang L, Fisher TE, Embrey M, Guare JP, Jr., Egbertson MS, Vacca JP, Huff JR, Felock PJ, Witmer MV, Stillmock KA, Danovich R, Grobler J, Miller MD, Espeseth AS, Jin L, Chen IW, Lin JH, Kassahun K, Ellis JD, Wong BK, Xu W, Pearson PG, Schleif WA, Cortese R, Emini E, Summa V, Holloway MK, Young SD. A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *PNAS,* **2004**; 101(31):11233-11238.

62. Shimura K, Kodama E, Sakagami Y, Matsuzaki Y, Watanabe W, Yamataka K, Watanabe Y, Ohata Y, Doi S, Sato M, Kano M, Ikeda S, Matsuoka M. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency

virus integrase inhibitor elvitegravir (JTK-303/GS-9137). *Journal of Virology,* **2008**; 82(2):764-774.

63. Tomasselli AG, Heinrikson RL. Targeting the HIV-protease in AIDS therapy: a current clinical perspective. *Biochimica et Biophysica Acta,* **2000**; 1477(1-2):189-214.

64. King NM, Prabu-Jeyabalan M, Nalivaika EA, Wigerinck P, de Bethune MP, Schiffer CA. Structural and thermodynamic basis for the binding of TMC114, a next-generation human immunodeficiency virus type 1 protease inhibitor. *Journal of Virology,* **2004**; 78(21):12012-12021.

65. Li LY, Stewart BH, Fleisher D. Oral delivery of HIV-protease inhibitors. *Critical Reviews in Therapeutic Drug Carrier Systems,* **2000**; 17(2):73-99.

66. Mathe G. Human obesity and thinness, hyperlipidemia, hyperglycemia, and insulin resistance associated with HIV1 protease inhibitors. Prevention by alternating several antiproteases in short sequences. *Biomedecine & Pharmacotherapie,* **1999**; 53(10):449-451.

67. Pozniak A, Opravil M, Beatty G, Hill A, de Bethune MP, Lefebvre E. Effect of baseline viral susceptibility on response to darunavir/ritonavir versus control protease inhibitors in treatment-experienced HIV type 1-infected patients: POWER 1 and 2. *AIDS Research and Human Retroviruses,* **2008**; 24(10):1275-1280.

68. Madruga JV, Berger D, McMurchie M, Suter F, Banhegyi D, Ruxrungtham K, Norris D, Lefebvre E, de Bethune MP, Tomaka F, De Pauw M, Vangeneugden T, Spinosa-Guzman S. Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial. *Lancet*, **2007**; 370(9581):49-58.

69. Shafer RW, Vuitton DA. Highly active antiretroviral therapy (HAART) for the treatment of infection with human immunodeficiency virus type 1. *Biomedecine & Pharmacotherapie,* **1999**; 53(2):73-86.

70. Inductivo-Yu I, Bonacini M. Highly active antiretroviral therapy-induced liver injury. *Current Drug Safety,* **2008**; 3(1):4-13.

71. Prakash M, Poreddy V, Tiyyagura L, Bonacini M. Jaundice and hepatocellular damage associated with nevirapine therapy. *The American Journal of Gastroenterology,* **2001**; 96(5):1571-1574.

72. Tarr PE, Telenti A. Toxicogenetics of antiretroviral therapy: genetic factors that contribute to metabolic complications. *Antiviral Therapy,* **2007**; 12(7):999-1013.

CHAPTER 2

PLANT-DERIVED NATURAL COUMARINS AND DERIVATIVES AS POTENT ANTI-HIV AGENTS

2.1 Introduction

The development of new anti-HIV agents focusing on novel structures and (or) new mechanisms of action is still urgently needed due to the limitations of current anti-HIV drugs. Natural products are typically secondary metabolites, which are produced by organisms in response to external stimuli such as nutritional changes, infection, and competition. They have been the source and inspiration for long-established medicinal agents and the majority of current FDA-approved drugs. According to statistical results, one third of all new chemical entities that were approved by the FDA between 1981-2006 fall into the categories of natural product and natural product-derived small molecules.[2] Undoubtedly, natural products will continue to provide a source and inspiration for new pharmacological entities.

In our laboratory, identification and development of plant-derived natural product lead compounds involves three steps: (1) bioactivity-guided isolation and characterization of active fractions or compounds; (2) rational drug design and analog synthesis; and (3) mechanism of action studies.[3-7] Examples of such compounds evaluated intensely in our laboratory are coumarins and their derivatives with potent *in vitro* anti-HIV activity.

2.2 Recent Advances in Coumarins as Anti-HIV Agents

Coumarins are an important class of oxygen heterocycles. The parent coumarin was isolated from Tanka beans in the 19th century. Since then, a number of naturally occurring coumarins have been isolated mainly from various plant sources in recent years. Extracts of these plants have been employed as traditional medicines in different areas of the world.[8] Naturally occurring coumarins have exhibited multiple biological activities, including anti-tumor, anti-fungal, anti-bacterial,[9] anti-inflammatory,[10] anti-hypertension, anti-arrhythmia, and anti-osteoporosis effects as well as pain relief and prevention of asthma and sepsis.

In recent years, studies have found that many naturally occurring coumarins or coumarin derivatives exhibit anti-HIV activity. Suksdorfin (**1**), a khellactone coumarin, was isolated from the fruit of *Lomatium suksdorfii* in our laboratory in 1994. It showed anti-HIV activity with an acceptable therapeutic index (TI) value in H9 lymphocyte cells.[11, 12] Also discovered in our laboratory in 2000, imperatorin (**2**), isolated from the root of *Rerula sumbul*, exhibited high anti-HIV activity against the H9 cell line.[13] Two 4-phenyl coumarins mesuol (**3**) and isomesuol A (**4**) were isolated from the leaves and twigs of *M. pluricostata*. Both compounds suppressed HIV-1 replication in Jurkat T cells.[14, 15] In 1992, a series of tetracyclic coumarins was isolated from the tree of *Calophyllum lanigerum*. (+)-Calanolide A (**5**) and (-)-calanolide B (**6**) exhibited inhibitory activity against HIV-1 RT.[16] In 2002, a screening study was performed on various 4-hydroxycoumarins identify HIV-1 PIs. Both phenprocoumon (**7**) and warfarin (**8**) were identified as potential PIs.[17] In another massive screening involving protein crystallography and molecular modeling,

PD099560 (**9**) was first identified as a nonpeptide competitive HIV-1 PI. [18] Modification of **9** based on computational study led to the discovery of the more potent **10**, which had an IC_{50} value of 0.52 μM in an HIV protease assay.[19]

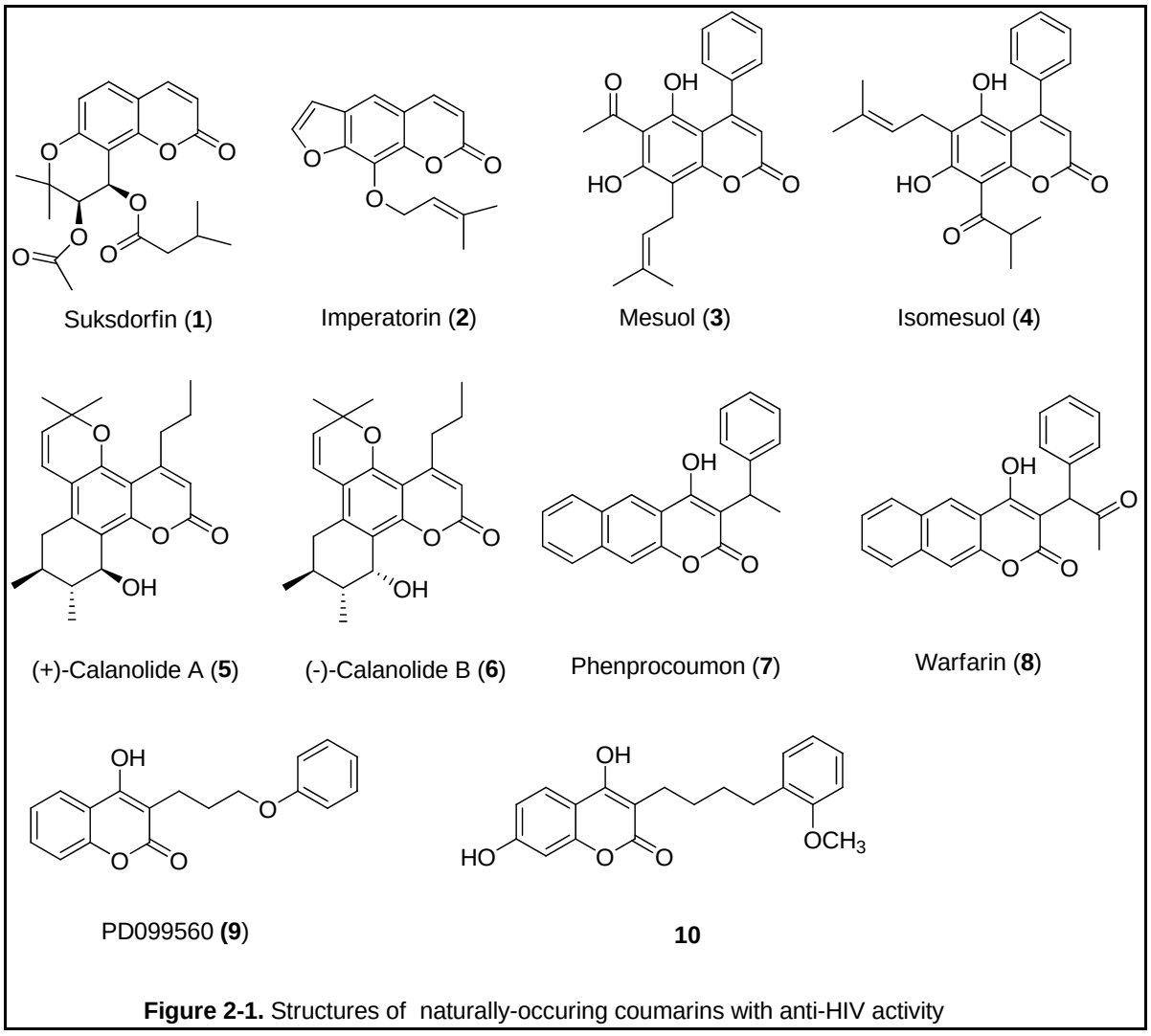


Figure 2-1. Structures of naturally-occuring coumarins with anti-HIV activity

2.3 Modification of Suksdorfin

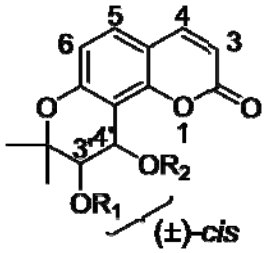
As mentioned above, suksdorfin (**1**) was first isolated and identified as a lead compound against HIV-1 in our laboratory in 1994. Suksdorin had an EC_{50} value of 2.6 μM and a therapeutic index (TI) of 30.6 against the infected H9 lymphocyte cell line. [11] In addition, suksdorfin was also suppressive during acute HIV-1 infections of

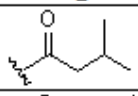
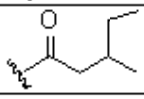
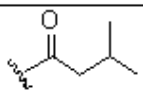
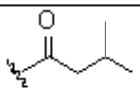
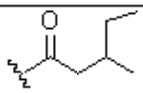
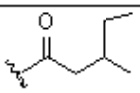
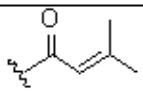
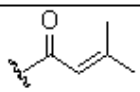
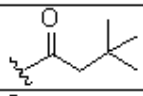
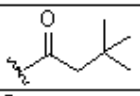
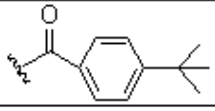
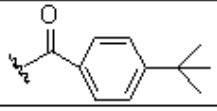
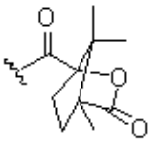
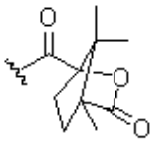
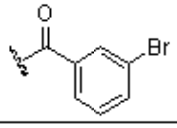
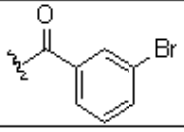
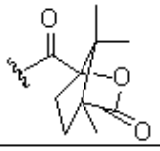
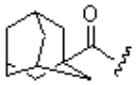
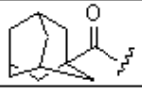
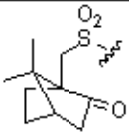
peripheral blood mononuclear cells, monocyte/macrophages and the promonocytic cell line U937. Compound **1** represents a novel class of potent anti-HIV agents, which is structurally unique compared with other known anti-AIDS drugs. In order to obtain more effective analogs and investigate the mechanism of action, **1** has been chosen as a lead compound for various structural modifications.

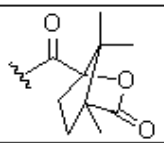
2.3.1 Modification of Dihydroxypyrano 3',4' Positions

Compound **1** contains two chiral centers at the 3' and 4' positions on the pyrano ring system, both with R configurations. Fourteen different dihydroseselin (khellactone) derivatives, including all four stereochemical isomers, were designed, synthesized, and evaluated for anti-HIV activity (Table 2-1).[11, 12] These compounds included khellactone itself (**12**), various acetyl-khellactones (**1**, **11**, **22, 24**), and khellactone diesters (**13**–**21**). The ester groups introduced at the 4' and (or) 3' positions included simple and branched alkyl systems, aromatic rings and large bulky groups. 3',4'-Di-(*O*)-(-)-camphanoyl-(+)-*cis*-khellactone (DCK, **18**) demonstrated the most promising inhibitory activity against HIV replication in H9 cells. With an EC_{50} value of 2.56×10^{-4} μM, DCK was 100-fold more potent than AZT (0.045 μM) in the same assay. The anti-HIV activity of DCK was highly sterospecific as the (-)-*cis*, (-)-*trans*, and (+)-*trans* isomers were much less active.[12, 20] In addition, other racemic khellactone analogs with diverse ester groups at the 3' and 4' positions were inactive or 10,000 times less active.

Table 2-1. Anti-HIV activity of khellactone derivatives in H9-lymphocytes.



Compound	R_1	R_2	EC_{50} [a] (μM)	TI [b]
1	Ac		2.6	30.6
11	Ac		NS [c]	NS
12	H	H	NS	NS
13			7.0	2.0
14			7.0	14.4
15			4.7	3.5
16			NS	NS
17			<1.4	>2.2
18* (DCK)			2.56×10^{-4}	136,719
19**			NS	NS
20*			NS	NS
21*			0.60	35.5
22*	Ac		1.14	16.67
23*		H	NS	NS

24*	AC		0.052	2230

[a] EC_{50} = concentration that inhibits viral replication by 50%. [b] TI = *in vitro* therapeutic index, ratio of IC_{50}/EC_{50}. IC_{50} = inhibitory concentration for cytotoxicity (not shown). [c] NS = No suppression. * Optically pure (+)-*cis*-khellactone compound; ** Optically pure (-)-*cis*-khellactone compound

2.3.2 Modifications of Substitutions on Khellactone

The prior research identified (+)-*cis*-DCK (**18**) as a new lead compound for further development. Over 30 DCK analogs with diverse functional groups on the coumarin ring system have been designed, synthesized, and evaluated in initial SAR studies against HIV-IIIB replication in H9 cells (Table 2-2).

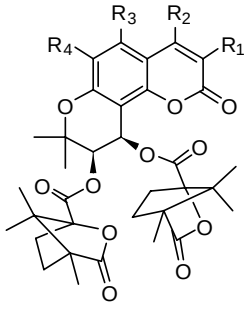
The SAR study of DCK analogs indicated that compounds with methyl or methyoxy groups at one or two of the 3-, 4- and 5-positions of DCK exhibited comparable (**29**–**31** and **33**–**35**) or even better (**25**–**27**) anti-HIV activity than DCK (**18**). 4-MethylDCK (4-MDCK, **26**) and 5-MDCK (**27**) exhibited the greatest anti-HIV activity. However; similar modifications at the 6-position (**28**, **32**) negatively influenced the anti-HIV activity. Larger groups, such as linear or branched propyl groups (**52**, **53**) and phenyl (**54**), on the chromone ring also adversely influenced the activity. These results indicated that a methyl group on the coumarin ring probably fits well into a hydrophobic cleft on the target's active surface and greatly increases both the agent's target affinity and the desired pharmacological response. Larger groups could affect the planarity of the ring system and, therefore, interfere with target binding. A phenyl substituent (**54**) may not fit into the binding site. [20, 21]

Compounds with CH_2OH and CH_2OAc groups at the 3-position (**37**–**38** and **42**–

43) maintained comparable or had better anti-HIV activity compared with DCK. (Note, a different assay system was being used, and the EC_{50} of DCK when screened in parallel with these compounds was 0.049 μM). On the other hand, aminomethyl-substituted derivatives (**39**, **40**) showed decreased activity. Most DCK derivatives displayed potency comparable or better than AZT against wild strain HIV-1 IIIB in H9 or CEM-SS cell lines. [22, 23]

However, most of the DCK analogs were active against HIV-RTMDR1 strains. HIV-RTMDR1 is a multi-drug resistant HIV strain that contains four RT mutations, (M41L, l74V, V106A, T215Y) and is resistant to most NRTIs and NNRTIs.

Table 2-2. Anti-HIV activity of DCKs in acutely infected H9 lymphocytes



	R_1	R_2	R_3	R_4	EC_{50}[a] (μM)	IC_{50}[b] (μM)	TI[c]
18 (DCK)	H	H	H	H	$2.56x10^{-4}$	35	$1.4x10^{5}$
25	CH_3	H	H	H	$5.25x10^{-5}$	>113	$>2.2x10^{6}$
26	H	CH_3	H	H	$1.83x10^{-6}$	>126	$>6.0x10^{7}$
27	H	H	CH_3	H	$2.39x10^{-7}$	>95	$>4.0x10^{8}$
28	H	H	H	CH_3	0.151	33	218
29	OCH_3	H	H	H	$2.38x10^{-3}$	>153	$>6.4x10^{4}$
30	H	OCH_3	H	H	$2.99x10^{-3}$	>153	$>1.5x10^{4}$
31	H	H	OCH_3	H	$1.92x10^{-4}$	>153	$>8.0x10^{5}$
32	H	H	H	OCH_3	15.8	>153	>9.7
33	CH_3	CH_3	H	H	$1.92x10^{-3}$	>154	$>3.7x10^{4}$
34	H	CH_3	CH_3	H	$4.19x10^{-3}$	>154	$>1.7x10^{4}$
35	H	CH_3	H	CH_3	$4.69x10^{-3}$	3.75	$1.3x10^{3}$

36*	CH_2Br	H	H	H	0.059	>11.1	>186
37*	CH_2OAc	H	H	H	0.017	11.6	676
38*	CH_2OH	H	H	H	0.029	23.0	806
39*	CH_2NH_2	H	H	H	0.667	>15.4	>23
40*	CH_2NEt_2	H	H	H	3.67	>14.1	>4
41*	CH_2Br	CH_3	H	H	$1.1x10^{-4}$	>20.9	$>1.9x10^5$
42*	CH_2OAc	CH_3	H	H	0.026	14.8	567
43*	CH_2OH	CH_3	H	H	0.0042	24.9	6000
44*	H	CH_3	H	CH_2Br	0.156	>13.7	>88
45*	H	CH_3	H	CH_2OAc	0.544	>14.1	>26
46*	H	CH_3	H	CH_2OH	0.111	>15.0	>102
47*	H	CH_3	H	CH_2NH_2	0.148	>15.0	>102
48	H	CH_3	OCH_3	H	0.076	>15.0	86
49	Cl	CH_3	H	H	$2.01x10^{-3}$	104	$5.2x10^4$
50*	Br	CH_3	H	H	0.155	>14.0	91
51*	COOH	H	H	H	NS	>15.0	NS
52	$CH_2CH_2CH_3$	H	H	H	$1.75x10^{-2}$	>200	$>8.6x10^3$
53	$CH_2(CH_3)_2$	H	H	H	$3.15x10^{-2}$	>128	$>4.8x10^3$
54*	C_6H_5	H	H	H	0.12	>143	$>1.2x10^3$
55*	CF_3	H	H	H	1.81	>145	>80.1
AZT					0.045	1875.0	$4.2x10^4$

[a] EC_{50} = concentration that inhibits viral replication by 50%. [b] IC_{50} = inhibitory concentration for cytotoxicity. [C] TI = *in vitro* therapeutic index, ratio of IC_{50}/EC_{50}. [d] NS = No suppression. * The activity was tested using a diverse screening assy. These compounds were screened initially in MT-2 cells and then in H9 lymphocytes with ELISA for confirmation. Under this assay system, DCK (**18**) exhibited an EC_{50} of 0.049 μM and TI of >328.

2.3.3 Development of Bio-isosteres of DCK Analogs

Based on the concept of bio-isosterism, new analogs were designed and synthesized (Figure 2-2). The thio-bioisosters of DCK (**56**, **57**) contain sulfur rather than oxygen in ring A, and retained anti-HIV activity in comparison to the substituted DCKs. In the CEM-SS cell line, **57** showed promising potency with an EC_{50} value of 0.064 μM and TI over 3149. It was more active than DCK (EC_{50} 0.14 μM and TI >100) in the same cell line. [24]

DCK lactam analogs were also synthesized asymmetrically and evaluated for

anti-HIV activity against HIV-1 in H9 lymphocytes. Both compounds (**58**, **59**) retained high anti-HIV activity. The EC_{50} values of **58** and **59** against HIV-IIIB in H9 cell line were $2.5x10^{-4}$ and $4.6x10^{-3}$ μM, respectively (EC_{50} of DCK was $2.56x10^{-4}$ μM).[25]

Modification on ring C was also conducted and 7-thia and 7-carbo DCK derivatives were designed and synthesized (**60**–**64**).[26, 27] Bioassay results indicated that both 7-thia and 7-carbo DCKs could be potential anti-HIV agents. Compound **60** exhibited potent inhibitory activity against HIV-1 replication with an EC_{50} value of 0.14 μM and TI value of 1110,[26] while compounds **62**–**64** were also potent with EC_{50} value ranging from 0.068-0.39 μM (EC_{50} of DCK was 0.049 μM in the same assay system). [27]

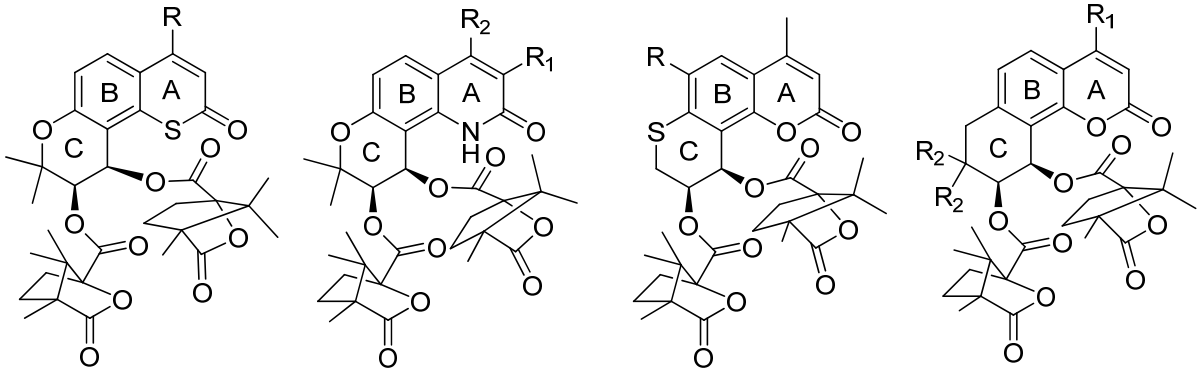


Figure 2-2. Bioisosteres of DCKs

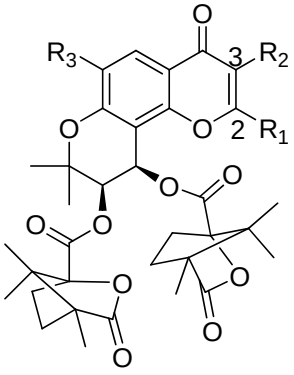
2.3.4 Development of Pyranochromone Derivatives (DCPs) as Novel Anti-HIV Agents

DCP (3',4'-di-*O*-(-)-camphanoyl-2',2'-dimethyldihydropyrano[2,3-*f*]chromone, **65**) was developed from DCK with the intention to further extend the SAR study.[28, 29] In order to introduce structural variety, the 2*H*–pyran-2-one system in DCK was

replaced with a 4*H*-pyran-4-one in DCP. Diverse substitutions (mostly alkyl groups) were introduced on the pyranochromone ring skeleton. Compared with DCK analogs, the synthesized DCP analogs, not only showed high potency against a wild-type HIV-IIIB strain, but most of them also exhibited activity against a multi-drug resistant HIV RTMDR-1 strain. (Table 3)

The initial SAR study of DCPs suggested that DCP analogs are more promising than DCK analogs due to their potential to inhibit the replication of HIV RTMDR-1, which is resistant to DCKs. Methyl and ethyl groups at the 2- or 3-position (**66**, **68**, **69**) of the DCP chromone ring system were critical for anti-HIV activity against both HIV strains. In fact, 2-EDCP (**69**) presented the best anti-HIV activity against both wild-type and drug-resistant HIV-1 strains. Larger substituents at the 2-, 3-, and 6-positions of the chromone ring dramatically decreased the anti-HIV activity against both wild-type and drug-resistant strains.

Table 2-3. Anti-HIV activity of DCP analogs



Compd	R_1	R_2	R_3	HIV-IIIB[a]		HIV-1 RTMDR1[b]	
				EC_{50}(μM)	TI	EC_{50}(μM)	TI
65	H	H	H	0.0013	$1.1x10^5$	NS[c]	NS
66	H	CH_3	H	$9.9x10^{-4}$	$1.5x10^5$	1.38	2.5
67	H	C_6H_5	H	1.54	23.3	NS	NS
68	CH_3	H	H	0.0031	8600	0.19	62.5
69	CH_2CH_3	H	H	$3.2x10^{-4}$	$1.2x10^5$	0.06	718
70	$CH_2CH_2CH_3$	H	H	0.02	1860	0.14	272

71	$CH(CH_3)_2$	H	H	0.07	483	0.14	>111
72	$CH_2OCH_2CH_3$	H	H	0.1	151	0.37	>34
73	C_6H_5	H	H	0.129	>277	0.17	71
74	CH_3	CH_3	H	0.007	1500	0.31	15
75	CH_2CH_3	H	$C(CH_3)_3$	1.62	22	7.36	1.9
DCK				0.049	>328	12.06	1.3
26				0.0059	>6660	9.43	1.7
AZT				0.044	$4.3x10^5$	0.1	>375

[a] This assay was performed in H9 lymphocytes by Panacos, Inc
[b] This assay was performed in the MT-4 cell line by Dr. Chin-Ho Chen, Duke University, NC
[c] NS = no suppression at the concentration of 10 μg/mL.

2.4 Studies on Mechanism of Action

Suksdorfin and DCK analogs are coumarin derivatives that exhibited potent anti-HIV activity. The chemical structure of DCK is unique compared with the drugs currently used in AIDS therapy. Although DCK is a potent inhibitor of many HIV-1 isolates, the compound is ineffective against an HIV-1 strain resistant to both NRTIs and NNRTI.[28] Because this resistant strain is also resistant to DCK, HIV-1 RT might be the target of these coumarin-derived compounds.

2.4.1 Structure of HIV-1 RT

HIV-RT is a main target of drugs used in the treatment of AIDS. It is part of the the HIV viral protein shell surrounding the nucleic acid core and is essential for the replication of the virus.

HIV-RT is a heterodimer enzyme composed of two related subunits, one with 66kDa-p66 and another with 51kDa-p51.The latter is a proteolytic cleavage product of p66 polypeptide, having the same sequence but adopting a different conformation. The additional residues at the carboxy terminus of p66 comprise the RNaseH domain. As p51 is a proteolytic product of p66, any amino acid mutations in the HIV

genome affect both subunits.[30] The p66 subunit includes four subdomains designated thumb, palm, finger, and connection, because of its similarity with a right hand.[1](Figure 2-3) The p51 interacts with the p66 subunit *via* the connection domains as well as through several other parts of the peptide chain. The p66 finger and thumb domains are very flexible, acquiring an open structure when the DNA fits between the fingers and the thumb.[31, 32]

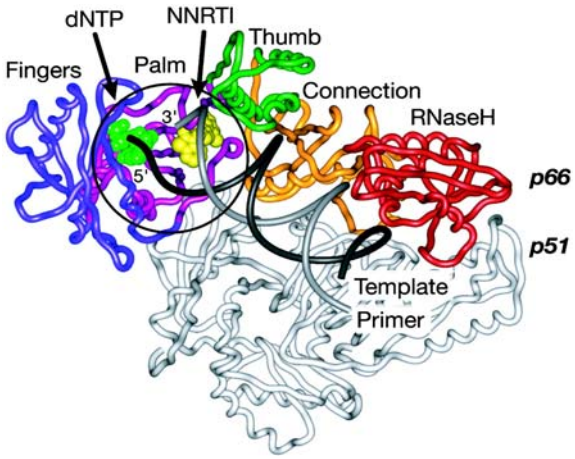


Figure 2-3. Model of HIV-1 RT with NNRTI, DNA primer/template, and incoming dNTP. Adapted from Pata et al. [1]

2.4.2 Mechanism Study of DCK and Its dDerivatives

Several mechanistic studies have been conducted on suksdorfin, DCK, and DCK derivatives. The earliest study in 1994 indicated that suksdorfin and DCK inhibit HIV-1 at a step after HIV-1 entry, but before integration of HIV-1 DNA in host chromosomes.[12] The compounds were inactive in an assay that primarily detects the RNA-dependent DNA polymerase activity of HIV-RT. (Figure 2-4, adapted from Huang, et al.[12]) These results suggested a unique mechanism of action for this compound series.

In 2005, further research was conducted at Duke University to explore the mechanism of action of DCK. [33] Initially, they evaluated the activity of DCK against HIV-1 strains that are resistant to multiple RT inhibitors (HIV-1/RTMDR1) and protease inhibitors (HIV-1/PRMDR) to determine whether DCK can inhibit pre-existing drug-resistant strains. The results were consistent with those of the previous assay. DCK was 2 log units less active agent to HIV-1/RTMDR1, but quite sensitive to HIV-1 PRMDR, which again indicated that HIV-1 RT might be the target of DCK (Figure 2-5). To take a closer look at the interaction between DCK and RT, a DCK-resistant HIV-1 mutant was obtained by growing wild-type HIV-1 in the presence of escalating doses of DCK. This mutant contains two mutations, E138K and G550K, (Figure 2-6 A) and was approximately 2 log units less sensitive to DCK. E138K was later identified to be fully responsible for the DCK resistance (Figure 2-6 B) and played a key role in the anti-HIV activity of DCK

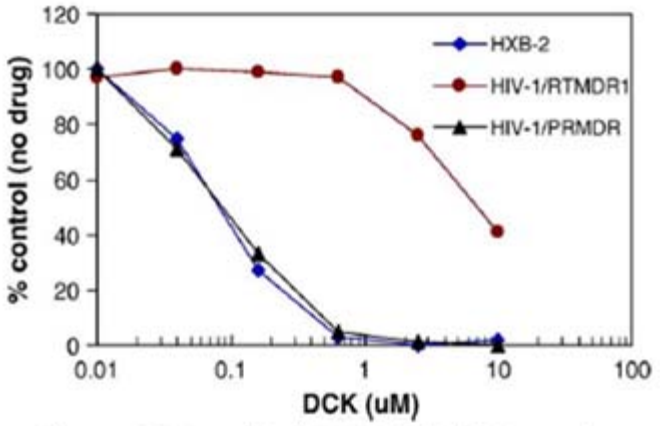


Figure 2-5. A multiple HIV-1 RT inhibitor-resistant strain is resistant to DCK **Adapted from Huang, et. al.**

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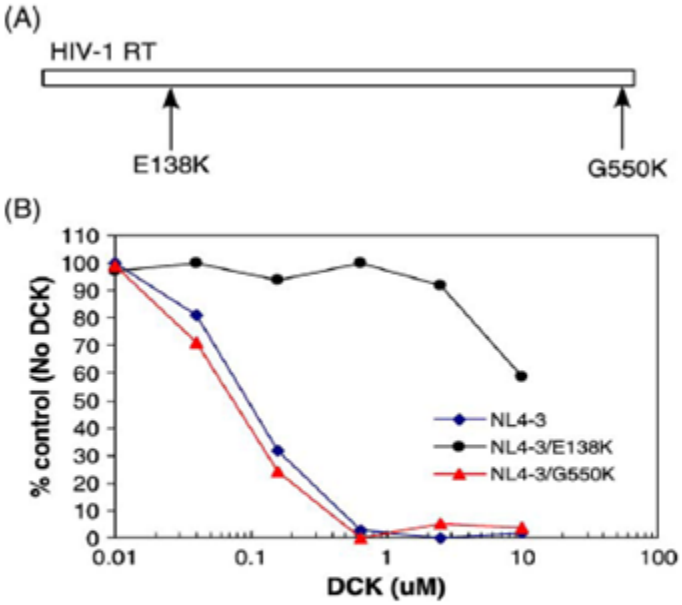


Figure 2-6. (A). Changes in amino acid residues in the HIV-1 RT of DCK escape variants. (B). An E138K mutation confers DCK-resistant phenotype. **Adapted from Huang, et. al.**

DCP and its analogs are DCK derivatives that were designed to improve the drug-resistance profile of DCK.[28] Compared with DCK, the major advantage of DCP analogs is their potential to overcome the drug-resistance problem against HIV-

1/RTMDR-1 strain. However, the DCK-resistant mutant, NL4-3/E138K, remains resistant to 2-EDCP (**69**). [33] These results strengthen the notion that the amino acid E138 in HIV-1 RT plays a key role in the anti-HIV-1 activity of DCK and 2-EDCP.

Finally, the ability of DCK to inhibit the DNA-dependent DNA polymerase (DDDP) activity of HIV-1 RT was determined. The results showed that DCK could inhibit the activity of HIV-1 when using poly-DNA as a template. (Figure 2-7)

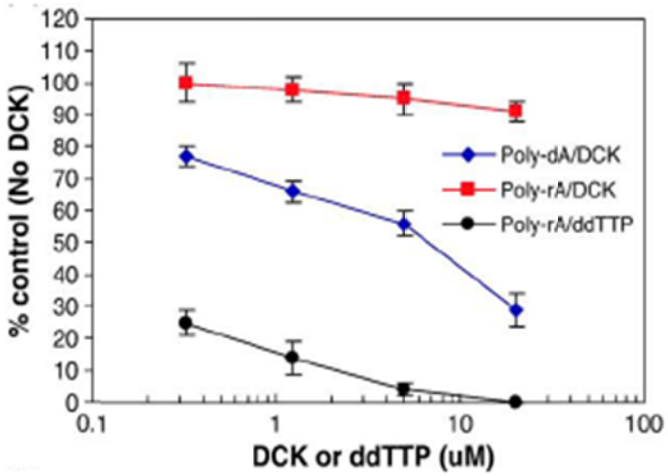


Figure 2-7. DCK inhibits the DNA-dependent DNA polymerase activity of HIV-1 RT. Poly-dA was used as templates to replace poly-rA in the HIV-1 RT assays. Adapted from Huang, et al.

From this series of mechanistic studies, the following conclusions were made.

(1) HIV-1 RT is the target of DCK and its derivatives;

(2) DCK could not inhibit RNA-dependent-DNA polymerase (RDDP) activity;

(3) DCK could inhibit DNA-dependent-DNA polymerase (DDDP) activity

2.5 Conclusions

Over several years of extensive research, great progress has been achieved in the discovery and modification of potential anti-HIV DCK and DCP derivatives. DCP

analogs displayed better potency than DCK against a HIV-RTMDR1 strain. The mechanism of action studies indicated that DCK and its derivatives target HIV-1 RT by interacting with the DNA-dependent DNA polymerase activity of HIV-RT, while most available NNRTIs function by inhibiting the RDDP activity of HIV-1. Therefore, DCK and DCP derivatives are structurally and mechanistically unique from clinically used anti-HIV agents. However, DCP derivatives show low water solubility and poor bioavailability, which limits their potential as drug candidates for clinical use. Therefore, it is necessary and urgent to discover and develop novel DCP analogs as drug candidates with potent anti-HIV activity and pharmaceutically desirable water solubility and bioavailability.

2.6 References

1. Pata, J. D.; Stirtan, W. G.; Goldstein, S. W.; Steitz, T. A., Structure of HIV-1 reverse transcriptase bound to an inhibitor active against mutant reverse transcriptases resistant to other nonnucleoside inhibitors. *Proc Natl Acad Sci U S A,* **2004,** 101, (29), 10548-10553.

2. Newman, D. J.; Cragg, G. M., Natural products as sources of new drugs over the last 25 years. *J Nat Prod,* **2007,** 70, (3), 461-77.

3. Lee, K. H., Current developments in the discovery and design of new drug candidates from plant natural product leads. *J Nat Pro,d* **2004,** 67, (2), 273-283.

4. Shi, Q.; Chen, K.; Morris-Natschke, S. L.; Lee, K. H., Recent progress in the development of tubulin inhibitors as antimitotic antitumor agents. *Curr Pharm Des,* **1998,** 4, (3), 219-248.

5. Wang, H. K.; Xia, Y.; Yang, Z. Y.; Natschke, S. L.; Lee, K. H., Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. *Adv Exp Med Biol,* **1998,** 439, 191-225.

6. Xia, Y.; Yang, Z. Y.; Morris-Natschke, S. L.; Lee, K. H., Recent advances in the discovery and development of quinolones and analogs as antitumor agents. *Curr Med Chem,* **1999,** 6, (3), 179-194.

7. Lee, K. H., Anticancer drug design based on plant-derived natural products. *J Biomed Sci ,***1999,** 6, (4), 236-250.

8. Kulkarni, M. V.; Kulkarni, G. M.; Lin, C. H.; Sun, C. M., Recent advances in coumarins and 1-azacoumarins as versatile biodynamic agents. *Curr Med Chem,* **2006,** 13, (23), 2795-2818.

9. Asha, K. N.; Chowdhury, R.; Hasan, C. M.; Rashid, M. A., Antibacterial activity and cytotoxicity of extractives from Uvaria hamiltonii stem bark. *Fitoterapia,* **2003,** 74, (1-2), 159-163.

10. Leal, L. K.; Ferreira, A. A.; Bezerra, G. A.; Matos, F. J.; Viana, G. S., Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. *J Ethnopharmacol,* **2000,** 70, (2), 151-159.

11. Lee, T. T.; Kashiwada, Y.; Huang, L.; Snider, J.; Cosentino, M.; Lee, K. H., Suksdorfin: an anti-HIV principle from Lomatium suksdorfii, its structure-activity correlation with related coumarins, and synergistic effects with anti-AIDS nucleosides. *Bioorg Med Chem,* **1994,** 2, (10), 1051-1056.

12. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K. H., Anti-AIDS agents. 15. Synthesis and anti-HIV activity of dihydroseselins and related analogs. *J Med Chem1* **1994,** 37, (23), 3947-3955.

13. Zhou, P.; Takaishi, Y.; Duan, H.; Chen, B.; Honda, G.; Itoh, M.; Takeda, Y.; Kodzhimatov, O. K.; Lee, K. H., Coumarins and bicoumarin from Ferula sumbul: anti-HIV activity and inhibition of cytokine release. *Phytochemistry,* **2000,** 53, (6), 689-697.

14. Reutrakul, V.; Leewanich, P.; Tuchinda, P.; Pohmakotr, M.; Jaipetch, T.; Sophasan, S.; Santisuk, T., Cytotoxic coumarins from Mammea harmandii. *Planta Med,* **2003,** 69, (11), 1048-1051.

15. Marquez, N.; Sancho, R.; Bedoya, L. M.; Alcami, J.; Lopez-Perez, J. L.; Feliciano, A. S.; Fiebich, B. L.; Munoz, E., Mesuol, a natural occurring 4-phenylcoumarin, inhibits HIV-1 replication by targeting the NF-kappaB pathway. *Antiviral Res,* **2005,** 66, (2-3), 137-145.

16. Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H., 2nd; McMahon, J. B.; Currens, M. J.; Buckheit, R. W., Jr.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R., The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, Calophyllum lanigerum. *J Med Chem,* **1992,** 35, (15), 2735-2743.

17. Kirkiacharian, S.; Thuy, D. T.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T., Structure-activity relationships of some 3-substituted-4-hydroxycoumarins as HIV-1 protease inhibitors. *Farmaco,* **2002,** 57, (9), 703-708.

18. Lunney, E. A.; Hagen, S. E.; Domagala, J. M.; Humblet, C.; Kosinski, J.; Tait, B. D.; Warmus, J. S.; Wilson, M.; Ferguson, D.; Hupe, D.; et al., A novel nonpeptide HIV-1 protease inhibitor: elucidation of the binding mode and its application in the design of related analogs. *J Med Chem,* **1994,** 37, (17), 2664-2677.

19. Janakiraman, M. N.; Mullen, C. R.; Strohback, J. W., Crystallography and the Design of Non-peptide HIV Protease nhibitors; 4-Hydroxycoumarins, 4-Hydroxy-2-pyrones, and Tetronic Acid. *American Crystallographic Association Annual Meeting,* **1995**.

20. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H., Anti-AIDS agents. 37. Synthesis and structure-activity relationships of (3'R,4'R)-(+)-cis-khellactone derivatives as novel potent anti-HIV agents. *J Med Chem,* **1999,** 42, (14), 2662-2672.

21. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H., Anti-AIDS agents. 33. Synthesis and anti-HIV activity of mono-methyl substituted 3',4'-di-O-(-)-camphanoyl-(+)-cis-khellactone (DCK) analogues. *Bioorg Med Chem Lett,* **1998,** 8, (16), 2151-2156.

22. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K. H., Anti-AIDS agents. 42. Synthesis and anti-HIV activity of disubstituted (3'R,4'R)-3',4'-di-O-(S)-camphanoyl-(+)-cis-khellactone analogues. *J Med Chem,* **2001,** 44, (5), 664-671.

23. Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H., Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med Res Rev,* **2003,** 23, (3), 322-345.

24. Xia, P.; Yin, Z. J.; Chen, Y.; Zhang, Q.; Zhang, B.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H., Anti-AIDS agents. Part 58: synthesis and anti-HIV activity of 1-thia-di-O-(-)-camphanoyl-(+)-cis-khellactone (1-thia-DCK) analogues. *Bioorg Med Chem Lett,* **2004,** 14, (12), 3341-3343.

25. Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, K. H., Anti-AIDS agents part 41: synthesis and anti-HIV activity of 3',4'-di-o-(-)-camphanoyl-(+)-cis-khellactone (DCK) lactam analogues. *Bioorg Med Chem Lett,* **2000,** 10, (10), 1003-1005.

26. Chen, Y.; Zhang, Q.; Zhang, B.; Xia, P.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H., Anti-AIDS agents. Part 56: Synthesis and anti-HIV activity of 7-thia-di-O-(-)-camphanoyl-(+)-cis-khellactone (7-thia-DCK) analogs. *Bioorg Med Chem,* **2004,** 12, (24), 6383-6387.

27. Wang, Y.; Huang, S. X.; Xia, P.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Morris-Natschke, S. L.; Lee, K. H., Anti-AIDS agents 72. Bioisosteres (7-carbon-DCKs) of the potent anti-HIV lead DCK. *Bioorg Med Chem Lett,* **2007,** 17, (15), 4316-4319.

28. Yu, D.; Chen, C. H.; Brossi, A.; Lee, K. H., Anti-AIDS agents. 60. Substituted 3'R,4'R-di-O-(-)-camphanoyl-2',2'-dimethyldihydropyrano[2,3-f]chromone (DCP) analogues as potent anti-HIV agents. *J Med Chem,* **2004,** 47, (16), 4072-4082.

29. Yu, D.; Brossi, A.; Kilgore, N.; Wild, C.; Allaway, G.; Lee, K. H., Anti-HIV agents. Part 55: 3'R,4'R-Di-(O)-(-)-camphanoyl-2',2'-dimethyldihydropyrano[2,3-f]chromone (DCP), a novel anti-HIV agent. *Bioorg Med Chem Lett,***2003,** 13, (9), 1575-1576.

30. Shafer, R.; Dupnik, K.; Winters, M.; Eshleman, S., Human Retrovirus and AIDS, Theoretical Biology and Biophysics. *Los Alamos National Laboratories,* **2001**, 1-51.

31. Lu, H.; Macosko, J.; Habel-Rodriguez, D.; Keller, R. W.; Brozik, J. A.; Keller, D. J., Closing of the fingers domain generates motor forces in the HIV reverse transcriptase. *J Biol Chem,* **2004,** 279, (52), 54529-54532.

32. Sarafianos, S. G.; Das, K.; Ding, J.; Boyer, P. L.; Hughes, S. H.; Arnold, E., Touching the heart of HIV-1 drug resistance: the fingers close down on the dNTP at the polymerase active site. *Chem Biol,* **1999,** 6, (5), R137-146.

33. Huang, L.; Yuan, X.; Yu, D.; Lee, K. H.; Chen, C. H., Mechanism of action and resistant profile of anti-HIV-1 coumarin derivatives. *Virology,* **2005,** 332, (2), 623-628.

CHAPTER 3

EFFICIENT MICROWAVE-ASSISTED ONE-POT PREPARATION OF ANGULAR 2,2-DIMETHYL-2*H*-CHROMONE CONTAINING COMPOUNDS

 Tetrahedron Letts. 2010, 33, 4382-4386.

3.1 Introduction

As introduced in Chapter 2, Suksdorfin (**1**), isolated from Lomatium *suksdorfii* was discovered in early 1994 to exhibit anti-HIV activity.[1] Continuing research led to the discovery of DCK and DCP analogs, some of which demonstrated much more significant anti-HIV activity than **1**. [2-5] 4-MDCK (**26**) and 2-EDCP (**69**) are potent representatives of these two series and were selected as lead compounds for further modification to investigate more potent and selective anti-HIV agents as clinical trial candidate. The skeletons of **1**, **26** and **69** share a similar motif, 2,2-dimethyl-2*H*-chromones (rings B and C shown in Figure 3-1). Since the synthesis of 2,2-dimethyl-2*H*-chromone motif is the first and the key step in the synthesis of DCK and DCP series, the efficiency of accomplishing this reaction dramatically affects the synthesis of the desired final products.

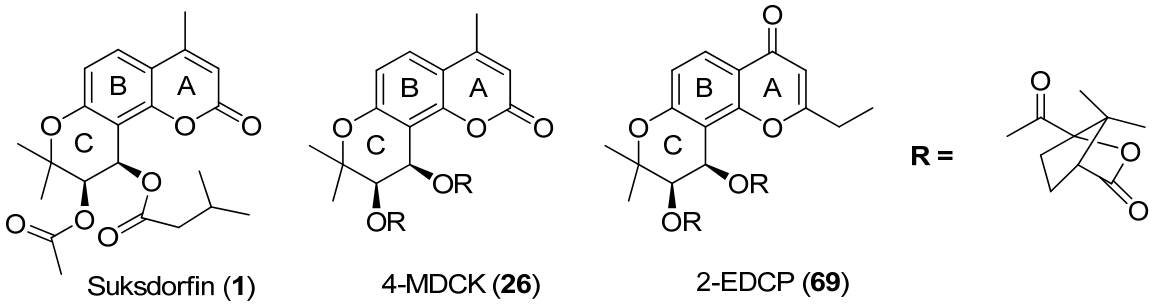


Figure 3-1. Structures of Suksdorfin (**1**), 4-MDCK (**26**) and 2-EDCP (**69**)

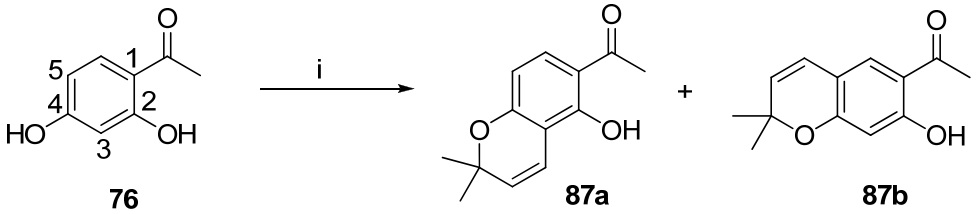
In our previous studies, different methods were utilized to construct **C** ring. In the investigation of DCKs, a two-step reaction was involved. a) nucleophilic substitution with 3-chloro-3-methyl-1-butyne, followed by b) Claisen rearrangement and cyclization in *N*,*N*-diethylaniline at reflux temperature over 200^{o}C.[3] This transformation generally needs over 48 hours and the yield averages below 40%. In the course of the investigation of DCP analogues, a one-pot synthesis succeeded, by which alkylation and cyclization were accomplished by addition of 4,4-dimethoxy-2-methyl-2-butanol gradually into the refluxing solution of 1-(2,4-dihydroxyphenyl) ethanone in pyridine (at approximately 140 ^{o}C). [5] The maximum yield of the desired product derived from this conversion, however, still remained low (< 40%) even with 48-hour reaction. Both conventional syntheses to make 2,2-dimethyl-2*H*-chromone were not time and yield efficient, partially attributing to the formation of a linear by-product (**b-** series as **87b** shown in scheme 3-1). Therefore, a more efficient synthetic approach is needed to shorten reaction time, increased yield of the desired product (**a**-series), and better control of the formation of the linear by-products in order for scaling-up synthesis,

Microwave (MW) synthesis has been considered as a useful approach in this study. One of benefits of MW synthesis is to manage the desired reaction under appropriate conditions (time, temperature, and pressure) to achieve the desired product in a reasonable high yield. In this paper, we report herein our recent study in design and conduction of a series of MW synthesis with variations of reaction duration, temperature, and reagent, in order to optimize the synthesis of angular 2,2-dimethyl-2*H*-chromones (**a**-series), a key and desired intermediate in our DCK and DCP analog synthesis.

3.2 Results

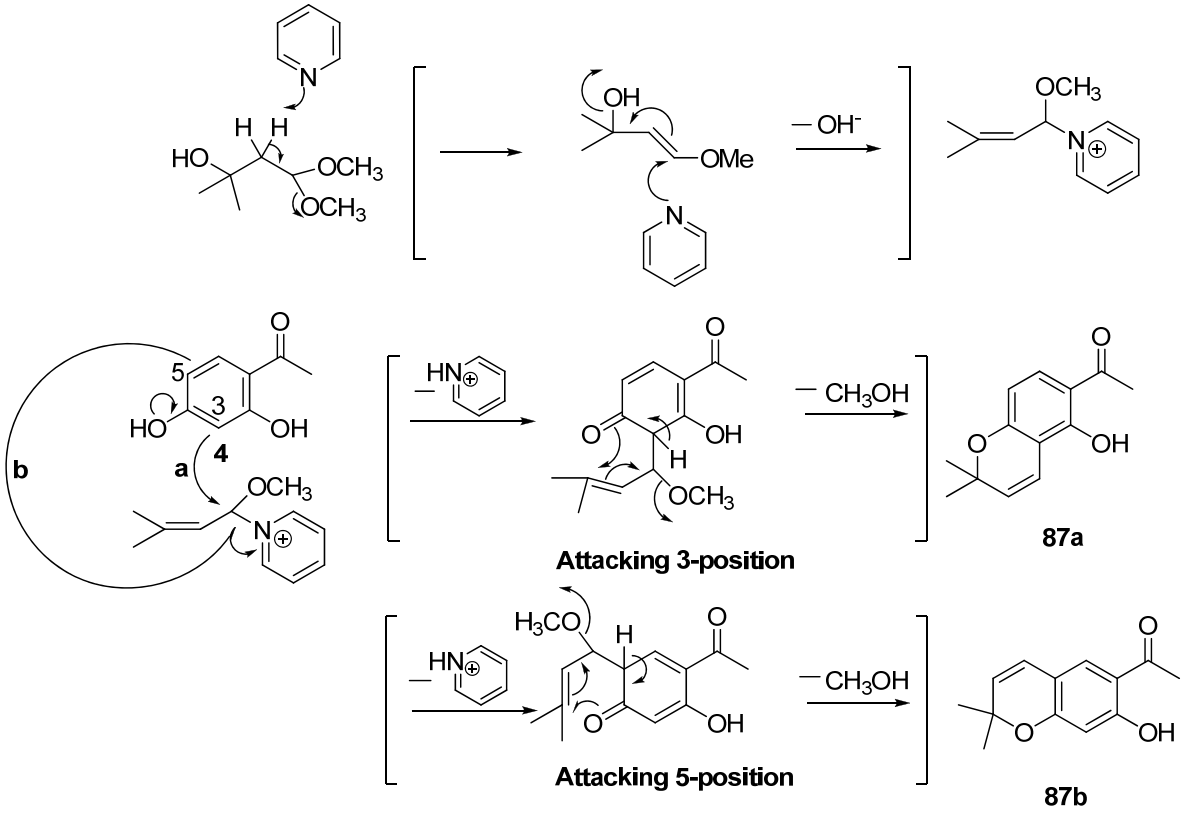
In our previous report, 2,2-dimethyl-2*H*-chromones, such as **87a** in Scheme 3-1, was synthesized by reaction of 1-(2,4-dihydroxyphenyl) ethanone with 4,4-dimethoxy-2-methyl-2-butanol in pyridine through alkylation and cyclization (38% of the best yield). The by-product **87b** was also obtained in 6.40 % (Scheme 3-1). The possible mechanism was proposed in scheme 3-2, which illustrated the formation of the desired angular product **87a** and the undesired by-product **87b**. The activated alkylation reagent, 4,4-dimethoxy-2-methyl-2-butanol initially attacks at electron-rich position 3 or 5 of the ethanone and leads the lone-pair electron of ketone oxo at 4-position to approach the electrophilic carbon, followed by the leaving of CH_3OH to form the angular (**a**-series) or linear (**b**-series) products. Using the same reagent, a series of experiments were designed and conducted in our MW synthesis study to search a better condition to selectively make **87a** than that from the conventional synthesis. The result was listed in Table 3-1. The reaction temperature was set up from 140 ^{o}C to 240 ^{o}C with 20 ^{o}C interval and the reaction duration was set up from

2 hours to 8 hours with two-hour interval. It was observed that the yields of **87a** and **b** increased with temperature rising and time extending. The best MW condition in this experiment is 220 ^{o}C / 4 hours, with the yield of **87a** up to 57.4%. Prolonging /shortening reaction time other than 4 hours or rising / lowering reaction temperature other than 220 ^{o}C resulted in lowered yield of **87a**. Although comparing with the best report reaction condition, at 140 ^{o}C for 48 hour, the yield of **87b** under MW condition also slightly increased (6.40% vs. 7.32%), this condition is still significantly improved and reasonably acceptable to reach the maximal amount of **87a**.



76 **87a** **87b**

Scheme 3-1. Synthesis of compounds **87 a-b**. Reagent and Condtions: (i) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave condition.



Scheme 3-2. Speculated mechanism for observed transformations

Table 3-1 . Yields of compounds 87a and 87b under varied microwave conditions

	Yield of compound **87a** (%)				Yield of compound **87b** (%)			
	2 h	4h	6h	8 h	2 h	4 h	6 h	8 h
140 ^{o}C	--- [a]	3.38	---	---	---	0.49	---	---
160 ^{o}C	---	15.2	23.1	23.8	---	1.40	2.33	2.47
180 ^{o}C	---	35.5	41.5	41.9	---	4.11	5.02	7.76
200 ^{o}C	---	45.7	49.3	54.0	---	6.06	6.27	7.14
220 ^{o}C	52.7	57.4	54.0	---	6.4	7.32	8.05	---
240 ^{o}C	---	38.0	35.8	---	---	5.92	5.60	---

[a]: not available

To verify the application of the optimized MW reaction condition, 3-hydroxy-9*H*-xanthane-9-one (**82**) was selected as the starting material to make the corresponding product (entry 7 in Table 3-3) under a series of MW conditions and the results were listed in Table 3-2. The best condition to achieve 55.7% of the

desired product **93a** was obtained at 220 ^{o}C for 4 hours as well, which is about 10 times better than the yield obtained from the traditional method utilizing the same alkylation reagent (4.34%, table 3-4, entry 7), suggesting that 220^{o}C/4h may be an applicable condition for different substances to make 2,2-dimethyl-2*H*-chromones related products, 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromones, 3,3-dimethyl-pyrano[2,3-*c*]xanthen-7(3*H*)-ones or other products, such as 3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acidin-7(12*H*)-one.

A scope of different substances which contain similar phenol moiety (B ring) and diverse fused A-rings were applied with the optimized MW condition. The results were listed in Table 3-3. It was observed that most reactions generated two new products besides the starting materials recovered, the desired product (**a**) and the undesired by-product (**b**)**,** except the entries 3 and 5 (Table 3-3), in which only the desired angular products (**a**) were monitored by TLC and MS. Generally, the desired angular products (**a**) were predominant compared to the undesired linear products **b** (Table 3-3), implying that the MW reaction condition is efficient and applicable to synthesize diverse desired products (**a**). The products, **a** and **b** were able to be separated over silica gel chromatograph and identified by NMR.

The data in table 3-3 further indicated that the reaction substances with alkyl groups, such as methyl and ethyl groups at 6-positoin of 1-(2,4-dihydroxyphenyl) ethanone (Table 3-3, entries 2-3) generated better yields of the desired products (66.4% for **88a** and 72.6% for **89a**, entries 2 and 3) than the yield of **87a** (57.4%, entry 1) though **88b** was a bit higher than **87b** . Interestingly, the undesired by-product **89b** was not detected under the applied reaction condition, with

approximately 25% of the starting material **78** recovered, suggesting a stereo favorable cyclization toward at the 3-position. With propan-1-one (**79**) as starting material (Table 3-3, entry 4), the desired **90a** was obtained in 63.5%, almost 6% higher than **87a** in entry 1, in which the ethan-1-one was used as the starting material, while the undesired **90b** was about 6% lower the undesired product **87b** in entry 1. Besides 3-hydroxy-9*H*-xanthane-9-one (**82**) mentioned above, a series of substances (**80-85**) with a fused A ring (Table 3-3, entries 5-10) were treated with 4,4-dimethoxy-2-methyl-2-butanol under the MW reaction condition. Corresponding products and the yields were listed in Table 3-3. Although the yields of the desired products were relatively low relative to the single ring reactants **76-79**, they are generally workable with much improved yields compared with the conventional reaction condition utilizing the same alkylation reagent, by which the yields of the desired products **91a, 92a**, and **93a** are 13.5%, 23.9%, and 4,34%, respectively (Table 3-4). Unexpectedly, **91b** was not detected under both conditions though the yield of **91a** was not comparably high. The starting material **80** was mainly recovered. The preparation of compound **94a** was reported previously by reaction of 1,3-dihydroxy-xanthen-9-one (**83**) with 2-chloro-2-methylbutyne. After a two-step reaction, the desired product was received in a considerable low yield of 12.5%.[6] With the MW initiation condition, the yield increased up to near 40%. A substantial mass of **b**-type product was also obtained (**94b,** Table 3-3), suggesting that the 3-hydroxyl group may play a role in assisting the formation of the liner **b**-type product. Substances with methyl substitution at 6 or 7 position (**84** and **85**) yielded less amount of **b**-products (**95b** and **96b**) relative to **94b**, though the yields of the desired

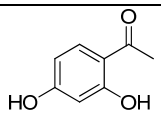
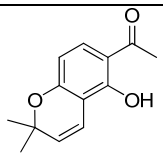
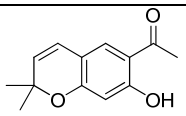
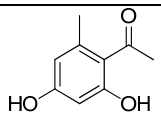
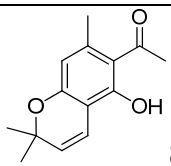
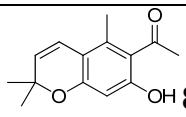
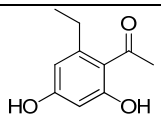
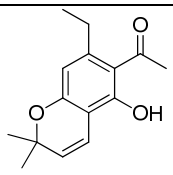
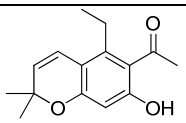
a-products (**95a** and **96a**) gave no significant change comparing to **94a**. The synthesis of compound **97a** was reported previously, starting with 2-methyl-3-butyn-2-ol through two or multiple-steps reactions, to have a total yield less than 20% (Table 3-4).[7, 8] Under the optimized MW condition, we successfully synthesized **97a** from 1,3-dihydroxy-10-methylacridin-9(10*H*)-one (**86**) in an improved yield of 36.2%.

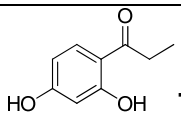
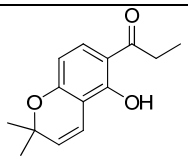
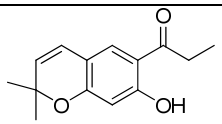
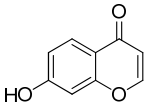
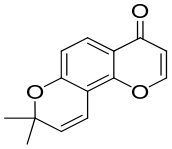
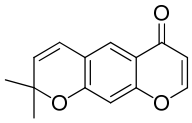
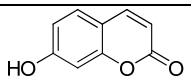
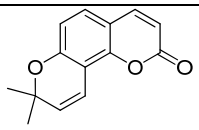
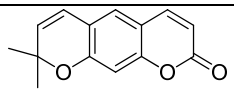
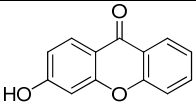
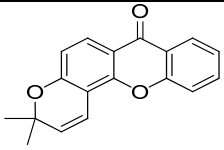
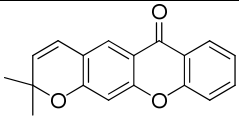
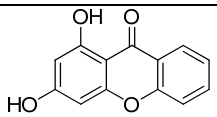
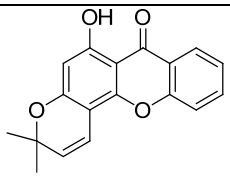
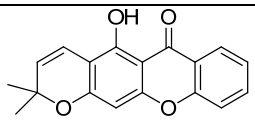
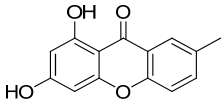
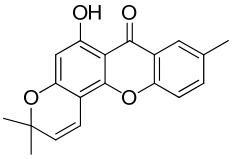
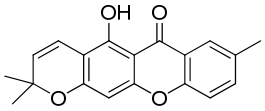
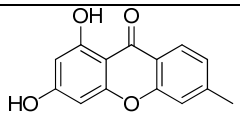
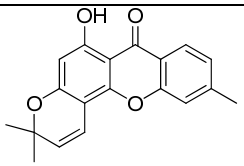
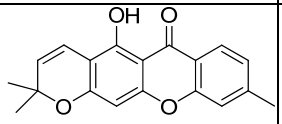
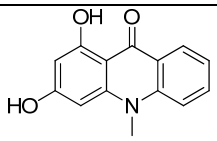
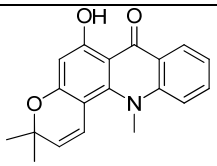
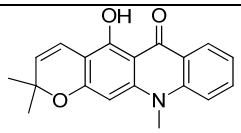
Table 3-2. Yileds of compounds 93a and 93b under varied temperature and reaction time.

	Yield of compound **93a** (%)		Yield of compound **93b** (%)	
	4 h	6 h	4 h	6 h
180	38.4	44.8	2.94	2.14
200	49.7	--- [a]	3.62	---
220	55.7	---	4.08	---
240	52.6	---	4.58	---

[a]: not available

Table 3-3. Products and yields derived from diverse starting materials under the microwave condition (220 ^{o}C, 4 hours)**.**

Entry	Starting Material	Angular product a	Linear product b	Yield of a	Yield of b
1	 **76**	 **87a**	 **87b**	57.4	7.32
2	 **77**	 **88a**	 **88b**	66.4	9.54
3	 **78**	 **89a**	 **89b**	72.6	N/A[a]

4	 **79**	 **90a**	 **90b**	63.5	2.65
5	 **80**	 **91a**	 **91b**	40.3	N/A
6	 **81**	 **92a**	 **92b**	31.6	5.47
7	 **82**	 **93a**	 **93b**	55.7	4.08
8	 **83**	 **94a**	 **94b**	38.9	27.2
9	 **84**	 **95a**	 **95b**	42.6	17.4
10	 **85**	 **96a**	 **96b**	38.1	7.05
11	 **86**	 **97a**	 **97b**	36.2	4.33

[a]: not detectable by MS and TLC

Table 3-4. Comparisons of conventional and microwave syntheses.

Entry / Products	Conventional heating system (140 °C/48h for entries 1, 5-7)	MW condition (220°C/4h) (%) [a]

	(%) [a]			
	Angular product **a**	Linear product **b**	Angular product **a**	Linear product **b**
1 / **86**	38	6.40	57.4	7.32
5 / **91**	13.6	N/A	40.3	N/A
6 / **92**	23.9	2.55	31.6	5.47
7 / **93**	4.34	1.2	55.7	4.08
8 / **94**[6, b]	12.5	6.3	38.9	27.2
14 / **97** [7, c]	20%	--- [d]	36.2	4.33

[a] alkylation reagent: 2,2-dimethoxy-2-methyl-2-butanol. [b] 2-chloro-2-methylbutyne, two-step reaction. [c] alkylation reagent: 2-methyl-3-butyn-2-ol, two-step reaction. [d] not available in the reference.

3.3 Discussion and Conclusions:

In this research work, we were able to first and successfully utilize microwave initiation method to synthesize the desired angular 2,2-dimethyl-2*H*-chromones, the key intermediate of the synthesis of DCP and DCK. Through alkylation and cyclyzation, with an appropriate starting substance and alkylation reagent, a series of desired 2,2-dimethyl-2*H*-chromones related products, 8,8-dimethyl-8*H*-pyrano[2,3-f]chromones, 3,3-dimethyl-pyrano[2,3-*c*]xanthen-7(3*H*)-ones or other products, such as 3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acidin-7(12*H*)-one were obtained in an one pot reaction. Comparing to literature reported methods, the newly developed microwave synthesis condition dramatically shortened the reaction time from 2 days to 4 hours with much higher to comparable yields. Increasing reaction temperature from 140 to 220 °C and extending reaction time favor the formation of both **a-** and **b-** products with a relatively less increasing rate for the undesirable **b**-product. Although the yield of the desired products are still not ideal, in comparison to literature reports with conventional heating conditions, the currently optimized MW condition demonstrated significant improvement in selectively synthesis of the desired products 2,2-dimethyl-

2*H*-chromones, 8,8-dimethyl-8*H*-pyrano[2,3-f]chromones and other products, such as 3,3-dimethyl-pyrano[2,3-*c*]xanthen-7(3*H*)-ones.

We also analyzed the factors that might affect the yield and regioselectivity in this reaction. Electronic effect on B ring influenced significantly on reaction yield as well as regioselectivity. With electron-donating groups, such as alkyl groups at 6-postion of 1-(2,4-dihydroxyphenyl) ethanone could increase the electron density at 3-position, which consequently enhanced alkylation reactivity. The lone-pair group on hydroxyl group introduced at 1-position of xanthenone (Table 3-1, entries 8-10) results in higher electron density at 2-position and therefore reduces the regioselectivity between **a-** and **b**- products. In addition, steric effect of the substituents may also play a role in the alkylation and cyclization. Introducing ethyl group on 6-position (Compound 6, table 3, entry 3) blocked the alkylation reagent occurring at 5 position, which led to the desired product **89a** predominately.

The significant advancement demonstrated in this study is that, the MW method with the optimized condition can be wildly utilized in diverse ring systems, including phenoyl, chromone, xanthenone as well as acridinone, which dramatically broadens the possibility of further efficiently exploring DCK and DCP analogs as novel anti-HIV agents. This work is currently on-going in authors' labs and the exciting results will be reported shortly.

3.4 Experimental Section

The proton nuclear magnetic resonance (1H NMR) spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The

solvent used was $CDCl_3$ unless indicated. Microwave reactions were performed with a Biotage initiator EXP US. Mass spectra were measured on Shimadzu LCMS-2010 (ESI-MS). Biotage Flash and Isco Companion systems were used as medium-pressure column chromatography. All other chemicals were obtained from Aldrich.

General procedure for microwave-assisted synthesis of 2,2-dimethyl-2*H*-chromone containing compounds.

Diverse ethanone,s bi or tri-cycling starting materials (**76**-**86**, 1 equiv) , 4,4-dimethoxy-2-methyl-2-butanol (2.5 equiv) and anhydrous pyridine were added into microwave vial and sealed. Pre-stir for 20sec then increase the reaction temperature to 220^{o}C for 4 hours under high microwave absorption condition. At completion, the reaction mixture was cooled to room temperature, diluted with EtOAc and washed with aqueous HCl (10%) and brine, separately. The organic layer was collected and the solvent was removed under vacuum. The residue was purified by column chromatography (hexane:EtOAc = 97:3) to afford corresponding products.

6-Acetyl-2,2-dimethyl-5-hydroxy-2*H*-chromone (87a). MS (ESI+) m/z (%) 219 (M^+ + 1, 100); ^{1}NMR δ 7.52 (1H, d, *J* = 8.7 Hz, H-7), 6.72 (1H, d, *J* = 7.5 Hz, H-4), 6.33 (1H, d, *J* = 8.7 Hz, H-8), 6.58 (1H, d, *J* = 7.5 Hz, H-3), 2.54 (3H, s, $COCH_3$-1), 1.45 (6H, s, CH_3-2,2).

1(7-Hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)ethanone (87b). MS (ESI+) m/z (%) 219 (M^+ + 1, 100); ^{1}NMR δ 7.31 (1H, s, H-8), 6.33 (1H, s, H-5), 6.28 (1H, d, *J* = 9.6 Hz, H-4), 5.58 (1H, d, *J* = 9.6 Hz), 2.54 (3H, s, $COCH_3$-6), 1.45, 1.44 (each 3H, CH_3-2,2).

6-Acetyl-2,2,7-trimethyl-5-hydroxy-2*H*-chromone (88a). mp 56-67 ^{o}C; MS (ESI+) m/z (%) 233 (M^{+} + 1, 100); ^{1}NMR δ 6.69 (1H, d, *J* = 10.2 Hz, H-4), 6.19 (1H, s, H-8), 5.52 (1H, d, *J* = 10.2 Hz, H-3), 3.31 (3H, s, $COCH_3$-1), 2.53 (3H, s, CH_3-7), 1.43 (6H, s, CH_3-2,2).

1-(7-Hydroxy-2,2,5-trimethyl-2H-chromen-6-yl)ethanone (88b). MS (ESI+) m/z (%) 233 (M^{+} + 1, 100); ^{1}NMR δ 6.54 (1H, d, *J* = 10.2 Hz, H-4), 6.27 (1H, s, H-8), 5.66 (1H, d, *J* = 10.2 Hz, H-3), 2.60 (3H, s, $COCH_3$-6), 2.49 (3H, s, CH_3-5), 1.42, 1.42 (each 3H, s, CH_3-2,2).

6-Acetyl-2,2-dimethyl-5-hydroxy-7-ethyl-2*H*-chromone (89a). MS (ESI+) m/z (%) 247 (M^{+} + 1, 100%); ^{1}H NMR δ 6.70 (1H, d, *J* = 10.2 Hz, H-4), 6.25 (1H, s, H-8), 6.52 (1H, d, *J* = 10.2 Hz, H-3), 2.86 (2H, q, *J* = 7.5 Hz, CH_2CH_3-7), 2.64 (3H, s, CH_3CO-6), 1.42, 1.41 (each 3H, s, CH_3-2,2), 1.26 (3H, t, *J* = 7.5 Hz, CH_2CH_3-7).

1-(5-Hydroxy-2,2-dimethylo-2H-chromen-6-yl)propan-1-one (90a). MS (ESI+) m/z (%); ^{1}H NMR δ 7.56 (1H, d, *J* = 9.0 Hz, H-7), 6.73 (1H, d, *J* = 9.6 Hz, H-4), 6.34 (1H, d, *J* = 9.0 Hz, H-8), 5.59 (1H, d, *J* = 9.6 Hz, H-3), 2.94 (2H, q, *J* = 7.5 Hz, $COCH_2CH_3$-6), 1.45, 1.44 (each 3H, s, CH_3-2,2), 1.23 (3H, t, J = 7.5 Hz, $COCH_2CH_3$-6).

2',2'-Dimethyl-pyrano[2,3,*f*]chromone (91a). MS (ESI+) m/z (%); ^{1}H NMR δ 7.98 (1H, d, *J* = 8.7 Hz, H-5), 7.81 (1H, d, *J* = 6.3 Hz, H-2), 6.85 (1H, d, *J* = 8.7 Hz, H-6), 6.79 (1H, d, *J* = 10.2 Hz, H-4'), 6.28 (1H, d, *J* = 6.3 Hz, H-3), 5.72 (1H, d, *J* = 10.2 Hz, H-3'), 1.49, 1.49 (each 3H, s, CH_3-2',2').

Seselin (92a). MS (ESI+) m/z (%); ^{1}H NMR δ 7.60 (1H, d, *J* = 9.6 Hz, H-4), 7.21 (1H, d, *J* = 8.8 Hz, H-5), 6.89 (1H, d, *J* = 10.0 Hz, H-10), 6.73 (1H, d, *J* = 8.8 Hz, H-6), 6.23 (1H, d, *J* = 9.6 Hz, H-3), 5.74 (1H, d, *J* = 10.0 Hz, H-9), 1.48, 1.48 (each 3H, s, CH_3-8,8).

8,8-Dimethylpyrano[3,2-*g*]chromen-2(8*H*)-one (92b). MS (ESI+) m/z (%); ^{1}H NMR δ 7.58 (1H, d, *J* = 9.2 Hz, H-4), 7.05 (1H, s, H-5), 6.72 (1H, s, H-10), 6.35 (1H, d, *J* = 10.0 Hz, H-6), 6.23 (1H, d, *J* = 9.2 Hz, H-3), 5.70 (1H, d, *J* = 10.0 Hz, H-7), 1.47, 1.47 (each 3H, s, CH_3-8,8).

3,3-Dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (93a). MS (ESI+) m/z (%); ^{1}H NMR δ 8.34 (1H, dd, *J* = 8.1, 1.5 Hz, H-8), 8.15 (1H, d, *J* = 8.7 Hz, H-6), 7.71 (1H, t, *J* = 8.1, 8.1 Hz, H-10), 7.50 (1H, d, *J* = 8.1 Hz, H-11), 7.38 (1H, t, *J* = 8.1, 8.1 Hz, H-9), 7.00 (1H, d, *J* = 9.9 Hz, H-1), 6.85 (1H, d, *J* = 8.7 Hz, H-5), 5.76 (1H, d, *J* = 9.9 Hz, H-2). 1.53, 1.53 (each 3H, s, CH_3-2,2).

2,2-Dimethylpyrano[3,2-*b*]xanthen-6(2*H*)-one (93b). MS (ESI+) m/z (%); ^{1}H NMR δ8.31 (1H, d, *J* = 8.0 Hz, H-7), 7.94 (1H, s, H-5), 7.68 (1H, t, *J* = 8.4, 7.2 Hz, H-9), 7.45 (1H, d, *J* = 8.4 Hz, H-10), 7.36 (1H, t, *J* = 8.0, 7.2 Hz, H-8), 6.81 (1H, s, H-12), 6.47 (1H, d, *J* = 10.0 Hz, H-4), 5.73 (1H, d, *J* = 10.0 Hz, H-3), 1.50, 1.50 (each 3H, s, CH_3-2,2).

6-Hydroxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (94a). MS (ESI+) m/z (%) ; ^{1}H NMR δ 12.97 (1H, s, OH-6), 8.26 (1H, dd, *J* = 8.1, 1.8 Hz, H-8), 7.72 (1H, t, *J* = 8.4, 6.9 Hz, H-10), 7.46 (1H, d, *J* = 8.4 Hz, H-11), 7.38 (1H, t, *J* = 6.9, 8.1

Hz, H-9), 6.85 (1H, d, *J* = 9.9 Hz, H-1), 6.27 (1H, s, H-5), 5.63 (1H, d, *J* = 9.9 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-2,2).

5-Hydroxy-2,2-dimethylpyrano[3,2-*b*]xanthen-6(2*H*)-one (94b). MS (ESI+) m/z (%) ; ^{1}H NMR *δ* 13.17 (1H, s, OH-5), 8.24 (1H, dd, *J* = 7.8, 1.5 Hz, H-7), 7.70 (1H, t, *J* = 8.4, 7.8 Hz, H-9), 7.44 (1H, d, *J* = 8.4 Hz, H-10), 7.37 (1H, t, *J* = 7.8,7.8 Hz, H-8), 6.75 (1H, d, *J* = 10.2 Hz, H-4), 6.36 (1H, s, H-12), 5.62 (1H, d, *J* = 10.2 Hz, H-3), 1.49, 1.49 (each 3H, s, CH_3-2,2).

6-Hydroxy-3,3,9-trimethylpyrano[2,3-c]xanthen-7(3*H*)-one (95a). MS (ESI+) m/z (%); ^{1}H NMR *δ* 13.0 (1H, s, OH-6), 8.02 (1H, d, *J* = 2.7 Hz, H-8), 7.53 (1H, dd, *J* = 8.4, 2.7 Hz, H-10), 7.37 (1H, d, *J* = 8.4 Hz, H-11), 6.84 (1H, d, *J* = 9.9 Hz, H-1), 6.26 (1H, s, H-5), 5.61 (1H, d, *J* = 9.9 Hz), 2.47 (3H, s, CH_3-9), 1.56, 1.49 (each 3H, s, CH_3-2,2).

5-Hydroxy-2,2,8-trimethylpyrano[3,2-*b*]xanthen-6(2*H*)-one (95b). MS (ESI+) m/z (%); ^{1}H NMR *δ* 13.23 (1H, s, OH-5), 8.01 (1H, d, *J* = 2.4 Hz, H-7), 7.51 (1H, dd, *J* = 8.7, 2.4 Hz, H-9), 7.33 (1H, d, *J* = 8.7 Hz, H-10), 6.75 (1H, d, *J* = 9.9 Hz, H-4), 6.34 (1H, s, H-12), 5.61 (1H, d, *J* = 9.9 Hz, H-3), 2.46 (3H, s, CH_3-8), 1.56, 1.48 (each 3H, s, CH_3-2,2).

6-Hydroxy-3,3,10-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (96a). MS (ESI+) m/z (%); ^{1}H NMR *δ* 13.02 (1H, s, OH-5), 8.10 (1H, d, *J* = 8.7 Hz, H-8), 7.24 (1H, s, H-11), 7.15 (1H, d, *J* = 8.7 Hz, H-9), 6.82 (1H, d, *J* = 9.9 Hz, H-1), 6.25 (1H, s, H-5), 5.61 (1H, d, *J* = 9.9 Hz, H-2), 2.50 (3H, s, CH_3-10), 1.49, 1.49 (each 3H, s, CH_3-2,2).

5-Hydroxy-2,2,9-trimethylpyrano[3,2-*b*]xanthen-6(2*H*)-one (96b). MS (ESI+) m/z (%); ^{1}H NMR δ 13.23 (1H, s, OH-5), 8.10 (1H, d, *J* = 8.1 Hz, H-7), 7.21 (1H, s, H-10), 7.18 (1H, d, *J* = 8.1 Hz, H-8), 6.74 (1H, d, *J* =10.2 Hz, H-4), 6.33 (1H, s, H-12), 5.61 (1H, d, *J* = 10.2 Hz, H-3), 2.49 (3H, s, CH_3-9), 1.48, 1.48 (each 3H, s, CH_3-2,2).

6-Hydroxy-3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acridin-7(12*H*)-one (97a). MS (ESI+) m/z (%);^{1}H NMR δ 13.14 (1H, s, OH-6), 8.47 (1H, d, *J* = 7.8 Hz, H-8), 7.72 (1H, t, *J* = 8.4, 4.2 Hz, H-10), 7.49 (1H, d, *J* = 8.4 Hz, H-11), 7.30 (1H, t, *J* = 7.8, 4.2 Hz, H-9), 6.81 (1H, d, *J* = 9.9 Hz, H-1), 6.32 (1H, s, H-5), 5.60 (1H, d, *J* =9.9 Hz, H-2), 3.80 (3H, s, CH_3-12), 1.49, 1.49 (each 3H, s, CH_3-2,2).

5-Hydroxy-2,2,11-trimethyl-2*H*-pyrano[3,2-*b*]acridin-6(11*H*)-one (97b). MS (ESI+) m/z (%);^{1}H NMR δ 13.20 (1H, s, OH-5), 8.34 (1H, d, *J* = 8.4 Hz, H-7), 7.68 (1H, t, *J* = 8.4, 4.2 Hz, H-9), 7.40 (1H, d, *J* = 8.4 Hz, H-10), 7.27 (1H, t, *J* = 8.4, 4.2 Hz, H-8), 6.53 (1H, d, *J* = 9.6 Hz, H-4), 6.23 (1H, s, H-12), 5.47 (1H, d, *J* = 9.6 Hz, H-3), 3.87 (3H, s, CH_3-11), 1.49, 1.49 (each 3H, s, CH_3-2,2).

3.5 Reference

1. Lee, T.; Kashiwada, Y.; Huang, L.; Snider, J.; Cosentino, M.; Lee, K. H. *Bioorg. Med. Chem.* **1994,** *2*, 1051-1056.

2. Xie, L.; Crimmins, M. T.; Lee, K. H. *Tetrahedron Lett.* **1995,** *36*, 4529-4532.

3. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2662-2672.

4. Yu, D.L.; Brossi, A.; Kilgore, N.; Wild, C.; Allaway, G.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003,** *13*, 1575-1576.

5. Yu, D.L.; Chen, C. H.; Brossi, A.; Lee, K. H. *J. Med. Chem.* **2004,** *47*, 4072-4082.

6. Schneider, J.; Evans, E. L.; Grunberg, E.; Fryer, R. I. *J. Med. Chem.* **1972,** *15*, 266-270.

7. Motiur Rahman, A. F.; Liang, J. L.; Lee, S. H.; Son, J. K.; Jung, M. J.; Kwon, Y.; Jahng, Y. *Arch. Pharm. Res.* **2008**, *31*, 1087-1093.

8. Reisch, J.; Voerste, A. A. W.; Top, M.; Dziemba, P. *Monatshefte fur chemie.* **1992**, *123*, 473-475.

CHAPTER 4

DESIGN, SYNTHESIS, MOLECULAR MODELING AND STRUCTURE-ACTIVITY RELATIONSHIP OF NOVEL DICAMPHANOYL-2',2'-DIMETHYLDIHYDROPYRANOCHROMONE (DCP) ANALOGS AS POTENT ANTI-HIV AGENTS

 Bioorg. Med. Chem. 2010, 18, 6678-6689.

4.1 Introduction

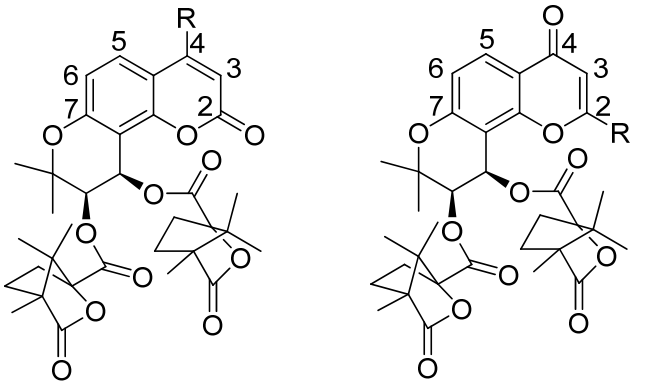
As introduced in Chapter 2, over 30 formulations have been approved by the US FDA to treat AIDS, however, drug resistance problems have dramatically reduced the efficacy of these current anti-HIV agents.[1] Therefore, research to find new anti-HIV agents with either higher potency or novel mechanisms has attracted great attention to overcome this problem. [2]

In our prior studies, DCK (**18**) and 4-MDCK, (**26**) showed high potency against HIV-1_{IIIB} replication in H9 lymphocytes. The EC_{50} and therapeutic index (TI) values were reported as 0.049 μM and 328 for DCK, and, 0.0059 μM and 6660 for 4-MDCK, respectively (Figure 4-1).[3, 4] More specifically, preliminary mechanism of action-related studies indicated that 4-MDCK inhibited the activity of HIV-RT through inhibition of DNA-dependent DNA polymerase activity, in contrast to currently available NNRTIs that block HIV-RT by inhibiting RNA-dependent DNA polymerization.[5] However, DCK had reduced activity against the multi-RT inhibitor resistant (RTMDR-1) strain. In the course of our continuing exploration of DCK analogs as potent anti-HIV agents, 4*H*-chrom-4-one derivatives (DCPs) were

designed and synthesized as DCK positional isomers (Figure 1).[6, 7] Compared with DCKs, DCP analogs not only retained high activity against wild-type HIV, but also showed potency against HIV/RTMDR-1.[7] Among the previously reported DCP derivatives, 2-EDCP (**69**) exhibited the best anti-HIV activity against both wild-type and drug-resistant strains with EC_{50} values of 0.070 and 0.11 μM and TI values of 94 and 60, respectively. The uniqueness of DCP analogs opens a new avenue for us to discover a distinct class of potent, effective anti-HIV drugs for AIDS therapy.

The structure-activity relationship (SAR) information provided from our previous study on the DCP series led to the following conclusions. Steric effects of substitutions on position-2, -3, and -6 of the chromone system could influence the anti-HIV activity. Bulky substituents at position-3 or -6 dramatically reduced anti-HIV activity. In addition, appropriate alkyl substitution at position-2 was crucial to maintain high activity against both wild-type and multi-RT inhibitor-resistant strains. 2-EDCP (**69**), with an ethyl group at position-2 of the chromone ring, exhibited the most potent activity against both virus strains.

However, the preliminary SAR information on DCPs was not extensive enough to establish a feasible pharmacological profile. Except for the steric effect, prior data could not illustrate how other factors such as electronic and hydrogen-bond effects might influence activity. In addition, all active DCP analogs synthesized had poor water-solubility. Therefore, additional DCP analogs with varying substituents, particularly different from the prior analogs, are needed in the search for an optimal anti-HIV-1 drug candidate from this compound class.



18 DCK, R = H
26 4-MDCK, R = CH_3

65 DCP, R = H
69 2-EDCP, R = CH_2CH_3

Figure 4-1. DCK and DCP analogs

In our present study, DCP analogs with different structural functionalities on the pyranochromone have been synthesized towards this aim. We first designed and synthesized several 5-alkyl-substituted DCPs to explore the steric effect at position-5, which was not a major focus in prior studies. Then, we introduced combinations of diverse functional groups at position-2, -3, -5 and -6, including halogen, cyano, and amino groups, to explore electronic and hydrogen-bonding effects. Furthermore, we introduced hydrophilic heterocyclic amine moieties at position-2 to generate compounds with better water solubility. All newly synthesized DCPs were evaluated for their activity against both wild-type and RTMDR-1 strains. Two of the active and more polar compounds, **98** and **115**, were selected for water solubility analysis in comparison with the active lead compound **69**.

A quantitative structure-activity relationship (QSAR) molecular modeling study was also performed in this research using Partial Least Square (PLS) method with QSAR-Model module of MOE 2009 to systematically study the structure-anti-HIV-activity relationships of DCP-class compounds. With this study, we aimed not only to

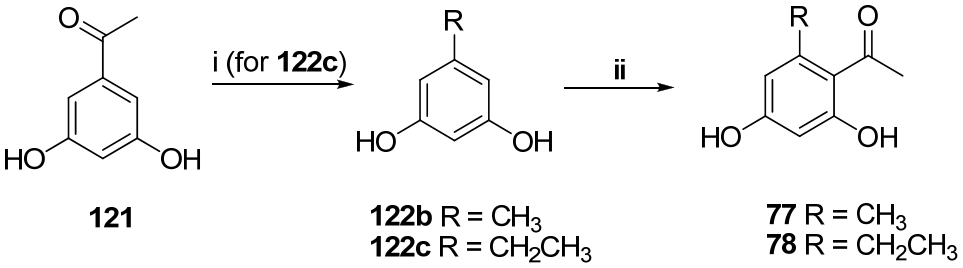
establish a pharmacological profile of DCPs, but also to study the DCP pharmacophores that play an important role in anti-HIV activity.

In this paper, we report and discuss the chemistry and synthesis of the newly synthesized DCP analogs, the results of anti-HIV activity evaluation, water solubility analysis, QSAR-model and pharmacophore studies, as well as structure-anti-HIV activity relationship conclusions resulting from the studies.

4.2 Results and Discussion

4.2.1 Chemistry

Scheme 4-1 illustrates the synthesis of 6-methyl- and 6-ethyl-2,4-dihydroxyphenyl ethanones (**77-78**, respectively). Compounds **122b** (commercially available) and **122c** [synthesized by reduction of 3′,5′-dihydroxyacetophenone (**121**)] were acylated through a Friedel-Crafts reaction in the presence of the Lewis acid $ZnCl_2$ to afford **77** and **78**, respectively (Scheme 4-1).[8, 9, 10]



121 **122b** R = CH_3; **122c** R = CH_2CH_3 **77** R = CH_3; **78** R = CH_2CH_3

Scheme 4-1. Synthesis of compounds **77, 78**. Reagents and conditions: (**i**) Pd/C, H_2, 4% HCl, rt.; (**ii**) CH_3CN, HCl (g), $ZnCl_2$, diethyl ether, 0°C.

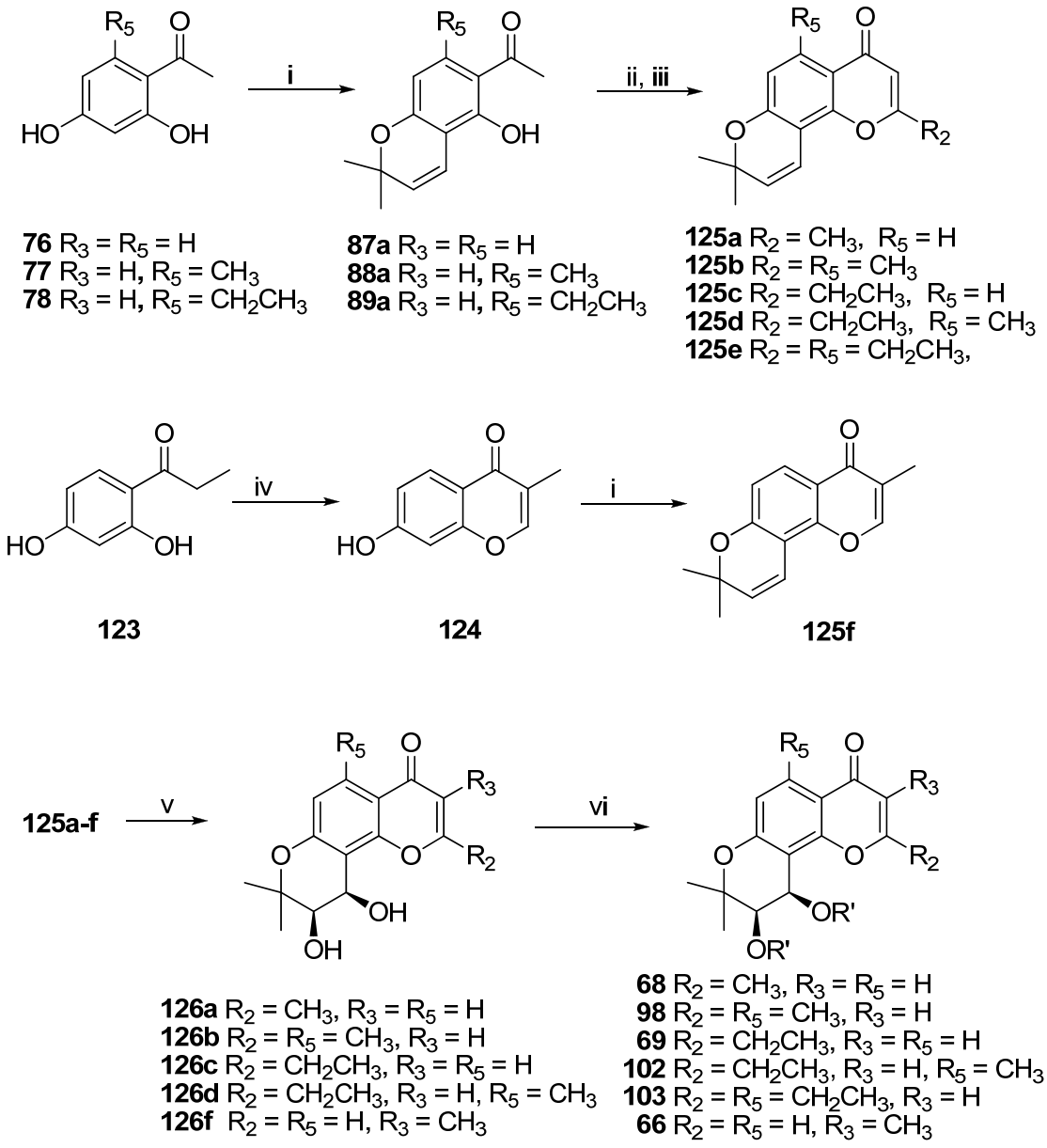
The synthesis of 2,3,5-alkyl substituted DCP analogs is shown in Scheme 4-2. Commercially available 1-(2,4-dihydroxyphenyl)ethanone (**76**) and the synthesized

77, **78** were converted to **87a**-**89a** by alkylation with 4,4-dimethoxy-2-methyl-2-butanol in pyridine. This reaction was conducted using the modified microwave-assisted method described in chapter 3 by reaction of ethanone and butanol in a microwave initiator at 220 °C for 4 h.[11] Compounds **66**, **68**-**69**, **98**, and **102**-**103** were synthesized following literature procedures.[7] Briefly**,** reaction of pyrano-ring-closure products with diverse ethyl alkanoates in the presence of NaH followed by hydrolysis with Amberlyst 15 resin in isopropanol afforded the chromone ring closure products (**125a**–**e**). 2',2'-Dimethyl-3-methylpyaranochrmone (**125f**) was synthesized from propiophenone (**123**) in two steps. Commercially available **123** was treated with methanesulfonyl chloride in dry DMF to afford 7-hydroxy-3-methyl-chromone (**124**).[12] Compound **124** was converted to the corresponding pyranochromone (**125f**) by alkylation with 4,4-dimethoxy-2-methyl-2-butanol in pyridine under microwave conditions. The asymmetric dihydroxylation of **125a**-**f** was accomplished using a catalytic Sharpless asymmetric dihydroxylation,[13,14] in which $K_2OsO_2(OH)_4$ served as catalyst and $(DHQ)_2PYR$ as chiral auxiliary.[14,15] After drying in vacuo overnight, the diols (**126a-f**) were reacted with excess (*S*)-camphanoyl chloride in anhydrous dichloromethane in the presence of excess DMAP at room temperature for 2 h to afford the target compounds **66, 68-69**, **98** and **102-103.**

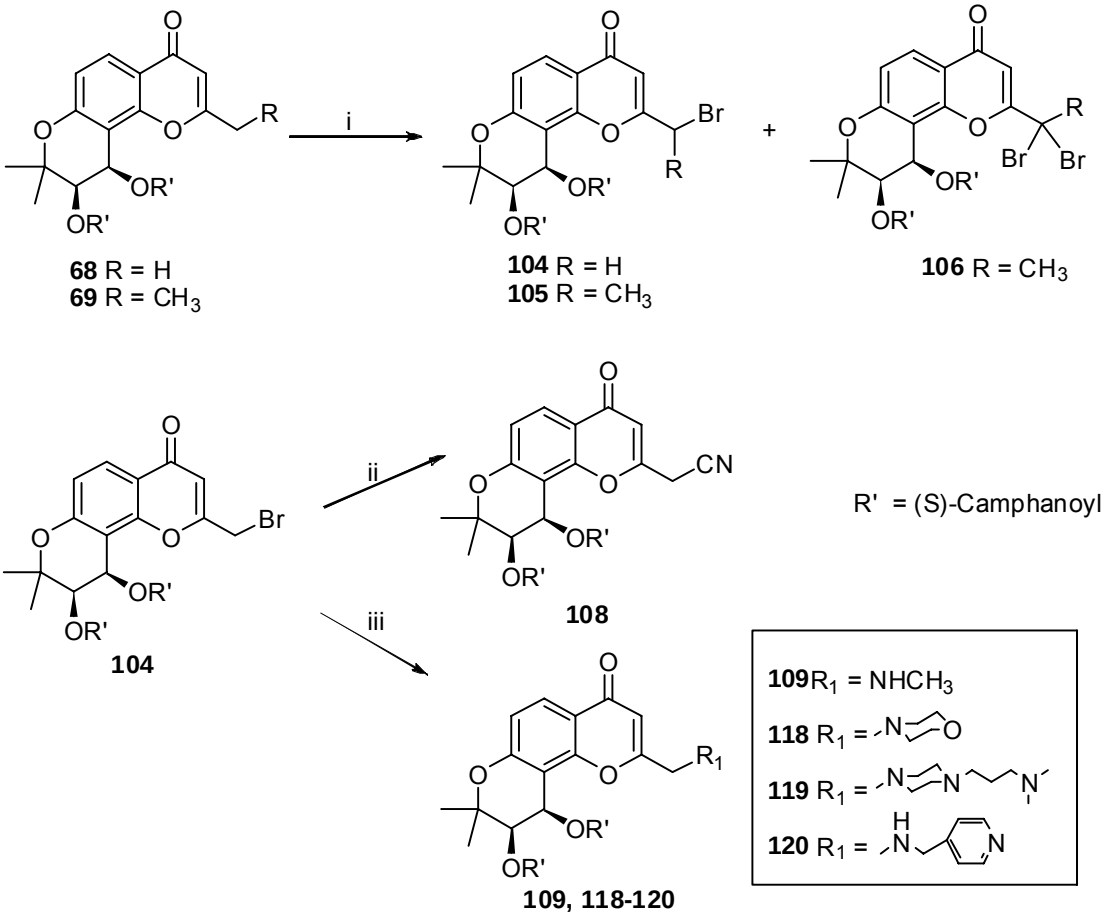
Scheme 4-3 illustrates the synthesis of novel DCP analogs with various functional groups at position-2. The synthesis of **104** and **105** was accomplished by benzylic bromination with NBS in anhydrous carbon tetrachloride in the presence of 3-chloroperbenzoic acid (MCPBA) as a radical initiator. Dibromo-substituted DCP analog (**106**) was also obtained during the reaction as previously reported.[16]

Compound **104** was treated with KCN under mild condition in DMF to give **108**.[17] Reaction of **104** with appropriate amine groups in THF at room temperature afforded compounds **109** and **118-120**.[18]

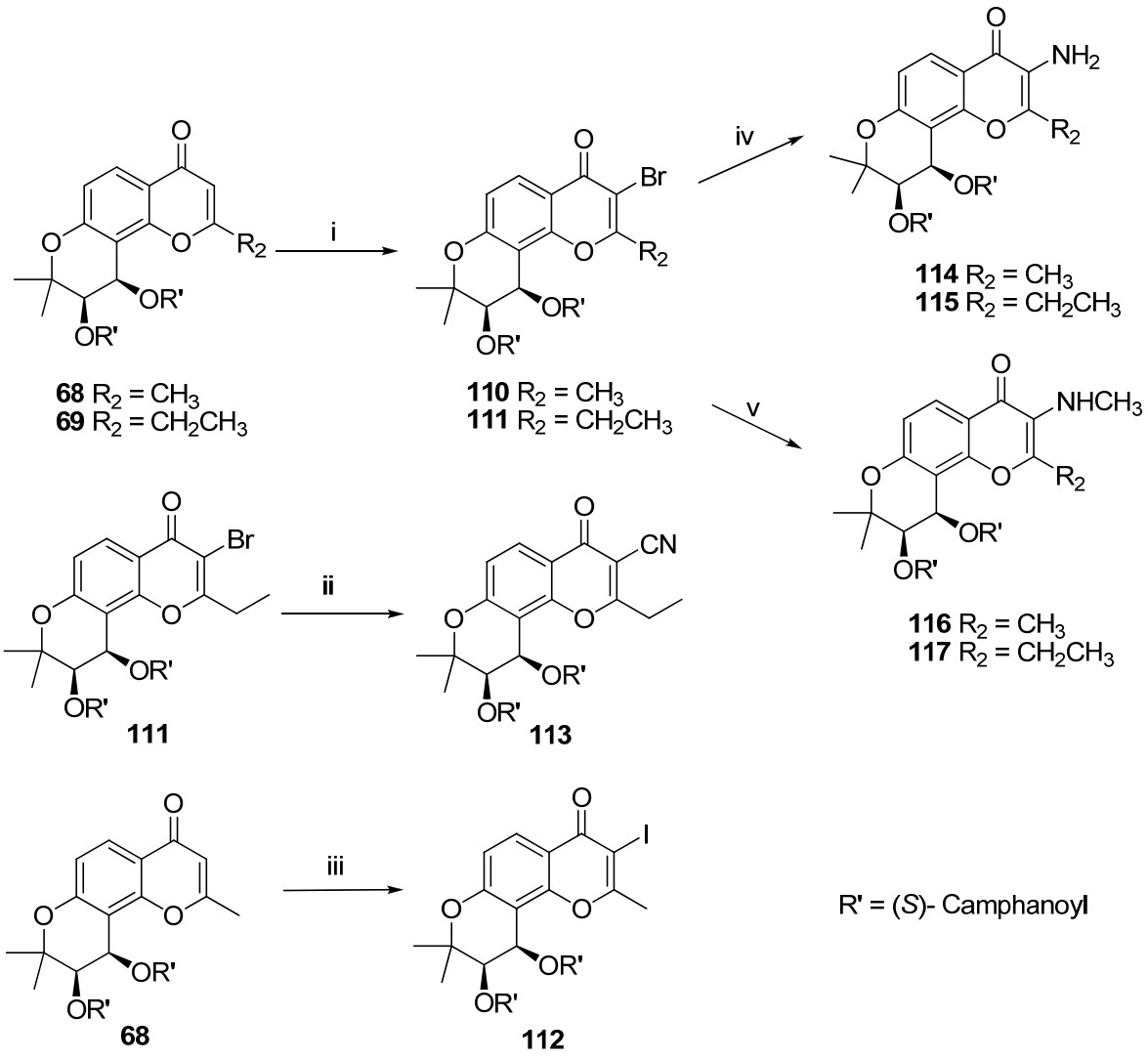
The synthesis of novel DCP analogs **110**-**117** with 3-substitutions is shown in Scheme 4-4. Selective bromination of **68** or **69** in acetonitrile gave **110** or **111**, respectively.[19] Compound **111** was subsequently converted to **113** in the presence of KCN in a mixture of DMF and 95% aq EtOH.[17] Reaction of **110** or **111** with 33% aqueous ammonium solution or methylamine at room temperature gave **114**-**117**.[18] Compound **368** was treated with I_2 in the presence of CF_3CO_2Ag as catalyst to obtain **112** in almost quantitative yield.[20]



Scheme 4-2. Synthesis of alkyl substituted DCP analogs. Reagents and conditions: (i) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave; (**ii**) ethyl alkanoates, NaH, THF, reflux; (**iii**) Amberlyst 15 resin, isopropanol, reflux; (iv) methanesulfonyl chloride, DMF; (v) $K_3Fe(CN)_6$, $(DHQ)_2PYR$, $K_2OsO_2(OH)_4$, K_2CO_3, *t*-butanol/H_2O, 0°C; (vi) (*S*)-camphanoyl chloride, DMAP, CH_2Cl_2.

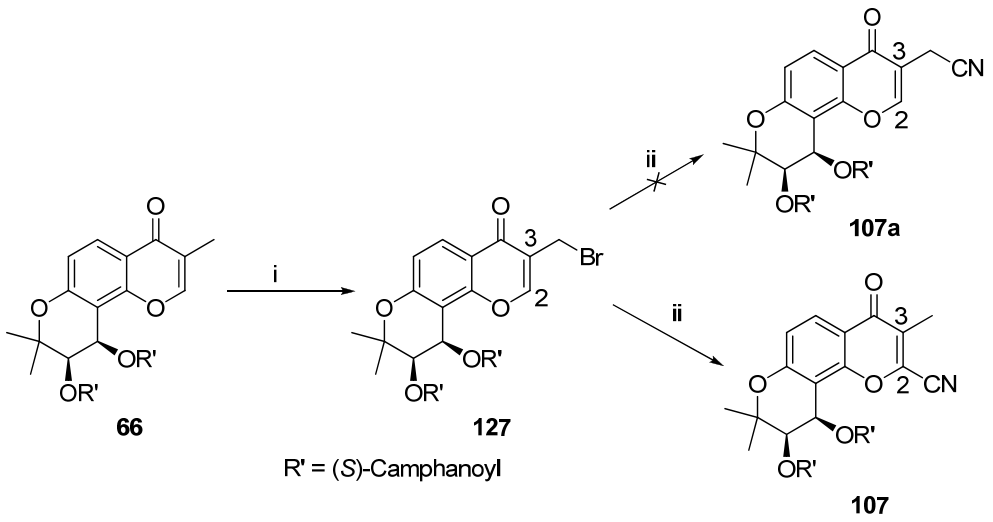


Scheme 4-3. Synthesis of novel 2-substituted DCP analogs (**104 -106, 108-109, 118 - 120**). Reagents and conditions: (i) NBS, 3-chloroperbenzoic acid, CCl_4, reflux; (ii) KCN, DMF, 95% aq EtOH; (iii) THF, diverse amine.

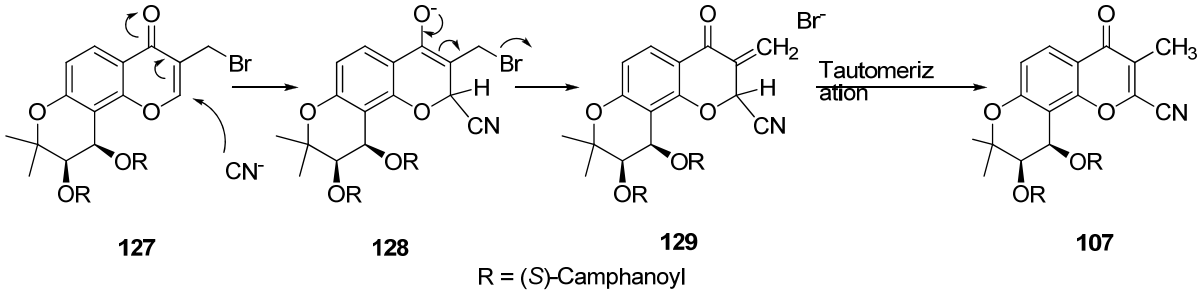


Scheme 4-4. Synthesis of novel 3-substituted DCP analogs (**110** - **117**). Reagents and conditions: (i) NBS, CH_2Cl_2, reflux; (ii) KCN, DMF, 95% aq EtOH; (iii) I_2, CF_3COOAg, CH_2Cl_2, 0°C; (iv) NH_4OH, THF; (v) $NHCH_3$ H_2O, THF.

The synthesis of 2-cyano-3-methyl-DCP (**107**) is given in Scheme 4-5. Stirring **66** with NBS in acetonitrile and heating to reflux gave **127**, which was further reacted with NaCN to give **107,** with a cyano substituent at position-2,[17] rather than displacement of bromide to give **107a**. The postulated Michael addition-elimination mechanism is illustrated in Scheme 4-6. Tautomerization of intermediate **128** regains resonance stabilization and produces compound **107**.

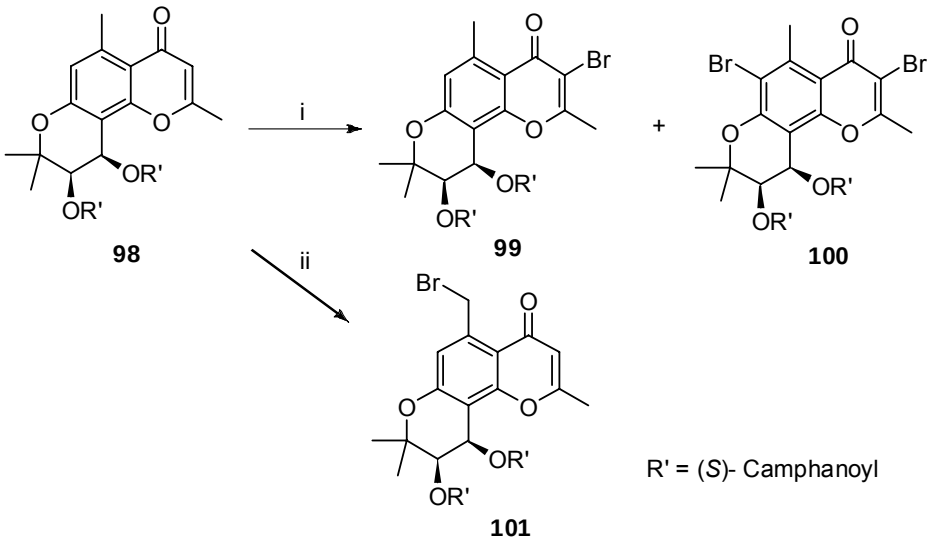


Scheme 4-5. Synthesis of compoud **107**. Reagents and conditions: (i) NBS, CH_3CN, Reflux; (ii) NaCN, DMF, 95% aq EtOH



Scheme 4-6. Speculated mechanism for production of **107**

The synthesis of **99**-**101** is shown in Scheme 4-7. Different solvents were used as mentioned above to selectively generate **99** and **101**.[16, 19] Compound **100** was obtained by using excess NBS.



Scheme 4-7. Synthesis of compounds **99-101**. Reagents and conditions: (i) NBS, CH_3CN, reflux; (ii) NBS, 3-chloroperbenzoic acid, CCl_4, reflux.

4.2.2 Biological Evaluation

All newly synthesized DCP analogs (**98** – **120**) were evaluated for anti-HIV activity against both HIV-1/NL4-3 and HIV-1/RTMDR1 in a single cycle infection assay using TZM-bl cells. The data are given in Table 4-1.

Compounds **98**-**103** are novel DCP analogs with short groups at both positions-2 and -5. Compounds **98**, **102**, and **103** with methyl and ethyl substituents at these positions exhibited promising anti-HIV activity against the non-drug-resistant strain HIV-1_{NL4-3}. They also showed comparable or greater TI values compared with both positive DCP reference standards **69** and **68**. Compound **98** (2-CH_3, 5-CH_3) had the highest potency (EC_{50} 0.036 μM, TI 420) among these six compounds, and was two times and 3 times more potent than **69** and **68** (**69**: EC_{50} 0.07 μM, TI 94; **68**: 0.10 μM, TI 110). However, changing the 5-CH_3 in **98** to 5-CH_2Br in **101** was unfavorable to anti-HIV activity and also decreased the TI value (**101**: 0.81 μM, TI 11). Likewise,

adding bromine at position-3 (**99**) or position-3 and -6 (**100**) led to decreased potency and TI.

Compounds **104**-**109** and **118**-**120** are novel 2-substituted DCPs. Similarly to **101**, bromination of the alkyl group at position-2 (**104**-**106**) was unfavorable to anti-HIV activity. The potency of **105** with bromoethyl substitution (EC_{50} 0.46 μM, TI 7.6) was six times lower than that of **69**; while **106** with dibromoethyl substitution, exhibited only mild potency (EC_{50} 1.0 μM, TI 1.8). Compound **107** with a cyano group at position-2 (EC_{50} 0.14 μM, TI 290) exhibited comparable anti-HIV activity and lower cytotoxicity compared with **68** and **69**. However, the anti-HIV activity of **108** with a cyanomethyl group at position 2 decreased significantly. With EC_{50} of 1.8 μM, **108** was ten times less potent than **107,** suggesting that a slight variation in the substitution at position-2 may result in a significant change in anti-HIV activity. It is postulated that expanding the conjugation of the chromone core structure by adding a cyano group at position-2 might contribute to high activity. Compound **109**, with 2-CH_2NHCH_3 substitution, also showed considerable anti-HIV activity (EC_{50} 0.29 μM, TI >100). These results suggest that analogs with polar groups, such as cyano and amino, introduced appropriately at position-2, can maintain anti-HIV activity. In addition, these groups should increase the compounds' polarity, which may improve water solubility. However, **118**–**120** contain large hydrophilic moieties at position-2, and showed either very weak (**118** and **119**) or no (**120**) activity.

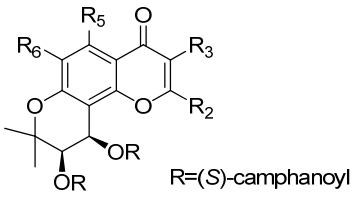
Compounds **110**-**117** are novel 3-substitued DCPs. Introduction of halogens such as bromine (**110**, **111**) and iodine (**112**) at position-3 reduced anti-HIV activity and TI. Compounds **110** (3-Br. EC_{50} 0.55 μM, TI 14) and **112** (3-I. EC_{50} 0.73 μM, TI

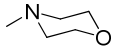
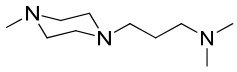
18) were five and seven times less potent, respectively, than the corresponding non-brominated **68** (EC_{50} 0.10 μM, TI 110). 3-Cyano-2-ethyl-DCP (**113**) showed moderate activity (EC_{50} 0.55 μM, TI >54), and analogs with NH_2 and $NHCH_3$ substituents at position-3 (**114**-**117**) maintained good anti-HIV activity. With a low EC_{50} value of 0.12 μM, **115** (3-NH_2) was equipotent with **69** (3-H) and three times more potent than **117** (3-$NHCH_3$). The 3-NH_2 analogs (**114**, **115**) showed comparable or greater potency and TI values than corresponding 3-$NHCH_3$ analogs (**116**, **117**).

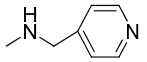
In summary, the influence of position-2 and -3 substituents on anti-HIV activity was generally equivalent. Electronic and hydrogen-bonding effects from halogen, cyano, and amino groups at these positions could influence both anti-HIV activity and TI. Halogens were not favorable and led to decreased anti-HIV potency and lower TI, while amino moieties resulted in both potent anti-HIV activity and high TI values. Analogs with a cyano substituent, particularly at position-2, maintained good anti-HIV-1 activity. In addition, the extended conjugation of the chromone ring system might be important to the high-potency, and led to the potency difference of **107** and **108**. Anti-HIV-1 activity was quite sensitive to the substituent size at position-2, and large moieties were not tolerable. Functional groups at position-5 of the chromone ring are also important for potent anti-HIV activity. Addition of a methyl group at this position led to increased anti-HIV activity, as exemplified by **98** and **102**. 2,5-Dimethyl DCP (**98**) had the highest TI values against both wild-type and drug-resistant HIV.

Table 4-1.

Anti-HIV activity of DCP analogs 98 – 120[a]



	R_2	R_3	R_5	R_6	IC_{50} (μM)[b]	NL 4-3		HIV-1 RTMDR1	
						EC_{50} (μM)	TI	EC_{50} (μM)	TI
98	CH_3	H	CH_3	H	15	0.036	420	0.049	310
99	CH_3	Br	CH_3	H	>14	0.59	>24	0.91	>15
100	CH_3	Br	CH_3	Br	>12	10	>1.2	>12	1
101	CH_3	H	CH_2Br	H	8.9	0.81	11	1.2	7.4
102	CH_2CH_3	H	CH_3	H	11	0.10	110	0.054	200
103	CH_2CH_3	H	CH_2CH_3	H	>30	0.25	>120	0.34	>88
104	CH_2Br	H	H	H	1.7	0.55	3.0	0.56	3.0
105	$CHBrCH_3$	H	H	H	3.5	0.46	7.6	0.24	15
106	$C(Br)_2CH_3$	H	H	H	1.8	1.0	1.8	0.45	4.0
107	CN	CH_3	H	H	40	0.14	290	1.9	21
108	CH_2CN	H	H	H	17	1.8	9.4	1.4	12
109	CH_2NHCH_3	H	H	H	>30	0.29	>100	0.69	>43
110	CH_3	Br	H	H	7.7	0.55	14	0.57	14
111	CH_2CH_3	Br	H	H	>27	1.1	>24	0.99	>28
112	CH_3	I	H	H	13	0.73	18	1.6	8.0
113	CH_2CH_3	CN	H	H	>30	0.55	>54	0.79	>37
114	CH_3	NH_2	H	H	32	0.25	130	0.47	68
115	CH_2CH_3	NH_2	H	H	23	0.12	190	0.31	74
116	CH_3	$NHCH_3$	H	H	>60	0.30	>200	0.45	>130
117	CH_2CH_3	$NHCH_3$	H	H	32	0.44	73	1.1	29
118		H	H	H	>28	2.0	>14	4.4	>6.3
119		H	H	H	17	3.7	4.5	7.5	2.2

120		H	H	H	>27	21	>1.3	21	>1.3
68	CH_3	H	H	H	11	0.10	110	0.19	58
69	CH_2CH_3	H	H	H	6.6	0.07	94	0.11	60

[a] All data presented in this table were averaged from at least three independent experiments. [b] Cytotoxicity was determined using a Promega CytoTox-Glo™ assay kit.

Most of the new DCP analogs were active against HIV RTMDR-1 strain, but were approximately two to three times less potent than against wild-type virus. Compounds **98** and **102** showed the most promising activity against HIV-$1_{RTMDR-1}$ with EC_{50} values of 0.049 and 0.054 μM and TI values of 310 and 200, respectively. These two compounds were approximately two-fold more potent than **69** against drug-resistant virus. Thus, the functional group at position-5 of the chromone ring is critical for potent activity against the drug-resistant strain. Halogen-substituted DCPs (**99**-**101**, **104**-**106**, **110**-**112**) showed reduced anti-HIV activity against HIV RTMDR-1 when compared with **68** and **69**. Among the halogen-substituted DCPs, **105** showed the best activity with EC_{50} of 0.24 μM. Amino-substituted DCP analogs (**109**, **114**-**117**) showed considerable activity against wild-type HIV-1_{NI4-3}, but reduced activity against the drug-resistant strain. The EC_{50} values of **109** and **115** against HIV-$1_{RTMDR-1}$ were 0.69 and 0.31 μM, respectively, which are approximately three times higher than EC_{50} against wild-type virus (0.29 μM and 0.12 μM). The SAR analysis of the synthesized DCP derivatives against the drug-resistant strain is similar to that for the wild-type virus.

4.2.3 Water Solubility (WS) Analysis

Because prior active DCP analogs showed poor water solubility, we were interested in improving this molecular parameter. We selected two active compounds from the preliminary SAR work for further analysis: **98**, which had the best anti-HIV activity against both virus strains, and **115**, which contains a hydrophilic amine group and maintains high anti-HIV activity against wild-type virus (Table 4-1). Both compounds showed lower predicted log P values than 2-EDCP (**69**) (Table 4-2), indicative of increased polarity that may improve the water solubility. We then performed a WS analysis with **98** and **115**, in comparison to **69**. We first established a standard curve of each tested compound by dissolution of the compound in acetonitrile at room temperature at various concentrations. The solubility in water could be determined by HPLC through the correlation between the saturated concentration of each compound in water and the correlating area detected by HPLC. With a solubility value less than 0.9 mg/L, 2-EDCP (**69**) showed the lowest WS among the three compounds. Compound **98** had an improved WS value (5.2 mg/L), and compound **115** presented the best WS value of 10.3 mg/L. (Table 4-2) This latter result confirmed that increasing the polarity of DCP analogs by introducing polar functional groups could result in improved water solubility. While both compounds **98** and **115** showed better WS than 2-EDCP (**69**), **98** also showed more potent anti-HIV activity than **69**, and thus, could merit further development study as a drug candidate.

Table 4-2. Log P values and water solubility results of 69, 98 and 115.

Compound	Predicted Log P value [a]	Water solubility (*mg*/L)
98	3.85	5.2
115	2.88	10.3

69 (2-EDCP)	4.01	<0.9

[a] calculated using ACD program

4.2.4 Molecular Modeling

4.2.4.1. Partial Least Square (PLS) QSAR

The PLS QSAR method was employed in the study using the QSAR-Model module of MOE 2009.[21] This method is relatively less sophisticated among those traditional available QSAR approaches. It was explored here to test if reliable models could be built for underlying data sets. A set of 2,489 theoretical molecular descriptors used in this calculation was computed using the software *Dragon v.5.5*.[22] The number of components was set to no limit on the degree of the fit. The maximum condition number of the principal component transform of the correlation matrix S, the condition limit, was set to be a very large number of 1.0*106.

We used the structures of the 25 DCP analogs listed in Table 4-1 and their anti-HIV activities (EC_{50} in μM) against both NL_{4-3} and RTMDR1 HIV strains to establish PLS models in the present study. The activity of each compound was transformed to the commonly used logarithm format and the $\log(1/EC_{50})$ ranged from -1.31 to 1.44 for the activity against NL_{4-3} HIV and from -1.31 to 1.31 for the activity against RTMDR1 HIV. The leave-one-out cross validation scheme was used to test the reliability and robustness of the resulting models. One of the 25 compounds was excluded, and a PLS model was developed for the remaining 24 compounds. Then the model was used to predict the anti-HIV activity of the excluded compound. This procedure was repeated 25 times for each type of activity until each compound was

used as the external test compound. From the leave-one-out cross validation procedure for the PLS model, the correlation coefficients (R^2) / mean absolute errors (MAE) for the wild type and drug resistant HIV strains were 0.67 / 0.30 (Figure 4-2a) and 0.60 / 0.35, respectively (Figure 4-2b). Compounds **118**, **119**, and **120** had relatively larger MAE than the remaining compounds, and compound **119** was a common outlier in both models. A probable reason is that these compounds have dissimilar R_2 substituents compared with the rest of the dataset. The R^2 and MAE values obtained from both models indicated that the newly established models can reliably be used to screen external chemical libraries in future studies.

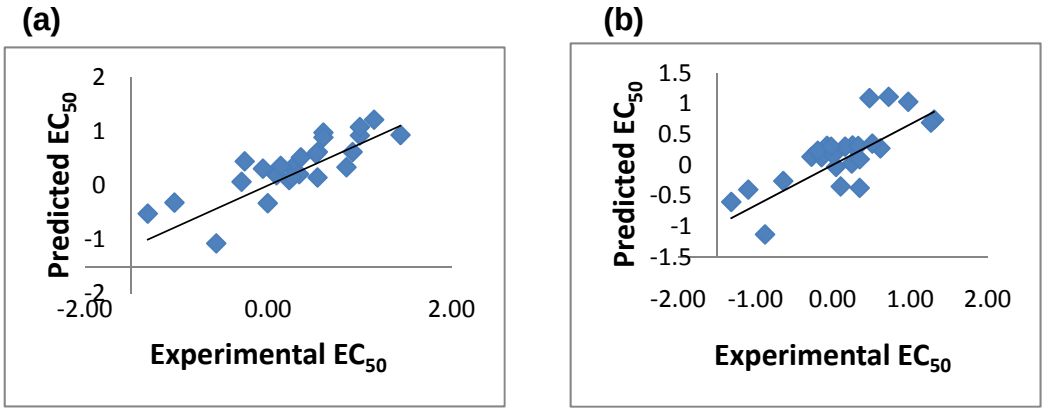


Figure 4-2. The correlation between experimental and predicted EC_{50} values obtained from leave one out cross validation for **(a)** NL4-3 HIV strain and **(b)** RTMDR1 HIV strain.

4.2.4.2. Pharmacophore Analysis

To explore DCP pharmacophores, the chemical structures of the three most potent compounds (**69**, **98**, and **102**) and the three weakest compounds (**100**, **119** and **120**) were energy minimized and superimposed using the Flexible Alignment of MOE 2009. Then the pharmacophore analysis was performed using the

Pharmacophore Query. The results are shown in Figures 4-3. The yellow balls shown in Figure 4-3 represent the identified pharmacophore. In both sets of compounds, the planar chromone ring, carbonyl group at position-4, and the oxygen at position-1′ were identified as part of the corresponding pharmacophore. However, in the most potent compounds (Figure 3a), the carbonyl group of the 4'-camphanoyl ester, which represents a hydrogen bond acceptor, was identified as a unique pharmacophore. In the three weakest compounds (Figure 3b), the orientation of both camphanoyl groups varied dramatically due to the introduction of bulky substitutions at position-2, which suggested that the orientations of the 3′- and 4′-camphanoyl groups might be critical for maintaining high anti-HIV activity.

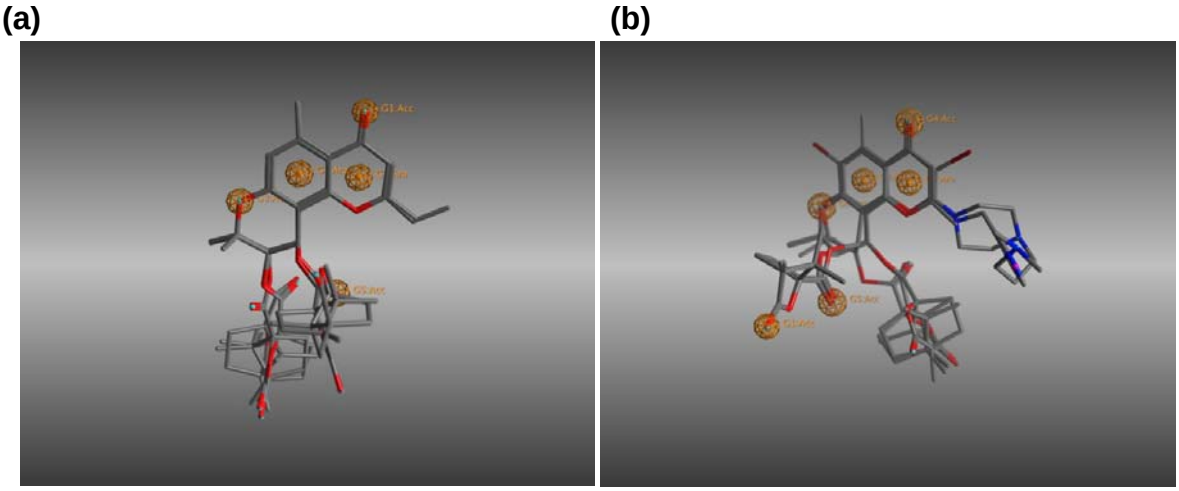


Figure 4-3. The pharamacophore analysis of the 3 most active compounds (**a**) and 3 most inactive compounds (**b**) using MOE 2009. The default setting was used except the tolerance (neighbor distance) and consensus score threshold (percentage of the compounds containing the pharmacophore) were changed to 0.5 and 100% respectively. Atom coloring: gray, carbon; blue, nitrogen; red, oxygen. Pharmacophore schemes: purple, hydrogen bond donor; light blue, hydrogen bond acceptor; yellow, aromatic center.

4.3 Conclusions

Our study identified a series of new DCP analogs with high anti-HIV potency against both wild-type and drug-resistant HIV-1 strains. The following SAR conclusions were drawn from these results.

(1) Position-5 of the DCP chromone ring system is critical for anti-HIV activity against both wild-type and drug-resistant HIV-1 strains, and appropriate alkyl groups on this position can improve anti-HIV activity against both virus strains.

(2) Electronic and hydrogen-bonding effects at position-2 and -3 can influence the anti-HIV activity as well as therapeutic index.

(3) The orientations of the 3′- and 4′-camphanoyl groups are critical to maintain high anti-HIV activity against both virus strain, and the carbonyl group in the 4′ position camphanoyl ester was identified as a potential hydrogen-bond acceptor by pharmacophore analysis.

We also analyzed the water solubility of selected newly synthesized DCP analogs and confirmed that increasing polarity can dramatically improve the water-solubility of DCP analogs.

In addition, we successfully established reliable PLS QSAR models. These models should help to predict the EC_{50} values of newly designed DCP analogs, which may be a useful tool for design of future new DCP analogs.

4.4 Experimental section

4. 4.1. Chemistry

Melting points were measured with a Fisher Johns melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were

measured on a 300MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Microwave reactions were performed with a Biotage initiator EXP US. Mass spectra were measured on Shimadzu LCMS-2010 (ESI-MS). Optical rotation was measured with a Jasco Dip-2000 digital polarimeter at 20^{o}C at the sodium D line. Thin-layer chromatography (TLC) was performed on PLC silica gel 60 F_{254} plates (0.5mm, Merck). Biotage Flash and Isco Companion systems were used as medium-pressure column chromatography. Shimadzu LC-20AT prominence liquid chromatography was used as HPLC system. Alltima 2.1mm x 100 mm C18 3u was used as HPLC column. Silica gel (200-400 mesh) from Aldrich, Inc. was used for column chromatography. All other chemicals were obtained from Aldrich, Inc. All final compounds are >95% pure on the basis of the two HPLC conditions.

5-ethylbenzene-1,3-diol (122c). A reaction mixture of 2 g (13.1 mmol) of 3′,5′-dihydroxyacetophenone (**121**), 1 g of Pd/C (10%) and 150 mL aqueous HCl (4%) was hydrogenated overnight (900 mL of H_2). The mixture was filtered and extracted with three portions of Et_2O. The dried solution was evaporated at reduced pressure. The residue was purified by column chromatography with hexanes:EtOAc = 10:1 to afford **122c** as a white solid. 80% yield; MS (ESI+) m/z (%) 137 (M^+ + 1, 100); ^{1}H NMR δ 6.26 (2H, s, H-4, 6), 6.18 (1H, s, H-2), 4.90 (2H, br, OH-1, 3), 2.53(2H, q, J = 7.5 Hz, CH_2CH_3-5), 1.20 (3H. t, J = 7.5 Hz, CH_2CH_3-5).

1-(2,4-dihydroxy-6-methylphenyl)ethanone (77). MeCN (0.7 mL, 20 mmol) and dry $ZnCl_2$ (1.36 g, 10 mmol) were added to a solution of 3,5-dihydroxytoluene **122b** (1.24 g, 10 mmol) in Et_2O (5 mL). Hydrogen chloride gas was then bubbled

through the mixture, and the resulting precipitate was filtered off and dissolved in water. This solution was neutralized by adding aqueous ammonia solution (33%) and was subsequently stirred for 30 min at 100 ^{o}C. The crude product was purified by column chromatography with hexanes:EtOAc = 7:3 to afford **77** (680 mg). 41% yield; MS (ESI-) m/z (%) 165 (M^- - 1, 100); ^{1}NMR δ 6.24 (1H, s, H-5), 6.23 (1H, s, H-3), 5.44 (2H, br, OH-2, 4), 2.62 (3H, s, $COCH_3$-1), 2.55 (3H, s, CH_3-6).

1-(2,4-dihydroxy-6-ethylphenyl)ethanone (78). The procedure was identical to that used for the preparation of **77**. 40% yield (starting with 2.36 g of **122c**); MS (ESI+) m/z (%) 181 (M^+ + 1, 100); ^{1}NMR δ 6.28 (1H, s, H-5), 6.19 (1H, s, H-3), 4.85 (2H, br, OH-2, 4), 2.91 (2H, q, *J* = 7.2 Hz, CH_2CH_3-6), 2.67 (1H, s, $COCH_3$-1), 1.30 (3H, t, *J* = 7.2 Hz, CH_2CH_3-6).

General procedure for the preparation of 87a-89a and 125f. A mixture of starting compound **76**, **77**or **78** (1 equiv) or **124**, 4,4-dimethoxy-2-methyl-2-butanol (1.5-2 equiv) and pyridine (2-3 mL) was heated at 220 °C for 4 h under high absorption microwave conditions. The reaction mixture was cooled to rt, diluted with EtOAc and washed with aqueous HCl (10%) and brine. The organic layer was separated, and solvent was removed in vacuo. The residue was purified by column chromatography with hexanes:EtOAc = 97:3 to afford **87a**-**89a** and **125f**.

6-Acetyl-2,2-dimethyl-5-hydroxy-2*H*-chromone (87a). 57.3 % yield (starting with 1 g of **76**); MS (ESI+) m/z (%) 219 (M^+ + 1, 100); ^{1}NMR δ 7.52 (1H, d, *J* = 8.7 Hz, H-7), 6.72 (1H, d, *J* = 7.5 Hz, H-4), 6.33 (1H, d, *J* = 8.7 Hz, H-8), 6.58 (1H, d, *J* = 7.5 Hz, H-3), 2.54 (3H, s, $COCH_3$-1), 1.45 (6H, s, CH_3-2,2).

6-Acetyl-2,2,7-trimethyl-5-hydroxy-2*H*-chromone (88a). 66.4% yield (starting with 77.2 mg of **77**); mp 56-67 ^{o}C; MS (ESI+) m/z (%) 233 (M^{+} + 1, 100); ^{1}NMR δ 6.69 (1H, d, *J* = 10.2 Hz, H-4), 6.19 (1H, s, H-8), 5.52 (1H, d, *J* = 10.2 Hz, H-3), 3.31 (3H, s, $COCH_3$-1), 2.53 (3H, s, CH_3-7), 1.43 (6H, s, CH_3-2,2).

6-Acetyl-2,2-dimethyl-5-hydroxy-7-ethyl-2*H*-chromone (89a). 72.4% yield (starting with 500 mg of **78**); MS (ESI+) m/z (%) 247 (M^{+} + 1, 100%); ^{1}H NMR δ 6.70 (1H, d, *J* = 10.2 Hz, H-4), 6.25 (1H, s, H-8), 6.52 (1H, d, *J* = 10.2 Hz, H-3), 2.86 (2H, q, *J* = 7.5 Hz, CH_2CH_3-7), 2.64 (3H, s, CH_3CO-6), 1.42, 1.41 (each 3H, s, CH_3-2,2), 1.26 (3H, t, *J* = 7.5 Hz, CH_2CH_3-7).

2',2',3-Trimethyl-pyrano[2,3,*f*]-chromone (125f). 60% yield (starting with 120 mg of **124**); mp 66-67 ^{o}C; MS (ESI+) m/z (%) 243 (M^{+} + 1, 100); ^{1}H NMR δ 7.98 (1H, d, *J* = 8.7 Hz, H-5), 7.73 (1H, s, H-2), 6.81 (1H, d, *J* = 8.7 Hz, H-6), 6.76 (1H, d, *J* = 9.9 Hz, H-4'), 5.67 (1H, d, *J* = 9.9 Hz, H-3'), 2.00 (3H, s, CH_3-3), 1.48 (6H, s, CH_3-2',2').

General procedure for the preparation of 125a-e. A mixture of **87a**, **88a** or **89a** and ethyl alkanoate in absolute THF was added slowly to a sodium hydride/THF suspension under nitrogen. The mixture was warmed to reflux temperature for 2-6 h monitored by TLC, followed by neutralization with 10% aqueous HCl, and extraction three times with CH_2Cl_2. The organic layer was collected and the solvent evaporated under reduced pressure. The residue and Amberlyst 15 resin were stirred in isopropanol at reflux temperature to give 2-substituted dimethylpranochromone **125a**-**e**.

2,2',2'-Trimethyl-pyrano[2,3,*f*]-chromone (125a). 56 % yield (starting with 558.2 mg of **87a**); mp 123-125 ^{o}C; MS (ESI+) m/z (%) 243 (M^+ + 1, 100); ^{1}H NMR δ 7.92 (1H, d, *J* = 8.7 Hz, H-5), 6.80 (1H, d, *J* = 8.7 Hz, H-6), 6.78 (1H, d, *J* = 9.9 Hz, H-4'), 6.09 (1H, s, H-3), 5.68 (1H, d, *J* = 9.9 Hz, H-3'), 2.36 (3H, s, CH_3-2), 1.48 (6H, s, CH_3-2',2').

2,2',2'-Trimethyl-5-methylpyrano[2,3,*f*]-chromone (125b). 38 % yield (starting with 770 mg of **88a**); mp 128 130 ^{o}C; MS (ESI+) m/z (%) 257 (M^+ + 1, 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 9.9 Hz, H-4'), 6.56 (1H, s, H-6), 6.00 (1H, s H-3), 5.64 (1H, d, *J* = 9.9 Hz, H-3'), 2.76 (3H, s, CH_3-5), 2.31 (3H, s, CH_3-2), 1.46 (6H, s, CH_3-2',2').

2',2'-Dimethyl-2-ethylpyrano[2,3,*f*]-chromone (125c). 66% yield (starting with 1.1 g of **87a**); mp 97-98^{o}C; MS (ESI+) m/z (%) 279 (M^+ + Na, 100); ^{1}H NMR δ 7.92 (1H, d, *J* = 8.7 Hz, H-5), 6.80 (1H, d, *J* = 8.7 Hz, H-6), 6.77 (1H, d, *J* = 10.2 Hz, H-4'), 6.10 (1H, s, H-3), 5.69 (1H, d, *J* = 10.2 Hz, H-3'), 2.65 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 1.44, 1.48 (each 3H, s, CH_3-2',2'), 1.30 (3H, t, *J* = 7.5 Hz, $CH_2$$CH_3$-2).

2',2'-Dimethyl-2-ethyl-5-methylpyrano[2,3-*f*]-chromone (125d). 45% yield (starting with 64.8 mg of **88a**); mp 123-124 ^{o}C; MS (ESI+) m/z (%) 271 (M^+ + 1, 100); ^{1}H NMR δ 7.75 (1H, d, *J* = 10.5 Hz, H-4'), 6.57 (1H, s, H-6), 6.01 (1H, s, H-3), 5.64 (1H, d, *J* = 10.5 Hz, H-3'), 2.77 (3H, s, CH_3-5), 2.60 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 1.59, 1.47 (each 3H, s, CH_3-2',2'), 1.29 (3H, t, *J* = 7.5 Hz, $CH_2$$CH_3$-2).

2',2'-Dimethyl-2,5-diethylpyrano[2,3,*f*]chromone (125e). 36% yield (starting with 120 mg of **89a**); MS (ESI+) m/z (%) 285 (M^+ + 1, 100); ^{1}H NMR δ 6.76 (1H, d, *J*

= 10.2 Hz, H-4'), 6.61 (1H, s, H-6), 6.01 (1H, s H-3), 5.64 (1H, d, *J* = 10.2 Hz, H-3'), 3.24 (2H, q, *J* = 7.5 Hz, CH_2CH_3-5), 2.58 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.48 (6H, s, CH_3-2',2'), 1.29 (3H, t, *J* = 7.5 Hz, CH_2CH_3-5), 1.22 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2).

Preparation of 7-hydroxy-3-methylchromone (124). The commercially available phenol **123** (400 mg, 2.41 mmol) in dry DMF (6 mL) was heated to 50 ^oC, and a solution of methanesulfonyl chloride (0.5 mL) in dry DMF (1mL) was added slowly. The mixture was then reacted at 60 ^oC for 6 h. After cooling, the reaction mixture was poured into a large volume of ice-cold aqueous sodium acetate (12 g/100 mL). The crude product was filtered off and purified by column chromatography with hexanes:EtOAc = 7:3 to afford **124** (120 mg). 28% yield; mp 155-157 ^oC; MS (ESI+) m/z (%) 199 (M^+ + Na, 100); ^1H NMR δ 10.72 (1H, s, OH-7), 8.11 (1H, s, H-2), 7.88 (1H, d, *J* = 9.0 Hz, H-5), 6.89 (1H, dd, *J* = 9.0, 2.4 Hz, H-6), 6.80 (1H, d, *J* = 2.4 Hz, H-8), 1.87 (3H, s, CH_3-3).

General procedure for the preparation of 126a-d and 126f. A mixture of $K_3Fe(CN)_6$ (3 equiv), K_2CO_3 (3 equiv), $(DHQ)_2$-PYR (2% equiv), and $K_2OsO_2(OH)_4$ (2% equiv) was dissolved in *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 ^oC and methanesulfonamide (1 equiv) was added with stirring. After 20 min, substituted pyranochromone (**125a-d** and **125f**) was added. The mixture was stirred at 0 ^oC for 1-2 days, monitored by TLC. At completion, $Na_2S_2O_5$ (excess), water and CH_2Cl_2 were added, and stirring was continued for 1 h at rt. The mixture was extracted with CH_2Cl_2 three times, and the combined organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by

column chromatography with hexanes:EtOAc = 3:7 to afford the pure substituted (+)-*cis*-3',4'-dihydroxypyranochromones (**126a-d** and **126f**).

3'*R*,4'*R*-Dihydroxy-2,2',2'-trimethylpyrano[2,3-*f*]chromone (126a). 66% yield (starting with 1.1 g of **125a**); mp 176-178 ^{o}C; MS (ESI+) m/z (%) 276 (M^{+} + 1, 100); ^{1}H NMR (DMSO) δ 7.95 (1H, d, J = 9.0 Hz, H-5), 6.84 (1H, d, J = 9.0 Hz, H-6), 6.10 (1H, s, H-3), 5.20 (1H, t, J = 4.2, 4.2 Hz, H-4'), 3.87 (1H, t, J = 7.2, 4.2 Hz, H-3'), 3.44 (1H, d, J = 4.2 Hz, OH-4'), 3.18 (1H, d, J = 7.2 Hz, OH-3'), 2.40 (3H, s, CH_3-2), 1.50, 1.44 (each 3H, s, CH_3-2',2').

3'*R*,4'*R*-Dihydroxy-2, 5, 2',2'-tetramethylpyrano[2,3-*f*]chromone (126b). 35% yield (starting with 325 mg of **125b**); mp 114-116 ^{o}C; MS (ESI+) m/z (%) 291 (M^{+} + 1, 100) ; ^{1}H NMR δ 6.61 (1H, s, H-6), 6.04 (1H, s, H-3), 5.15 (1H, t, J = 3.9, 4.5 Hz, H-4'), 3.85 (1H, dd, J = 4.5, 6.6 Hz, H-3'), 3.08 (1H, d, J = 3.9 Hz, OH-4'), 3.05 (1H, d, J = 6.6 Hz, OH-3'), 2.74 (3H, s, CH_3-5), 2.35 (3H, s, CH_3-2), 1.46, 1.43 (each 3H, s, CH_3-2',2').

3'*R*,4'*R*-Dihydroxy-2',2'-dimethyl-2-ethylpyrano[2,3-*f*]chromone (126c). 28% yield (starting with 120 mg of **125c**); mp 153-155 ^{o}C; MS (ESI+) m/z (%) 291 (M^{+} + 1, 100); ^{1}H NMR (DMSO) δ 7.80 (1H, d, J = 9.0 Hz, H-5), 6.83 (1H, d, J = 9.0 Hz, H-6), 6.13 (1H, s, H-3), 4.97 (1H, t, J = 4.8, 4.2 Hz, H-4'), 3.64 (1H, t, J = 6.6, 4.8 Hz, H-3'), 3.08 (1H, d, J = 4.2 Hz, OH-4'), 2.99 (1H, d, J = 6.6 Hz, OH-3'), 2.58 (2H, q, J = 7.5 Hz, $CH_2$$CH_3$-2), 1.38, 1.37 (each 3H, s, CH_3-2',2'), 1.26 (3H, t, J = 7.5 Hz, CH_2CH_3-2).

3'*R*, 4'*R*-Dihydroxy-5,2',2'-trimethyl-2-ethylpyrano[2,3-*f*]chromone (126d). 40% yield (starting with 272 mg of **125d**); mp 114-116 ^{o}C; MS (ESI+) m/z (%) 305 (M^+ + 1, 100); ^{1}H NMR δ 6.62 (1H, s, H-6), 6.06 (1H, s, H-3), 5.15 (1H, dd, *J* = 3.6, 5.1 Hz, H-4'), 3.86 (1H, dd, *J* = 5.1, 6.9 Hz, H-3'), 3.01 (1H, d, *J* = 6.9 Hz, OH-3'), 2.98 (1H, d, *J* = 3.9 Hz, OH-4'), 2.76 (3H, s, CH_3-5), 2.64 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.46, 1.42 (each 3H, s, CH_3-2',2'), 1.31 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2).

3'*R*,4'*R*-Dihydroxy-3,2',2'-trimethylpyrano[2,3-*f*]chromone (126f). 55% yield (starting with 1.3 g of **125f**); mp 180-182 ^{o}C; MS (ESI+) m/z (%) 277 (M^+ + 1, 100); ^{1}H NMR δ 7.98 (1H, d, *J* = 8.7 Hz, H-5), 7.74 (1H, d, *J* = 1.0 Hz, H-2), 6.82 (1H, d, *J* = 8.7 Hz, H-6), 6.15 (1H, dd, *J* = 5.4, 3.6 Hz, H-4'), 3.85 (1H, dd, *J* = 5.7, 5.4 Hz, H-3'), 3.43 (1H, d, *J* = 3.6 Hz, OH-4'), 3.19 (1H, d, *J* = 5.7 Hz, OH-3'), 1.99 (3H, d, *J* = 1.0 Hz, CH_3-3), 1.41, 1.42 (each 3H, s, CH_3-2',2').

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-3-bromomethyl-dihydropyrano [2,3-*f*]chromone (127). The mixture of **66** (40 mg, 0.06 mmol), NBS (18 mg, 0.1 mmol), and MeCN (2ml) was heated to reflux for 4 h, monitored by TLC. At completion, the mixture was concentrated and purified by PTLC with an eluent of hexanes:EtOAc = 5:4 to afford pure **127** (30 mg): 70% yield; MS-ESI+ (*m*/*z*, %) 715 (M^+ + 1, 100); ^{1}H NMR δ 8.21 (1H, d, *J* = 9.0 Hz, H-5), 7.96 (1H, s, H-2), 6.98 (1H, d, *J* = 9.0 Hz, H-6), 6.72 (1H, d, *J* = 4.8 Hz, H-4'), 5.38 (1H, d, *J* = 4.8 Hz, H-3'), 4.35 (2H, s, CH_2Br-3), 2.43, 2.20, 1.93, 1.85 (each 2H, m, camphanoyl CH_2), 1.53, 1.49 (each 3H, s, CH_3-2',2'), 1.13, 1.10, 1.09, 1.02, 0.98, 0.91 (each 3H, s, camphanoyl CH_3).

General procedure for the preparation of 66, **68-69**, **98, 102.** The substituted 3'*R*,4'*R*-dihydroxypyranochromones (**126a-d** and **126f**), (*S*)-(-)-camphanic chloride (3 equiv), and DMAP (4 equiv) were stirred in CH_2Cl_2 for 1-2 h at rt, monitored by TLC. At completion, the mixture was diluted with CH_2Cl_2 and washed by water and brine. The solvent was then removed under reduced pressure and the residue was purified by PTLC with hexanes:EtOAc = 3:2 to afford the appropriately alkyl-substituted 3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyldihydroprano[2,3-*f*]chromones (**66, 68-69, 98, 102**).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2,2',2'-trimethyldihydropyrano[2,3-*f*]chromone (68). 70% yield (starting from 100 mg of **126a**); mp 146-148 °C; MS-ESI+ (*m*/*z*, %) 659 (M^+ + Na, 100); ^{1}H NMR *δ* 8.11 (1H, d, *J* = 8.8 Hz, H-5), 6.90 (1H, d, *J* = 8.8 Hz, H-6), 6.75 (1H, d, *J* = 4.6 Hz, H-4'), 6.12 (1H, s, H-3), 5.37 (1H, d, *J* = 4.6 Hz, H-3'), 2.46, 2.12, 1.92, 1.70 (each 2H, m, camphanoyl CH_2), 2.27 (3H, s, CH_3-2), 1.53, 1.46 (each 3H, s, CH_3-2'), 1.11, 1.10, 1.07, 1.00, 0.97, 0.94 (each 3H, s, camphanoyl CH_3); 60% de. [R]D -69.6° (*c*) 0.25, $CHCl_3$).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-3,2',2'-trimethyldihydropyrano[2,3-*f*]chromone (66). 75% yield (starting from 200 mg of **126f**); mp 146-148 °C; MS-ESI+ (*m*/*z*, %) 659 (M^+ + Na, 100); ^{1}H NMR *δ* 8.16 (1H, d, *J* = 9.0 Hz, H-5), 7.61 (1H, s, H-2), 6.91 (1H, d, *J* = 9.0 Hz, H-6), 6.70 (1H, d, *J* = 4.8 Hz, H-4'), 5.36 (1H, d, *J* = 4.8 Hz, H-3'), 2.56, 2.32, 2.24, 1.85 (each 2H, m, camphanoyl CH_2), 2.12 (3H, s, CH3-3), 1.64, 1.59 (each 3H, s, CH3-2'), 1.32, 1.24, 1.22, 1.12, 1.10, 1.00 (each 3H, s, camphanoyl CH_3); 90% de. $[\alpha]_D$ -36.2° (*c*) 0.23, $CHCl_3$).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyldihydropyrano[2,3-*f*]chromone (69). 71% yield (starting with 146 mg of **126c**); mp 90-92 °C; MS-ESI+ (*m*/*z*, %) 645 (M^+ + Na, 100); ^{1}H NMR δ 8.15 (1H, d, *J* = 9.0 Hz, H-5), 7.69 (1H, d, *J* = 6.3 Hz, H-2), 6.94 (1H, d, *J* = 9.0 Hz, H-6), 6.72 (1H, d, *J* = 4.8 Hz, H-4'), 6.32 (1H, d, *J* = 6.3 Hz, H-3), 5.37 (1H, d, *J* = 4.8 Hz, H-3'), 2.46, 2.20, 1.90, 1.74 (each 2H, m, camphanoyl CH_2), 1.52, 1.47 (each 3H, s, CH_3-2'), 1.11, 1.10, 1.08, 1.02, 0.99, 0.89 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -95.3° (*c* = 0.17, $CHCl_3$).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2,5,2',2'-tetramethyldihydropyrano[2,3-*f*]chromone (98). 54% yield (starting with 290 mg of **126b**); mp 144-145 °C; MS-ESI+ (*m*/*z*, %) 645 (M^+ + 1, 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 4.8 Hz, H-4'), 6.67 (1H, s, H-6), 6.05 (1H, s, H-3), 5.37 (1H, d, *J* = 4.8 Hz, H-3'), 2.81 (3H, s, CH_3-5), 2.50, 2.20, 1.95, 1.85 (each 2H, m, camphanoyl CH_2), 2.24 (3H, s, CH_3-2), 1.54, 1.47 (each 3H, s, CH_3-2',2'), 1.14, 1.13, 1.10, 1.01, 1.00, 0.96 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -71.2° (*c* = 0.002, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyl-5-methyldihydropyrano [2,3-f]chromone (102). 60% yield (starting with 100 mg of **126d**); mp 133-134 °C; MS-ESI+ (*m*/*z*, %) 665 (M^+ + 1, 100); ^{1}H NMR δ 6.70 (1H, d, *J* = 4.5 Hz, H-4'), 6.64 (1H, s, H-6), 6.04 (1H, s H-3), 5.36 (1H, d, *J* = 4.5 Hz, H-3'), 2.78 (3H, s, CH_3-5), 2.50 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 2.50, 2.14, 1.91, 1.71 (each 2H, m, camphanoyl CH_2), 1.52, 1.44 (each 3H, s, CH_3-2',2'), 1.21 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.11, 1.10, 1.07, 0.99, 0.97, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -55.0° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2,5,2',2'-tetramethyl-3-bromodihydropyrano [2,3-*f*]chromone (99). A mixture of **98** (80 mg, 0.12 mmol), NBS (32.0 mg, 0.18 mmol) and MeCN (2 mL) was heated to 110 ^{o}C for 3 h under high-absorption microwave conditions. At completion, the mixture was concentrated and purified by PTLC with an eluent of hexanes:EtOAc = 1:1 to afford pure **99** (28 mg). 32% yield; mp 146-147 ^{o}C; MS-ESI+ (*m*/*z*, %) 729 (M^+ , 100); ^{1}H NMR δ 6.72 (1H, d, *J* = 4.8 Hz, H-4'), 6.71 (1H, s, H-6), 5.36 (1H, d, *J* = 4.8 Hz, H-3'), 2.81 (3H, s, CH_3-5), 2.49 (3H, s, CH_3-2), 2.50, 2.15, 1.95, 1.72 (each 2H, m, camphanoyl CH_2), 1.53, 1.47 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.09, 1.00, 0.98, 0.95 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -65.8° (*c* = 0.018, CH_3Cl).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2,5,2',2'-tetramethyl-3,6-dibromodihydro pyrano[2,3-*f*]chromone (100). The procedure was identical to that used for the preparation of **99**. 10% yield (starting with 80 mg of **98**); mp 148-150 ^{o}C; MS-ESI+ (*m*/*z*, %) 809 (M^+ , 100); ^{1}H NMR δ 6.74 (1H, d, *J* = 4.5 Hz, H-4'), 5.39 (1H, d, *J* = 4.5 Hz, H-3'), 3.05 (3H, s, CH_3-5), 2.49 (3H, s, CH_3-2), 2.50, 2.16, 1.92, 1.73 (each 2H, m, camphanoyl CH_2), 1.56 (6H, s, CH_3-2',2'), 1.13, 1.12, 1.09, 1.00, 0.99, 0.96 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -67.2° (*c* = 0.018, CH_3Cl).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2,2',2'-trimethyl-5-bromomethyldihydro-pyrano[2,3-*f*]chromone (101). A mixture of **98** (100 mg, 0.15 mmol), NBS (29.4 mg, 0.17 mmol), and 3-chloroperbenzoic acid (2.6 mg, 0.015 mmol), dissolved in 2 mL of anhydrous CCl_4 was heated to 100 ^{o}C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes:EtOAc = 1:1 to afford pure **101** (38mg).

35% yield; mp 128-130 ^{o}C; MS-ESI+ (*m*/*z*, %) 729 (M^+, 100); ^{1}H NMR *δ* 6.97 (1H, s, H-6), 6.72 (1H, d, *J* = 4.5 Hz, H-4'), 6.15 (1H, s, H-3), 5.39 (1H, d, *J* = 4.5 Hz, H-3'), 5.29 (1H, d, *J* = 61.2 Hz, CH_2Br-5), 5.12 (1H, d, *J* = 61.2 Hz, CH_2Br-5), 2.50, 2.18, 1.94, 1.71 (each 2H, m, camphanoyl CH_2), 2.26 (3H, s, CH_3-2), 1.54, 1.48 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.09, 1.02, 0.99, 0.97 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -66.9° (*c* = 0.018, CH_3Cl).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2,5-diethyldihydropyrano[2,3-f]chromone (103). Compound **125e** (50 mg) was dihydroxylated using the identical procedure described above for 2 days. At completion, the mixture was extracted with CH_2Cl_2, and the combined organic layer was concentrated under reduced pressure to give crude 3'*R*,4'*R*-dihydroxyl-DCP. Without purification, the crude product was stirred with camphanic chloride (3 equiv) and DMAP (4 equiv) at rt for 2 h to give 30 mg of **103**. 30% yield; mp 108-110 ^{o}C; MS-ESI+ (*m*/*z*, %) 665 (M^+ + 1, 100); ^{1}H NMR *δ* 6.70 (1H, d, *J* = 4.5 Hz, H-4'), 6.69 (1H, s, H-6), 6.04 (1H, s H-3), 5.37 (1H, d, *J* = 4.5 Hz, H-3'), 3.25 (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-5), 2.50, (2H, q, *J* = 7.5 Hz, $CH_2$$CH_3$-2), 2.50, 2.15, 1.92, 1.70 (each 2H, m, camphanoyl CH_2), 1.53, 1.45 (each 3H, s, CH_3-2',2'), 1.24 (3H, t, *J* = 7.5 Hz, CH_2CH_3-5), 1.21 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.12, 1.11, 1.07, 1.01, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -6.5° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-bromomethyldihydropyrano [2,3-*f*]chromone (104). A mixture of **68** (200 mg, 0.31 mmol), NBS (60.6 mg, 0.34 mmol), and 3-chloroperbenzoic acid (5.4 mg, 0.031 mmol), dissolved in 2mL of anhydrous CCl_4 was heated to 100 ^{o}C for 5 h under high-absorption microwave

conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes:EtOAc = 1:1 to afford pure **104** (50 mg). 23% yield; mp 180-182 ^{o}C; MS-ESI+ (*m*/*z*, %) 717 (M^+ + 1, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.93 (1H, d, *J* = 9.0 Hz, H-5), 6.74 (1H, d, *J* = 4.5 Hz, H-4'), 6.40 (1H, s, H-3), 5.42 (1H, d, *J* = 4.5 Hz, H-3'), 4.12 (2H, s, CH_2Br-2), 2.50, 2.17, 1.90, 1.74 (each 2H, m, camphanoyl CH_2), 1.56, 1.47 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.09, 1.07, 1.02, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -13.9° (*c* = 0.01, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-(1-bromoethyl)dihydropyrano [2,3-f]chromone (105). The procedure was identical to that used for the preparation of **104**: 25% yield (starting with 50 mg of **69**); mp 158-159 ^{o}C; MS-ESI+ (*m*/*z*, %) 731 (M^+ + 1, 100); ^{1}H NMR δ 8.12 (1H, d, *J* = 9.0 Hz, H-5), 6.92 (1H, d, *J* = 9.0Hz, H-5), 6.74 (1H, d, *J* = 4.5 Hz, H-4'), 6.34 (1H, s, H-3), 5.42 (1H, d, *J* = 4.5 Hz, H-3'), 4.76 (1H, t, *J* = 7.2, *CH*BrCH$_3$-2), 2.50, 2.19, 1.90, 1.74 (each 2H, m, camphanoyl CH_2), 1.57, 1.47 (each 3H, s, CH_3-2',2'), 1.27 (3H, d, *J* = 7.2, CHBr*CH$_3$*-2), 1.12, 1.11, 1.08, 1.05, 1.03, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -31.9° (*c* = 0.005, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-(1-dibromoethyl)dihydro-pyrano [2,3-f]chromone (106). A mixture of **69** (50 mg, 0.08 mmol), NBS (28.5 mg, 0.16 mmol), and 3-chloroperbenzoic acid (2 mg, 0.01 mmol), dissolved in 1 mL of anhydrous CCl_4 was heated to 100 ^{o}C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with an eluent of hexanes:EtOAc = 1:1 to afford pure **13** (8mg). 12% yield; mp 166-168 ^{o}C; MS-ESI+ (*m*/*z*, %) 809 (M^+ + 1, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.94 (1H, d, *J* = 9.0Hz, H-5), 6.80 (1H, d, *J* = 4.2 Hz, H-4'), 6.67 (1H,

s, H-3), 5.42 (1H, d, *J* = 4.2 Hz, H-3'), 2.81 (3H, s, $C(Br)_2CH_3$-2), 2.50, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.57, 1.47 (each 3H, s, CH_3-2',2'), 1.12, 1.11, 1.08, 1.05, 1.03, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -48.1° (*c* = 0.004, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-3,2',2'-trimethyl-2-cyanodihydropyrano[2,3-f]chromone (107). A solution of sodium cyanide in 95% EtOH (aqueous) was cooled in an ice-bath. Compound **127** (50 mg, 0.07 mmol) in 0.5 mL DMF was added slowly to the above solution over a 15 to 20 min period. The mixture was stirred at rt and monitored by TLC. At completion, the mixture was poured into ice-water and extracted with EtOAc three times. The combined organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in *vacuo* and the residue purified by PTLC to afford pure **107** (10 mg). 22% yield; mp 196-198 °C; MS-ESI+ (*m*/*z*, %) 684 (M^+ + Na, 100); ^{1}H NMR *δ* 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.99 (1H, d, *J* = 9.0 Hz, H-6), 6.61 (1H, d, *J* = 4.5 Hz, H-4'), 5.41 (1H, d, *J* = 4.5 Hz, H-3'), 2.55, 2.20, 1.96, 1.85 (each 2H, m, camphanoyl CH_2), 2.28 (3H, s, CH_3-3), 1.52, 1.48 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.10, 1.09, 1.05, 0.98 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -39.0° (*c* = 0.002, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-methylcyanodihydropyrano [2,3-f]chromone (108). The procedure was identical to that used for the preparation of **107**: 27% yield (starting with 20 mg of **104**); mp 102-104 °C; MS-ESI+ (*m*/*z*, %) 684 (M^+ + Na, 100); ^{1}H NMR *δ* 8.14 (1H, d, *J* = 9.0 Hz, H-5), 6.97 (1H, d, *J* = 9.0Hz, H-6), 6.72 (1H, d, *J* = 4.2 Hz, H-4'), 6.49 (1H, s, H-3), 5.40 (1H, d, *J* = 4.2 Hz, H-3'), 3.63 (2H, t, *J* = 25.5 Hz, CH_2CN-2), 2.50, 2.10, 1.95, 1.70 (each 2H, m, camphanoyl

CH_2), 1.56, 1.49 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.09, 1.03, 1.00, 0.98, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -27.9° (*c* = 0.002, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2,2',2'-trimethyl-3-bromodihydropyrano[2,3-f]chromone (110). The procedure was identical to that used for the preparation of **99**. 52% yield (starting from 21 mg of **68**); mp 160-161 °C; MS-ESI- (*m*/*z*, %) 713 (M^- - 1, 100); ^{1}H NMR δ 8.16 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.75 (1H, d, *J* = 4.5 Hz, H-4'), 5.38 (1H, d, *J* = 4.5 Hz, H-3'), 2.53 (3H, s, CH_3-2), 2.46, 2.16, 1.92, 1.73 (each 2H, m, camphanoyl CH_2), 1.54, 1.48 (each 3H, s, CH_3-2',2'), 1.13, 1.12, 1.09, 1.01, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -47.7° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyl-3-bromodihydropyrano [2,3-f]chromone (111). The procedure was identical to that used for the preparation of **99**. 60% yield (starting from 20 mg of **68**); mp 166-168 °C; MS-ESI+ (*m*/*z*, %) 730 (M^+ + 1, 100); ^{1}H NMR δ 8.18 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.74 (1H, d, *J* = 4.8 Hz, H-4'), 5.41 (1H, d, *J* = 4.8 Hz, H-3'), 3.02, 2.85 (each 1H, m, CH_2CH_3-2), 2.45, 2.10, 1.95, 1.85 (each 2H, m, camphanoyl CH_2), 1.55, 1.47 (each 3H, s, CH_3-2',2'), 1.24 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.13, 1.11, 1.081.03, 0.98, 0.98 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -34.1° (*c* = 0.006, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2,2',2'-trimethyl-3-iododihydropyrano[2,3-f]chromone (112). An anhydrous CH_2Cl_2 solution of **68** (40 mg, 0.06 mmol) and CF_3CO_2Ag (13.g mg, 0.06 mmol) was cooled to 0 °C in an ice-bath. I_2 (17.6 mg, 0.07 mmol) was added slowly under N_2 protection. The reaction mixture was stirred at 0

^{o}C for 2 h, monitored by TLC. At completion, the mixture was concentrated and purified by PTLC to give pure **112** (43 mg): 90% yield; mp 179-180 ^{o}C; MS-ESI+ (*m*/*z*, %) 785 (M^{+} + Na, 100); ^{1}H NMR δ 8.17 (1H, d, *J* = 9.0 Hz, H-5), 6.95 (1H, d, *J* = 9.0 Hz, H-6), 6.76 (1H, d, *J* = 4.5 Hz, H-4'), 5.39 (1H, d, *J* = 4.5 Hz, H-3'), 2.65 (3H, s, CH_3-2), 2.50. 2.15, 1.96, 1,85 (each 2H, m, camphanoyl CH_2), 1.55, 1.49 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.09, 1.02, 0.99, 0.96 (each 2H, s, camphanoyl CH_3); $[\alpha]_D$ -37.5° (*c* = 0.002, CH_2Cl_2).

3'*R*,4'*R*-di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyl-3-cyanodihydropyrano [2,3-f]chromone (113). The procedure was identical to that used for the preparation of **107**: 25% yield (starting with 50 mg of **111**); mp 193-194 ^{o}C; MS-ESI- (*m*/*z*, %) 674 (M^{-} - 1, 100); ^{1}H NMR δ 7.75 (1H, d, *J* = 9.0 Hz, H-5), 6.78 (1H, d, *J* = 9.0 Hz, H-6), 6.65 (1H, d, *J* = 4.5 Hz, H-4'), 5.38 (1H, d, *J* = 4.5 Hz, H-3'), 2.51 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 2.40, 2.16, 1.92, 1.74 (each 2H, m, camphanoyl CH_2), 1.55, 1.49 (each 3H, s, CH_3-2',2'), 1.24 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2), 1.21, 1.10, 1.07, 0.99, 0.98, 0.96, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -31.5° (*c* = 0.002, CH_2Cl_2).

General procedure for the preparation of amino-substituted DCP derivatives (109, 114-120). A THF solution of bromo-substituted DCP analogs (**104**, **110** or **111**) (1 equiv), various amines or aqueous amine solution (2.5 equiv) was stirred at rt for 3.5 h. The mixture was poured into water (excess) and extracted with EtOAc. After the usual workup, the crude product was purified by PTLC with an eluent of hexanes:EtOAc = 7:1 to afford corresponding amino-substituted DCP analogs (**109**, **114**-**120**).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-(methylamino)methyldihydro-pyrano[2,3-*f*]chromone (109). 80% yield (starting from 50 mg of **104**); mp 137-138 ^{o}C; MS-ESI+ (*m*/*z*, %) 666 (M^+ + 1, 100); ^{1}H NMR δ 8.14 (1H, d, *J* = 9.0 Hz, H-5), 6.92 (1H, d, *J* = 9.0 Hz, H-6), 6.75 (1H, d, *J* = 4.5 Hz, H-4'), 6.34 (1H, s, H-3), 5.40 (1H, d, *J* = 4.5 Hz, H-3'), 3.60, 3.55 (each 1H, d, *J* = 8.7 Hz, CH_2NHCH$_3$-2), 2.85 (1H, s, CH$_2$*NH*CH$_3$-2), 2.45, 2.14, 1.95, 1.71 (each 2H, m, camphanoyl CH$_2$), 2.45 (3H, s, CH$_2$NH*CH$_3$*-2), 1.55, 1.48 (each 3H, s, CH$_3$-2',2'), 1.13, 1.11, 1.08, 1.02, 0.99, 0.97, (each 3H, s, camphanoyl CH$_3$); $[\alpha]_D$ -35.8° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2,2',2'-trimethyl-3-aminodihydropyrano[2,3-*f*]chromone (114). 50% yield (starting from 18 mg of **110**); mp 137-138 ^{o}C; MS-ESI+ (*m*/*z*, %) 652 (M^+ + 1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.7 Hz, H-5), 6.75 (1H, d, *J* = 4.8 Hz, H-4'), 6.70 (1H, d, *J* = 8.7 Hz, H-6), 5.39 (1H, d, *J* = 4.8 Hz, H-3'), 2.46 (2H, s, NH$_2$-3), 2.45, 2.20, 1.95, 1.85 (each 2H, m, camphanoyl CH$_2$), 2.17 (3H, s, CH$_3$-2), 1.48, 1.47 (each 3H, s, CH$_3$-2',2'), 1.24, 1.10, 1.07, 0.99, 0.97, 0.86 (each 3H, s, camphanoyl CH$_3$; $[\alpha]_D$ -8.0° (*c* = 0.004, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyl-3-aminodihydropyrano [2,3-*f*]chromone (115). 40% yield (starting from 100 mg of **111**); mp 145-146 ^{o}C; MS-ESI+ (*m*/*z*, %) 652 (M^+ + 1, 100); ^{1}H NMR δ 7.70 (1H, d, *J* = 8.7 Hz, H-5), 6.73 (1H, d, *J* = 4.5 Hz, H-4'), 6.68 (1H, d, *J* = 8.7 Hz, H-6), 5.38 (1H, d, *J* = 4.5 Hz, H-3'), 5.0 (2H, br, NH$_2$-3), 2.50 (2H, q, *J* = 7.5 Hz, CH_2CH$_3$-2), 2.40, 2.20, 1.91, 1.60 (each 2H, m, camphanoyl CH$_2$), 1.52, 1.46 (each 3H, s, CH$_3$-2',2'), 1.21 (3H, t, *J* = 7.5 Hz, CH$_2$*CH$_3$*-2), 1.11, 1.10, 1.07, 0.99, 0.97, 0.85, (each 3H, s, camphanoyl CH$_3$); $[\alpha]_D$ -10.0° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-3,2',2'-trimethyl-3-methylaminodihydropyrano [2,3-*f*]chromone (116). 80% yield (starting from 22.2 mg of **110**); mp 122-124 ^{o}C; MS-ESI+ (*m*/*z*, %) 666 (M^+ + 1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.4 Hz, H-5), 6.76 (1H, d, *J* = 4.8 Hz, H-4'), 6.70 (1H, d, *J* = 8.4 Hz, H-6), 5.40 (1H, d, *J* = 4.8 Hz, H-3'), 3.06 (3H, s, NH*CH*$_3$-3), 2.54 (1H, s, *NH*CH_3-3), 2.40, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 2.20 (3H, s, CH_3-2), 1.52, 1.47 (each 3H, s, CH_3-2',2'), 1.12, 1.10, 1.06, 0.98, 0.95,0.83 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -21.4° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-ethyl-3-methylaminodihydro-pyrano[2,3-*f*]chromone (117). 75% yield (starting from 100 mg of **111**); mp 163-164 ^{o}C; MS-ESI+ (*m*/*z*, %) 680 (M^+ + 1, 100); ^{1}H NMR δ 7.73 (1H, d, *J* = 8.4 Hz, H-5), 6.76 (1H, d, *J* = 4.5 Hz, H-4'), 6.69 (1H, d, *J* = 8.4 Hz, H-6), 5.39 (1H, d, *J* = 4.5 Hz, H-3'), 3.08 (3H, s, NH*CH*$_3$-3), 2.63 (2H, q, *J* = 7.5 Hz, *CH*$_2$$CH_3$-2), 2.48, 2.20, 1.94, 1.77 (each 2H, m, camphanoyl CH_2), 1.55, 1.48 (each 3H, s, CH_3-2',2'),1.13, 1.11, 1.07, 0.99, 0.96 ,0.87 (each 3H, s, camphanoyl CH_3), 1.08 (1H, br, *NH*CH_3-3); $[\alpha]_D$ -24.6° (*c* = 0.013, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-morpholinomethyldihydro-pyrano[2,3-*f*]chromone (118). 30% yield (starting from 30 mg of **104**); mp 140-142 ^{o}C; MS-ESI+ (*m*/*z*, %) 722 (M^+ + 1, 100); ^{1}H NMR δ 8.13 (1H, d, *J* = 9.0 Hz, H-5), 6.92 (1H, d, *J* = 9.0 Hz, H-6), 6.74 (1H, d, *J* = 4.5 Hz, H-4'), 6.48 (1H, s, H-3), 5.38 (1H, d, *J* = 4.5 Hz, H-3'), 3.72 (4H, t, *J* = 4.5 Hz, 4.8 Hz, morpholine CH_2), 3.26, 3.42 (each 1H, d, *J* = 16.5 Hz, CH_2), 2.54 (4H, t, *J* = 4.5 Hz, 4.8 Hz, morpholine CH_2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 1.54, 1.48 (each 3H, s, CH_3-

2',2'), 1.13, 1.11, 1.08, 1.00, 0.98, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -28.5° (*c* = 0.011, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-(dimethylaminopropyl-piperazin1ylmethyl)–dihydropyrano[2,3-*f*]chromone (119). 15% yield (starting from 30 mg of **104**); mp 132-133 °C; MS-ESI+ (*m*/*z*, %) 806 (M^+, 100); ^{1}H NMR *δ* 8.13 (1H, d, *J* = 8.7 Hz, H-5), 6.92 (1H, d, *J* = 8.7 Hz, H-6), 6.73 (1H, d, *J* = 4.5 Hz, H-4'), 6.47 (1H, s, H-3), 5.38 (1H, d, *J* = 4.5 Hz, H-3'), 3.30, 3.44 (each 1H, d, *J* = 15.0 Hz, CH_2-2), 2.50 (8H, m, piperazine CH_2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 2.41 (4H, m, amino-propylpiperazine CH_2), 2.28 (6H, s, dimethylamino-propylpiperazine CH_3), 1.70 (2H, m, amino-propylpiperazine CH_2), 1.54, 1.48 (each 3H, s, CH_3-2',2'), 1.13, 1.11, 1.08, 1.01, 0.98, 0.95, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -20.3° (*c* = 0.003, CH_2Cl_2).

3'*R*,4'*R*-Di-*O*-(-)-camphanoyl-2',2'-dimethyl-2-(pyridin-4-ylmethylamino)-methyldihydropyrano[2,3-*f*]chromone (120). 25% yield (starting from 30 mg of **104**); mp 116-118 °C; MS-ESI+ (*m*/*z*, %) 806 (M^+ + 1, 100); ^{1}H NMR *δ* 8.55 (2H, d, *J* = 6.0 Hz, pyridine CH), 8.13 (1H, d, *J* = 9.0 Hz, H-5), 7.29 (2H, d, *J* = 6.0 Hz, pyridine CH), 6.92 (1H, d, *J* = 9.0 Hz, H-6), 6.74 (1H, d, *J* = 4.5 Hz, H-4'), 6.36 (1H, s, H-3), 5.39 (1H, d, *J* = 4.5 Hz, H-3'), 3.85 (1H, s, CH_2*NH*-2), 3.59, 3.68 (each 1H, d, *J* = 15.9 Hz, CH_2-2), 2.50, 2.15, 1.95, 1.70 (each 2H, m, camphanoyl CH_2), 1.55, 1.48 (each 3H, s, CH_3-2'), 1.13, 1.11, 1.07, 0.99, 0.96, 0.94, (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -16.9° (*c* = 0.003, CH_2Cl_2).

4.4.2 HIV-1 Infectivity Assay

Anti-HIV-1 activity was measured as reductions in Luc reporter gene expression after a single round of virus infection of TZM-bl cells. HIV-1 at 200 $TCID_{50}$ and various dilutions of test samples (eight dilutions, 4-fold stepwise) were mixed in a total volume of 100 μL growth medium in 96-well black solid plates (Corning-Costar). After 48-h incubation, culture medium was removed from each well and 100 μL of Bright Glo luciferase reagent was added to each culture well. The luciferase activity in the assay wells was measured using a Victor 2 luminometer. The 50% inhibitory dose (IC_{50}) was defined as the sample concentration that caused a 50% reduction in Relative Luminescence Units (RLU) compared to virus control wells after subtraction of background RLU.

4.4.3 Cytotoxicity Assay

The general procedure was performed according to CytoTox-Glo™ cytotoxicity assay instructions for using product G9290, G9291 and G9292. (Promega)

4.4.4 Water Solubility Assay

Each tested compound was added in excess to 1.5 mL Eppendorf tubes containing 1 mL of HPLC grade water. The tubes were placed into Branson 5510 ultrasonic tank at room temperature for 1 h. The excess solid was separated from the solution through a PTFE syringe filter (0.2 μM diameter). The supernatant was dispensed into glass HPLC vials. The concentration of the samples was determined with HPLC, on an Alltima C18 3u column (2.1 mm × 100 mm) and a flow rate of 200 μL/min. The samples (5 μL) were injected and run with a solution of 35% water and

65% MeCN. For each compound, a standard curve consisting of five concentrations (5-fold stepwise) in MeCN was established initially.

4.5 References

1. Kilmarx, P. H. Global epidemiology of HIV. *Curr Opin HIV AIDS* **2009,** 4, 240-246.

2. Hupfeld, J.; Efferth, T. Review. Drug resistance of human immunodeficiency virus and overcoming it by natural products. *In Vivo* **2009,** 23, 1-6.

3. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K. H. Anti-AIDS agents. 15. Synthesis and anti-HIV activity of dihydroseselins and related analogs. *J Med Chem* **1994,** 37, 3947-3955.

4. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. Anti-AIDS agents. 37. Synthesis and structure-activity relationships of (3'R,4'R)-(+)-cis-khellactone derivatives as novel potent anti-HIV agents. *J Med Chem* **1999,** 42, 2662-2672.

5. Huang, L.; Yuan, X.; Yu, D.; Lee, K. H.; Chen, C. H. Mechanism of action and resistant profile of anti-HIV-1 coumarin derivatives. *Virology* **2005,** 332, 623-628.

6. Yu, D.; Brossi, A.; Kilgore, N.; Wild, C.; Allaway, G.; Lee, K. H. Anti-HIV Agent 55: 3'R, 4'R-Di-*O*-(-)-Camphanoyl-2',2'-Dimethyldihydropyrano[2,3,*f*]chromone (DCP), a Novel Anti-HIV Agent. *Bioorg. Med. Chem. Lett.* **2003,** 13, 1575-1576.

7. Yu, D.; Chen, C. H.; Brossi, A.; Lee, K. H. Anti-AIDS agents. 60. Substituted 3'R,4'R-di-O-(-)-camphanoyl-2',2'-dimethyldihydropyrano[2,3-f]chromone (DCP) analogues as potent anti-HIV agents. *J Med Chem* **2004,** 47, 4072-4082.

8. Linusson, A.; Gottfries, J.; Olsson, T.; Ornskov, E.; Folestad, S.; Norden, B.; Wold, S. Statistical molecular design, parallel synthesis, and biological evaluation of a library of thrombin inhibitors. *J Med Chem* **2001,** 44, 3424-3439.

9. Ding, K.; Wang, S. Efficient Synthesis of Isoflavone Analogues via a Suzuki Coupling Reaction. *Tetrahedron Letters* **2005,** 46, 3707-3709.

10. Appel, B.; Saleh, N.; Langer, P. Domino Reactions of 1,3-bis-silyl enol ethers with Benzopyrylium triflates Efficient Synthesis of Fluorescent 5*H*-Benzo[c]chromen-6-ones, Dibenzo[c,d]chromen-6-ones, and 2,3-Dihydro-1*H*-4,6-Dioxachrysen-5-ones. *Chemistry - A European Journal* **2006,** 12, 1221-1236.

11. Zhou, T.; Shi, Q.; Lee, K. H. Anti-AIDS Agents 83. Efficient Microwave-assisted One-pot Preparation of Angular 2,2-Dimethyl-2*H*-chromone Containing Compounds. *Tetrahedron Lett.* **2010,** 51, 4382-4386.

12. Wahala, K.; Hase, T. A. Expedient Synthesis of Polyhydroxyisoflavones. *J. Chem. Soc., Perkin Trans. 1* **1991,** 12, 3005-3008.

13. Kolb, H. C.; VanNieuwenhze, M. S.; Shaprless, K. Catalytic Asymmetric Dihydroxylation. *Chem. Rev.* **1994,** 94.

14. Mehltretter, G. M.; Dobler, C.; Sundermeier, U.; Beller, M. An Improved Version of the Sharpless Asymmetric Dihydroxylation. *Tetrahedron Lett.* **2000,** 41, 8083-8087.

15. Xie, L.; Crimmins, M. T.; Lee, K. H. Anti-AIDS Agents 22. Asymmetric Synthesis of 3',4'-Di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (DCK), a Potent Anti-HIV Agent. *Tetrahedron Lett.* **1995,** 36, 4529-4532.

16. Djerassi, C. Brominations with N-Bromosuccinimide and Related Compounds, The Wohl-Ziegler Reaction. *Chem. REv.* **1948,** 43, 271-317.

17. Sundaresan, A. K., Ramamurthy, V. Making a Difference on Excited-State Chemistry by Controlling Free Space within a Nanocapsule: Photochemistry of 1-(4-Alkylphenyl)-3-phenylpropane-2-ones. *Org. Letts.* **2007,** 9, 3575-3578.

18. Miyake, H.; Nichino, S.; Nishimura, A.; Sasaki, M. New Synthesis of 3-Bromoflavones via Bromination of 1-(2-Hydroxyphenyl)-3-Arylpropane-1,3-dione by $CuBr_2$. *Chem. Letts.* **2007,** 36, 522-523.

19. Dallavalle, S.; Gattinoni, S.; Mazzini, S.; Scaglioni, L.; Merlini, L.; Tinelli, S.; Beretta, G.; Zunino, F. Synthesis and Cytotoxic Activity of a New Series of Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2008,** 18, 1484-1489.

20. Tang, G.; Ding, K.; Nikolovska-Coleska, Z.; Yang, C. Y.; Qiu, S.; Shangary, S.; Wang, R.; Guo, J.; Gao, W.; Meagher, J.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Wang, S. Structure-based design of flavonoid compounds as a new class of small-molecule inhibitors of the anti-apoptotic Bcl-2 proteins. *J Med Chem* **2007,** 50, 3163-3166.

21. Montreal, Q., Canada. MOE. *Chemical Compouting Group* **2007**.

22. (Italy), T. s. r. l. M. DRAGON for Windows (Software for Molecular Descriptor Calculations). **2006,** Version 5.4.

CHAPTER 5

DESIGN, SYNTHESIS AND EVALUATION OF 1*R*,2*R*-3,3-DIMETHYLDIHYDROPYRANO- [2,3-*c*]XANTHEN-7(1*H*)-ONE (DCX) DERIVATIVES AS NOVEL ANTI-HIV AGENTS

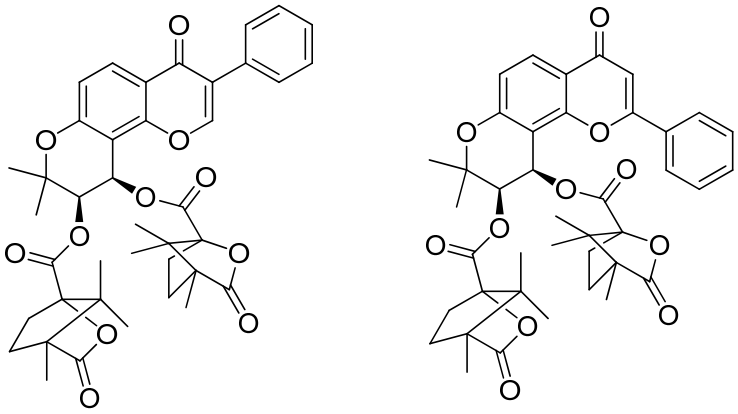
5.1 Introduction

As introduced in Chapter 2, compounds (**67**, **73**) with a benzyl ring substituted at 2 or 3 position of DCP have been synthesized and evaluated. (Figure 5-1)[1] However, compounds with an additional aromatic ring fused to the chromone ring system have never been studied.

Previous SAR study suggested that the planar chromone ring system in DCPs is critical to maintain anti-HIV potential against both wide type and multi-drug resistant HIV strains. Pharmacophore analysis described in chapter 4 also identified the planar ring system as a potential pharmacophore.[2] We speculated that an extend planar system by constructing a fused aromatic ring to chromone ring can help keeping a more rigid planer system and the extended conjugation ring may sustain or even increase the π-π stacking interaction between the ligand and the target protein.

Therefore, in order to better understand the interaction between the planar ring system of drug molecules and binding pocket, we designed a series of tri-aryl

conjugated compounds, with a xanthen-9-one moiety replacing the chromone ring in DCK and DCP series.(Figure 5-2) We hope this modification could enhance the interaction between the drug molecules and the target proteins, and therefore, improve the anti-HIV activity profile.



67 **73**

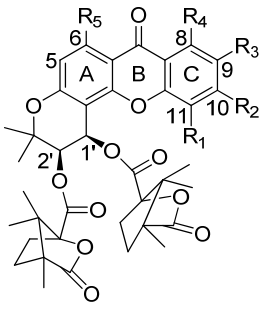
Figure 5-1. Structures of DCP analogs **67** and **73**

5.2 Design

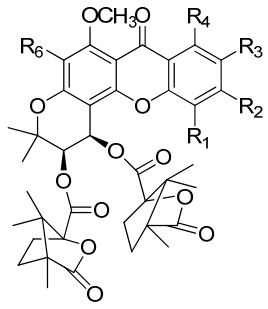
Inspired from previous study, introducing appropriate alkyl or O-alkyl groups on the planar ring system dramatically improved the anti-HIV activity. 4-MDCK (**26**) and 2-EDCP (**69**) exhibited 5- and 4-time better anti-HIV activity than the parent DCK (**18**) and DCP (**65**), respectively (Chapter 2); 5-methyl substituted DCP (compounds **98** and **102**) presented the best potency against both wild-type and drug-resistant HIV strains among the tested compounds. In our current study, we first designed 1*R*,2*R*-3,3-dimethyldihydropyranoxanthone (DCX) analogs with a series of small alkyl and O-alkyl groups substituted on the C ring (R_1 – R_4) and A ring (R_5) to evaluate the effects of alky substitutions (Figure 5-2, compounds **130-131**, **133-140** and **148**) We then introduced a group of other functional moieties such as halogen, cyano groups

with different physicochemical properties to explore how these functionalities affect the anti-HIV activity. The cyano group might also expand the conjugation of xanthenone ring besides its electron withdrawing effect, and results in the potent anti-HIV activity, as speculated in chapter 4. (Figure 5-2, compounds **142-147**, **149-150** and **152**)

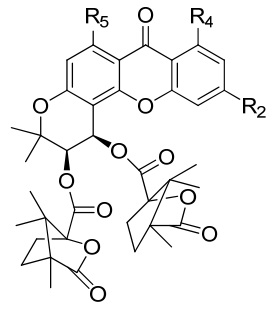
To increase compounds' polarity and solve the water-solubility problem, we proposed to build some small polar functional groups, such as hydroxyl, amine into the ring system. (Figure 5-2, compounds **132**, **141** and **151**) Both hydroxy and amine moieties not only can manipulate the polarity of the drug molecule but also are able to be derivatized to make water soluble salt.



130 $R_1 = R_2 = R_3 = R_4 = R_5 = H$
131 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OCH_3$
133 $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$, $R_5 = OCH_3$
134 $R_2 = CH_3$, $R_1 = R_3 = R_4 = H$, $R_5 = OCH_3$
135 $R_3 = CH_3$, $R_1 = R_2 = R_4 = H$, $R_5 = OCH_3$
136 $R_4 = CH_3$, $R_1 = R_2 = R_3 = H$, $R_5 = OCH_3$
137 $R_1 = R_5 = OCH_3$, $R_2 = R_3 = R_4 = H$
138 $R_2 = = R_5 = OCH_3$, $R_2 = R_3 = R_4 = H$
139 $R_3 = R_5 = OCH_3$, $R_1 = R_2 = R_4 = H$
140 $R_4 = R_5 = OCH_3$, $R_1 = R_2 = R_3 = H$
148 $R_2 = R_5 = OCH_3$, $R_4 = CH_3$, $R_1 = R_3 = H$



142 $R_1 = F$, $R_2 = R_3 = R_4 = R_6 = H$
143 $R_3 = F$, $R_1 = R_2 = R_4 = R_6 = H$
144 $R_4 = F$, $R_1 = R_2 = R_3 = R_6 = H$
145 $R_2 = Br$, $R_1 = R_3 = R_4 = R_6 = H$
146 $R_3 = Br$, $R_1 = R_2 = R_4 = R_6 = H$
147 $R_6 = Br$, $R_1 = R_2 = R_3 = R_4 = H$
149 $R_6 = Br$, $R_1 = R_2 = R_4 = H$, $R_3 = CH_3$
150 $R_3 = CH_2Br$, $R_1 = R_2 = R_4 = R_6 = H$
152 $R_2 = CN$, $R_1 = R_3 = R_4 = R_6 = H$



132 $R_5 = OH$, $R_2 = R_4 = H$
141 $R_4 = OH$, $R_2 = H$, $R_5 = OCH_3$
151 $R_2 = NH_2$, $R_4 = H$, $R_5 = OCH_3$

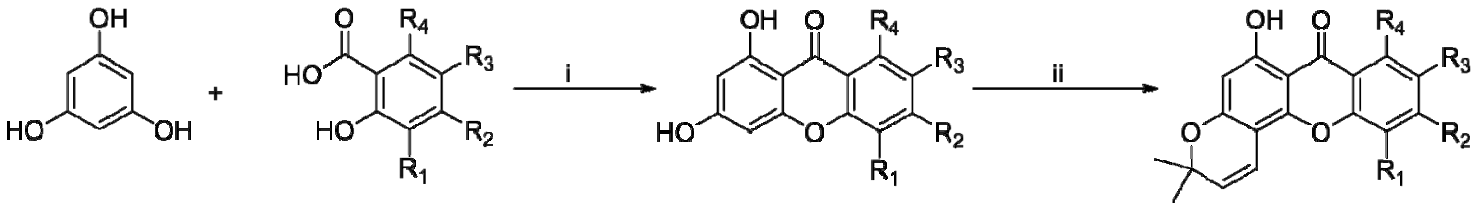
Figure 5-2. Structures of Novel DCX analogs

5.3 Chemistry

The general synthetic route to obtain pyrano-xanthones is shown in scheme 5-1. Hydroxylated xanthones (**169b-l, k-q**) were synthesized through the cyclization

reaction between phloroglucinol (**153**) and appropriate substituted salicylic acids (**154**-**168**), in the presence of Eaton's reagent (phosphorus pentoxide solution in methanesulfonic acid) as a catalytic condensation agent. [3] A slight excess amount of phloroglucinol was used to complete the desired reaction and avoid the formation of side products, hydroxychromeno[3,2-*b*]xanthenesdiones. The desired products (**169b-l, k-q**) as brownish solids were obtained after precipitation in ice-water and collected by filtration, which were carried to the next step reaction without further purification. 6-Hydroxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-ones (**94**-**96a, 170**-**181**) were synthesized by alkylation and cyclization from xanthones (**169b-l, k-q**) by the microwave-assisted method, described in chapter 3.[4]

Methylation of the resulting compounds, 6-hydorxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-ones (**94a-96a, 170-181**) with MeI and K_2CO_3 yielded 6-methoxy-3,3-dimethylpyranoxanthones (**182b-q**).[5] (DCX) analogs, **130**-**131**, **133**-**146** and **148** were synthesized by following the literature procedures[1] through Sharpless asymmetric dihydroxylations [6, 7] and esterification[7, 8] with excess (*S*)-camphanoyl chloride.

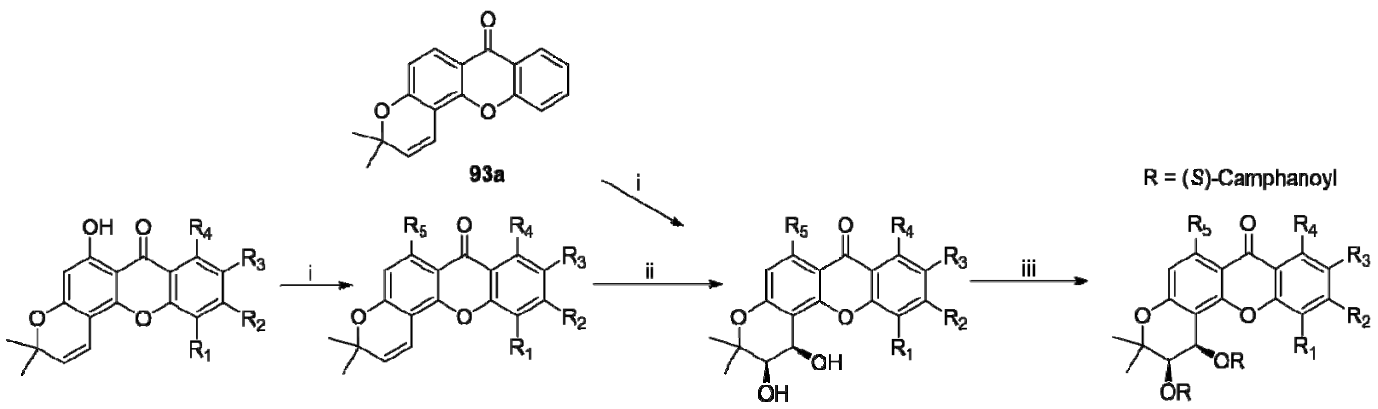


153 **154** $R_1 = R_2 = R_3 = R_4 = H$
155 $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
156 $R_2 = CH_3$, $R_1 = R_3 = R_4 = H$
157 $R_3 = CH_3$, $R_1 = R_2 = R_4 = H$
158 $R_4 = CH_3$, $R_1 = R_2 = R_3 = H$
159 $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
160 $R_2 = OCH_3$, $R_2 = R_3 = R_4 = H$
161 $R_3 = OCH_3$, $R_1 = R_2 = R_4 = H$
162 $R_4 = OCH_3$, $R_1 = R_2 = R_3 = H$
163 $R_1 = F$, $R_2 = R_3 = R_4 = H$
164 $R_3 = F$, $R_1 = R_2 = R_4 = H$
165 $R_4 = F$, $R_1 = R_2 = R_3 = H$
166 $R_2 = Br$, $R_1 = R_3 = R_4 = H$
167 $R_3 = Br$, $R_1 = R_2 = R_4 = H$
168 $R_2 = OCH_3$, $R_4 = CH_3$, $R_1 = R_3 = H$

169b $R_1 = R_2 = R_3 = R_4 = H$
169c $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
169d $R_2 = CH_3$, $R_1 = R_3 = R_4 = H$
169e $R_3 = CH_3$, $R_1 = R_2 = R_4 = H$
169f $R_4 = CH_3$, $R_1 = R_2 = R_3 = H$
169g $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
169h $R_2 = OCH_3$, $R_2 = R_3 = R_4 = H$
169i $R_3 = OCH_3$, $R_1 = R_2 = R_4 = H$
169k $R_4 = OH$, $R_1 = R_2 = R_3 = H$
169l $R_1 = F$, $R_2 = R_3 = R_4 = H$
169m $R_3 = F$, $R_1 = R_2 = R_4 = H$
169n $R_4 = F$, $R_1 = R_2 = R_3 = H$
169o $R_2 = Br$, $R_1 = R_3 = R_4 = H$
169p $R_3 = Br$, $R_1 = R_2 = R_4 = H$
169q $R_2 = OCH_3$, $R_4 = CH_3$, $R_1 = R_3 = H$

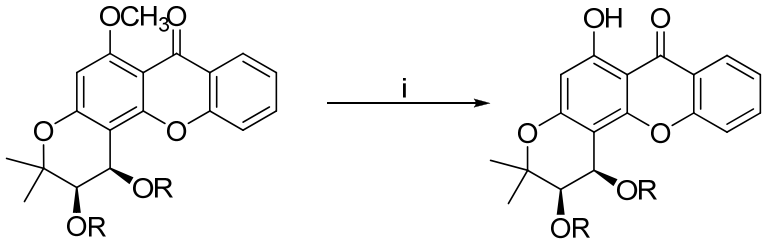
94a $R_1 = R_2 = R_3 = R_4 = H$
170 $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
96a $R_2 = CH_3$, $R_1 = R_3 = R_4 = H$
95a $R_3 = CH_3$, $R_1 = R_2 = R_4 = H$
171 $R_4 = CH_3$, $R_1 = R_2 = R_3 = H$
172 $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
173 $R_2 = OCH_3$, $R_2 = R_3 = R_4 = H$
174 $R_3 = OCH_3$, $R_1 = R_2 = R_4 = H$
175 $R_4 = OH$, $R_1 = R_2 = R_3 = H$
176 $R_1 = F$, $R_2 = R_3 = R_4 = H$
177 $R_3 = F$, $R_1 = R_2 = R_4 = H$
178 $R_4 = F$, $R_1 = R_2 = R_3 = H$
179 $R_2 = Br$, $R_1 = R_3 = R_4 = H$
180 $R_3 = Br$, $R_1 = R_2 = R_4 = H$
181 $R_2 = OCH_3$, $R_4 = CH_3$, $R_1 = R_3 = H$

Scheme 5-1. Synthesis of compounds **94a-96a**, **170-181**. Reagents and conditions:(i) Eaton's reagent, reflux; (ii) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave, 220°C/4h.



Scheme 5-2. Synthesis of compounds **130-131, 133-146, and 148.** Reagents and conditions:(i) MeI, K_2CO_3, acetone, reflux; (ii) $K_3Fe(CN)_6$, $(DHQ)_2PYR$, $K_2OsO_2(OH)_4$, K_2CO_3, methanesulfonamide, *t*-butanol/H_2O, 0°C; (iii) (S)-camphanoyl chloride, DMAP, CH_2Cl_2, rt.

Demethylation of 6-methoxy DCX (**131**) with 48% hydrogen bromide acid solution yielded 6-hydroxy DCX (**132**) in 63% yield.[5] (Scheme 5-3)

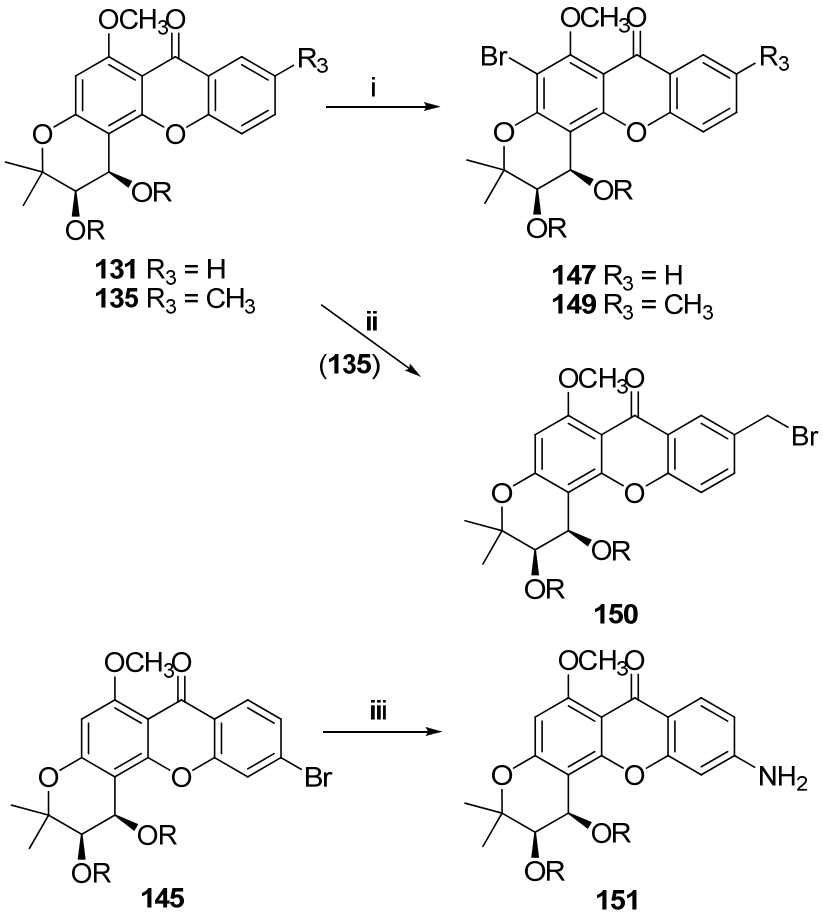


131 R = (*S*)-Camphanoyl **132**

Scheme 5-3. Synthesis of compound **132**. Reagents and conditions:
(i) HBr/CH_3COOH, reflux

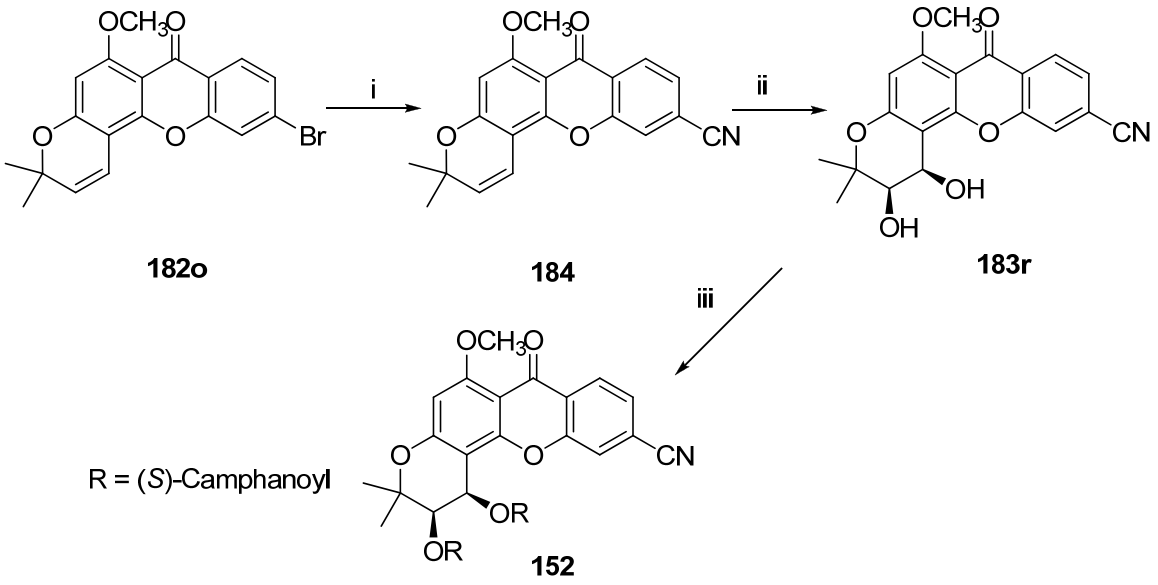
Compounds **147**, **149** and **150** were synthesized by bromination of of **131** and **135** with NBS under appropriate conditions. Bromination at 5-position was accomplished in dichloromethane under microwave condition [9] to generate compounds **147** and **149**; While benzylic bromination of **135** with NBS in anhydrous carbon tetrachloride in the presence of MCPBA as a radical initiator[2] led to compound **150**. Reaction of **145** with excess NH_3 aqueous solution afforded compound **151**.[10] (Scheme 5-4)

Scheme 5-5 illustrates the synthetic pathway of 6-methoxy-10-cyano DCX (**152**). Compound **182o** was treated with zinc cyanide in the presence of tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] as a catalyst to afford compound **184**.[11] After dihydroxylation and esterification by following the existing procedures discussed before [6.7.8] to yield the compound **152**.



R = (*S*)-Camphanoyl

Scheme 5-4. Synthesis of compounds **147**, **149**-**151**. Reagents and conditions: (i) NBS, CH_2Cl_2, microwave 100°C/2h; (**ii**) MCPBA, NBS, CCl_4, reflux; (**iii**) NH_4OH (33% aq.), THF, rt.



Scheme 5-5. Synthesis of compound **152**. Reagents and conditions: (i) $Zn(CN)_2$, tetrakis (triphenylphosphine)palladium(0), DMF, 160°C; (ii) $K_3Fe(CN)_6$, K_2CO_3, $(DHQ)_2PYR$, $K_2OsO_2(OH)_4$, methanesulfonamide, *t*-butanol/H_2O, 0°C; (iii) (s)-camphanoyl chloride, DMAP, CH_2Cl_2, rt.

5.4 Results and Discussion

All synthesized DCX analogs were screened against wild type NL4-3 HIV strain in a single cycle infection assay using TZM-bl cells and the result is shown in Table 5-1.

The structures of **130**-**132** are little different from each other with substitutions at 6-position. This slight structural differentiation resulted in significant activities deviation. Compound **131** with a methoxy group at 6-position showed 5-fold more potent anti-HIV activity than **130** with EC_{50} value as low as 0.063 μM and 1.5-fold more active than 2-EDCP (**69**, EC_{50} at 0.089 μM), a positive comparison. **131** also presented a two-fold better TI value than 2-EDCP, suggesting a better selectivity of HIV-infected cells over normal cells. In contrast, compound **132**, a 6-hydroxyl DCX analogue failed to present notable selectivity (an average TI value less than 4 was

observed) perhaps due to the intramolecular HB between C6-OH and C7 carbonyl oxo, which might in turn affect the H-bonding interaction between C7 carbonyl oxo and the target protein. These findings suggested that introducing a hydrophobic substituent at C6 position, is a good strategy to maintain or enhance anti-HIV activity. Unlike **132**, compound **141**, with an OH group at C8 position (R4), still remained certain levels of anti-HIV activity and sensitivity, suggesting an unequal HB effect resulted from two similar adjacent OHs and the carbonyl oxo, probably due to unequal molecular substitution surroundings and/or points by points interactions of the substituents on the drug molecule and the target protein, which may, in turn, weaken the intramolecular interaction of C8-OH.

With a bromide at 5-position (R_6), both compounds **147** and **149** showed significantly reduced anti-HIV activity and TI values (TI below 4), in comparison to their parent compounds **131** and **135**.

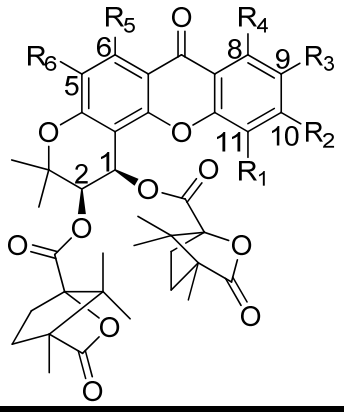
The size of substitution at C11 (R_1) of DCX series also impacts anti-HIV activity . Larger substitutions at C11 seemed to adversely influence the activity in our preliminary study. Compound **131,** with only C_{11}-H, exhibited higher potency than **142,** a 11-fluorine DCX , which is more potent than 11-methyl-DCX (**133**) (EC_{50} of **131**, **142** and **133** are 0.063, 0.23 and 1.52 μM, respectively). 11-Methoxy-DCX (**137**) showed no detectable anti-HIV activity and selectivity against wild type HIV_{NL4-3} strain. It may be due to the fact that the substitution R_1 at C11 influences the orientation of 1-camphanoyl group, as observed in the DCP series.[2]

As mentioned in chapter 3, the 2 and 3-positions of chromone system in DCP analogs, displayed equivalent characteristics when substituted with the same functionalities.[2] In this study, introducing substitutions at 9 (R_3) or 10 (R_2) position showed different anti-HIV activity profile. 9-methylated (**135**) and 9-methoxylated (**139**) DCXs exhibited better anti-HIV activity than their analogs 10-methylated (**134**) and 10-methoxylated (**138**) DCXs. The EC_{50} of **135** and **134** are 0.065 vs 0.095 μM and the EC_{50} of **139** and **138** are 0.12 vs 0.362 μM, respectively. The anti-HIV effect of C9 or C10 methylated DCXs is comparable to **131,** and better than 2-EDCP. However, methoxy group at 9- or 10-position decreased the activity compared to **131**.

The effect of the substitution at 8-position (R_4) on anti-HIV activity was studied and ranked as 8-F > 8-CH_3 > 8-OH > 8-OCH_3. 8-Fluoro-substituted DCX **144** presented the best anti-HIV activity and selectivity of the four compounds (**136, 140, 141** and 2-EDCP), with EC_{50} value of 0.062 μM and TI value greater than 224, which is , and comparable active to **131**. The finding indicated that there is non-substitution with size larger than proton H required at C8 position to maintain or enhance anti-HIV activity. F atom with the similar size to H exhibits the similar activity.

Introducing N-containing groups at position 10 (R_2) resulted in compounds **151** and **152. 151,** with a primary amine group, appeared an inactive compound, while **152** with a C10-cyano moiety, well sustained considerable anti-HIV activity, with EC_{50} ~ 0.20 μM.

Table 5-1. Anti-HIV activity of DCX analogs (130-152) against NL4-3 HIV strain.[a]



	R_1	R_2	R_3	R_4	R_5	R_6	CC_{50}[b] μM	EC_{50}[c] μM	TI[d]
130	H	H	H	H	H	H	>29.8	0.308	>96.8
131	H	H	H	H	OCH_3	H	>14.3	0.063	>227.0
132	H	H	H	H	OH	H	N/A	N/A	---[e]
133	CH_3	H	H	H	OCH_3	H	4.32	1.52	2.84
134	H	CH_3	H	H	OCH_3	H	>14.0	0.095	>147.4
135	H	H	CH_3	H	OCH_3	H	11.4	0.065	175.4
136	H	H	H	CH_3	OCH_3	H	11.6	0.15	77.3
137	OCH_3	H	H	H	OCH_3	H	N/A	N/A	---
138	H	OCH_3	H	H	OCH_3	H	>13.7	0.362	>37.8
139	H	H	OCH_3	H	OCH_3	H	10.8	0.12	88.8
140	H	H	H	OCH_3	OCH_3	H	11.6	1.70	6.83
141	H	H	H	OH	OCH_3	H	8.1	0.33	24.5
142	F	H	H	H	OCH_3	H	7.8	0.23	33.9
143	H	H	F	H	OCH_3	H	26.0	0.10	260
144	H	H	H	F	OCH_3	H	>13.9	0.062	>224.2
145	H	Br	H	H	OCH_3	H	N/A	N/A	---
146	H	H	Br	H	OCH_3	H	9.23	1.47	6.83
147	H	H	H	H	OCH_3	Br	N/A	N/A	---
148	H	OCH_3	H	CH_3	OCH_3	H	>26.8	0.14	>191.5
149	H	H	CH_3	H	OCH_3	Br	N/A	N/A	---
150	H	H	CH_2Br	H	OCH_3	H	>25.2	4.53	>5.55
151	H	NH_2	H	H	OCH_3	H	N/A	N/A	---
152	H	CN	H	H	OCH_3	H	9.08	0.20	45.4
69 2EDCP							12.1	0.089	136.0

[a] All data presented in this table were averaged from at least three independent experiments.
[b] Cytotoxicity was determined using a Promega CytoTox-Glo™ assay kit.
[c] This assay was performed in TZM-bl cell infected with NL4-3 HIV strain.
[d] Therapeutic index = CC_{50} / EC_{50}.
[e] No selective anti-HIV activity (CC_{50} / EC_{50} < 4)
[f] No suppression at 10 μg/mL

New DCX analogs which showed considerable anti-HIV activity against wild-type HIV strain were selected and screened the effectiveness against drug-resistant HIV RTMDR1 strain. The data are listed in Table 5-2. Interestingly, all selected DCX analogs (**130-131**, **134-136**, **139** and **143-144**) that are active against wild type HIV_{NL4-3} strain also showed activity against drug-resistant strain. Both EC_{50} and TI values supported that the compound **144** is most active compound followed by **131**>**135**>**143**. The activity profile recorded from anti-drug-resistant HIV strain is consistent to that derived from drug sensitive wild-type HIV strain.

In conclusion, the bioassay data generated from this study clearly demonstrated that the new class of DCX analogs is a series of potent and promising anti-HIV agents compared with DCP and DCK series. The SARs derived from this study are summarized as follows:

1. The planar ring extension from a two-conjunct ring system (pyranochromone in DCP) to a three-conjunct ring system (pyrano-xanthone in DCX) well remains or even increases the anti-HIV activity against both wild-type and drug-resistant strains, suggesting that a conjugated planar ring system may be essential to interact with the target protein through a ring stacking interaction and a larger DCX enables to occupy more space in binding pocket , and as a result interacting more efficiently with amino acids residues inside the binding pocket.
2. Substitutions at the different positions of the molecule dramatically affect the anti-HIV activity against both viral strains. A methyl group at the C11 (R_1) position generated an almost inactive compound (**133**), while at C9 (R_3) or

C10 (R_2) achieved two potent anti-HIV agents **134** and **135**. For the methoxy substitution, besides necessity at C6 (R_5), C9-OMe substituted compound **139** exhibited better activity than the C10-OMe and C8-OMe substituted compounds **138** and **140**. Although with a hydroxyl group at C6, compound **132** showed no anti-HIV activity and sensitivity, compound **141**, with a C8-OH, appeared relatively active with EC_{50} value of 0.3 μM.

3. The properties of the substitutions at the positions also play an important role. A free OH group at C6 resulted in an inactive compound **132**. Masked with a C6-methoxyl, compound **131** ranked up to the one of the most active compounds. Generally, methyl substituted compounds showed more potent anti-HIV activity than the compounds with a methoxyl substitution, except compound **149**, which bears an additional C5 (R6) bromide. Introducing polar groups, such as amine or cyano influenced the anti-HIV profile variably. A cyano group at the C10 well maintained the activity, while an amino group ruined the activity completely.

Table 5-2. Anti-HIV activity of DCX analogs against drug-resistant RTMDR1 HIV strain.

	CC_{50} (μM)	**EC_{50}[a] (μM)**	**TI[b]**
130	>29.8	0.546	>54.6
131	>14.3	0.074	>193.2
134	>14.0	0.363	>38.6
135	11.4	0.081	140.7
136	11.6	0.37	31.4
139	10.8	0.42	25.7
143	26.0	0.16	162.5
144	>13.9	0.065	>213.8
69	12.1	0.11	110

[a] This assay was performed in TZM-bl cell infected with HIV RTMDR1 strain.

[b] Therapeutic index = CC_{50} / EC_{50}.

5.5 Conclusion

Our study indentified a new entity, DCX, and a series of DCX analogs, as potent anti-HIV agents. Most of DCX analogs are active against both wild-type and drug-resistant HIV strains. Compared to the control 2-EDCP (**69**), three compounds (**131**, **135** and **144**) showed better activity in both HIV strains, and four other compounds (**134**, **139**, **143** and **148**) exhibited comparable anti-HIV potency. The compounds that presented high anti-HIV potency also presented high therapeutic index. Six analogs (**131**, **134-135**, **143-144** and **148**) showed enhanced TI values in comparison to the control. We also established SAR study based on anti-HIV on both wild-type and drug-resistant HIV strains. Further modification is currently under-going to further improve anti-HIV activity and pharmacological profile for this type of compounds.

5.6 Experimental Section

5.6.1 Chemistry

1H NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Microwave reactions were performed with a Biotage initiator EXP US. Mass spectra were measured on Shimadzu LCMS-2010 (ESI-MS). Biotage Flash and Isco Companion systems were used as medium-pressure column chromatography. All other chemicals were obtained from Aldrich.

Synthesis of substituted 1,3-dihydroxy-9*H*-xanthen-9-one (169). To a mixture of commercially available phlorolgucinol (1.2 equiv), and appropriate substituted salicylic acid (1 equiv) was added slowly 20 mL of Eaton's reagent (P_2O_5-CH_3SO_3H). The mixture was stirred for 3 hours at 80 ^oC, cooled to r.t., and poured onto ice. After vigorous stirring at r.t. for 2 h, brownish solid precipitated. The solid was collected by filtration, washed with water (pH ~ 6), and dried at 60^oC to give 1,3-dihydorxy-9*H*-xanthen-9-ones as a brownish solid. In most condition, the brownish solid was used without further purification.

1,3-Dihydroxy-9*H*-xanthen-9-one (169b). ^{1}NMR δ 8.15 (1H, d, J = 8.1 Hz, H-8), 7.85 (1H, t, J = 8.1, 6.9 Hz, H-6), 7.61 (1H, d, J = 8.1 Hz, H-5), 7.49 (1H, t, J = 8.1, 6.9 Hz, H-7), 6.41 (1H, s, H-4), 6.23 (1H, s, H-2).

1,3-Dihydroxy-7-methyl-9*H*-xanthen-9-one (169e). ^{1}NMR δ 12.88 (1H, s, OH-1), 11.03 (1H, s, OH-3), 7.92 (1H, s, H-8), 7.68 (1H, d, J = 8.4 Hz, H-5), 7.52 (1H, d, J = 8.4 Hz, H-6), 6.41 (1H, s, H-4), 6.22 (1H, s, H-2), 2.44 (3H, s, CH_3-7).

1,3,8-Trihydroxy-9*H*-xanthen-9-one (169k). ^{1}NMR δ 11.86 (1H, s, OH-1), 11.80 (1H, s, OH-8), 7.68 (1H, t, J = 8.4, 8.4 Hz, H-6), 7.01 (1H, d, J = 8.4 Hz, H-5), 6.80 (1H, d, J = 8.4 Hz, H-7), 6.39 (1H, d, J = 2.1 Hz, H-4), 6.23 (1H, d, J = 2.1 Hz, H-2).

Synthesis of 6-hydroxy-3,3-dimethylpyrano[2,3-*c*]-7(3*H*)-ones(170-181). A mixture of starting compounds **169c-i**, **169k-q** (1equiv), 4,4-dimethoxy-2-methyl-2-butanol (1.5-2 equiv) and anhydrous pyridine (2-3 mL) was heated at 220 ^oC for 4 h under high-absorption microwave condition. The reaction mixture was cooled to r.t.,

diluted with EtOAc and washed with aqueous HCl (10%), and brine. The organic layer was separated, and solvent was removed in vacuo. The residue was purified by column chromatography with Hexane:EtOAc = 97:3 to afford **170**-**181**.

6-Hydroxy-3,3,11-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (170). 43.6% yield (starting from 1.0 g crude **169c**); ^{1}NMR δ 13.22 (1H, s, OH-6), 8.09 (1H, d, J = 7.6 Hz, H-8), 7.54 (1H, d, J = 7.2 Hz, H-10), 7.25 (1H, t, J = 7.6, 7.2 Hz, H-9), 6.83 (1H, d, J = 9.6 Hz, H-1), 6.27 (1H, s, H-5), 5.63 (1H, d, J = 9.6 Hz, H-2), 2.58 (3H, s, CH_3-11), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6-Hydroxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (171). 30.0% yield (starting from 1.0 g crude **169f**); ^{1}NMR δ 13.32 (1H, s, OH-6), 7.53 (1H, t, J = 8.0, 8.0 Hz, H-10), 7.30 (1H, d, J = 8.0 Hz, H-9), 7.11 (1H, d, J = 8.0 Hz, H-11), 6.82 (1H, d, J = 9.6 Hz, H-1), 6.24 (1H, s, H-5), 5.50 (1H, d, J = 9.6 Hz, H-2), 2.90 (3H, s, CH_3-8), 1.48, 1.48 (each 3H, s, CH_3-3,3).

6-Hydroxy-3,3-dimethyl-11-methoxy-pyrano[2,3-*c*]xanthen-7(3*H*)-one (172). 28.0% yield (starting from 1.5 g crude **169g**); ^{1}NMR δ 12.93 (1H, s, OH-6), 7.81 (1H, d, J = 8.4 Hz, H-8), 7.29 (1H, d, J = 8.0 Hz, H-10), 7.24 (1H, t, J = 8.4, 8.0 Hz, H-9), 6.92 (1H, d, J = 10.0 Hz, H-1), 6.28 (1H, s, H-5), 5.62 (1H, d, J = 10.0 Hz, H-2), 4.02 (3H, s, OCH_3-11), 1.48, 1.48 (each 3H, s, CH_3-3,3).

6-Hydroxy-3,3-dimethyl-10-methoxy-pyrano[2,3-*c*]xanthen-7(3*H*)-one (173). 12.8% yield (starting from 1.0 g crude **169h**); ^{1}NMR δ 13.07 (1H, s, OH-6), 8.11 (1H, d, J = 9.3 Hz, H-8), 6.92 (1H, d, J = 9.3 Hz, H-9), 6.82 (1H, s, H-11), 6.81 (1H, d, J =

9.9 Hz, H-1), 6.24 (1H, s, H-5), 5.61 (1H, d, *J* = 9.9 Hz, H-2), 3.93 (3H, s, OCH_3-10), 1.48, 1.48 (each 3H, s, $_{CH3}$-3,3).

6-Hydroxy-3,3-dimethyl-9-methoxy-pyrano[2,3-*c*]xanthen-7(3*H*)-one (174). 42.0% yield (starting from 2.0 g crude **169i**); ^{1}NMR *δ* 12.98 (1H, s, OH-6), 7.61 (1H, d, *J* = 3.0 Hz, H-8), 7.41 (1H, d, *J* = 9.3 Hz, H-11), 7.32 (1H, dd, *J* = 9.3, 3.0 Hz, H-10), 6.84 (1H, d, *J* = 9.9 Hz, H-1), 6.27 (1H, s, H-5), 5.61 (1H, d, *J* = 9.9 Hz, H-2), 3.91 (3H, s, OCH_3-9), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6,8-Dihydroxy-3,3 –dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (175). 28.0% yield (starting from 1.0 g crude **169k**); ^{1}NMR *δ* 12.07, 11.95 (each 1H, s, OH-6,8), 7.56 (1H, t, *J* = 8.1, 8.7 Hz, H-10), 6.90 (1H, d, *J* = 8.1 Hz, H-9), 6.81 (1H, d, *J* = 10.2 Hz, H-1), 6.79 (1H, d, *J* = 8.7 Hz, H-11), 6.26 (1H, s, H-5), 5.63 (1H, d, *J* = 10.2 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6 -Hydroxy-11-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (176). 30.0% yield (starting from 1.0 g crude **169l**); ^{1}NMR *δ* 12.77 (1H, s, OH-6), 8.01 (1H, d, *J* = 8.4 Hz, H-8), 7.51 (1H, t, *J* = 8.4, 9.6 Hz, H-9), 7.29 (1H, d, *J* = 9.6 Hz, H-10), 6.86 (1H, d, *J* = 10.4 Hz, H-1), 6.30 (1H, s, H-5), 5.63 (1H, d, *J* = 10.4 Hz, H-2), 1.50, 1.50 (each 3H, s, CH_3-3,3).

6 -Hydroxy-9-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (177). 20.0% yield (starting from 1.0 g crude **169m**); ^{1}NMR *δ* 12.77 (1H, s, OH-6), 7.90 (1H, dd, *J* = 8.0, 2.8 Hz, H-10), 7.43-7.46 (2H, m, H-8,11), 6.83 (1H, d, *J* = 10.0 Hz, H-1), 6.29 (1H, s, H-5), 5.63 (1H, d, *J* = 10.0 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6-Hydroxy-8-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (178). 46.0% yield (starting from 1.0 g crude **169n**); ^{1}NMR δ 12.94 (1H, s, OH-6), 7.64 (1H, t, J = 8.1, 8.4 Hz, H-10), 7.24 (1H, d, J = 8.4 Hz, H-11), 7.04 (1H, d, J = 8.1 Hz, H-9), 6.78 (1H, d, J = 10.2 Hz, H-1), 6.25 (1H, s, H-5), 5.63 (1H, d, J = 10.2 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6-Hydroxy-10-bromo-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (179). 50.0% yield (starting from 1.8 g crude **169o**); ^{1}NMR δ 12.79 (1H, s, OH-6), 8.10 (1H, d, J = 8.4 Hz, H-8), 7.67 (1H, s, H-11), 7.50 (1H, d, J = 8.4 Hz, H-9), 6.79 (1H, d, J = 10.0 Hz, H-1), 6.28 (1H, s, H-5), 5.64 (1H, d, J = 10.0 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6-Hydroxy-9-bromo-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (180). 20.0% yield (starting from 1.0 g crude **169p**); ^{1}NMR δ 12.72 (1H, s, OH-6), 8.24 (1H, d, J = 2.4 Hz, H-8), 7.78 (1H, dd, J = 8.8, 2.4 Hz, H-10), 7.35 (1H, d, J = 8.8 Hz, H-11), 6.80 (1H, d, J = 6.4 Hz, H-1), 6.27 (1H, s, H-5), 5.63 (1H, d, J = 6.4 Hz, H-2), 1.49, 1.49 (each 3H, s, CH_3-3,3).

6-Hydroxy-10-methoxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (181). 30.0% yield (starting from 1.0 g crude **169q**); ^{1}NMR δ 13.44 (1H, s, OH-6), 6.76 (1H, d, J = 10.0 Hz, H-1), 6.67 (1H, s, H-9), 6.62 (1H, s, H-11), 6.18 (1H, s, H-5), 5.56 (1H, d, J = 10.0 Hz, H-2), 3.87 (3H, s, OCH_3-10), 2.81 (3H, s, CH_3-8), 1.45,1.45 (each 3H, s, CH_3-3,3).

Synthesis of 6-methoxy-3,3-dimethylpyrano[2,3-c]xanthen-7(3*H*)-ones (182b-q). A mixture of compounds (**94-96a**, **170-181**) (1 equiv), K_2CO_3 (3 equiv) in

anhydrous acetone (20 mL) was heated to reflux temperate for 3 hours, then allowed to cool to r,t. MeI (2-3 equiv) was added at r.t and the reaction was kept stirring overnight, monitored by TLC. At completion, the reaction mixture was filtered, and the filtrate was evaporated to dryness. The crude products **182j** and **182n** weredirectly carried to the dihydroxylation reaction without purification. The remaining crude product was purified by column chromatography using EtOAc and Hexane to give compounds **182b-l**, **k-m** and **o-q**.

6-Methoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182b). 95% yield (starting from 150 mg of **94a**); ESI+ (*m/z*, %), 309 (M^+, 100);^{1}NMR δ 8.29 (1H, d, *J* = 8.1 Hz, H-8), 7.62 (1H, t, *J* = 7.2, 7.8 Hz, H-10), 7.39 (1H, d, *J* = 7.8 Hz, H-11), 7.32 (1H, t, *J* = 7.2, 8.1 Hz, H-9), 6.89 (1H, d, *J* = 9.9 Hz, H-1), 6.31 (1H, s, H-5), 5.62 (1H, d, *J* = 9.9 Hz, H-2), 3.98 (3H, s, OCH_3-6), 1.50, 1.50 (each 3H, s, CH3-3,3).

6-Methoxy-3,3,11-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182c). 78% yield (starting from 300 mg of **170**); ESI+ (*m/z*, %), 323 (M^++1, 100);^{1}NMR δ 8.14 (1H, d, *J* = 7.6 Hz, H-8), 7.48 (1H, d, *J* = 7.6 Hz, H-10), 7.22 (1H, t, *J* = 7.6, 7.6 Hz, H-9), 6.88 (1H, d, *J* = 9.6 Hz, H-1), 6.31 (1H, s, H-5), 5.62 (1H, d, *J* = 9.6 Hz, H-2), 3.98 (3H, s, OCH_3-6), 2.54 (3H, s, CH_3-11), 1.51, 1.51 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3,10-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182d). 100% yield (starting from 200 mg of **96a**); ESI+ (*m/z*, %), 323 (M^++1, 100);^{1}NMR δ 8.17 (1H, d, *J* = 8.1 Hz, H-8), 7.20 (1H, s, H-11), 7.14 (1H, d, *J* = 8.1 Hz, H-9), 6.89 (1H, d, *J* = 9.9 Hz, H-1), 6.30 (1H, s, H-5), 5.62 (1H, d, *J* = 9.9 Hz, H-2), 3.97 (3H, s, OCH_3-6), 2.48 (3H, s, CH_3-10), 1.50, 1.50 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3,9-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182e). 86% yield (starting from 399 mg of **95a**); ESI+ (*m/z*, %), 323 (M^++1, 100);^{1}NMR δ 8.06 (1H, s, H-8), 7.45 (1H, d, *J* = 8.4 Hz, H-11), 7.31 (1H, d, *J* = 8.4 Hz, H-10), 6.90 (1H, d, *J* = 9.9 Hz, H-1), 6.30 (1H, s, H-5), 5.62 (1H, d, *J* = 9.9 Hz, H-2), 3.98 (3H, s, OCH_3-6), 2.45 (3H, s, CH_3-9), 1.50, 1.50 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182f). 84% yield (starting from 700 mg of **171**); ^{1}NMR δ 7.45 (1H, t, *J* = 8.0, 7.6 Hz, H-10), 7.24 (1H, d, *J* = 8.0 Hz, H-9), 7.06 (1H, d, *J* = 7.6 Hz, H-11), 6.87 (1H, d, *J* = 10.0 Hz, H-1), 6.28 (1H, s, H-5), 5.60 (1H, d, *J* = 10.0 Hz, H-2), 3.98 (3H, s, OCH_3-6), 2.90 (3H, s, CH_3-8), 1.50, 1.50 (each 3H, s, CH_3-3,3).

6,11-Dimethoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182g). 40% yield (starting from 110 mg of **172**); ESI+ (*m/z*, %), 339 (M^++1, 100);^{1}NMR δ 7.87 (1H, d, *J* = 8.0 Hz, H-8), 7.24 (1H, t, *J* = 8.0, 8.0 Hz, H-9), 7,15 (1H, d, *J* = 8.0 Hz, H-10), 6.97 (1H, d, *J* = 10.0 Hz, H-1), 6.32 (1H, s, H-5), 5.63 (1H, d, *J* = 10.0 Hz, H-2), 4.01, 3.98 (each 3H, s, OCH_3-6,11), 1.51, 1.51 (each 3H, s, CH_3-3,3).

6,10-Dimethoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182h). 95% yield (starting from 110 mg of **173**); ^{1}NMR δ 8.17 (1H, d, *J* = 8.1 Hz, H-8), 7.20 (1H, s, H-11), 7.15 (1H, d, *J* = 8.1 Hz, H-9), 6.89 (1H, d, *J* = 9.9 Hz, H-1), 6.30 (1H, s, H-5), 5.62 (1H, d, *J* = 9.9 Hz, H-2), 3.97 (3H, s, OCH_3-6), 2.48 (3H, s, OCH_3-10), 1.50. 1.50 (each 3H, s, CH_3-3,3).

6,9-Dimethoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182i). 72% yield (starting from 400 mg of **174**); ESI+ (*m/z*, %), 377 (M^++K, 100); ^{1}NMR δ 7.66

(1H, d, *J* = 3.0 Hz, H-8), 7.32 (1H, d, *J* = 9.0 Hz, H-11), 7.20 (1H, dd, *J* = 9.0, 3.0 Hz, H-10), 6.86 (1H, d, *J* = 9.9 Hz, H-1), 6.27 (1H, s, H-5), 5.59 (1H, d, *J* = 9.9 Hz, H-2), 3.95 (3H, s, OCH_3-6), 3.87 (3H, s, OCH_3-9), 1.47, 1.47 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3-dimethyl-8-hydroxy-pyrano[2,3-*c*]xanthen-7(3*H*)-one (182k). 95% yield (starting from 100 mg of **175**); ^{1}NMR *δ* 13.21 (1H, s, OH-8), 7.49 (1H, t, *J* = 8.4, 8.0 Hz, H-10), 6.85 (1H, d, *J* = 9.6 Hz, H-1), 6.83 (1H, d, *J* = 8.4 Hz, H-9), 6.74 (1H, d, *J* = 8.0 Hz, H-11), 6.31 (1H, s, H-5), 5.63 (1H, d, *J* = 9.6 Hz, H-2), 4.03 (3H, s, OCH_3-6), 1.51, 1.51 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3-dimethyl-11-fluoro-pyrano[2,3-*c*]xanthen-7(3*H*)-one (182l). 40% yield (starting from 303 mg of **176**); ^{1}NMR *δ* 8.04 (1H, d, *J* = 8.4 Hz, H-8), 7.41 (1H, t, *J* = 8.4, 9.6 Hz, H-9), 7.24 (1H, d, *J* = 9.6 Hz, H-10), 6.93 (1H, d, *J* = 10.0 Hz, H-1), 6.33 (1H, s, H-5), 5.64 (1H, d, *J* = 10.0 Hz, H-2), 3.98 (3H, s, OCH_3-6), 1.51, 1.51 (each 3H, s, CH_3-3,3).

6-Methoxy-3,3-dimethyl-9-fluoro-pyrano[2,3-*c*]xanthen-7(3*H*)-one (182m). 61% yield (starting from 188 mg of **177**); ^{1}NMR *δ* 7.92 (1H, d, *J* = 8.4 Hz, H-10), 7.27-7.41 (2H, m, H-8,11), 6.87 (1H, d, *J* = 10.4 Hz, H-1), 6.31 (1H, s, H-5), 5.62 (1H, d, *J* = 10.4 Hz, H-2), 3.98 (3H, s, OCH_3-6), 1.51, 1.51 (each 3H, s, CH_3-3,3).

6-Methoxy-10-bromo-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182o). 95% yield (starting from 295 mg of **179**); ^{1}NMR *δ* 8.13 (1H, d, *J* = 8.4 Hz, H-8), 7.60 (1H, s, H-11), 7.44 (1H, d, *J* = 8.4 Hz, H-9), 6.83 (1H, d, *J* = 10.0 Hz, H-1), 6.31 (1H, s, H-5), 5.63 (1H, d, *J* = 10.0 Hz, H-2), 3.97 (3H, s, OCH_3-6), 1.50, 1.50(each 3H, s, CH_3-3,3).

6-Methoxy-9-bromo-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182p). 72% yield (starting from 70 mg of **180**); ESI+ (*m/z*, %), 387 (M^+, 100);^{1}NMR δ 8.36 (1H, d, J = 2.4 Hz, H-8), 7.69 (1H, dd, J = 8.8, 2.4 Hz, H-10), 7.28 (1H, d, J = 8.8 Hz, H-11), 6.84 (1H, d, J = 10.0 Hz, H-1), 6.30 (1H, s, H-5), 5.61 (1H, d, J = 10.0 Hz, H-2), 3.96 (3H, s, OCH_3-6), 1.48, 1.48 (each 3H, s, CH_3-3,3).

6,10-Dimethoxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (182q). 54% yield (starting from 295 mg of **181**); ^{1}NMR δ 6.85 (1H, d, J = 10.0 Hz, H-1), 6.67 (1H, s, H-9), 6.63 (1H, s, H-11), 6.28 (1H, s, H-5), 5.59 (1H, d, J = 10.0 Hz, H-2), 3.96 (3H, s, OCH_3-6), 3.88 (3H, s, OCH_3-10), 2.86 (3H, s, CH_3-8), 1.49, 1.49 (each 3H, s, CH_3-3,3).

General procedure for the preparation of 183a-q. A mixture of $K_3Fe(CN)_6$ (3 equiv), K_2CO_3 (3 equiv), $(DHQ)_2$-PYR (2% equiv), and $K_2OsO_2(OH)_4$ (2% equiv) was dissolved in *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 ^oC and methanesulfonamide (1 equiv) was added with stirring. After 20 min, substituted pyranochromone (**93a** and **182b-q**) was added. The mixture was stirred at 0 ^oC for 1-2 days, monitored by TLC. At completion, $Na_2S_2O_5$ (excess), water and CH_2Cl_2 were added, and stirring was continued for 1 h at rt. The mixture was extracted with CH_2Cl_2 three times, and the combined organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography with hexanes:EtOAc = 3:7 to afford the pure substituted (+)-*cis*-3',4'-dihydroxypyranochromones (**183a-b, d-n, p-q**). The crude products of **183c** and **183o** were carried to the next step without purification.

(1*R*,2*R*)-1,2-dihydroxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183a). 22% yield (starting from 70 mg of **93a**); ESI+ (*m/z*, %), 313 (M^++1, 100);[1]NMR *δ* 8.35 (1H, d, *J* = 7.8 Hz, H-8), 8.20 (1H, d, *J* = 9.3 Hz, H-6), 7.71 (1H, t, *J* = 8.1, 8.1 Hz, H-10), 7.49 (1H, d, *J* = 8.1 Hz, H-11), 7.41 (1H, t, *J* = 8.1, 7.8 Hz, H-9), 6.89 (1H, d, *J* = 9.3 Hz, H-5), 5.35 (1H, d, *J* = 5.4 Hz, H-1), 3.93 (1H, br, H-2), 3.34, 3.10 (each 1H, br, OH-1,2), 1.52, 1.46 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183b). 75% yield (starting from 188mg of **182b**); ESI+ (*m/z*, %), 343 (M^+, 100); [1]NMR *δ* 8.30 (1H, d, *J* = 7.8 Hz, H-8), 7.63 (1H, t, *J* = 6.9, 8.1 Hz, H-10), 7.38 (1H, d, *J* = 8.1 Hz, H-11), 7.36 (1H, t, *J* = 7.8, 6.9 Hz, H-9), 6.30 (1H, s, H-5), 5.25 (1H, br, H-1), 3.95 (3H, s, OCH_3-6), 3.89 (1H, br, H-2), 3.37, 3.24 (each 1H, br, OH-1,2), 1.52, 1.48 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-3,3,10-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183d). 45% yield (starting from 200 mg of **182d**); [1]NMR *δ* 8.15 (1H, d, *J* = 8.4 Hz, H-8), 7.14-7.16 (2H, br, H-9,11), 6.27 (1H, s, H-5), 5.23 (1H, br, H-1), 3.94 (3H, s, OCH_3-6), 3.88 (1H, s, H-2), 3.55 (H, d, *J* = 3.6 Hz, OH-1), 3.30 (1H, d, *J* = 6.9 Hz, OH-2), 2.46 (3H, s, CH_3-10), 1.55, 1.49 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-3,3,9-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183e). 59% yield (starting from 250 mg of **182e**); [1]NMR *δ* 7.97 (1H, s, H-8), 7.34 (1H, d, *J* = 8.4 Hz, H-10), 7.19 (1H, d, *J* = 8.4 Hz, H-11), 6.20 (1H, s, H-5), 5.20 (1H, t, *J* = 4.5, 4.5 Hz, H-1), 3.90 (3H, s, OCH_3-6), 3.87 (1H, t, *J* = 4.5, 4.5 Hz,

H-2), 3.84 (1H, d, *J* = 4.5 Hz, OH-1), 3.47 (1H, d, *J* = 4.5 Hz, OH-2), 2.42 (3H, s, CH_3-9), 1.55, 1.48 (each 3H, s, CH_3-3,3),

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183f). 40% yield (starting from 180 mg of **182f**); ESI+ (*m/z*, %), 357 (M^++1, 100); ^{1}NMR *δ* 7.42 (1H, t, *J* = 8.4, 7.6 Hz, H-10), 7.20 (1H, d, *J* = 8.4 Hz, H-9), 7.07 (1H, d, *J* = 7.6 Hz, H-11), 6.26 (1H, s, H-5), 5.22 (1H, d, *J* = 4.8 Hz, H-1), 3.95 (3H, s, OCH_3-6), 3.87 (1H, d, *J* = 4.8 Hz, H-2), 3.32, 3.22 (each 1H, s, OH-1,2), 2.05 (3H, s, CH_3-8), 1.50, 1.46 (each 3H, s, CH_3-3,3).

183g 60% yield (starting from 35mg mg crude **182g**); ESI+ (*m/z*, %), 339 (M^++1, 100); ESI+ (*m/z*, %), 373 (M^++1, 100);

(1*R*,2*R*)-6,10-Methoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183h). 30% yield (starting from 110 mg of **182h**); ESI+ (*m/z*, %), 373 (M^++1, 100);^{1}NMR *δ* 8.18 (1H, d, *J* = 8.7 Hz, H-8), 6.90 (1H, d, *J* = 8.7 Hz, H-9), 6.71 (1H, s, H-11), 6.26 (1H, s, H-5), 5.20 (1H, br, H-1), 3.92 (3H, s, OCH_3-6), 3.90 (3H, s, OCH_3-10), 3.50 (1H, br, H-2), 3.30 (2H, br, OH-1,2), 1.55, 1.48 (each 3H, s, CH_3-3,3),

(1*R*,2*R*)-1,2-dihydroxy-6,9-dimethoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183i). 40% yield (starting from 200 mg of **182i**); ESI+ (*m/z*, %), 373 (M^++1, 100); ^{1}NMR *δ* 7.65 (1H, s, H-8), 7.31 (1H, d, *J* = 9.0 Hz, H-11), 7.22 (1H, d, *J* = 9.0 Hz, H-10), 6.27 (1H, s, H-5), 5.23 (1H, br, H-1), 3.94 (3H, s, OCH_3-6), 3.90 (3H, s, OCH_3-9), 3.35 (1H, br, H-2), 3.23, 3,23 (each 1H, s, OH-1,2), 1.51, 1.46 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6,8-dimethoxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183j). 50% yield (starting from 60 mg of **182j**); ESI+ (*m/z*, %), 373 (M^++1, 100); ^{1}NMR δ 7.51 (1H, t, *J* = 8.7, 8.4 Hz, H-10), 6.96 (1H, d, *J* = 8.7 Hz, H-11), 6.79 (1H, d, *J* = 8.4 Hz, H-9), 6.26 (1H, s, H-5), 5.22 (1H, br, H-1), 3.97, 3.91 (each 3H, s, OCH_3-6,8), 3.19 (1H, br, H-2), 1.49, 1.45 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-8-hydroxy-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183k). 45% yield (starting from 50 mg of **182k**); ESI+ (*m/z*, %), 357 (M^++1, 100);^{1}NMR δ 13.14 (1H, s, OH-8), 7.52 (1H, t, *J* = 8.4, 8.4 Hz, H-10), 6.96 (1H, d, *J* = 8.4 Hz, H-9), 6.79 (1H, d, *J* = 8.4 Hz, H-11), 6.32 (1H, s, H-5), 5.23 (1H, d, *J* = 4.8 Hz, H-1), 3.99 (3H, s, OCH_3-6), 3.89 (1H, d, *J* = 4.8 Hz, H-2), 3.11, 3.10 (each 1H, s, OH-1,2), 1.50, 1.47 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-11-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183l). 50% yield (starting from 107 mg of **182l**); ESI+ (*m/z*, %), 361 (M^++1, 100);^{1}NMR δ 8.06 (1H, d, *J* = 8.0 Hz, H-8), 7.44 (1H, t, *J* = 8.0, 9.6 Hz, H-9), 7.30 (1H, d, *J* = 9.6 Hz, H-10), 6.34 (1H, s, H-5), 5.29 (1H, d, *J* = 4.4 Hz, H-1), 3.98 (3H, s, OCH_3-6), 3.91 (1H, s, OH-1), 3.39 (1H, d, *J* = 4.4Hz, H-2), 3.21 (1H, s, OH-2), 1.51, 1.46 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-9-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183m). 50% yield (starting from 110 mg of **182m**); ESI+ (*m/z*, %), 361 (M^++1, 100); ^{1}NMR δ 7.92 (1H, d, *J* = 8.4 Hz, H-10), 7.56 (1H, m, H-8), 7.39 (1H, m, H-11), 6.34 (1H, d, *J* = 4.0 Hz, H-1), 6.33 (1H, s, H-5), 5.24 (1H, d, *J* =

4.0 Hz, H-2), 3.97 (2H, s, OCH_3-6), 3.60, 3.40 (each 1H, s, OH-1,2), 1.49, 1.47 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-8-fluoro-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183n). 60% yield (starting from 50 mg of **182n**); ESI+ (*m/z*, %), 361 (M^++1, 100); ^{1}NMR δ 7.56 (1H, d, *J* = 9.0 Hz, H-11), 7.21 (1H, d, *J* = 9.0 Hz, H-9), 6.98 (1H, t, *J* = 9.0, 9.0 Hz, H-10), 6.28 (1H, s, H-5), 5.2 (1H, br, H-1), 3.93 (3H, s, OCH_3-6), 3.90 (1H, br, H-2), 3.12, 3.11 (each 1H, s, OH-1,2), 1.50, 1.57 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6-methoxy-9-bromo-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183p). 45% yield (starting from 50 mg **182p**); ESI+ (*m/z*, %), 421 (M^+, 100); ^{1}NMR δ 8.34 (1H, d, *J* = 1.6 Hz, H-8), 7.69 (1H, dd, *J* = 8.8, 1.6 Hz, H-10), 7.25 (1H, d, *J* = 8.8 Hz, H-11), 6.27 (1H, s, H-5), 5.20 (1H, d, *J* = 4.4 Hz, H-1), 3.92 (3H, s, OCH_3-6), 3.86 (1H, d, *J* = 4.4 Hz, H-2), 3.20, 3.12 (each 1H, s, OH-1,2), 1.49, 1.45 (each 3H, s, CH_3-3,3).

(1*R*,2*R*)-1,2-dihydroxy-6,10-dimethoxy-3,3,8-trimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (183q). 50% yield (starting from 50 mg **182q**); ^{1}NMR δ 6.67 (1H, s, H-9), 6.67 (1H, s, H-11), 6.28 (1H, s, H-5), 5.23 (1H, d, *J* = 4.4 Hz, H-1), 4.10 (1H, d, *J* = 4.4 Hz, H-2), 3.93 (3H, s, OCH_3-6), 3.89 (3H, s, OCH_3-10), 3.13, 3.11 (each 1H, s, OH-1,2), 2.87 (3H, s, CH_3-8), 1.49, 1.46 (each 3H, s, CH_3-3,3).

6-Methoxy-10-cyano-3,3-dimethylpyrano[2,3-*c*]xanthen-7(3*H*)-one (184). A mixture of **182o** (10 mg, 0.025 mmoL), zinc cyanide (3.5 mg, 0.03mmoL) and $Pd(PPh_3)_4$ (12 mg, 0.011mmoL) was dissolved into anhydrous DMF (0.5 mL) and

heated to 130^{o}C for 16 hours, monitored by TLC. At completion, the reaction mixture was filtered over celite and the crude product was purified by PTLC with EtOAc : Hexane = 1:3 to afford pure **184** as yellow solid. 90% Yield; MS-ESI+ (*m*/*z*, %) 334 (M^+ + 1, 100); ^{1}NMR δ 8.35 (1H, d, *J* = 8.0 Hz, H-8), 7.72 (1H, s, H-11), 7.55 (1H, d, *J* = 8.0 Hz, H-9), 6.82 (1H, d, *J* = 10.0 Hz, H-1), 6.32 (1H, s, H-5), 5.64 (1H, d, *J* = 10.0 Hz, H-2), 3.96 (3H, s, OCH_3-6), 1.57, 1.49 (each 3H, s, CH_3-2,2).

General procedure for the preparation of 130-131, 133-146, 148 and 152. The substituted 1*R*,2*R*-dihydroxypyranoxanthones (**183a-r**) (1 equiv), (*S*)-(-)-camphanic chloride (3 equiv), and DMAP (4 equiv) were stirred in CH_2Cl_2 for 1-2 h at rt, monitored by TLC. At completion, the mixture was diluted with CH_2Cl_2 and washed by water and brine, seperately. The solvent was then removed under reduced pressure and the residue was purified by PTLC with hexanes:EtOAc = 3:2 to afford the appropriately substituted 1*R*,2*R*-di-*O*-(-)-camphanoyl-3,3-dimethyldihydroprano [2,3-*c*]xanthones (**130**-**131**, **133**-**146**, **148** and **152**).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (130). 85% yield (starting from 15 mg of **183a**); white solid; mp = 146-148 ^{o}C; ESI+ (*m/z*, %), 695 (M^++Na, 100); ^{1}NMR δ 8.33 (1H, d, *J* = 7.8 Hz, H-8), 8.31 (1H, d, *J* = 9.0 Hz, H-6), 7.67 (1H, t, *J* = 7.2, 8.4 Hz, H-10), 7.39 (1H, t, *J* = 7.2, 7.8 Hz, H-9), 7.35 (1H, d, *J* = 8.4 Hz, H-11), 6.94 (1H, d, *J* = 9.0 Hz, H-5), 6.93 (1H, d, *J* = 4.5 Hz, H-1), 5.43 (1H, d, *J* = 4.5 Hz, H-2), 2.52, 2.322 1.90, 1.75 (each 2H, m, camphanoyl-CH_2), 1.57, 1.50 (each 3H, s, CH_3-3,3), 1.14, 1.12, 1.03, 1.00, 0.93, 0.89 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -22.7 ^{o} (*c* = 0.0015, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-methoxy-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (131). 88% yield (starting from 52 mg of **183b**); white solid; mp = 134-135 ^{o}C; ESI+ (*m/z*, %), 703 (M^{+}+1, 100); ^{1}NMR δ 8.40 (1H, d, *J* = 7.8 Hz, H-8), 7.72 (1H, t, *J* = 7.5, 8.1 Hz, H-10), 7.46 (1H, t, *J* = 7.8, 7.5 Hz, H-9), 7.39 (1H, d, *J* = 8.1 Hz, H-11), 6.98 (1H, d, *J* = 4.5 Hz, H-1), 6.46 (1H, s, H-5), 5.53 (1H, d, *J* = 4.5 Hz, H-2), 4.12 (3H, s, OCH_3-6), 2.60, 2.33, 2.10, 1.80 (each 2H, m, camphanoyl-CH_2), 1.69, 1.61 (each 3H, s, CH_3-3,3), 1.25, 1.25, 1.15, 1.12, 1.02, 1.01 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -40.9 ^{o} (*c* = 0.0069, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3,11-trimethyl-6-methoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (133). 85% yield (starting from 41 mg of **183c**); white solid; mp = 174-175 ^{o}C; ESI+ (*m/z*, %), 717 (M^{+}+1, 100); ^{1}NMR δ 8.12 (1H, d, *J* = 7.6 Hz, H-8), 7.36 (1H, d, *J* = 7.2 Hz, H-10), 7.24 (1H, t, *J* = 7.6, 7.2 Hz, H-9), 6.87 (1H, d, *J* = 4.4 Hz, H-1), 6.34 (1H, s, H-5), 5.44 (1H, d, *J* = 4.4 Hz, H-2), 4.01 (3H, s, OCH_3-6), 2.45, 2.25, 1.90, 1.67 (each 2H, m, camphanoyl-CH_2), 2.39 (3H, s, CH_3-11), 1.60, 1.49 (each 3H, s, CH_3-3,3), 1.14, 1.14, 1.03, 1.00, 0.93, 0.89 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -40.9 ^{o} (*c* = 0.0069, CH_3Cl); $[\alpha]_D$ -42.5 ^{o} (*c* = 0.0051, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3,10-trimethyl-6-methoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (134). 80% yield (starting from 50 mg of **183d**); white solid; mp = 174-175 ^{o}C; ESI+ (*m/z*, %), 739 (M^{+}+Na, 100); ^{1}NMR δ 8.15 (1H, d, *J* = 8.1 Hz, H-8), 7.13 (1H, d, *J* = 8.1 Hz, H-9), 7.07 (1H, s, H-11), 6.87 (1H, d, *J* = 4.8 Hz, H-1), 6.32 (1H, s, H-5), 5.39 (1H, d, *J* = 4.8 Hz, H-2), 3.99 (3H, s, OCH_3-6), 2.41 (3H, s, CH_3-10), 2.45, 2.20, 1.95, 1.80 (each 2H, m, camphanoyl-CH_2), 1.56, 1.49

(each 3H, s, CH_3-3,3), 1.14, 1.13, 1.04, 1.00, 0.90, 0.87 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -37.2 ^{o} (*c* = 0.0018, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3,9-trimethyl-6-methoxy-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (135). 77% yield (starting from 100 mg of **183e**); Light yellow solid; mp = 179-180 ^{o}C; ^{1}NMR δ 8.05 (1H, s, H-8), 7.41 (1H, d, *J* = 8.7 Hz, H-11), 7.18 (1H, d, *J* = 8.7 Hz, H-10), 6.86 (1H, d, *J* = 4.5 Hz, H-1), 6.32 (1H, s, H-5), 5.40 (1H, d, *J* = 4.5 Hz, H-2), 4.00 (3H, s, OCH_3-6), 2.44 (3H, s, CH_3-9), 2.45, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl-CH_2), 1.57, 1.49 (each 3H, s, CH_3-3,3), 1.14, 1.13, 1.03, 1.00, 0.90, 0.88 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -34.6 ^{o} (*c* = 0.0028, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3,8-trimethyl-6-methoxy-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (136). 75% yield (starting from 70 mg of **183f**); white solid; mp = 240-242 ^{o}C; ESI+ (*m/z*, %), 717 (M^++1, 100); ^{1}NMR δ 7.39 (1H, t, *J* = 8.4, 7.6 Hz, H-10), 7.09 (1H, d, *J* = 8.4 Hz, H-9), 7.06 (1H, d, *J* = 7.6 Hz, H-11), 6.82 (1H, d, *J* = 4.8 Hz, H-1), 6.29 (1H, s, H-5), 5.37 (1H, d, *J* = 4.8 Hz, H-2), 3.98 (3H, s, OCH_3-6), 2,86 (3H, s, CH_3-8), 2.46, 2.18, 1.90, 1.65 (each 2H, m, camphanoyl CH_2), 1.56, 1.55 (each 3H, s, CH_3-3,3), 1.11, 1.10, 1.01, 1.00, 0.88, 0.86 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -29.3 ^{o} (*c* = 0.003, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6,11-dimethoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (137). 53% yield (starting from 25 mg of **183g**); white solid; mp = 179-180 ^{o}C; ESI+ (*m/z*, %), 733 (M^++1, 100); ^{1}NMR δ 7.82 (1H, d, *J* = 8.0 Hz, H-8), 7.25 (1H, t, *J* = 8.0, 8.0 Hz, H-9), 7.09 (1H, d, *J* = 8.0 Hz, H-10), 6.82

(1H, d, *J* = 4.4 Hz, H-1), 6.33 (1H, s, H-5), 5.44 (1H, d, *J* = 4.4 Hz, H-2), 4.00, 3.91 (each 3H, s, OCH_3-6,11), 2.44, 2.27, 1.95, 1,70 (each 2H, m, camphanoyl-CH_2), 1.57, 1.47 (each 3H, s, CH_3-3,3), 1.13, 1.13, 1.03, 1.01, 0.91, 0.87 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -40.8 ^{o} (*c* = 0.0013, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6,10-dimethoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (138). 43% yield (starting from 10 mg of **183h**); white solid; mp = 177-178 ^{o}C; ESI+ (*m/z*, %), 733 (M^++1, 100); ^{1}NMR δ 8.12 (1H, d, *J* = 8.7 Hz, H-8), 6.84 (1H, dd, *J* = 8.7, 2.4 Hz, H-9), 6.79 (1H, d, *J* = 4.5 Hz, H-1), 6.59 (1H, d, *J* = 2.4 Hz, H-11), 6.26 (1H, s, H-5), 5.33 (1H, d, *J* = 4.5 Hz, H-2), 3.92 (3H, s, OCH_3-6), 3.77 (3H, s, OCH3-10), 2.40, 2.15, 1.95, 1.65 (each 2H, m, camphanoyl-CH_2), 1.50, 1.42 (each 3H, s, CH_3-3,3), 1.07, 1.06, 0.97, 0.93, 0.85, 0.81 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -16.2 ^{o} (*c* = 0.0052, CH_3Cl).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6,9-dimethoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (139). 60% yield (starting from 20 mg of **183i**); white solid; mp = 168-170 ^{o}C; ESI+ (*m/z*, %), 732 (M^+, 100); ^{1}NMR δ 7.66 (1H, s, H-8), 7.21, 7.21 (each 1H, d, H = 9.2 Hz, H-10,11), 6.85 (1H, d, *J* = 4.8 Hz, H-1), 6.32 (1H, s, H-5), 5.40 (1H, d, J = 4.8 Hz, H-2), 4.00 (3H, s, OCH_3-6), 3.88 (3H, s, OCH_3-9), 2.5, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl-CH_2), 1.57, 1.49 (each 3H, s, CH_3-3,3), 1.14, 1.13, 1.03, 1.00, 0.90, 0.88 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -35.3^o (*c* = 0.0024, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6,8-dimethoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (140). 52% yield (starting from 10 mg of **183j**); white solid;

mp = 155-156 ^{o}C; ESI+ (*m/z*, %), 733 (M^{+}+1, 100); ^{1}NMR *δ* 7.44 (1H, t, *J* = 8.4, 8.4 Hz, H-10), 6.82 (1H, d, *J* = 8.4 Hz, H-11), 6.80 (1H, d, *J* = 4.4 Hz, H-1), 6.75 (1H, d, *J* = 8.4 Hz, H-9), 6.27 (1H, s, H-5), 5.36 (1H, d, *J* = 4.4 Hz, H-2), 3.94, 3.93 (each 3H, s, OCH_3-6.8), 2.50, 2.20, 1.90, 1.70 (each 2H, camphanoyl-CH_2), 1.54, 1.46 (each 3H, s, CH_3-3,3), 1.13, 1.11, 1.03, 1.00, 0.88, 0.85 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -131.7 ^{o} (*c* = 0.003, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-methoxy-8-hydroxy-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (141). 30% yield (starting from 10 mg of **183k**); white solid; mp = 155-157 ^{o}C; ESI+ (*m/z*, %), 719 (M^{+}+1, 100); ^{1}NMR *δ* 12.94 (1H, s, OH-8), 7.43 (1H, t, *J* = 8.0, 8.4 Hz, H-10), 6.78 (1H, d, *J* = 4.8 Hz, H-1), 6.74 (1H, d, *J* = 8.0 Hz, H-9), 6.67 (1H, d, *J* = 8.4 Hz, H-11), 6.31 (1H, s, H-5), 5.36 (1H, d, *J*= 4.8 Hz, H-2), 3.99 (3H, s, OCH_3-6), 2,45, 2.18, 1.90, 1.65 (each 2H, m, camphanoyl-CH_2), 1.52, 1.45 (each 3H, s, CH_3-3,3), 1.10, 1.09, 1.00, 0.96, 0.91, 0.88 (each 2H, s, camphanoyl-CH_3); $[\alpha]_D$ -62.5 ^{o} (*c* = 0.0018, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-trimethyl-6-methoxy-11-fluoro-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (142). 80% yield (starting from 45 mg of **183l**); white solid; mp = 183-184 ^{o}C; ESI+ (*m/z*, %), 721 (M^{+}+1, 100); ^{1}NMR *δ* 8.05 (1H, d, *J* = 8.0 Hz, H-8), 7.39 (1H, t, *J* = 8.0, 8.4 Hz, H-9), 7.28 (1H, d, *J* = 8.4 Hz, H-10), 6.76 (1H, d, *J* = 4.4 Hz, H-1), 6.36 (1H, s, H-5), 5.43 (1H, d, *J* = 4.4 Hz, H-2), 4.01 (3H, s, OCH_3-6), 2.51, 2.20, 1.92, 1.75 (each 2H, m, camphanoyl-CH_2), 1.58, 1.48 (each 3H, s, CH_3-3,3), 1.13, 1.13, 1.06, 1.04, 1.00, 0.99 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -41.2 ^{o} (*c* = 0.0052, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-trimethyl-6-methoxy-9-fluoro-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (143). 48% yield (starting from 45 mg of **183m**); white solid; mp = 204-205 ^{o}C; ESI+ (*m/z*, %), 721 (M^{+}+1, 100); ^{1}NMR δ 7.92 (1H, d, J = 8.0 Hz, H-10), 7.28-7.36 (2H, m, H-9,11), 6.85 (1H, d, *J* = 4.4 Hz, H-1), 6.35 (1H, s, H-5), 5.40 (1H, d, *J* = 4.4 Hz, H-2), 4.00 (3H, s, OCH_3-6), 2.49, 2.19, 1.90, 1.68 (each 2H, m, camphanoyl-CH_2), 1.56, 1.49 (each 3H, s, CH_3-3,3), 1.14, 1.13, 1.04, 1.00, 0.90, 0.90 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -23.6 ^{o} (*c* = 0.0025, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-trimethyl-6-methoxy-8-fluoro-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (144). 80% yield (starting from 5 mg of **183n**); white solid; mp = 148-150 ^{o}C; ESI+ (*m/z*, %), 743 (M^{+}+Na, 100); ^{1}NMR δ 7.44 (1H, t, *J* = 8.7, 8.4 Hz, H-10), 7.01 (1H, d, *J* = 8.4 Hz, H-11), 6.92 (1H, d, *J* = 8.7 Hz, H-9), 6.76 (1H, d, *J* = 4.5 Hz, H-1), 6.26 (1H, s, H-5), 5.32 (1H, d, *J* = 4.5 Hz, H-2), 3.91 (3H, s, OCH_3-6), 2.40, 2.12, 1.91, 1.67 (each 2H, m, camphanoyl-CH_2), 1.51, 1.42 (each 3H, s, CH_3-3,3), 1.07, 1.05, 0.97, 0.93, 0.83, 0.83 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -17.1^{o} (*c* = 0.0035, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-methoxy-10-bromo-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (145). 52% yield (starting from 35 mg of **183o**); white solid; mp = 159-160 ^{o}C; ESI+ (*m/z*, %), 781 (M^{+}, 100); ^{1}NMR δ 8.11 (1H, d, *J* = 8.4 Hz, H-8), 7.48 (1H, d, *J* = 1.6 Hz, H-11), 7.44 (1H, dd, *J* = 8.4 Hz, H-9), 6.80 (1H, d, *J* = 4.8 Hz, H-1), 6.32 (1H, s, H-5), 5.46 (1H, d, *J* = 4.8 Hz, H-2), 3.97 (3H, s, OCH_3-6), 2.45, 2.08, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.61, 1.61 (each 3H, s, CH_3-3,3), 1.46, 1.14, 1.12, 1.05, 1.04, 1.02 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -25.3 ^{o} (*c* = 0.0035, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-9-bromo-6-methoxy-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (146). 50% yield (starting from 10 mg of **183p**); white solid; mp = 159-160 ^{o}C; ESI+ (*m/z*, %), 781(M^{+}, 100); ^{1}NMR δ 8.35 (1H, d, *J* = 2.8 Hz, H-8), 7.67 (1H, dd, *J* = 8.8, 2.8 Hz, H-10), 7.20 (1H, d, *J* = 8.8 Hz, H-11), 6.81 (1H, d, *J* = 4.4 Hz, H-1), 6.32 (1H, s, H-5), 5.47 (1H, d, *J* = 4.4 Hz, H-2), 3.98 (3H, s, OCH_3-6), 2.40, 2,10, 1.90, 1.60 (each 2H, m, camphanoyl-CH_2), 1.56, 1.46 (each 3H, s, CH_3-3,3), 1.14, 1.12, 1.03, 1.02, 1.01, 0.77 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -13.0 ^{o} (*c* = 0.004, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3,8-trimethyl-6, 10-dimethoxy-1,2-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (148). 52% yield (starting from 10 mg of **183q**); white solid; mp = 183-184 ^{o}C; ESI+ (*m/z*, %), 781(M^{+}, 100); ^{1}NMR δ 6.85 (1H, d, *J* = 4.8 Hz, H-1), 6.64 (1H, d, *J* = 2.4 Hz, H-9), 6.53 (1H, d, *J* = 2.4 Hz, H-11), 6.30 (1H, s, H-5), 5.39 (1H, d, *J* = 4.8 Hz, H-2), 3.99 (3H, s, OCH_3-6), 3.81 (3H, s, OCH_3-10), 2.84 (3H, s, CH_3-8), 2.45, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.56, 1.48 (each 3H, s, CH_3-3,3), 1.13, 1.13, 1.04, 1.00, 0.91, 0.88 (each 2H, s, camphanoyl CH_3); $[\alpha]_D$ -23.6 ^{o} (*c* = 0.0012, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-methoxy-10-cyano-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (152). 80% yield (starting from 36 mg of **183r**); white solid; mp = 164-165 ^{o}C; ESI+ (*m/z*, %), 728(M^{+}+1, 100); ^{1}NMR δ 8.36 (1H, d, *J* = 8.0 Hz, H-8), 7.60 (1H, d, *J* = 8.0 Hz, H-9), 7.55 (1H, s, H-11), 6.78 (1H, d, *J* = 4.4 Hz, H-1), 6.35 (1H, s, H-5), 5.39 (1H, d, *J* = 4.4 Hz, H-2), 3.99 (3H, s, OCH_3-6), 2.50, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl-CH_2), 1.56, 1.47 (each 3H, s, CH_3-3,3),

1.12, 1.11, 1.05, 1.02, 0.98, 0.95 (each 3H, s, camphanoyl-CH_3); $[\alpha]_D$ -30.0 ^{o} (*c* = 0.0012, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-hydroxy-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (132). Compound **131** (20mg, 0.028 mmoL) was heated with 48% HBr (2.0 mL) at reflux temperature for 12 h. The reaction mixture was allowed to cool, diluted with water, filtered and the residue was thoroughly washed with water. The crude product was purified by prepare TLC with hexanes:EtOAc = 1:1 to yield **132** as a white solid. 63% yield; MS (ESI+) m/z (%) 689 (M^+ + 1, 100); ^{1}NMR *δ* 13.67 (1H, s, OH-6), 8.25 (1H, d, *J* = 7.2 Hz, H-8), 7.74 (1H, d, *J* = 8.8, 8.4 Hz, H-10), 7.46 (1H, d, *J* = 8.4 Hz, H-11), 7.41 (1H, d, *J* = 8.8, 7.2 Hz, H-10), 6.63 (1H, d, *J* = 4.8 Hz, H-1), 6.44 (1H, s, H-5), 5.36 (1H, d, *J* = 4.8 Hz, H-2), 2.50, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.50, 1.47 (each 3H, s, CH_3-3,3), 1.17, 1.11, 1.08, 1.07, 0.98, 0.97 (each 3H, s, camphanoyl CH_3).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-5-bromo-6-methoxy-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (147). A mixture of **131** (50 mg, 0.071 mmoL), NBS (17.0 mg, 0.10 mmol) and CH_2Cl_2 (2 mL) was heated to 100 ^{o}C for 2 h under high-absorption microwave conditions. At completion, the mixture was concentrated and purified by PTLC with an eluent of hexane:EtOAc = 3:2 to afford pure **147** as white solid (43.7 mg). 78.9% yield; mp = 139-140 ^{o}C; MS (ESI+) m/z (%) 803, 805 (M^++Na-1, 50; M^+ + Na+1, 50); ^{1}NMR *δ* 8.29 (1H, d, *J* = 8.0 Hz. H-8), 7.65 (1H, t, *J* = 7.6, 7.6 Hz, H-10), 7.39 (1H, t, *J* = 8.0, 7.6 Hz, H-9), 7.29 (1H, d, *J* = 7.6 Hz, H-11), 6.90 (1H, d, *J* = 4.8 Hz, H-1), 5.44 (1H, d, *J* = 4.8 Hz, H-2), 4.05 (3H, s, OCH_3-6),

2.53, 2.21, 1.95, 1.75 (each 2H, m, camphanoyl CH_2), 1.14, 1.13, 1.05, 1.01, 0.94, 0.93 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -51.3 ^{o} (*c* = 0.0023, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3,9-trimethyl-5-Bromo-6-methoxy-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (149). The procedure was identical to that used for the preparation of **147**. 56% yield (starting from 50 mg of **135**); white solid; mp = 170-171 ^{o}C; MS (ESI+) m/z (%) 795, 797 (M^+-1, 50; M^+ +1, 50); ^{1}NMR δ 8.06 (1H, s, H-8), 7.45 (1H, d, *J* = 8.4 Hz, H-10), 7.19 (1H, d, *J* = 8.4 Hz, H-11), 6.88 (1H, d, *J* = 4.4 Hz, H-1), 5.43 (1H, d, *J* = 4.4 Hz, H-2), 4.04 (3H, s, OCH_3-6), 2.44 (3H, s, CH_3-9), 2.50, 2.20, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.58, 1.58 (each 3H, s, CH_3-3,3), 1.41, 1.13, 1.04, 1.01, 0.93, 0.91 (each 3H, s, camphanoyl CH_3); $[\alpha]_D$ -10.8 ^{o} (*c* = 0.0078, $CHCl_3$).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-hydroxy-9-bromomethyl-1,2-dihydropyrano[2,3-*c*]xanthen-7(1*H*)-one (150). A mixture of **135** (50 mg, 0.07 mmoL), NBS (18.6 mg, 0.105 mmoL), and 3-chloroperbenzoic acid (2 mg, 0.01 mmoL), dissolved in 1 mL of anhydrous CCl_4 was heated to 100 ^{o}C for 5 h under high-absorption microwave conditions. At completion, the mixture was concentrated and the residue was purified by PTLC with hexane:EtOAc = 1:1 to afford pure **150** (31.3 mg, white solid). 56.1% yield; MS (ESI+) m/z (%) 795, 797 (M^+-1, 50; M^+ +1, 50); ^{1}NMR δ 13.62 (1H, s, OH-6), 8.20 (1H, d, *J* = 2.0 Hz, H-8), 7.74 (1H, dd, *J* = 8.8, 2.0 Hz, H-10), 7.48 (1H, d, *J* = 8.8 Hz, H-11), 6.89 (1H, s, H-5), 5.30, 5.30 (each 1H, s, H-1,2), 2.44 (2H, s, CH_2Br-9), 2.40, 2.15, 1.90, 1.70 (each 2H, m, camphanoyl CH_2), 1.56, 1.55 (each 3H, s, CH_3-3,3), 1.11, 1.10, 1.04, 1.03, 0.98, 0.85 (each 3H, s, camphanoyl CH_3).

1*R*,2*R*-(-)-dicamphanoyl-3,3-dimethyl-6-methoxy-10-amine-1,2-dihydro pyrano[2,3-*c*]xanthen-7(1*H*)-one (151). A THF solution of **144** (10 mg, 0.013 mmoL), 25% ammonium hydroxide solution (0.004 mL, 0.03 mmoL) was stirred at rt for 3.5 h. The mixture was poured into water (excess) and extracted with EtOAc. The crude product was purified by PTLC with hexane:EtOAc = 7:1 to afford pure **151** as white solid. 15% yield; MS (ESI+) m/z (%) 741 (M^+ +Na, 100); ^{1}NMR δ 8.12 (1H, d, J = 8.4 Hz, H-8), 7.50 (1H, s, H-11), 7.45 (1H, d, J = 8.4 Hz, H-9), 6.64 (1H, d, J = 5.2 Hz, H-1), 6.31 (1H, s, H-5), 5.46 (1H, d, H-2), 3.96 (3H, s, OCH_3-6), 2.60 (2H, s, NH_2-10), 2.40, 2.10, 1.90, 1.65 (each 2H, m, camphanoyl-CH_2), 1.54, 1.46 (each 3H, s, CH_3-3,3), 1.14, 1.12, 1.06, 1.05, 1.02, 0.89 (each 3H, s, camphanoyl-CH_3).

5.6.2 HIV-1 Infectivity Assay

Anti-HIV-1 activity was measured as reductions in Luc reporter gene expression after a single round of virus infection of TZM-bl cells. HIV-1 at 200 $TCID_{50}$ and various dilutions of test samples (eight dilutions, 4-fold stepwise) were mixed in a total volume of 100 μL growth medium in 96-well black solid plates (Corning-Costar). After 48-h incubation, culture medium was removed from each well and 100 μL of Bright Glo luciferase reagent was added to each culture well. The luciferase activity in the assay wells was measured using a Victor 2 luminometer. The 50% inhibitory dose (IC_{50}) was defined as the sample concentration that caused a 50% reduction in Relative Luminescence Units (RLU) compared to virus control wells after subtraction of background RLU.

5.6.3 Cytotoxicity Assay

The general procedure was performed according to CytoTox-Glo™ cytotoxicity assay instructions for using product G9290, G9291 and G9292. (Promega)

5.7 References

1. Yu, D.; Chen, C. H.; Brossi, A.; Lee, K. H., Anti-AIDS agents. 60. Substituted 3'R,4'R-di-O-(-)-camphanoyl-2',2'-dimethyldihydropyrano[2,3-f]chromone (DCP) analogues as potent anti-HIV agents. *J. Med. Chem.* **2004,** 47, (16), 4072-4082.

2. Zhou, T.; Shi, Q.; Chen, C. H.; Zhu, H.; Huang, L.; P., H.; Lee, K. H., Anti-AIDS Agents 79. Design, Synthesis, Molecular Modeling and Structure-Activity Relationships of Novel Dicamphanoyl-2',2'-dimethyldihydropyranochromone (DCP) Analogs as Potent Anti-HIV Agents. *Bioorg. Med. Chem. Lett.* **2010,** 18, 6678-6689.

3. Moreau, S.; Varache-Lembege, M.; Larrouture, S.; Fall, D.; Neveu, A.; Deffieux, G.; Vercauteren, J.; Nuhrich, A., (2-Arylhydrazonomethyl)-substituted xanthones as antimycotics: synthesis and fungistatic activity against Candida species. *Eur. J. Med. Chem.* **2002,** 37, (3), 237-253.

4. Zhou, T.; Shi, Q.; Lee, K.-H., Anti-AIDS Agents 83. Efficient Microwave-assisted One-pot Preparation of Angular 2,2-Dimethyl-2*H*-chromone Containing Compounds. *Tetrahedron Lett.* **2010,** 51, (33), 4382-4386.

5. Reisch, J.; Herath, H. M. T.; Kumar, N. S., Convenient Synthesis of Isoacronycine and Some Other New Acridone Derivatives. *Liebigs Ann. Chem.* **1991**, (7), 685-689.

6. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B., Catalytic Asymmetric Dihydroxylation. *Chemical reviews* **1994,** 94, 2483-2547.

7. Mehltretter, G. M.; Dobler, C.; Sundermeier, U.; Beller, M., An Improved Version of the Sharpless Asymmetric Dihydroxylation. *Tetrahedron Lett.* **2000,** 41, 8083-8087.

8. Xie, L.; Crimmins, M. T.; Lee, K. H., Anti-AIDS Agents 22. Asymmetric synthesis of 3',4'-Di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (DCK), a Potent Anti-HIV Agent. *Tetrahedron Lett.* **1995,** 36, 4529-4532.

9. Dallavalle, S.; Gattinoni, S.; Mazzini, S.; Scaglioni, L.; Merlini, L.; Tinelli, S.; Beretta, G.; Zunino, F., Synthesis and Cytotoxic Activity of a New Series of Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2008,** 18, 1484-1489.

10. Miyake, H.; Nichino, S.; Nishimura, A.; Sasaki, M., New Synthesis of 3-Bromoflavones via Bromination of 1-(2-Hydroxyphenyl)-3-Arylpropane-1,3-dione by $CuBr_2$. *Chem. Letts.* **2007,** 36, 522-523.

11. Lynch, J. K.; Freeman, J. C.; Judd, A. S.; Iyengar, R.; Mulhern, M.; Zhao, G.; Napier, J. J.; Wodka, D.; Brodjian, S.; Dayton, B. D.; Falls, D.; Ogiela, C.; Reilly, R. M.; Campbell, T. J.; Polakowski, J. S.; Hernandez, L.; Marsh, K. C.; Shapiro, R.; Knourek-Segel, V.; Droz, B.; Bush, E.; Brune, M.; Preusser, L. C.; Fryer, R. M.; Reinhart, G. A.; Houseman, K.; Diaz, G.; Mikhail, A.; Limberis, J. T.; Sham, H. L.; Collins, C. A.; Kym, P. R., Optimization of chromone-2-carboxamide melanin concentrating hormone receptor 1 antagonists: assessment of potency, efficacy, and cardiovascular safety. *J. Me.d Chem.* **2006,** 49, (22), 6569-6584.

CHAPTER 6

DESIGN, SYNTHESIS AND SAR STUDY OF 3',4'-DI-SUBSTITUTED-2',2'-DIMETHYLDIHYDROPYRANO[2,3-*f*]CHROMONE (DSP) ANALOGS AS POTENT CHEMOSENSITIZERS TO OVERCOME MULTIDRUG RESISTANCE IN CANCER TREATMENT

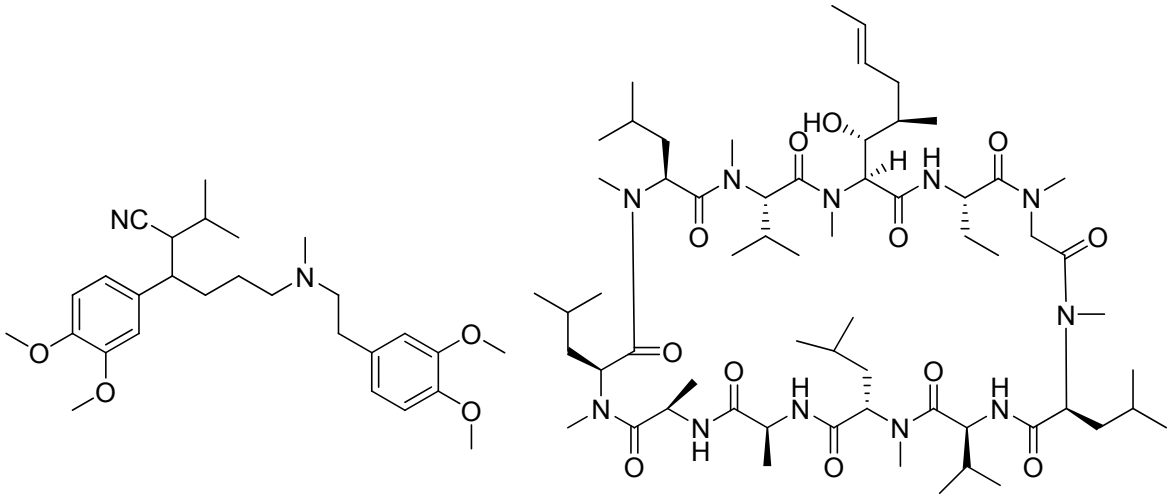
6.1 Introduction

Resistance of cancer cells to anticancer drugs remains one of the major obstacles in achieving an effective treatment for cancer. Three primary mechanisms of anti-cancer drug resistance have been identified by applying cellular and molecular biology methods, including decreased uptake of water-soluble drugs, such as nucleoside analogues and cisplatin, which require transporters to enter cells; various changes in cells that affect the capacity of cytotoxic drugs to kill cells; and the most commonly encountered, increased energy-dependent efflux of hydrophobic cytotoxic drugs by one of a family of energy-dependent transporters, such as P-glycoprotein (P-gp, also known as MDR1).[1] One approach used in clinical research to reverse multi-drug resistance (MDR) is focused on the development of chemosensitiziers especially those drugs that target to P-gp and inhibit its activity. Verapamil and cyclosporine A (Figure 6-1) are examples of first generation chemosensitizers that inhibit the activity of P-gp and were evaluated in the clinic as adjuvants for chemotherapy. Both compounds are able to increase the intracellular

concentration of cytotoxic agents, such as vincristine (VCR), cyclophosphamide, dexamethasone, doxorubicin in MDR cells. [2, 3] Moreover, both verapamil and cyclosporine A are wildly used medications as either a calcium channel blocker (verapamil) to treat hypertension or an immunosuppressant (cyclosporine) to reduce the activity of the patient's immune system in post-allogeneic organ transplant. However, clinical studies showed both were generally toxic at the doses required to attenuate P-gp function. Since then, several potent and selective next generation inhibitors have been developed and investigated yet significant clinical benefit of any of them have yet to emerge. The approach to inhibit P-gp may be problematic since drug distribution and elimination can be affected by these agents however it is also possible that the ideal chemotype for a chemosensitizer has yet to be identified and developed .[4]

It has been reported that naturally existing pyranocumarin (±)-praeruptorin A (PA) and its khellactone analogs, with 3',4'-modifications can reverse P-gp-mediated MDR.[5, 6] (Figure 6-2) (±)-3'-*O*-4'-*O*-bis(3,4-Dimethoxycinnamoyl)-*cis*-khellactone (DMDCK, **187**) showed the highest potency among its analogs . **187** is able to increase the activity of the chemotherapy drug vinblastine over 110-fold at the concentration of 4 μM.[6] However, even at such high concentration, compound **187** is unable to completely reverse vinblastine resistance in the liver cancer cell system used. [6] In addition, it was also reported that the side chains at 3' and 4' positions of khellactone system play an important role in maintaining high activity. Unfortunately, there are only few khellactone analogs with 3',4' modifications reported to show potent chemosensitizing activity so detailed SAR information is unavailable.

Therefore, continuing research to develop novel active analogs is worthwhile and is the basis for the work on the 3'R,4'R-di-substituted-pyranochomons (DSP) series reported herein.



Verapamil **Cyclosporine A**

Figure 6-1

6.2 Design

Even before the structure of P-gp protein was resolved, a general pharmacophore model of verapamil essential for P-gp binding and inhibition was proposed.[7] The pharmacophore consists of a butterfly-shaped molecule with two hydrophobic motifs (aromatic ring system) connected with a linker that contains some HB acceptors and/or donors. DMDCK (**187**) was subsequently shown to fit the pharmacophore and could be well-docked into the verapamil hypothetical binding site on the protein subunit of P-gp.[6, 7]

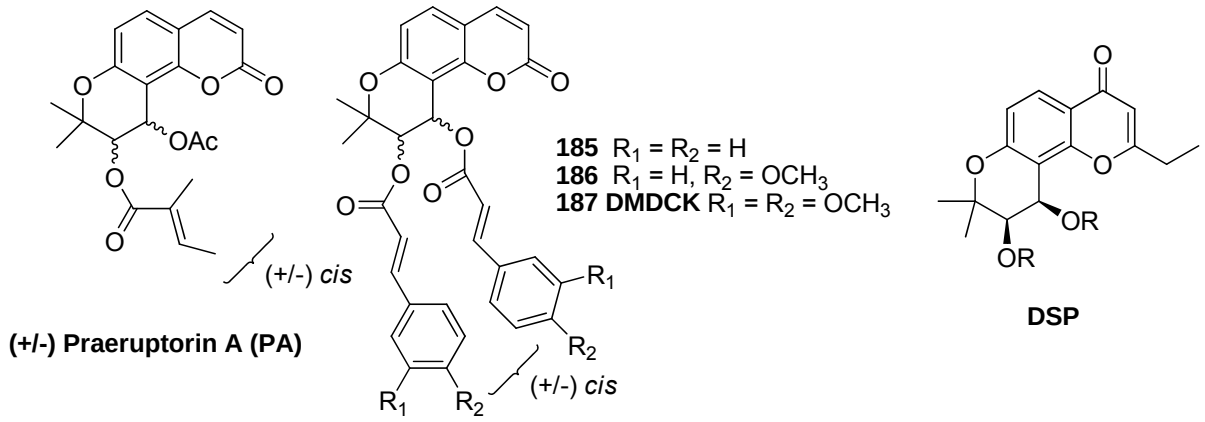


Figure 6-2. Structures of (+/-) Praeruptorin A, khellactone analogs **185-187** and DSP

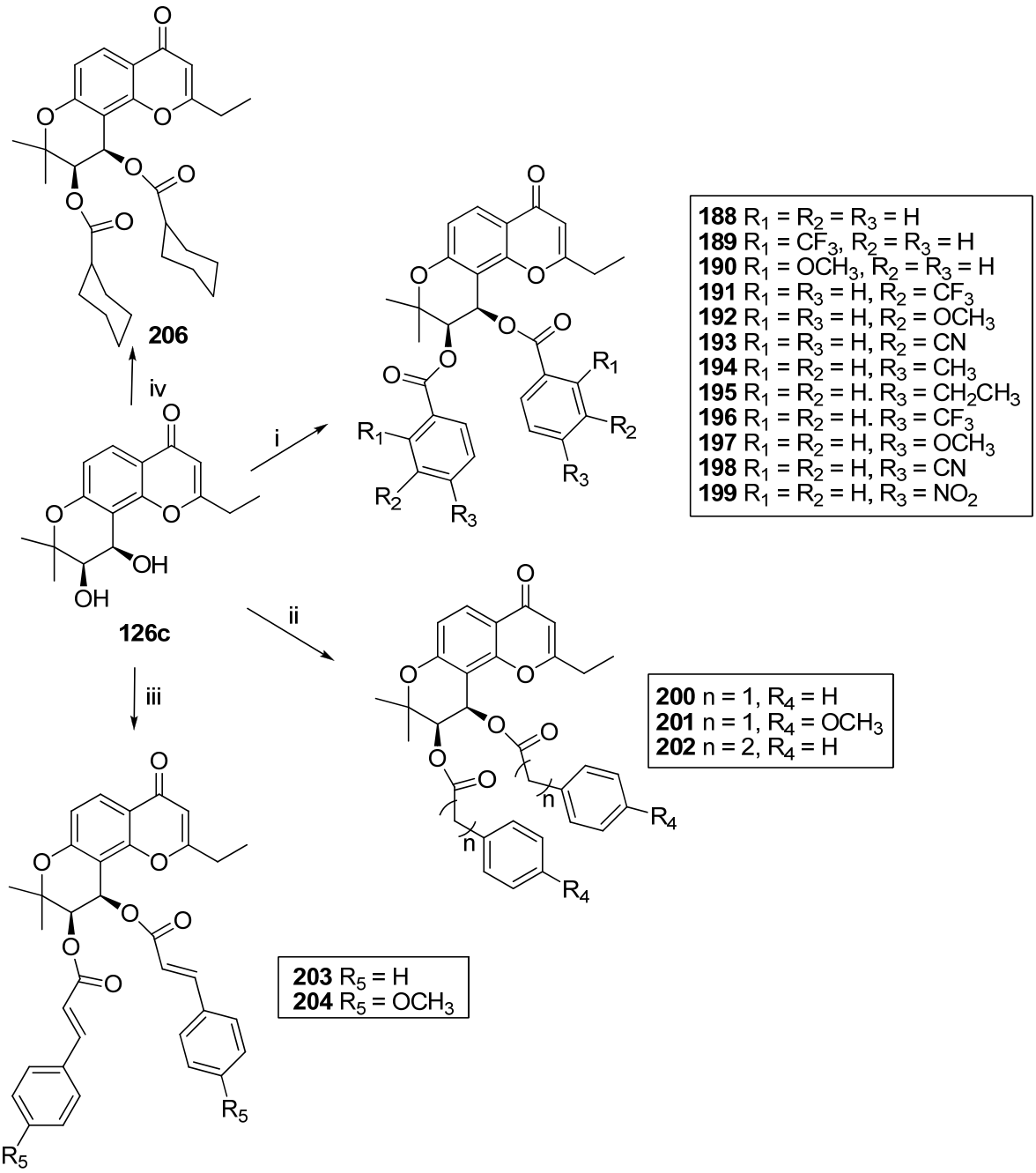
DSPs (Figure 6-2) share certain structural similarity to DMDCK (**187**). This prompted us to design and synthesize a series of DSP derivatives as new chemosensitizing agents. It is likely that the pyranochromone moiety has similar HB properties to those of khellactone in DMDCK. Incorporation of two side chains at 3' and 4' positions with substitutions containing aromatic moieties could well-mimic the hydrophobic moieties in verapamil and DMDCK (**187**). Based on this rationale, a series of DSP derivatives with varied 3' and 4' side chains were designed, synthesized, and screened against the MDR human esophageal cancer cell line (KB-Vin) with or without combination of the chemotherapy drug VCR. This approach allowed us to evaluate the intrinsic cytotoxicity of DSP analogs as well as their ability to reverse MDR.

6.3 Chemistry

Compound **126c**, 3'*R*,4'*R*-2',2'-dimethyl-pyranodihydrochromone, is a core structure of all the target compounds and a key intermediate of all the synthetic pathways. The synthesis of **126c** has been illustrated in Scheme 4-2 (Chapter 4). A

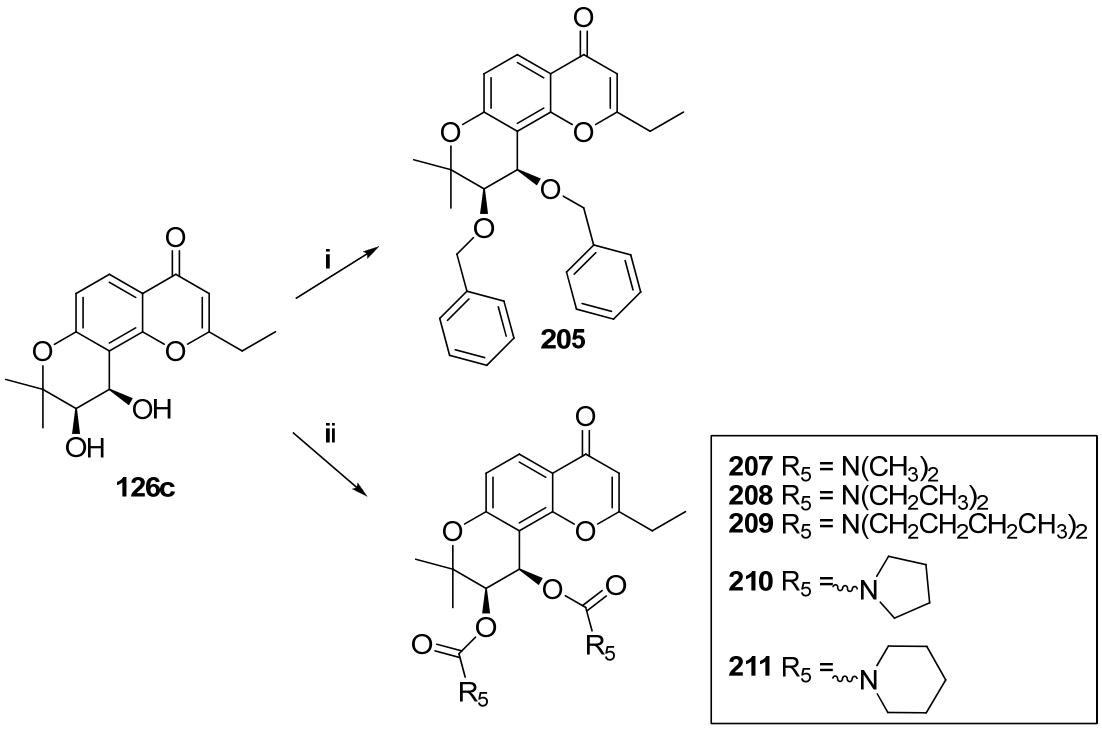
series of side chains with varied chain length and aromatic or non-aromatic functional groups at the chain end were introduced to the 3'and 4' positions of the core scaffold (Schemes 6-1, 6-2).

The synthesis of 3',4'-dibenzoyl-pyranodihydrochromone derivateves (**188** – **204** and **206**) is showed in Scheme 6-1. 3',4'-dihydroxy-pyranochrmone (**126c**) stirred with substituted benzoyl chloride or cyclohexal carbonyl chloride afforded compound **188** - **199** and **206**, in the presence of DMAP at room temperature. Reacting phenylacetyl chlorides or 3- phenylpropanoyl chloride with compound **126c** obtained compounds **200** – **202**. Reaction of **126c** with *trans*-cinnomoyl chlorides afforded compounds **203**- **204**.



Scheme 6-1. Synthesis of DSP derivatives (**188 - 204** and **206**). Reagents and Condition: (**i**) diverse benzoyl chloride, DMAP, anhydrous CH_2Cl_2, rt ;(**ii**) phenylacetyl chlorides or 3-phenylpropanoyl chloride, DMAP, anhydrous CH_2Cl_2, rt; (**iii**) *trans*-4-methoxycinnamoyl chloride, DMAP, CH_2Cl_2, rt; (iv) cyclohexanecarbonyl chloride, DMAP, anhydrous CH_2Cl_2, rt.

3',4'- Di-benzyloxy substituted DSP (**205**) was also generated by reacting compound **126c** with benzyl bromide in the presence of NaH in DMF under N_2 atmosphere. (Scheme 6-2) [8]



Scheme 6-2. Synthesis of DSP derivatives (**205**, **207**-**211**). Reagents and Condition: (i) benzyl bromide, NaH/DMF, N_2, 0°C; (ii) diverse dialkylcarbamyl chloride or cyclocarbamyl chloride, NaH, rt.

The synthesis of carbamates **207** - **211** was accomplished upon treatment of **126c** with *N*,*N*-dialkylcarbamylchloride, or pyrrolidine and piperidine carbonyl chlorides respectively, in anhydrous tetrahydrofuran, in the presence of NaH (Scheme 6-2). [9]

6.4 Results and Discussion

6.4.1 Chemosensitization Activity *In Vitro*

VCR is a potent mitotic inhibitor, and is widely used in the combination chemotherapy of acute leukemias and some solid tumors. The GI_{50} of VCR against KB cell line (drug-sensitive) is 0.009 μg/mL (Table 6-1). In KB-Vin cell line, a multi-

drug resistant cancer cell line, the cytotoxic effect of VCR dramatically decreased over 270-fold, with GI_{50} at 2.44 μg/mL (Table 6-1). In order to detect the potential of DSPs to reverse MDR in KB-Vin cells, the cytotoxic effect of VCR was evaluated with or without the combination of each newly synthesized DSP analog.

In order to exclude the possibility that cytotoxicity of the combination treatment with VCR and DSP might result from the cytotoxic effect of DSPs, all newly synthesized DSPs were screened individually against KB-Vin cells and the results are shown in Table 6-1. In general, all DSP analogs displayed low intrinsic cytotoxic activity and similar to the prototype first generation P-gp inhibitor verapamil.

The chemosensitizing activity of DSPs was then evaluated by measuring reversal of the cytotoxic effect of VCR in the presence of DSP analogs at 1 μg/mL and using verapamil as the positive control. The results shown in the table 6-2 reveal that most DSP analogs could reverse VCR resistance by lowing GI_{50} values of VCR in combination treatment in the range of 12-349 folds, compared to verapamil which increased VCR sensitivity 141- fold.

Compounds **188** – **199** are pyranochromones with benzoyloxy groups substituted at 3' and 4' positions. Different functional groups were selectively introduced on the *o*, *m* or *p* position of benzoyl rings. As shown in Table 6-2, non-subsituted or *o, m*-substiuted benzoyloxy-DSPs (**188** and **189**-**192**) displayed only weak chemosensitizing activity. Compounds **188**, **189**, and **191** failed to decrease the GI_{50} of VCR below 0.2 μg/ml. *o* and *m*-Methoxy-substituted benzoyloxy-DSPs (**190** and **192**) displayed only moderate chemosensitizing activity and increased the

potency of VCR around 28 times. **193** with a *m*-CN substituent, exhibit a better chemosensitizing activity (160- fold).

DSP analogs with *p*-substituted benzoyloxy rings (**194**-**199)** showed substitution-dependent activity. GI_{50} values of the combination of **194** and **197** with VCR were 0.007 and 0.0095 *μ*g/mL, respectively, corresponding to 349- and 257-fold increased potency relative to VCR alone. The improved potency was greater than that with verapamil. Interestingly compound **195**, with an ethyl group had a substantially lower effect (20.3-fold VCR increase), and **196**, substituted with a trifluoromethyl group, improved VCR activity by only 16.3-fold. Compounds **198**-**199**, with electron-withdrawing groups at *p*-position, also showed enhancements of approximately 200-fold, which was better than that of verapamil. These results suggested that, *p*-substitutions are generally preferred over *o*- or *m*-substituents for chemosensitizing activity. Substitutions with extended conjugation, e.g., CN or NO_2 groups, and smaller less sterically hindered groups may benefit the aefficacy of DSPs as new potent chemosensitizers.

Compounds **200**-**204** were designed, synthesized, and evaluated in order to understand the effects of adding a linker between the ester carbonyl group and the aromatic substituents in the side chains. Introducing a saturated linker slightly increased the chemosensitizing ability, with improved GI_{50} values of VCR-DSP combination from over 0.2 *μ*g/mL for **188** ($-O_2CC_6H_5$), 0.03 *μ*g/mL for **200** ($-O_2CCH_2C_6H_5$), and 0.023 *μ*g/mL for **202** ($-O_2CCH_2CH_2C_6H_5$). Unexpectedly, replacing the phenyl group of **200** with a *p*-methoxyphenyl in **201** resulted in an 2.4-fold reduction in chemosensitizing efficacy, and 7.5 times reduction compared with

197, which also contains a *p*-methoxyphenyl group, but linked directly to the ester carbonyl moiety. Compounds **203** and **204** have a conjugated double bond between the ester carbonyl group and the phenyl ring. Interestingly, combination of either compound with VCR led to significantly enhanced activity. Compound **203**, with an unsaturated vinyl group, showed a twofold increased efficacy compared with **202**, with a saturated ethylene group. **204**, with a *p*-methoxycinnamoyloxy 3',4' side chains, was able to fully restore the cytotoxicity of VCR. The GI_{50} value of the VCR-**204** combination was 0.0085 μg/mL, which was comparab to the values with **194** and **197**. Thus, cinnamic and *p*-methoxycinnamic esters linked to the core dihydropyranochromone structure favored MDR reversal activity.

In order to investigate the impact of the carbonyl group in the active analogs, **205** was synthesized. In this compound, the carbonyl moieties at 3' and 4' side chains have been replaced with methylene groups. With an ether rather than ester side chain, **205** will likely be chemically and metabolically more stable than its analog **188**. Interestingly, **205** showed better capability to reverse MDR than **188**. This finding shows that the carbonyl group may not be essential for activity. Further SAR studies are needed to confirm this hypothesis.

To further investigate the importance of the aromatic substitution at the end of 3',4'-side chains, compounds **206**-**211** were designed and synthesized. Most compounds without an aromatic substitutions at the side chains showed either none or marginal chemosensitizing activity, except **208**, which showed ten times better activity than its counterparts, **207**and **209**. **208** also presented 5-fold more potency than its cyclic analogs **210** and **211**, suggesting that the orientation of the N-alkyl

substitutions at 3' and 4' positions affect the interactions of the drug molecules and the binding pocket on the target protein, for example, the different orientation between chain alkyl goups (**208**) and cyclo-alkyl groups (**210**, **211**), which, in turn affects the activity.

In summary, three compounds, **194**, **197** and **204** were found to be the most potent chemosensitizers of VCR among 24 newly synthesized compounds. At non-toxic concentration, they are able to fully restore the activity of VCR against KB-Vin so that the MDR cell line was as sensitive as the parent KB cell line. They are also over two times better than positive control, verapamil (Table 6-2). While most of the active compounds with 3',4'-ester side chains ending in an aromatic ring, **208**, which contains two carbamate side chains and no aromatic system, also exhibited considerable potency. The dicarbamate **208** should also exhibit increased polarity and different physicochemical properties, which may benefit to further develop new analogs with interesting biological activity.

The SAR study on 3',4' side chains suggested the following conclusions. 1. A terminal aromatic ring is important but not necessary to maintain the high chemosensitizing capability; 2. Substitutions on the aromatic rings can significant impact on the activity. Moieties that have increased electron density, extended conjugation system, or HB effects could benefit the activity; 3. The characteristic of the linker between the core structure and the aromatic ring system also affect the chemosensitizing potential. Extending the conjugation system by adding double bond(s) benefits the activity.

Previous modification of PA and khellactone analogs revealed that the aromatic rings at 3' and 4' side chains of khellactone benefit the chemosensitizing activity[6] and in addition, methoxy group substituted on cinnamoyl side chains could impact the activity. Those results are mostly, but not fully, consistent with those reported here for pyranochromone-based DSP active analogs. For example, we found that **208**, without an aromatic ring in the 3',4'-side chains displayed good chemosensitizing ability, and thus, an aromatic ring is not essential for activity. In addition, our studies showed both positive (**197** vs **188**, **204** vs **203**) and negative (**201** vs **200**) effects for the addition of *p*-methyl group on a phenyl ring. Fong *et al.* also reported that **186** exhibited a decreased potency compared to**185**. [6]

Due to the different cell lines and MDR substrates, it is difficult to directly compare the activities between prior khellacton analogs and DSP analogs reported here. However, in both HepG2/Dox and KB-V1 cell lines, the best reported DMDCK (**187**) at 4 μM, could not completely restore the cytotoxicity of vinblastine, an alkaloid analog of VCR. In contrast, DSP analogs **194**, **197** and **204** completely restored the activity of VCR suggesting they are more efficacious and more potent than **187**.

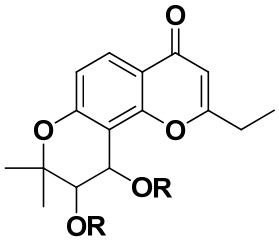
Table 6-1 Cytotoxicity of DSP derivatives, verapamil and VCR

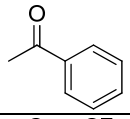
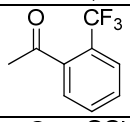
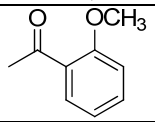
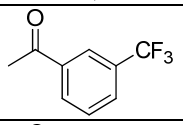
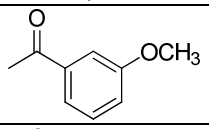
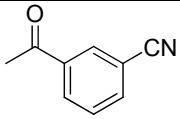
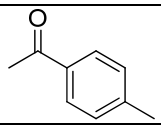
Compound	GI_{50} (μg/mL) [a]	Compound	GI_{50} (μg/mL)
188	8.79	**201**	13.3
189	> 31.5	**202**	10.1
190	>31.5	**203**	9.44
191	> 31.5	**204**	>36.4
192	> 35.8	**205**	>15.7
193	> 2.5	**206**	>20
194	20.6	**207**	>20
195	> 13.6	**208**	>20
196	>31.5	**209**	>20

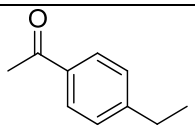
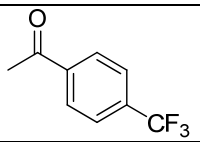
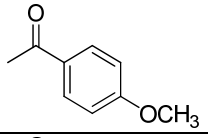
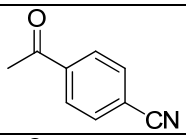
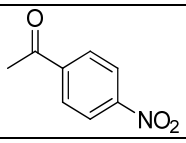
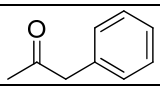
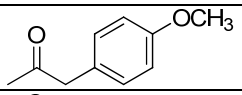
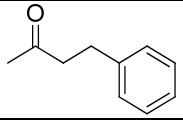
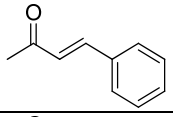
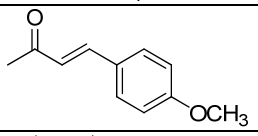
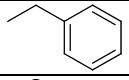
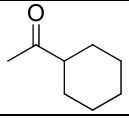
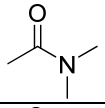
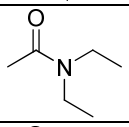
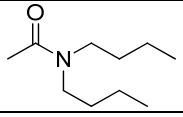
197	9.0	**210**	>20
198	>5.5	**211**	>20
199	>34.0	**verapamil**	>31.5
200	8.33	**VCR**	2.44
		VCR[b] (in KB cell)	0.009

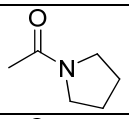
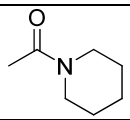
[a] Cytotoxicity as GI_{50} values for KB-Vin cell line or as indicated, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.
[b] cytotoxicity for KB cell line

Table 6-2 Cytotoxicity of VCR + DSP formula



Compound used in combination	R	GI_{50} of VCR against KB-Vin (μg/mL) [a]	Cytotoxicity fold increase of VCR
188		>0.2	<12.2
189		>0.2	< 12.2
190		0.087	28.1
191		>0.2	< 12.2
192		0.09	27.1
193		0.0152	160.5
194		0.007	348.6

195		0.12	20.3
196		0.15	16.3
197		0.0095	256.8
198		0.013	187.7
199		0.012	203.3
200		0.03	81.3
201		0.071	34.4
202		0.023	106.1
203		0.011	221.8
204		0.0085	287.1
205		0.060	40.7
206		>0.2	< 12.2
207		>0.2	< 12.2
208		0.021	116.2
209		>0.2	< 12.2

210		0.098	24.9
211		0.11	22.2
verapamil		0.017	141.2

[a] Cytotoxicity as GI_{50} values for KB-Vin cell line, the concentration of VCR + DSP (1μg/mL) that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.

6.4.2 Effect on Cellular P-gp Activity

The acquired VCR-resistance in KB-Vin cell line is due to enhanced drug efflux via P-gp over-expression.[10] Our research results demonstrate that addition of DSPs could restore VCR's cytotoxicity against KB-Vin, suggesting that DSP analogs might function like verapamil as P-gp inhibitors. To test this hypothesis, the effect of DSP analogs on efflux of the P-gp substrate Calcein-AM was investigated using the established and widely used assay for cellular P-gp function [11]. Inhibition of P-gp leads to the accumulation of Calcein-AM in the cytoplasm and concomitant degredation by esterase action to give a fluorescent derivative that can be conveniently monitored as a quatitative measurement of P-gp inhibition. Four DSP analogs (**191**, **196**-**198**) were selected and their ability to facilitate Calcein-AM influx was examined and compared to verapamil as the positive inhibitor control. The results are shown in Figure 6-3. Compounds **198** and **197** which showed improved activity versus verapamil in the chemosensitization assay (Table 6-2) also exerted more potent inhibition of cellular P-gp based on the functional assay results. Compound **196** which showed weaker chemosensitizing activity versus verapamil (16- and 142- fold respectively, Table 6-2) was correspondingly less active as a P-gp

inhibitor. Compound **191** exerted no effect on Calcein-AM efflux, which is consistent with its lack of chemosensitization activity. These results demonstrate that the rank-order of chemosensitizing efficacy of DSP analogs is consistent with their relative inhibitory activity toward cellular P-gp. Thus, DSP analogs most likely act as pump inhibitors in the KB-MDR cell system.

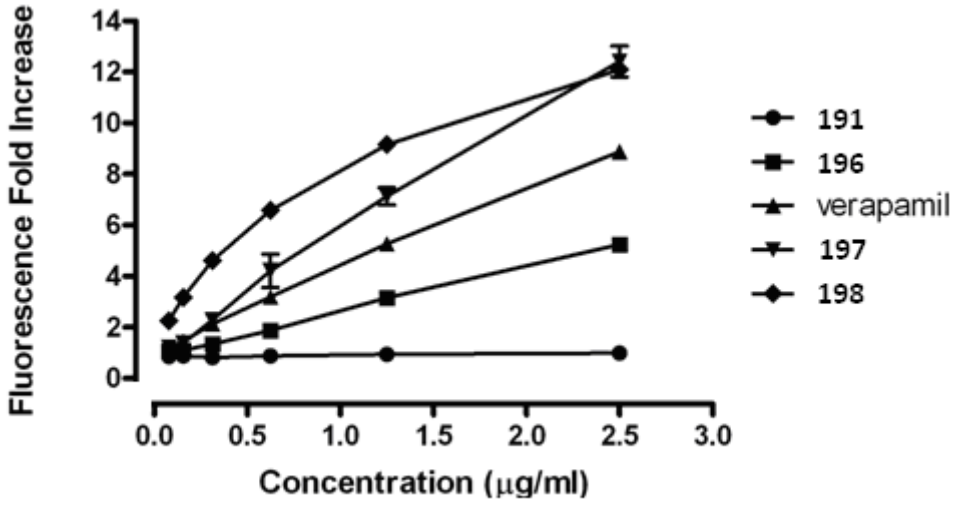


Figure 6-3. Effect on cellular P-gp activity of DSP analogs and verapamil

6.5 Conclusion

Novel DSP analogs with significant chemosensitizing activity in a MDR human cancer cell line were discovered. A SAR study on the 3' and 4' side chains of the pyranochormone ring was conducted with the following conclusions. *p*-Substitution on the aromatic ring in the side chain is better than *o*- and *m*-substitution. Small substitutions that can increase electron density or provide HB capacity at the putative interaction domain on P-gp benefit the activity. The length and identity of the linker is also important and a conjugated double-bond is favorable. Compared with verapamil, a prototype first generation chemosensitizer, nine DSPs exhibited

improved or comparable chemosensitizing potential. Compounds **194**, **197**, and **204** fully overcame VCR resistance in KB-Vin cancer cells at a concentration of 1μg/mL (1.63-1.89 μM range). Compared to recent work on khellactone-based chemosensitizers our study provides a more comprehensive SAR and the discovered DSP analogs with more promising potency at lower concentration. Preliminary work indicates that the chemosensitizing activity of DSP analogs results from P-gp inhibition. Detailed mechanistic studies are currently under ongoing.

6.6 Experimental Section

6.6.1 Chemistry

Melting points were measured with a Fisher Johns melting apparatus without correction. 1H NMR spectra were measured on a 300MHz varian Gemini 2000 spectrometer and varian Inova 400 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Microwave reactions were performed with a Biotage initator EXP US. Mass spectra were measured on Shimazu LCMS-2010 (ESI-MS). Optical rotation was measured with a Jasco Dip-2000 digital polarimeter at 20^{o}C at the sodium D line. Thin-layer chromatography (TLC) was performed on PLC silica Gel 60 F254 plate (20 x 20, Merck). Biotage Flash and Isco companion systems were used as medium-pressure column chromatography. Silica 40 μM columns from Grace Inc. were used for column chromatography. All final compounds are > 95% pure on the basis of the two HPLC conditions.

Synthesis of compounds 188-204 and 206. A mixture of compound **126c** (1 equiv), substituted benzoyl cholorde, cyclohenxal chloride, phenylcaetyl chlorides,

3-phenylpropanoyl chloride, or *trans*-cinnamoyl chlorides (3 equiv), and DMAP (4 equiv) were stirred in CH_2Cl_2 for 1-2 H at rt, monitored by TLC. At completion, the mixture was diluted with CH_2Cl_2 and washed by water and brine. The solvent was then removed under reduced pressure and the residue was purified by PTLC with hexane:EtOAc = 3:2 to afford the appropriatel compounds (**188**-**204** and **206**).

3'*R*,4'*R*-Di-benzoyloxy-2',2'-dimethyl-2-ethyldihydropyrano[2,3-*f*] chromone (188). 85% yield (starting from 30 mg of **126c**); MS-ESI + (*m/z*, %) 521 (M^+ + Na, 100); ^{1}NMR *δ* 8.15 (1H, d, *J* = 8.8 Hz, H-5), 8.10-8.13 (1H, m, benzoyl-3',4'), 7.87-7.90, 7.46-7.61, 7.32-7.46 (each 3H, m, bezoyl-3',4'), 7.01 (1H, d, *J* = 4.8 Hz, H-4'), 6.99 (1H, d, *J* = 8.8 Hz, H-6), 6.08 (1H, s, H-3), 5.66 (1H, d, *J* = 4.8 Hz, H-3'), 2.34 (2H, q, *J* = 8.0 Hz, CH_2CH_3-2), 1.69, 1.54 (each 3H, s, CH_3-2'), 1.02 (3H, t, *J* = 8.0 Hz, CH_2CH_3-2); $[\alpha]_D$ -82.9 ^{o} (*c* = 0.0035, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 499.1757, found: 499.1735.

3'*R*,4'*R*-Di-(2-trifluoromethylbenzoyloxy)-2',2'-dimethyl-2-ethyldihydro pyrano[2,3-*f*]chromone (189). 88% yield (starting from 30 mg of **126c**); MS-ESI + (*m/z*, %) 634 (M^+, 100); ^{1}NMR *δ* 7.95 (1H, d, *J* = 8.8 Hz, H-5), 7.50-7.80 (8H, m, benzoyl-3',4'), 6.65 (1H, d, *J* = 8.8 Hz, H-6), 6.30 (1H, s, H-3), 5.85 (1H, d, *J* = 4.8 Hz, H-4'), 5.50 (1H, d, *J* = 4.8 Hz, H-3'), 2.34 (2H, q, *J* = 8.0 Hz, CH_2CH_3-2), 1.69, 1.54 (each 3H, s, CH_3-2'), 1.02 (3H, t, *J* = 8.0 Hz, CH_2CH_3-2); $[\alpha]_D$ -34.7 º (*c* = 0.0019, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 635.1504, found: 635.1493.

3'*R*,4'*R*-Di-(2-methoxybenzoyloxy)-2',2'-dimethyl-2-ethyldihydropyrano [2,3-*f*]chromone (190). 80% yield (starting from 30 mg of **126c**); MS-ESI + (*m/z*, %)

559 (M^+ + 1, 100); ^{1}NMR δ 8.18, 7.81, 7.63, 7.07 (each 1H, d, J = 7.6 Hz, methoxybenzoyl-3',4'), 8.11 (1H, d, J = 8.8 Hz, H-5), 7.44, 7,42, 6.93, 6.90 (each 1H, t, J = 7.6 Hz, methoxybenzoyl-3',4'), 7.00 (1H, d, J = 4.8 Hz, H-4'), 6.94 (1H, d, J = 9.0, H-5), 6.09 (1H, s, H-3), 5.62 (1H, d, *J* = 4.8 Hz, H-3'), 3.77, 3.70 (each 3H, s, methoxybenzoyl-OCH_3-3',4'), 2.48 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.63, 1.56 (each 3H, s, CH_3-2'), 1.14 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2); $[\alpha]_D$ -40.0 º (*c* = 0.0007, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 559.1963, found: 559.1937.

3'*R*,4'*R*-Di-(3-trifluoromethylbenzoyloxy)-2',2'-dimethyl-2-ethyldihydro pyrano[2,3-*f*]chromone (191). 80% yield (starting from 100 mg of **126c**); MS-ESI + (*m/z*, %) 635 (M^+ + 1, 100); ^{1}NMR δ 8.15 (1H, d, *J* = 8.8 Hz, H-5), 8.02-8.07 (4H, m, benzoyl-3',4'), 7.77-7.82 (2H, m, benzoyl-3',4'), 7.49-7.55 (2H, m, benzoyl-3',4'), 6.99 (1H, d, *J* = 4.2 Hz, H-4'), 6.98 (1H, d, *J* = 8.8 Hz, H-6), 6.06 (1H, s, H-3), 5.67 (1H, d, *J* = 4.2 Hz, H-3'), 2.33 (2H, q, *J* = 7.6 Hz, CH_2CH_3-2), 1.68, 1.54 (each 3H, s, CH_3-2'), 1.02 (3H, t, *J* = 7.6 Hz, CH_2CH_3-2); $[\alpha]_D$ -74.6 º (*c* = 0.0013, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 635.1504, found: 635.1514.

3'*R*,4'*R*-Di-(3-methoxybenzoyloxy)-2',2'-dimethyl-2-ethyldihydropyrano [2,3-*f*]chromone (192). 80% yield (starting from 30 mg of **126c**); MS-ESI + (*m/z*, %) 559 (M^+ + 1, 100); ^{1}NMR δ 8.09 (1H, d, *J* = 8.4 Hz, H-5), 7.71, 7.68 (each 1H, d, *J* = 7.6 Hz, benzoyl-3',4'), 7.61 (2H, s, benzoyl-3',4'), 7.38 (2H, t, *J* = 8.4, 7.6 Hz, benzoyl-3',4'), 7.15 (2H, *J* = 8.4 Hz, benzoyl-3',4'), 6.93 (1H, d, *J* = 8.4 Hz, H-6), 6.17 (1H, s, H-3), 5.53 (1H, d, *J* = 4.8 Hz, H-4'), 5.41 (1H, d, *J* = 4.8 Hz, H-3'), 3.87, 3.85 (each 3H, s, 3-methoxybenzoyl-OCH_3-3',4'), 2.65 (2H, q, *J* = 7.6 Hz, CH_2CH_3-

2), 1.61, 1.49 (each 3H, s, CH_3-2'), 1.30 (3H, t, *J* = 7.6 Hz, CH_2CH_3-2); $[\alpha]_D$ -116.3 º (*c* = 0.0008, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 559.1968, found: 559.1986.

3'*R*,4'*R*-Di-(3-cyanobenzoyloxy)-2',2'-dimethyl-2-ethyl-dihydropyrano[2,3-*f*]chromone (193). 65% yield (starting from 100 mg of **126c**); MS-ESI + (*m/z,* %) 549 (M^+ + 1, 100); ^{1}NMR *δ* 8.19 (1H, d, *J* = 9.0 Hz, H-5), 8.11 (4H, m, benzoyl-3',4'), 7.86, 7.58 (each 2H, m, benzoyl-3',4'), 7.02 (1H, d, *J* = 9.0 Hz, H-6), 7.00 (1H, d, *J* = 5.4 Hz, H-4'), 6.16 (1H, s, H-3), 5.66 (1H, d, *J* = 5.4 Hz, H-3'), 2.36 (2H, q, *J* = 7.8 Hz, CH_2CH_3-2), 1.69, 1.57 (each 3H, s, CH_3-2'), 1.03 (3H, t, *J* = 7.8 Hz, CH_2CH_3-2); $[\alpha]_D$ -125.0 º (*c* = 0.0004, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 549.1662, found: 549.1666.

3'*R*,4'*R*-Di-(4-methylbenzoyloxy)-2',2'-dimethyl-2-ethyldihydropyrano[2,3-*f*]chromone (194). 80% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 527 (M^+ + 1, 100); ^{1}NMR *δ* 8.13 (1H, d, *J* = 9.0 Hz, H-5), 7.78, 7.78 (each 2H, d, *J* = 8.1 Hz, $COOC(CHCH)_2CCH_3$-3',4'), 7.17, 7.17 (each 2H, d, J = 8.1 Hz, $COOC(CHCH)_2CCH_3$-3',4'), 6.97 (1H, d, *J* = 9.0 Hz, H-6), 6.96 (1H, d, *J* = 4.8 Hz, H-4'), 6.07 (1H, s, H-3), 5.62 (1H, d, *J* = 4.8 Hz, H-3'), 2.39, 2.38 (each 3H, s, methylbenzoyl-CH_3-3',4'), 2.34 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.67, 1.53 (each 3H, s, CH_3-2'), 1.02 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2); $[\alpha]_D$ -81.3 º (*c* = 0.0015, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 527.2070, found: 527.2063.

3'*R*,4'*R*-Di-(4-ethylbenzoyloxy)-2',2'-dimethyl-2-ethyldihydropyrano[2,3-*f*]chromone (195). 70% yield (starting from 65 mg of **126c**); MS-ESI + (*m/z*, %) 555 (M^+ + 1, 100); ^{1}NMR *δ* 8.13 (1H, d, *J* = 9.0 Hz, H-5), 7.80, 7.80 (each 2H, d, *J* = 7.2

Hz, COOC(*CH*CH)$_2$CCH$_2$CH$_3$-3',4'), 7.20, 7.17 (2H, d, *J* = 7.2 Hz, COOC(CH*CH*)$_2$CCH$_2$CH$_3$-3',4'), 6.98 (1H, d, *J* = 9.0 Hz, H-6), 6.97 (1H, d, *J* = 4.8 Hz, H-4'), 6.07 (1H, s, H-3), 5.63 (1H, d, *J* = 4.8 Hz, H-3'), 2.69, 2.68 (each 2H, q, *J* = 7.5 Hz, ethylbenzoyl-*CH$_2$*CH$_3$-3',4'), 2.32 (2H, q, *J* = 7.5 Hz, *CH$_2$*CH$_3$-2), 1.68, 1.53 (each 3H, s, H-2'), 1.24(3H, t, *J* = 7.5 Hz, CH$_2$*CH$_3$*-2), 1.23, 1.03 (each 3H, t, *J* = 7.2 Hz ethylbenzoyl-CH$_2$*CH$_3$*-3',4'); $[\alpha]_D$ -93.6 º (*c* = 0.01, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 555.2383, found: 555.2366.

3'*R*,4'*R*-Di-(4-trifluoromethylbenzoyloxy)-2',2'-dimethyl-2-ethyl-dihydro pyrano[2,3-*f*]chromone (196). 60% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 635 (M^+ + 1, 100); ^{1}NMR *δ* 8.22 (2H, d, *J* = 8.1 Hz, trifluoromethylbenzoyl-H), 8.16 (1H, d, *J* = 8.7 Hz, H-5), 8.99 (2H, d, *J* = 8.4 Hz, trifluoromethylbenzoyl-H), 7.74, (2H, d, *J* = 8.1 Hz, trifluoromethylbenzoyl-C(*CH*CH)$_2$CCF$_3$-3',4'), 7.65 (2H, d, *J* = 8.4 Hz, trifuoromethylbenzoyl- C(CH*CH*)$_2$CCF$_3$-3',4'), 7.00 (1H, d, *J* = 8.7 Hz, H-6), 7.00 (1H, d, *J* = 4.8 Hz, H-4'), 6.12 (1H, s, H-3), 5.67 (1H, d, *J* = 4.8 Hz, H-3'), 2.33 (2H, q, *J* = 7.8 Hz, *CH$_2$*CH$_3$-2), 1.68, 1.56 (each 3H, s, CH$_3$-2'), 1.02 (3H, t, *J* = 7.8 Hz, CH$_2$*CH$_3$*-2); $[\alpha]_D$ -74.0 º (*c* = 0.0025, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 635.1504, found: 635.1488.

3'*R*,4'*R*-Di-(4-methoxybenzoyloxy)-2',2'-dimethyl-2-ethyl-dihydropyrano [2,3-*f*]chromone (197). 80% yield (starting from 100 mg of **126c**); MS-ESI + (*m/z*, %) 559 (M^+ + 1, 100); ^{1}NMR *δ* 8.13 (1H, d, *J* = 8.7 Hz, H-5), 7.85, 7.85 (each 2H, d, *J* = 8.7 Hz, COOC(*CH*CH)$_2$COCH$_3$-3',4'), 6.97 (1H, d, *J* = 8.7 Hz, H-6), 6.95 (1H, d, *J* = 4.8 Hz, H-4'), 6.85, 6.82 (each 2H, d, *J* = 8.7 Hz, COOC(CH*CH*)$_2$COCH$_3$-3',4'), 6.06 (1H, s, H-3), 5.61 (1H, d, *J* = 4.8 Hz, H-3'), 3.85, 3.84 (each 3H, s,

methoxybenzoyl-OCH_3-3',4'), 2.33 (2H, q, *J* = 7.2 Hz, CH_2CH_3-2), 1.66, 1.53 (each 3H, s, CH_3-2'), 1.03 (3H, t, *J* = 7.2 Hz, CH_2CH_3-2); $[\alpha]_D$ -105.0 º (*c* = 0.0012, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 559.1968, found: 559.1954.

3'*R*,4'*R*-Di-(4-cyanobenzoyloxy)-2',2'-dimethyl-2-ethyl-dihydropyrano[2,3-*f*]chromone (198). 80% yield (starting from 100 mg of **126c**); MS-ESI + (*m/z*, %) 549 (M^+ + 1, 100); ^{1}NMR *δ* 8.17 (1H, d, *J* = 8.7 Hz, H-5), 7.98, 7.97 (each 2H, d, *J* = 8.7 Hz, benzoyl-COC(*CH*CH)$_2$CCN-3',4'), 7.71, 7.68 (each 2H, d, *J* = 8.7 Hz, benzoyl-COC(CH*CH*)$_2$CCN-3',4'), 6.99 (1H, d, *J* = 8.7 Hz, H-6), 6.98 (1H, d, *J* = 5.1 Hz, H-4'), 6.08 (1H, s, H-3), 5.66 (1H, d, *J* = 5.1 Hz, H-3'), 2.34 (2H, t, *J* = 7.8 Hz, CH_2CH_3-2), 1.67, 1.56 (each 3H, s, CH_3-2'), 1.01 (3H, t, *J* = 7.8 Hz, CH_2CH_3-2); $[\alpha]_D$ -104.8 º (*c* = 0.0014, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 549.1662, found: 549.1648.

3'*R*,4'*R*-Di-(4-nitrobenzoyloxy)-2',2'-dimethyl-2-ethyl-dihydropyrano[2,3-*f*]chromone (199). 50% yield (starting from 50 mg of **126c**); MS-ESI + (*m/z*, %) 589 (M^+ + 1, 100); ^{1}NMR *δ* 8.23, 8.22 (each 2H, d, *J* = 9.0 Hz, COOC(CH*C*H)$_2$$CNO_2$-3',4'), 8.14 (1H, d, *J* = 8.4 Hz, H-5), 8.06, 8.04 (each 2H, d, *J* = 9.0 Hz, COOC(*CHCH*)$_2$$CNO_2$-3',4'), 6.99 (1H, d, *J* = 8.4 Hz, H-6), 6.98 (1H, d, *J* = 4.8 Hz, H-4'), , 6.06 (1H, s, H-3), 5.67 (1H, d, *J* = 4.8 Hz, H-3'), 2.33 (2H, q, *J* = 7.2 Hz, CH_2CH_3-2), 1.68, 1.57 (each 3H, s, CH_3-2'), 1.00 (3H, t, *J* = 7.2 Hz, CH_2CH_3-2); $[\alpha]_D$ -74.7 º (*c* = 0.0015, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 589.1458, found: 589.1437.

3'*R*,4'*R*-Di-(2-phenylacetoxy)-2',2'-dimethyl-2-ethyl-dihydropyrano[2,3-*f*] chromone (200). 53% yield (starting from 30 mg of **126c**); MS-ESI + (*m/z*, %) 549, 527 (M^+ + Na, 60; M^+ + 1, 40); ^{1}NMR δ 8.07 (1H, d, *J* = 8.8 Hz, H-5), 7.30 (10H, m, phenyl-3',4'), 6.85 (1H, d, *J* = 8.8 Hz, H-6), 6.58 (1H, d, *J* = 4.8 Hz, H-4'), 6.05 (1H, s, H-3), 5.20 (1H, d, *J* = 4.8Hz, H-3'), 3.53, 3.46 (each 2H, d, *J* = 15.9 Hz, acetoxy-$COOCH_2$-3',4'), 2.25 (2H, q, *J* = 7.6 Hz, CH_2CH_3-2), 1.32, 1.25 (each 3H, s, CH_3-2'), 1.12 (3H, t, *J* = 7.6 Hz, CH_2CH_3-2); $[\alpha]_D$ -60.0 º (*c* = 0.0015, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 527.2070, found: 527.2060.

3'*R*,4'*R*-Di-[2-(4-methoxyphenylacetoxy)]-2',2'-dimethyl-2-ethyl-dihydro pyrano[2,3-*f*]chromone (201). 50% yield (starting from 25 mg of **126c**); MS-ESI - (*m/z*, %) 585 (M^- - 1, 100); ^{1}NMR δ 8.07 (1H, d, *J* = 9.0 Hz, H-5), 7.20, 7.17 (each 2H, d, *J* = 8.7 Hz, phenyl-C(CHCH)$_2$$COCH_3$-3',4'), 6.87, 6.85 (each, 2H, d, *J* = 8.7 Hz, phenyl-C(CHCH)$_2$$COCH_3$-3',4'), 6.78 (1H, d J = 9.0 Hz, H-6), 6.44 (1H, d, *J* = 4.8 Hz, H-4'), 6.10 (1H, s, H-3), 4.03 (1H, d, *J* = 4.8 Hz, H-3'), 2.25 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.49, 1.34 (each 3H, s, CH_3-2'), 1.13 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2); $[\alpha]_D$ -47.7 º (*c* = 0.0013, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 587.2281, found: 587.2266.

3'*R*,4'*R*-Di-(3-phenylpropanoyloxy)-2',2'-dimethyl-2-ethyl–dihydropyrano [2,3-*f*]chromone (202). 70% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 555 (M^+ + 1, 100); ^{1}NMR δ 8.09 (1H, d, *J* = 9.0 Hz, H-5), 7.22 (10H, m, phenyl-C(CHCH)$_2$CH-3',4'), 6.88 (1H, d, J = 9.0 Hz, H-6), 6.59 (1H, d, *J* = 4.8 Hz, H-4'), 6.09 (1H, s, H-3), 5.26 (1H, d, *J* = 4.8 Hz, H-3'), 2.95, 2.95 (4H, t, *J* = 7.6 Hz, propanoyl-CH_2CH_2-3'.4'), 2.67, 2.58(each 2H, t, *J* = 7.6 Hz, propanoyl-CH_2CH_2-3',4'), 2.46 (2H,

q, *J* = 7.5 Hz, *CH*$_2$CH$_3$-2), 1.43, 1.33 (each 3H, s, CH$_3$-2'), 1.67 (3H, t, *J* = 7.5 Hz, CH$_2$*CH*$_3$-2); [α]$_D$ -74.8 º (*c* = 0.0023, CHCl$_3$); HRMS for (M$^+$ + H): calcd m/z 555.2393, found: 555.2378.

3'*R*,4'*R*-Di-(*E*)-phenylacryloyloxy-2',2'-dimethyl-2-ethyl-dihydropyrano [2,3-*f*]chromone (203). 65% yield (starting from 50 mg of **126c**); MS-ESI + (*m/z*, %) 551 (M$^+$ + 1, 100); ^{1}NMR *δ* 8.05 (1H, d, *J* = 9.0 Hz, H-5), 7.63, 7.63 (each 1H, d, *J* = 15.9 Hz COO*CH*CH-3',4'), 7.36-1.40 (5H, m, phenyl-3',4'), 1.23-1.29 (5H, m, phenyl-3',4'), 6.88 (1H, d, *J* = 9.0 Hz, H-6), 6.75 (1H, d, *J* = 4.8 Hz, H-4'), 6.38 (2H, d, *J* = 15.9 Hz, COOCH*CH*-3'.4'), 6.02 (1H, s, H-3), 5.42 (1H, d, *J* = 4.8 Hz, H-3'), 2.42 (2H, q, *J* = 6.9 Hz, *CH*$_2$CH$_3$-2), 1.52, 1.43 (each 3H, s, CH$_3$-2'), 1.17 (3H, t, *J* = 6.9 Hz, CH$_2$*CH*$_3$-2); [α]$_D$ -75.3 º (*c* = 0.0017, CHCl$_3$); HRMS for (M$^+$ + H): calcd m/z 551.2070, found: 551.2074.

3'*R*,4'*R*-Di-(*E*)-4-methoxyphenylacryloyloxy-2',2'-dimethyl-2-ethyl-dihydro pyrano[2,3*f*]chromone (204). 50% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 611 (M$^+$ + 1, 100); ^{1}NMR *δ* 8.10 (1H, d, *J* = 9.0 Hz, H-5), 7.66, 7.65 (each 1H, d, *J* = 15.3 Hz, (*E*)-acryloxy-OCOCH*CH*-3',4'), 7.40 (4H, dd, *J* = 8.7, 3.0 Hz, 4-methoxyphenyl-C(*CH*CH)$_2$COCH$_3$-3',4'), 6.93 (1H, d, *J* = 9.0 Hz, H-6), 6.85 (4H, dd, *J* = 8.7, 3.0 Hz, 4-methoxyphenyl-C(CH*CH*)$_2$COCH$_3$-3',4'), 6.80 (1H, d, J = 4.8 Hz, H-4'), 6.33, 6.32 (each 1H, d, *J* = 15.3 Hz, (*E*)-acryloxy-OCO*CH*CH-3',4'), 6.10 (1H, s, H-3), 5.47 (1H, d, *J* = 4.8 Hz, H-3'), 6.65 (6H, s, OCH$_3$-3',4'), 2.47 (2H, q, *J* = 7.5 Hz, CH$_2$CH$_3$-2), 1.58, 1.49 (each 3H, s, CH$_3$-2'), 1.59 (3H, t, *J* = 7.5 Hz, CH$_2$*CH*$_3$-2); [α]$_D$ -70.0 º (*c* = 0.0043, CHCl$_3$); HRMS for (M$^+$ + H): calcd m/z 611.2281, found: 611.2260.

3'*R*,4'*R*-Di-(cyclohexanecarbonyloxy)-2',2'-dimethyl-2-ethyl – dihydropyrano [2,3-*f*]chromone (206). 20 % yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 511 (M^+ + 1, 100); ^{1}NMR δ 8.08 (1H, d, *J* = 9.0 Hz, H-5), 6.88 (1H, d, *J* = 9.0 Hz, H-6), 6.61 (1H, d, *J* = 4.5 Hz, H-4'), 6.12 (1H, s, H-3), 5.27 (1H, d, *J* = 4.5 Hz, H-3'), 2.56 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 2.35 (2H, m, cyclohexanecarbonyl-3',4'), 2.80 (12H, m, cyclohexanecarbonyl-3',4'), 1.49, 1.42 (each 3H, s, CH_3-2'), 1.35 (8H, m, cyclohexanecarbonyl-3',4'), 1.23 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2); $[\alpha]_D$ -15.4 º (*c* = 0.005, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 511.2696, found: 511.2713.

Synthesis of 3'*R*,4'*R*-Dibenzyloxy-2',2'-dimethyl-2-ethyl-dihydropyrano [2,3-*f*]chromone (205). NaH (20.4 mg, 0.51 mmoL) was suspended in dry DMF. To this suspension were added at 0 ^oC a solution of compound **126c** (50 mg, 0.17 mmoL) in dry DMF (0.5 mL), and benzyl bromide (0.05 mL, 0.43 mmoL), under the protection of N_2. After 1h, the mixture was poured into 5% aqueous $NaHCO_3$ solution and extracted with Et_2O for 3 times. The organic layers were combined and dried over Na_2SO_4. The crude product was purified utilizing preparing TLC with hexane : EtOAc = 1 : 1 to afford **205** (16.2 mg). 57.3 % yield; MS-ESI + (*m/z*, %) 493 (M^+ + Na, 100); ^{1}NMR δ 8.00 (1H, d, *J* = 8.7 Hz, H-5), 7.41, 7.28 (each 5H, m, benzyl-3',4'), 6.81(1H, d, *J* = 8.7 Hz, H-6), 6.13 (1H, s, H-3), 5.10, 4.95, 4.85, 4.75 (each 1H, d, *J* = 11.4 Hz, benzyl-CH_2-3',4')5.09 (1H, d, *J* = 4.2 Hz, H-4'), 3.75 (1H, d, *J* = 4.2 Hz, H-3'), 2.60 (2H, q, *J* = 7.5 Hz, CH_2CH_3-2), 1.57, 1.50 (each 3H, s, CH_3-2'), 1.28 (3H, t, *J* = 7.5 Hz, CH_2CH_3-2); $[\alpha]_D$ -40.9 º (c = 0.01, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 471.2171, found: 471.2155.

Synthesis of compounds 207-211. NaH (5 equiv) was added to a solution of compound **126c** in dry THF at ice-bath condition and diverse carbamyl chlorides (3 equiv) were added. Stir at room temperature and monitored by TLC. At completion, the reaction mixture was poured carefully onto ethyl acetate and saturated aqueous $NaHCO_3$. The organic layer was washed with water for 3 times, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The crude product was purifed utilizing prepare TLC.

3'*R*,4'*R*-Di(dimethylcarbamoyloxy)-2',2'-dimethyl-2-ethyl–dihydropyrano [2,3-*f*]chromone (207). 30% yield (starting from 100 mg of **126c**); MS-ESI + (*m/z*, %) 433 (M^+ + 1, 100); ^{1}NMR δ 8.07 (1H, d, *J* = 8.8 Hz, H-5), 6.88 (1H, d, *J* = 8.8 Hz, H-6), 6.52 (1H, d, *J* = 4.8 Hz, H-4'), 6.11 (1H, s, H-3), 5.22 (1H, d, *J* = 4.8 Hz, H-3'), 2.97, 2.85 (each 6H, m, dimethylcarbamoyl-CH_2-3',4'), 2.55 (2H, q, *J* = 7.2 Hz, *CH_2*CH_3-2), 1.49, 1.46 (each 3H, s, CH_3-2'), 1.24 (3H, t, *J* = 7.2 Hz, CH_2*CH_3*-2); $[\alpha]_D$ -28.6 º (*c* = 0.01, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 433.1957, found: 433.195.

3'*R*,4'*R*-Di(diethylcarbamoyloxy)-2',2'-dimethyl-2-ethyl–dihydropyrano [2,3-*f*]chromone (208). 25% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 489 (M^+ + 1, 100); ^{1}NMR δ 8.07 (1H, d, *J* = 8.8 Hz, H-5), 6.88 (1H, d, *J* = 8.8 Hz, H-6), 6.59 (1H, d, *J* = 4.8 Hz, H-4'), 6.11 (1H, s, H-3), 5.25 (1H, d, *J* = 4.8 Hz, H-3'), 3.35, 3.35, 3.18, 3.18 (each 2H, q, *J* = 6.8 Hz, diethylcarbamoyl-CH_2-3',4'), 2.55 (2H, q, *J* = 7.6 Hz, *CH_2*CH_3-2), 1.49, 1.47 (each 3H, s, CH_3-2'), 1.23 (3H, t, *J* = 7.6 Hz, CH_2*CH_3*-2), 1.16 (6H, t, *J* = 6.8 Hz, diethylcarbamoyl-CH_3-4'), 1.09, 0.98 (each 3H, t, *J* = 6.8 Hz, diethylcarbamoyl-CH_3-3'); $[\alpha]_D$ -30.0 º (*c* = 0.001, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 489.2601, found: 489.2594.

3'*R*,4'*R*-Di(dibutylcarbamoyloxy)-2',2'-dimethyl-2-ethyl–dihydropyrano [2,3-*f*]chromone (209). 30% yield (starting from 50 mg of **126c**); MS-ESI + (*m/z*, %) 601 (M^+ + 1, 100); ^{1}NMR δ 8.06 (1H, d, *J* = 8.8 Hz, H-5), 6.86 (1H, d, *J* = 8.8 Hz, H-6), 6.58 (1H, d, J = 4.8 Hz, H-4'), 6.11 (1H, s, H-3), 5.23 (1H, d, *J* = 4.8 Hz, H-3'), 3.25 (8H, m, dibutylcarbamoyl-3',4'), 2.56 (2H, q, *J* = 7.6 Hz, CH_2CH_3-2), 1.57 (6H, m, dibutylcarbamoyl-CH_2), 1.47, 1.46 (each 3H, s, CH_3-2'), 1.33 (8H, m, dibutylcarbamoyl-CH_2), 1.25 (3H, t, *J* = 7.6 Hz, CH_2CH_3-2), 1.08 (2H, q, *J* = 7.2 Hz, dibutylcarbamoyl-CH_2-3'), 0.95, 0.94 (each 3H, t, *J* = 7.6 Hz, dibutylcarbamoyl-CH_3-4'), 0.86, 0.72 (each 3H, t, *J* = 7.2 Hz, dibutylcarbamoyl-CH_3-3'); $[\alpha]_D$ -13.5 º (*c* = 0.012, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 601.3853, found: 601.3833.

3'*R*,4'*R*-Di(pyrrolidine-1-carbonyloxy)-2',2'-dimethyl-2-ethyl–dihydro pyrano[2,3-*f*]chromone (210). 35% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 485 (M^+ + 1, 100); ^{1}NMR δ 8.05 (1H, d, *J* = 8.8 Hz, H-5), 6.87 (1H, d, *J* = 8.8 Hz, H-6), 6.54 (1H, d, *J* = 4.8 Hz, H-4'), 6.11 (1H, s, H-3), 5.23 (1H, d, *J* = 4.8 Hz, H-3'), 3.48, 3.26 (each 4H, m, pyrrolidine-N$(CH_2)_2$$(CH_2)_2$-3',4'), 2.55 (2H, q, *J* = 7.6 Hz, CH_2CH_3-2), 1.86 (8H, m, pyrrolidine-N$(CH_2)_2$$(CH_2)_2$-3',4'), 1.50, 1.47 (each 3H, s, CH_3-2'), 1.21 (3H, t, *J* = 7.6 Hz, CH_2CH_3-2); $[\alpha]_D$ -23.8 º (*c* = 0.011, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 485.2288, found: 485.2277.

3'*R*,4'*R*-Di-(piperidine-1-carbonyloxy)-2',2'-dimethyl-2-ethyl–dihydro pyrano[2,3-*f*]chromone (211). 32% yield (starting from 25 mg of **126c**); MS-ESI + (*m/z*, %) 513 (M^+ + 1, 100); ^{1}NMR δ 8.06 (1H, d, *J* = 8.8 Hz, H-5), 6.87 (1H, d, *J* = 8.8 Hz, H-6), 6.56 (1H, d, *J* = 4.8 Hz, H-4'), 6.11 (1H, s, H-3), 5.24 (1H, d, *J* = 4.8 Hz, H-3'), 3.77 (8H, m, piperidine-N$(CH_2)_2$$(CH_2)_2CH_2$-3',4'), 2.57 (2H, q, *J* = 7.6 Hz,

*CH_2*CH_3-2), 1.58 (12H, m, piperidine-N$(CH_2)_2$*$(CH_2)_2CH_2$*-3',4'), 1.47, 1.46 (each 3H, s, CH_3-2'), 1.26 (3H, t, *J* = 7.6 Hz, CH_2*CH_3*-2); $[\alpha]_D$ -28.2 º (*c* = 0.0017, $CHCl_3$); HRMS for (M^+ + H): calcd m/z 513.2601, found: 513.2620.

6.6.2 Chemosensitization (MDR Modulation) Assay:

Three thousand drug-resistant cells, KB-vin cells (MDR-1) selected using VCR[12] were treated for 2 or 3 days with test agent in the presence or absence of VCR . The end-point is cellular protein measured using the Sulforhodamine B (SRB) method developed at NIH-NCI for *in vitro* anticancer drug screening [13]. The degree of chemoreversal was determined by fixing the concentration of test agent and measuring the alteration in dose-response of cells to VCR toxicity. Verapamil was used as chemosensitizer control and data from replicate experiments was plotted and the apparent GI_{50} value were determined using statistical software (GraphPad Prizm, CA).

6.6.3 Calcein-AM Loading Assay:

10,000 KB-Vin (MDR) cells per well were seeded into 96-well plates with medium containing 5% FBS and incubated for 1 day for adhesion and equilibration. Treatment involved supplementing cultures with distinct concentrations of test compounds for 30 minutes at 37^{o}C. After removing medium, fresh medium with both 1 μM Calcein-AM (Alexis Biochemicals) and test compounds as needed. Following incubation for 30 minutes at 37 medium was removed and wells washed gently and quickly with cold PBS buffer in low ambient light condition.. Cell were lysed using 20 mM Tris buffer and fluorescence measured using a BioTek TLx800 ELISA reader

set to Ex. 494 nm and Em. 517 nm. Composite data obtained from two or more independent experiments were analyzed using Prizm™ (Graphpad Software, San Diego, CA)[11]

6.7 References:

1. Dalton, W. S., Is p-glycoprotein a potential target for reversing clinical drug resistance? *Curr Opin Oncol* **1994,** 6, (6), 595-600.

2. Hindenburg, A. A.; Baker, M. A.; Gleyzer, E.; Stewart, V. J.; Case, N.; Taub, R. N., Effect of verapamil and other agents on the distribution of anthracyclines and on reversal of drug resistance. *Cancer Res* **1987,** 47, (5), 1421-1425.

3. Thomas, H.; Coley, H., Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibitinf P-glycoprotein. *Cancer Control* **2003,** 10, 159-165.

4. Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M., Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* **2006,** 5, (3), 219-234.

5. Wu, J. Y.; Fong, W. F.; Zhang, J. X.; Leung, C. H.; Kwong, H. L.; Yang, M. S.; Li, D.; Cheung, H. Y., Reversal of multidrug resistance in cancer cells by pyranocoumarins isolated from Radix peucedani. *Eur J Pharmacol,* **2003,** 473, (1), 9-17.

6. Fong, W. F.; Shen, X. L.; Globisch, C.; Wiese, M.; Chen, G. Y.; Zhu, G. Y.; Yu, Z. L.; Tse, A. K.; Hu, Y. J., Methoxylation of 3',4'-aromatic side chains improves P-glycoprotein inhibitory and multidrug resistance reversal activities of 7,8-pyranocoumarin against cancer cells. *Bioorg. Med. Chem.* **2008,** 16, (7), 3694-3703.

7. Pajeva, I. K.; Wiese, M., Pharmacophore Model of Drugs Involved in P-glycoprotein Multidrug Resistace: Explanation of Structural Variety (Hypothesis). *J. Med. Chem.* **2002,** 45, 5671-5686.

8. Baba, T.; Huang, G.; Minoru, I., Synthesis of the JKLM-ring Fragment of Ciguatoxin. *Tetrahedron* **2003,** 59, 6851-6872.

9. Nguyen, T. M.; Sittisombut, C.; Boutefnouchet, S.; Lallemand, M.-C.; Michel, S.; Koch, M.; Tillequin, F.; Mizinghien, R.; Lansiaux, A.; David-Cordonnier, M.-H.; Pfeiffer, B.; Kraus-Berthier, L.; Leonce, S.; Pierre, A., Synthesis, Antitumor Activity, and Mechanism of Action of Benzo[*a*]pyrano[3,2-*h*]acridin-7-one Analogues of Acronycine. *J. Med. Chem.* **2006,** 49, 3383-3394.

10. Gouaze, V.; Yu, J. Y.; Bleicher, R. J.; Han, T. Y.; Liu, Y. Y.; Wang, H.; Gottesman, M. M.; Bitterman, A.; Giuliano, A. E.; Cabot, M. C., Overexpression of glucosylceramide synthase and P-glycoprotein in cancer cells selected for resistance to natural product chemotherapy. *Mol Cancer Ther* **2004,** 3, (5), 633-639.

11. http://www.biosciencetechnology.com/ShowPR.aspx?PUBCODE=090&ACCT=9000009885&ISSUE=0404&RELTYPE=PR&Cat=SubCat=1&PRODCODE=00005949&PRODLETT=A&CALLFROM=PR&CommonCount=0.

12. Lunney, E. A.; Hagen, S. E.; Domagala, J. M.; Humblet, C.; Kosinski, J.; Tait, B. D.; Warmus, J. S.; Wilson, M.; Ferguson, D.; Hupe, D.; et al., A novel nonpeptide HIV-1 protease inhibitor: elucidation of the binding mode and its application in the design of related analogs. *J. Med. Chem.* **1994,** 37, (17), 2664-2677.

13. Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R., Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer. Inst.* **1990,** 82, (13), 1113-1118.

CHAPTER 7

SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

7.1 General Conclusions

In this research work, we first optimized the synthetic methodology for the 2,2-dimethyl-2*H*-chromone motif that provides key intermediates to synthesize DCK and DCP analogs. The resulting microwave-assisted method dramatically shortened the reaction time and also increased the yields of the desired angular 2.2-dimethyl-2*H*-chromone products compared with linear side products.

Using the optimized synthetic method, we next designed and synthesized 23 novel DCP analogs to further improve anti-HIV activity against both wild-type and drug-resistant HIV strains and to improve water-solubility. A more comprehensive SAR was generated for DCP analogs, and two compounds with increased activity against both virus strains were identified with improved water-solubility. A reliable QSAR model was established based on novel DCP analogs, and pharmacophore analysis identified potential pharmacophores in DCP that opened a new avenue to design new analogs.

Next, a new anti-HIV entity, 1,2-dicamphanoyl-pyranoxanthone (DCX), was developed, synthesized, and evaluated. Skeleton-modified DCX analogs maintained anti-HIV activity against both wild-type and drug-resistant HIV strains. A SAR study

of the substitutions on the planar xanthone ring system was conducted, and Three DCX analogs exhibited better anti-HIV activity against both virus strains than the control, 2-EDCP. Six DCXs presented enhanced TI than 2-EDCP.

Finally, 3',4'-di-substituted-2',2'-dimethyldihydropyranochromone (DSP) analogs were designed and synthesized as potent chemosensitizers against multi-drug resistant cancer cells. A comprehensive SAR study concerning the 3' and 4' side chains was conducted, and three DSP analogs were identified with improved chemosensitizing potential to reverse MDR in the KB-Vin human esophageal cancer cell line compared with verapamil, the first identified chemosensitizer. A preliminary mechanism of action study revealed that DSP analogs might function as P-gp inhibitors to reverse MDR.

7.1.1 Development of Efficient Microwave-assisted One-pot Reaction to Form Angular 2,2-Dimethyl-2*H*-chromone Containing Compounds

Conventional synthesis of angular 2,2-dimethyl-2*H*-chromones, which are key intermediates in the preparation of DCK and DCP analogs, was not efficient in reaction time or yield, partially due to the low selectivity of the desired angular **a**-product. (Figure 7-1) In this research work, we were able to develop the first microwave initiation method to synthesize these key intermediates. We also tested this method on a broad range of starting materials with diverse ring systems. Through alkylation and cyclization of an appropriate starting material with 4,4-dimethoxy-2-methyl-2-butanol as the alkylating reagent, the desired 2,2-dimethyl-2*H*-chromone-related products, 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromenes, 3,3-dimethyl-pyrano[2,3-*c*]xanthen-7(3*H*)-ones, or 3,3,12-trimethyl-3*H*-pyrano[2,3-

c]acidin-7(12*H*)-ones were obtained in a one-pot reaction. Compared with reported literature methods, the newly developed microwave synthesis conditions shortened the reaction time dramatically from 2 days to 4 hours with much higher to comparable yields. Increasing the reaction temperature from 140 to 220 °C and extending the reaction time favored the formation of both **a-** and **b-**products with relatively better selectivity of the desirable **a-**product. (Figure 7-1)

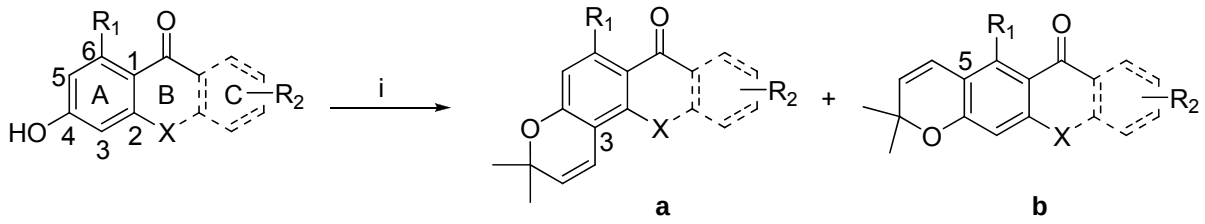


Figure 7-1. Synthesis of 2,2-dimethyl-2*H*-chromone containing compounds. Conditions and reagents: (i) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave 220^{o}C/4h.

The factors that might affect the yield and regioselectivity in this reaction were also analyzed:

1. Electronic effects on the A-ring significantly influenced the reaction yield as well as regioselectivity. Electron-donating groups on the A-ring could increase the electron density at the 3-position, which consequently enhanced alkylation reactivity. (Figure 7-1)

2. The lone-electron pair on the hydroxy group introduced at the R_1-position of xanthone resulted in higher electron density at the next open position and, therefore, reduced the regioselectivity between **a-** and **b**-products.

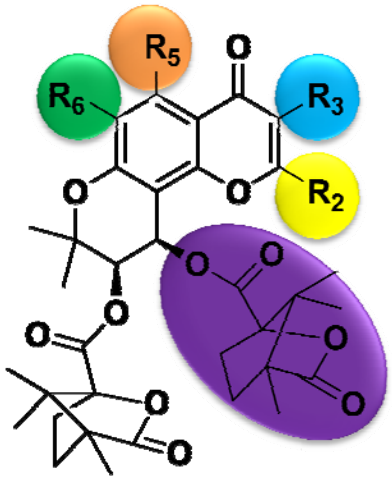
3. Steric effects of the substituents may also play a role in the alkylation and cyclization. Large groups on the 6-position blocked alkylation from occurring at

the 5-position, which led predominately to the desired angular product.

The significant advancement of this study is that the microwave method, with appropriately optimized conditions, can be widely applied to diverse ring systems, including phenoyl, chromone, and xanthone, as well as acridinone, which dramatically broadens the possibility of efficiently exploring DCK and DCP analogs as novel anti-HIV agents.

7.1.2 Design, Synthesis, Molecular Modeling, and SAR of Novel Dicamphanoyl-2',2'-dimethyldihydropyranochromone (DCP) Analogs as Potent Anti-HIV Agents

Our study designed, synthesized, and evaluated a series of new DCP analogs to explore the structural requirements of dihydropyranochromones for anti-HIV activity, especially against HIV/RTMDR1 drug-resistant virus, and to improve water solubility. Twenty-three novel DCPs were obtained, and compounds **98**, **102**, **107** and **115** exhibited comparable or even better anti-HIV activity than the reported best the lead, 2-EDCP (**69**), against HIV_{NL4-3} virus with EC_{50} values ranging from 0.036 – 0.14 μM. (EC_{50} of **69** is 0.07 μM). (Figure 7-2) Compounds **98** and **102** also presented two-fold higher potency than 2-EDCP against the drug-resistant HIV_{RTMDR1} strain with EC_{50} values of 0.049 and 0.054 μM, respectively. In addition, the water-solubility assay indicated that compounds **98** and **115** are 5-fold and 10-fold more water-soluble than 2-EDCP (**69**). The following SAR conclusions were drawn from this study:



98 $R_2 = R_5 = CH_3$, $R_3 = R_6 = H$
102 $R_2 = CH_2CH_3$, $R_5 = CH_3$, $R_3 = R_6 = H$
107 $R_2 = CN$, $R_3 = CH_3$, $R_5 = R_6 = H$
115 $R_2 = CH_2CH_3$, $R_3 = NH_2$, $R_5 = R_6 = H$

Figure 7-2. Structures of DCP analogs

(1) R_5 of the DCP chromone ring system is critical for anti-HIV activity against both wild-type and drug-resistant HIV-1 strains, and appropriate alkyl groups on this position can improve anti-HIV activity against both virus strains.

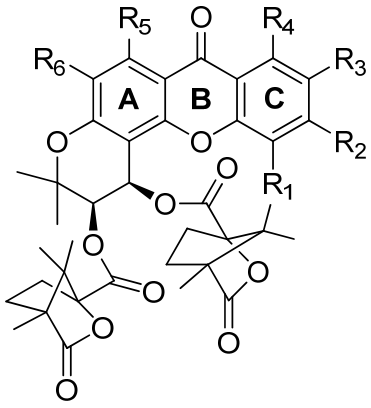
(2) Electronic and hydrogen-bonding effects at R_2 and R_3 can influence the anti-HIV activity as well as therapeutic index.

(3) The orientations of the 3′- and 4′-camphanoyl groups are critical to maintain high anti-HIV activity against both virus strains, and the carbonyl group in the 4′ position camphanoyl ester was identified as a potential hydrogen-bond acceptor by pharmacophore analysis.

In addition, we successfully established reliable PLS QSAR models. These models should help to predict the EC_{50} values of newly designed DCP analogs, which may be a useful tool for design of future new DCP analogs.

7.1.3 Design, Synthesis, and SAR of Dicamphanoyl-dihydropyrano-[2,3-c]xanthen-7(1*H*)-one (DCX) Derivatives as Novel Anti-HIV Agents

SAR studies of DCK and DCP analogs suggested that the planar ring system in DCK and DCP analogs might interact with the binding pocket through π-π interaction or DNA chelation. To investigate such interactions, a new chemical entity, 1,2-dicamphanoyl-pyranoxanthone (DCX), was designed, synthesized, and evaluated. Most of the synthesized DCX analogs were active against both wild-type and drug-resistant HIV strains. Compared to the control 2-EDCP (**69**), three compounds (**131**, **135**, and**144**) showed better activity against both HIV strains, and four additional compounds (**134**, **139**, **143**, and **148**) exhibited comparable anti-HIV potency to 2-EDCP. Six analogs showed enhanced TI values in comparison to the control.



131 $R_1 = R_2 = R_3 = R_4 = R_6 = H$, $R_5 = OCH_3$
135 $R_1 = R_2 = R_4 = R_6 = H$, $R_3 = CH_3$, $R_5 = OCH_3$
144 $R_1 = R_2 = R_3 = R_6 = H$, $R_4 = F$, $R_5 = OCH_3$

134 $R_1 = R_3 = R_4 = R_6 = H$, $R_2 = CH_3$, $R_5 = OCH_3$
139 $R_1 = R_2 = R_4 = R_6 = H$, $R_3 = R_5 = OCH_3$
143 $R_1 = R_2 = R_4 = R_6 = H$, $R_3 = F$, $R_5 = OCH_3$
148 $R_1 = R_3 = R_6 = H$, $R_4 = CH_3$, $R_2 = R_5 = OCH_3$

Figure 7-3. Structures of DCXs with high anti-HIV potency

The following SAR conclusions were drawn: (Figure 7-4)

1. The planar ring extension from pyranochromone (DCP) to pyrano-xanthone (DCX) resulted in maintained anti-HIV activity against both wild-type and drug-resistant strains, which confirmed our hypothesis that the conjugated planar ring is significant when interacting with the binding pocket.

2. Substitution at R_5 of the DCX xanthone ring is critical for anti-HIV activity against both viral strains. A methoxy group influenced the anti-HIV activity in a positive way, while a hydroxy group dramatically decreased the efficacy and selectivity.
3. The R_1-position could not tolerate large functional groups. Compounds with R_2 and R_3 substituents, especially R_3 with small alkyl or O-alkyl substitution, retained a high level of anti-HIV activity against both wild-type and drug-resistant strains. (compounds **134-136**, **138-140**, and **148**)
4. A bromine group on the xanthone ring was generally not favorable to anti-HIV activity and TI value; while a fluorine group was acceptable for sustained activity.
5. Polar substitutions on different positions resulted in different anti-HIV profiles. A hydroxy group at R_4 resulted in much better anti-HIV activity than the same group at R_5. A cyano group at R_2 led to maintained activity; however, an amino group at the same position abolished anti-HIV efficacy completely.

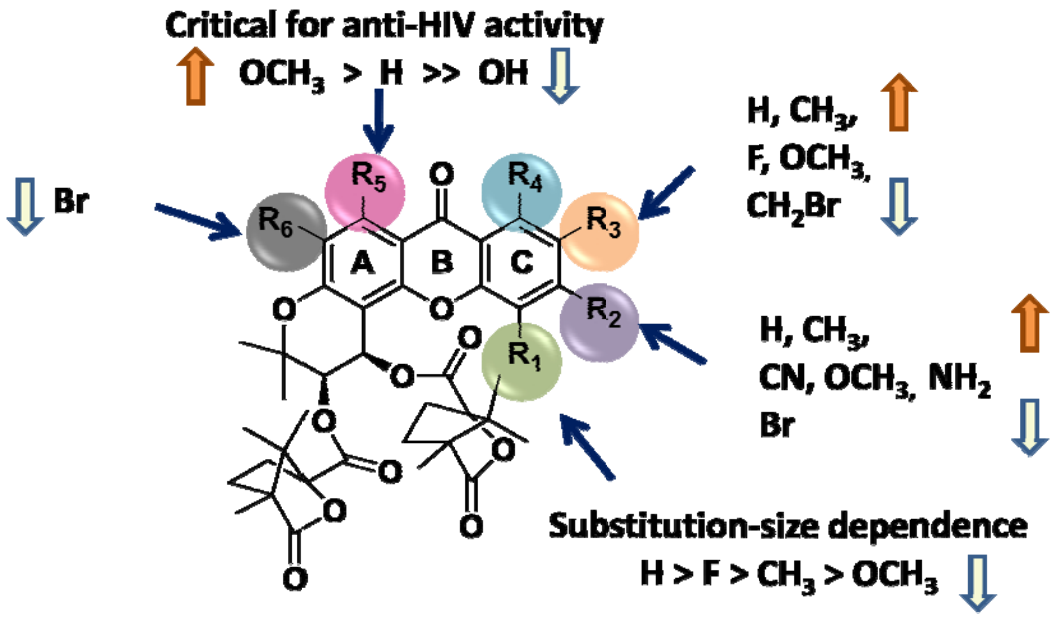


Figure 7-4. SAR model of DCX analogs

7.1.4 Design, Synthesis, and SAR Study of 3',4'-Disubstituted-2',2'-dimethyldihydropyranochromone (DSP) as Potent Chemosensitizers to Overcome MDR in Cancer Treatment

We designed and synthesized a series of DSP derivatives as a new class of chemosensitizers in the treatment of MDR cancer cells. Nine compounds presented comparable or better chemosensitizing activity in comparison with verapamil, the prototype first generation chemosensitizer. Compounds **194**, **197**, and **204** fully resolved the vincristine resistance in the KB-Vin human esophageal cancer cell line and recovered the cytotoxicity of vincristine to as low as 0.008 μg/mL. Surprisingly, compound **208** with carbamate moieties rather than aromatic side chains at 3' and 4' positions also exhibited considerable activity and could regenerate the cytotoxicity of vincristine to 0.021 μg/mL. (Figure 7-5)

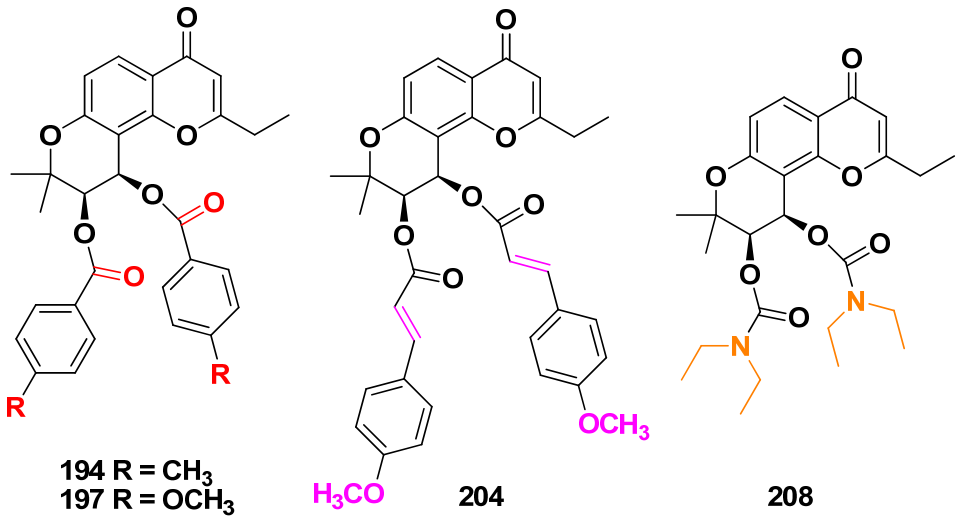


Figure 7-5. Structures of selected DSP derivatives

The SAR study of 3' and 4' side chains suggested the following conclusions:

1. Aromatic rings at the 3' and 4' positions are important but not necessary to maintain high chemosensitizing activity.

2. Substitutions on the aromatic rings have significant impacts on the activity. The moieties that can increase electron density, such as in compounds **194** and **197**, extend the conjugation system, or provide HB acceptors could benefit the activity.

3. The characteristics of the linker between the pyranochromone and the aromatic side chains influenced the chemosensitizing potential. Adding double bond(s), such as in compound **204**, benefited the activity.

Finally, we conducted a mechanism of action study related to DSP analogs, and the results indicated that the chemosensitizing efficacy of DSP analogs was

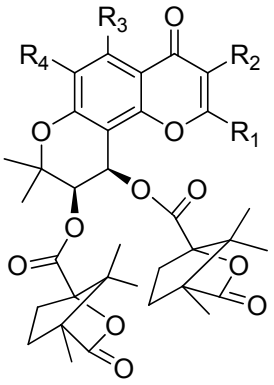
consistent with their relative inhibitory capability toward cellular P-gp. The result demonstrated that DSPs act as pump inhibitors in the KB-Vin cell system.

7.2 Future Directions

7.2.1 Further Design and Synthesis of Water-soluble DCP and DCX Analogs.

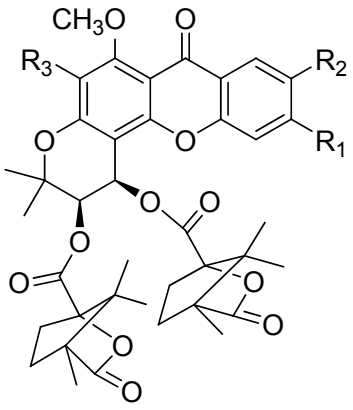
Previous studies to improve the anti-HIV activity and water-solubility of DCPs coupled with SAR study of DCXs suggested that introducing small polar groups on the pyranochromone ring in DCP or pyranoxanthone ring in DCX could result in preserved high anti-HIV activity, while also benefiting the pharmacological profile and consequently improving the water solubility. Therefore, in our future study directions, we have designed new series of novel DCP and DCX analogs to follow up on these results. Various small polar groups are substituted on the 2, 3, 5, or 6-position of newly designed DCPs (Figure 7-6), while the polar groups are incorporated at R_1 to R_3 in DCX analogs (Figure 7-7). We hope to identify new anti-HIV agents as clinical drug candidates in this future study.

The proposed synthetic pathways for the newly designed DCP analogs are shown in Schemes 7-1 to 7-3.



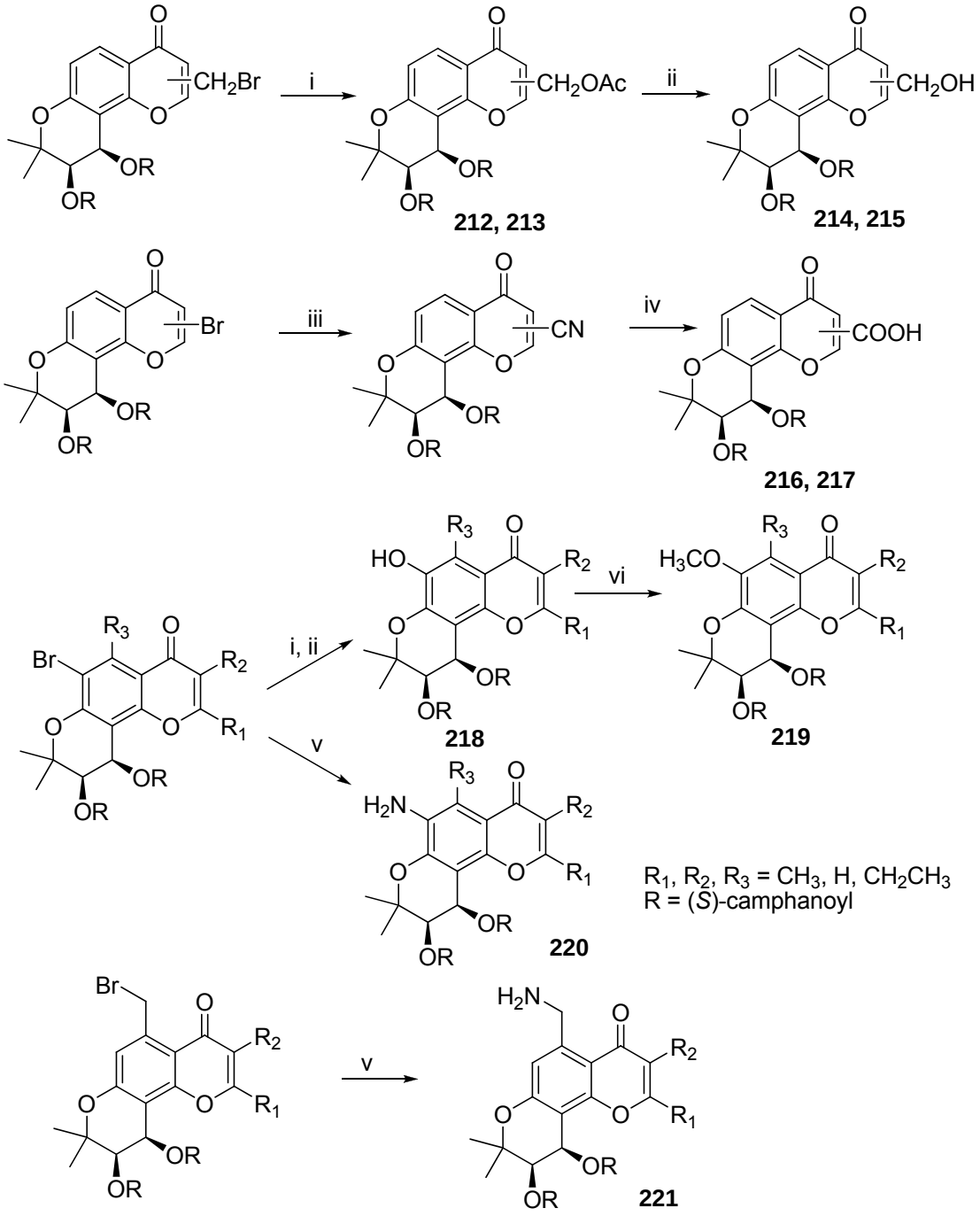
R_1 = **R_2** = CH_2OH, CH_2OAc, $COOH$, OCH_3, NO_2
R_3 = OH, CH_2OH, NH_2, CH_2NH_2, $COCH_3$, OCH_3
R_4 = NH_2, OH,CH_2OH

Figure 7-6. Newly designed DCP analogs

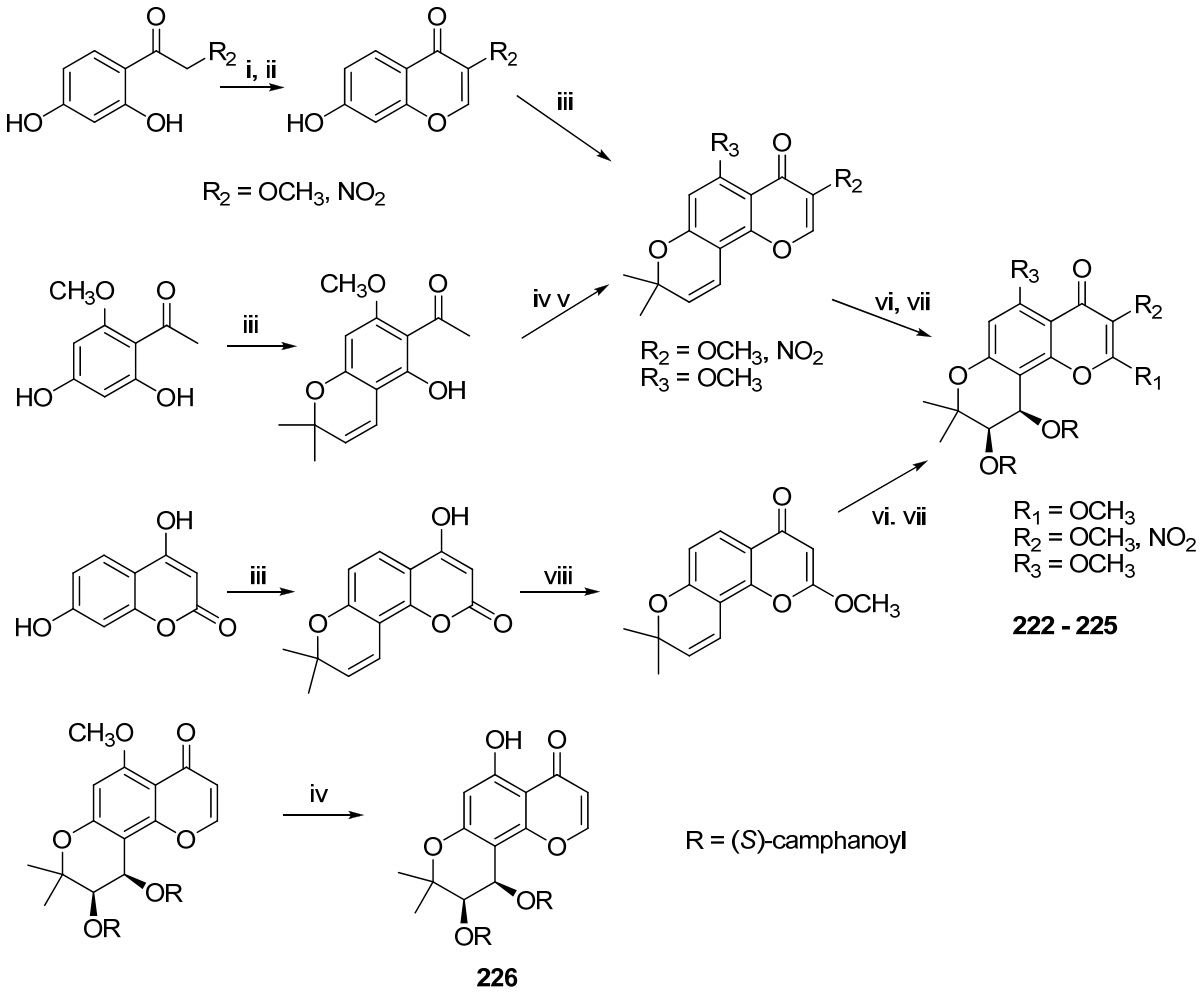


R_1 = OH, OAc, $COOH$,NO_2
R_2 = OH, OAc, $COOH$, CN, NH_2, NO_2
R_3 = OH, CH_2OH, NH_2, OCH_3, CN

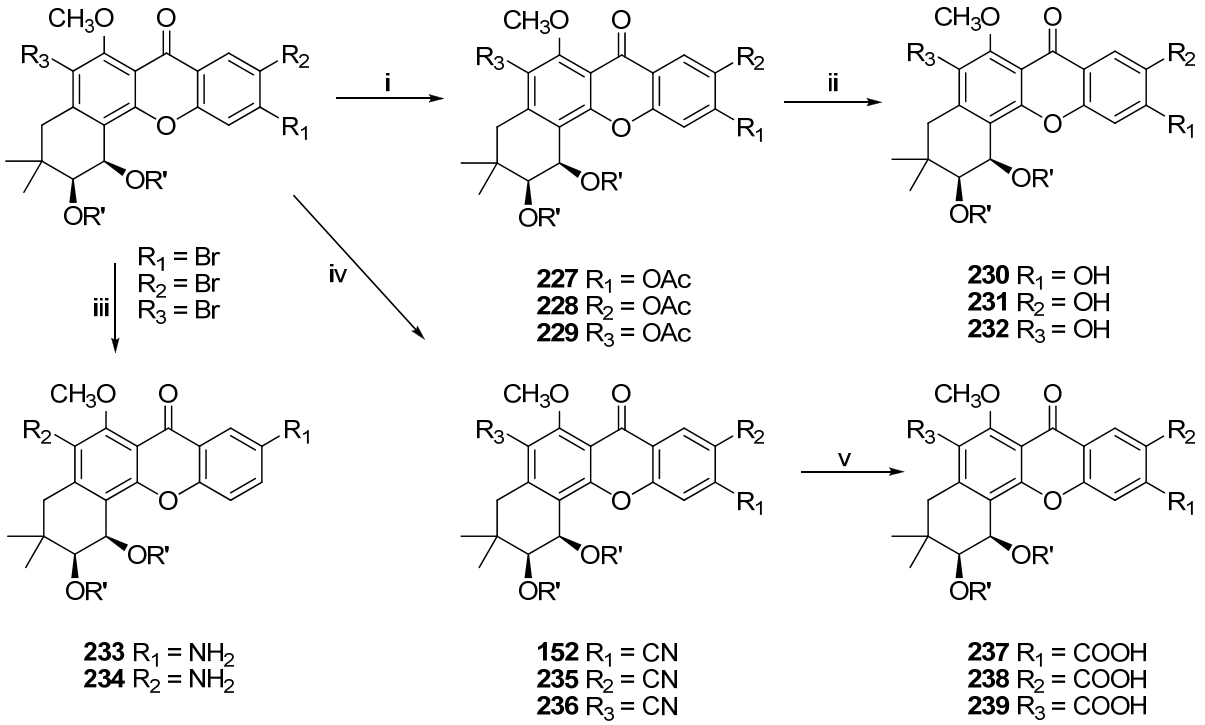
Figure 7-7. Newly designed DCX analogs



Scheme 7-1. Synthetic pathway for newly designed DCP--- part A: Reagent and Conditions: (i) acetic anhydride, NaOAc, reflux; (ii) HCl (2N) in EtOH, reflux; (iii) NaCN, DMF, 0^{o}C; (iv) H_3PO_4, 155^{o}C, 4h; (v) NH4OH, THF, rt; (vi) MeI, K_2CO_3, acetone.



Scheme 7-2. Synthetic pathway of newly designed DCPs— part B. Conditions and reagents: (i) 70% perchloric acid, triethyl orthoformate, 40 min; (ii) H_2O, reflux, 5 min; (iii) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave, 220°C/4h; (iv)alkyl alkonoate, NaH, THF, reflux; (v) Amberlyst 15 resin, isopropanol, reflux; (vi)$K_3F_3(CN)_6$, $(DHQ)_2PYR$, $K_2OsO_2(OH)_4$, K_2CO_3, *t*-butanol, 0°C; (vii) (S)-camphanoyl chloride, DMAP, CH_2Cl_2; (viii) CH_2N_2, Et_2O; (ix) HBr solution in acetic acid, reflux.



Scheme 7-3. Synthesis pathway for newly designed DCX analogs. Reagents and conditions: (i) acetic anhydride, NaOAc, reflux; (ii) HCl (2N) in EtOH, reflux; (iii) NH_4OH, THF, rt; (iv) NaCN, DMF/EtOH, 0°C; (v) H_3PO_4, 155°C, 4h

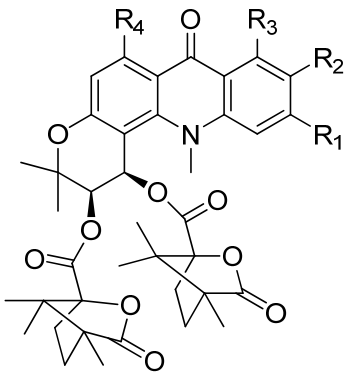
All newly synthesized DCP and DCX analogs will be screened for anti-HIV-1 activity against both HIV-1/NL4-3 and HIV/RTMDR1 strains. The method has been described in detail in Chapter 4.4.2.

To test whether the newly designed compounds show improved water-solubility, those with high anti-HIV potential will be tested for their water-solubility following the method mentioned in Chapter 4.4.3.

7.2.2 Design and Synthesis of Dihydropyrano[2,3-*c*]acridinone Derivatives as Anti-HIV Agents

Bioisosteric modification may profoundly affect pharmacological action, metabolism, activity, and toxicity. As we described in Chapter 2.3.3, this basic principle of analog design was successfully applied in DCK modifications. DCK

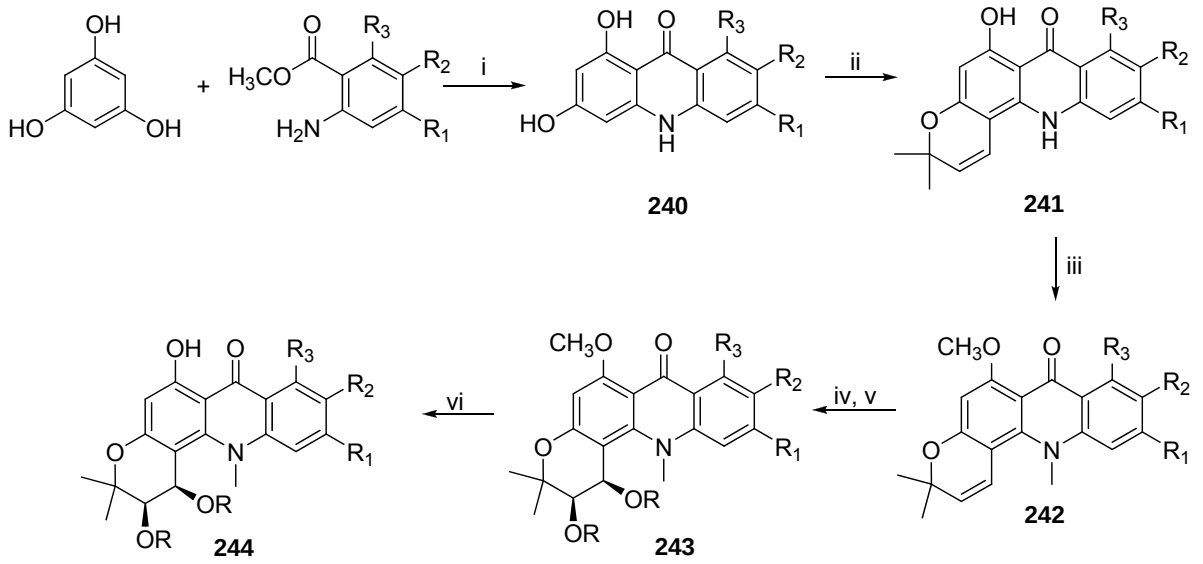
lactam analogs, e.g., compound **57**, showed better anti-HIV activity than 4-MDCK (**26**).[1] We have designed a series of DCX lactam analogs, (1R,2R)-dicamphanoyl-1,2-dihydroxy-3,3,12-trimethyl-2,3-dihydro-1*H*-pyrano[2,3-*c*]acridin-7(12*H*)-ones (Figure 7-8), modeled after this successful DCK modification, The novel DCX lactam analogs with new core structures should provide more insight into the SAR of this molecular scaffold.



R_1 - R_4 = H, CH_3, OCH_3, OH, CN, NO_2, NH_2, $NHCH_3$

Figure 7-8. Structures of DCX lactam analogs

The synthetic route to DCX lactam derivatives is shown in Scheme 7-4. A mixture of methyl anthranilate, phloroglucinol, and *p*-toluenesulfonic acid in hexanol heated at reflux gives ring closure acridin-9(10*H*)-one products (**240**). Pyrano ring closure product **241** is prepared using an optimized microwave-assisted one-pot reaction, as described in Chapter 3. Methylation using $(CH_3)_2SO_4$ adds methyl groups on both 10-NH and 6-OH groups to give **242**. The subsequent reaction steps are similar to those used to synthesize DCXs. Demethylation of the 6-methoxy group in **243** should give the 6-hydroxy compound (**244**).



R_1 - R_3 = H, CH_3, OCH_3, OH, CN, NO_2, NH_2, $NHCH_3$.
R =(*S*)-camphanoyl

Scheme 7-4. Synthesis route of DCX lactam. (i) *p*-toluenesulfonic acid, hexanol, reflux; (ii) 4,4-dimethoxy-2-methyl-2-butanol, pyridine, microwave, 220°C/4h; (iii) $(CH_3)_2SO_4$, NaH, DMF, 45°C; (iv) $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, $(DHQ)_2PYR$, K_2CO_3, 0°C; (v) (s)-camphanoyl chloride, DMAP, CH_2Cl_2, rt. (vi) 48% HBr solution in acetic acid, reflux

In our most recent study, compound **243** (R_1 = R_2 = R_3 = H, Scheme7-4) was successfully synthesized utilizing this method, which confirmed that the synthetic pathway shown in Scheme 7-4 is workable. More analogs will be synthesized as shown in Scheme 7-4 to discover new leads, establish the SAR and explore the mechanism of action.

6-Hydroxy-3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acridin-7(12*H*)-one (**241**, R_1 = R_2 = R_3 = H). 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.77 (1H, d, *J* = 7.2 Hz, H-8), 7.61 (1H, t, *J* = 7.2, 7.2 Hz, H-10), 7.52 (1H, t, *J* = 7.2, 7.2 Hz, H-9), 7.43 (1H, d, *J* = 7.2 Hz, H-11),

6-Methoxy-3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acridin-7(12*H*)-one (**242**, R_1 = R_2 = R_3 = H, R_4 = OCH_3). 10% yield. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.40 (1H, d,

J = 8.1 Hz, H-8), 7.62 (1H, t, *J* = 8.1, 7.2 Hz, H-10), 7.35 (1H, d, *J* = 8.1 Hz, H-11), 7.24 (1H, t, *J* = 7.2, 8.1 Hz, H-9), 6.55 (1H, d, *J* = 9.3 Hz, H-1), 6.31 (1H, s, H-5), 5.52 (1H, d, *J* = 9.3 Hz, H-2), 3.97 (3H, s, OCH_3-6), 3.83 (3H, s, NCH_3-12), 1.54, 1.54 (each 3H, s, CH_3-2,2).

1*R*,2*R*-Dicamphanoyl-6-methoxy-3,3-dimethyl-2,3-dihydro-1*H*-pyrano[2,3-*c*]acridin-7(12*H*)-one (**243,** $R_1 = R_2 = R_3 = H$). ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 8.34 (1H, d, *J* = 8.7 Hz, H-8), 7.63 (1H, t, *J* = 6.6, 8.7 Hz, H-10), 7.41 (1H, d, *J* = 8.7 Hz, H-9), 7.24 (1H, t, *J* = 8.7, 6.6 Hz, H-9), 6.26 (1H, s, H-5), 5.46 (1H, d, *J* = 3.0 Hz, H-1), 5.28 (1H, d, *J* = 3.0 Hz, H-2), 4.07 (3H, s, NCH_3-12), 3.96 (3H, s, OCH_3-6), 2.50, 2.20, 2.06, 1.90 (each 2H, m, camphanoyl-CH_2), 1.58, 1.49 (each 3H, s, CH_3-2,2), 1.13, 1.12, 1.06, 1.03, 0.94, 0.92 (each 3H, s, camphanoyl-CH_3).

All newly synthesized DCX lactam analogs will be screened for anti-HIV-1 activity against both HIV-1/NL4-3 and HIV/RTMDR1 strains. The method has been described in detail in Chapter 4.4.2.

7.2.3 Development of Pyranochromone Analogs with Dual-functions to Improve Outcome of HAART

HAART regiments have some limitations, the major one being the failure to eradicate HIV even after several years of therapy. One reason for this failure is that, despite potent antiretroviral treatment, compartments of replication-competent virus persist, suggesting that antiretroviral agents do not reach all of the infected cells. In addition, all PIs suffer from poor pharmacokinetic properties.

7.2.3.1 Expression of P-gp and Outcome of HAART

Membrane transporters (efflux and influx) are now recognized as playing an important role in anti-HIV drug absorption and disposition, and they explain, at least in part, the broad diversity in intracellular concentrations of drugs. As mentioned in Chapter 6, P-gp is the most well characterized ATP-binding cassette (ABC) transporter by far, and has the ability to reduce intracellular concentrations of many anticancer agents, such as vincristine, doxorubicine, etc. This protein is widely expressed in the many barrier tissues, such as intestine, liver, kidney , blood-brain barrier (BBB), [2-4] and lymphocytes.[5, 6] Recent studies revealed that expression and function of P-gp also impact the intracellular concentrations of many anti-HIV agents.

It is now well established that most PIs are substrates of P-gp, which is expressed in the GI tract and the liver, and acts with CYP3A to reduce the bioavailability of PIs.[7] In combination treatment, the PI booster ritonavir inhibits both CYP3A and P-gp and markedly increased the bioavailability of PIs.

P-gp is also expressed on lymphocytes, including CD4+ lymphocytes and may reduce cellular accumulation of anti-HIV agents in HIV-infected lymphocytes. An earlier study showed that HIV-infected T cell and monocytic cell lines have increased P-gp expression, and accumulate significantly less AZT in comparison to uninfected cells.[8]

The central nervous system (CNS) has been cited as a reservoir for the HIV-1 virus due to incomplete suppression of the viral replication.[9] Suboptimal concentrations of anti-HIV-1 drugs in the CNS can provide ideal conditions for the selection of more virulent mutants.[10] P-gp is highly expressed on the (BBB.[2] As

substrates of P-gp, the limited ability of PIs to transverse the BBB is attributed to the activity of this efflux transporter. Another *in vivo* brain distribution study showed that P-gp may play a significant role in restricting the distribution of abacavir, which is a NRTI and also a P-gp substrate, to the CNS. [11]

Although the NNRTIs nevirapine, efavirenz and delavirdine are not substrates of P-gp, all of these drugs acted as P-gp modulators to induce the expression and function of P-gp in the LS180 human colon adenocarcinoma cell line. The observed induction could be reversed by the potent P-gp inhibitor verapamil. [12]

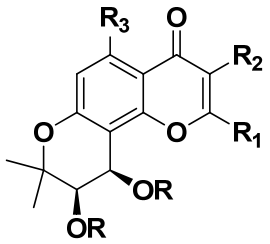
Collectively, these studies suggested that the selective inhibition of P-gp may facilitate the entry of PIs and certain NRTIs across the BBB and into HIV infected lymphocytes and, therefore, enhance the concentration of anti-HIV drugs in these sites to therapeutic levels. Inhibition of P-gp may also help increase the bioavailability of PIs.

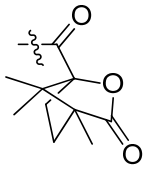
7.2.3.2 Preliminary Studies of DCP and DCX analogs to Overcome P-gp Mediated MDR in Cancer Cells

The pyranochromone in DCP is a critical pharmacophore for the chemosensitizing activity of novel DSP analogs (Chapter 6) against multidrug resistant cancer cells. The structural similarity between DCPs and DSPs suggests that DCP analogs and their DCX derivatives might also reverse MDR in cancer cells, by functioning as P-gp inhibitors. Therefore, these analogs might act as dual-inhibitors in the treatment of AIDS. In addition to inhibiting the proliferation of HIV-1, they could also block the P-gp efflux potential and, as a result, increase the cellular concentration of other anti-HIV agents, such as PIs, in HAART.

In order to test our hypothesis, selected DCP and DCX analogs that exhibited high anti-HIV activity against both wild-type and drug resistant HIV strains were screened in combination with VCR against KB-Vin cells. The cytotoxicity data for the combinations, (VCR+DCP) or (VCR+DCX), are listed in Tables 7-1 and 7-2. Many compounds, such as **98**, **131**, **134**, and **135**, which showed considerable anti-HIV activity against both wild-type and drug-resistant HIV strains, also exhibited extremely high potency in the combination formula against the KB-Vin cell line.

Table 7-1. Anti-HIV and chemosensitizing data of selected DCP analogs.

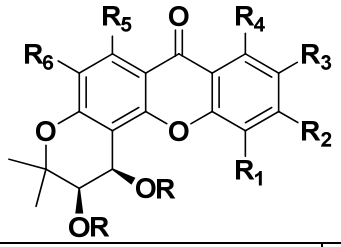


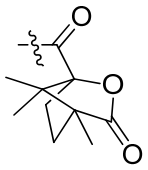
R = 

Compd.	Structure	EC_{50} against HIV_{NL4-3} (μM)	GI_{50} of (VCR + 4 μM DCPs)
69	$R_1 = CH_2CH_3$	0.089	0.022
98	$R_1 = R_3 = CH_3$	0.036	0.0068
102	$R_1 = CH_2CH_3$, $R_3 = CH_3$	0.10	>0.23
verapamil			0.044
VCR			2.64
VCR in KB[c]			0.0020

[a] This assay was performed in TZM-bl cell infected with HIV/NL4-3 strain.
[b] Cytotoxicity as GI_{50} values for KB-Vin cell line, the concentration of VCR + 4μM DCP that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.
[c] Cytotoxicity of VCR against KB parent cell line detected using sulforhodamine B assay.

Table 7-2. Anti-HIV and chemosensitizing data of selected DCX analogs.



R = 

Compd.	Structure	EC_{50} against HIV_{NL4-3} (μM)[a]	GC_{50} of (VCR + 4 μM DCX) (μM) [b]
130	R_1 –R_5 = H	0.308	0.0023
131	R_5 = OCH_3	0.063	0.0096
134	R_2 = CH_3, R_5 = OCH_3	0.095	0.0081
135	R_3 = CH_3, R_5 = OCH_3	0.065	0.0074
138	R_2 = R_5 = OCH_3	0.362	0.0020
139	R_3 = R_5 = OCH_3	0.121	0.0083
142	R_1 = F, R_5 = OCH_3	0.23	0.0033
verapamil		---	0.044
VCR			2.64
VCR in KB[c]			0.0022

[a] This assay was performed in TZM-bl cell infected with NL4-3 HIV strain.
[b] Cytotoxicity as GI_{50} values for KB-Vin cell line, the concentration of VCR + DSP (1μg/mL) that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.
[c] Cytotoxicity of VCR against KB parent cell line detected using sulforhodamine B assay.

7.2.3.3 SAR Study of DCP and DCX Analogs as Dual-functional Molecules

The preliminary results shown above provide a promising start for further investigation. To further improve the potential of DCP and DCX analogs as dual-functional molecules, a detailed SAR study is needed. All DCP and DCX analogs (synthesized and to be synthesized) will been screened for their chemosensitizing activity applying the method described in Chapter 6.6.2. We will next combine the SAR information from both anti-HIV activity and chemosensitizing activity and locate our best lead that exhibits strong efficacy in both functions. The exploration and synthesis of new DCP and DCX analogs were described in Section 7.2.1.

7.2.3.4. Effect of DCP and DCX Analogs on Cellular P-gp Activity

In order to test that the ability of identified DCPs and DCXs to regenerate the cytotoxicity of VCR is due to the inhibition of P-gp, four dual-functional DCP and DCX analogs (**98**, **131**, **134** and **135**) will be tested in the calcein-AM loading assay to detect their potential against cellular P-gp function. Ten thousand KB-Vin (MDR) cells per well are seeded into 96-well plates with medium containing 5% RBS and incubated for 1 day for adhesion and equilibration. Treatment involves supplementing cultures with distinct concentrations of test DCP or DCX compounds (initial concentration of 20 μM with two-fold dilutions for eight concentrations of each compound) for 30 min at 37 ^{o}C. After removing medium, fresh medium with both 1μM calcein-AM and test compounds as needed is added for 100 μL. Following incubation for 30 min, medium is again removed, and wells washed gently and quickly with cold PBS buffer in low ambient light condition (200 μL). Cell are lysed using 20 mM Tris buffer and fluorescent measured using a BioTek TLx800 ELISA reader set to Ex.494 nm and Em.517 nm. Composite data from two or more independent experiments are analyzed using Prizm^{TM}.

7.2.3.5 Effect of Combined Usage of Dual-functional Agent and PIs on HIV-1 Replication

We will further explore whether identified dual-functional agents can in fact promote the efficacy of PIs, which are P-gp substrates. We will treat the HIV-1/NL4-3 infected PBMC cell line with a combined formula of dual functional agent + saquinavir (PI, also a P-gp substrate[13]). Anti-HIV-1 activity is measured by p24

antigen capture. If test compound successfully inhibits the function of P-gp and increases the cellular concentration of saquinavir, the effect of the combined drug formula would be characterized as a synergistic effect; otherwise, an additive effect would be detected.

First of all, we need to detecte the MDR-reversal potency of DCP/DCX n order to locate the best concentraion of DCP/DCX which could be used in both chemosensiting and anti-HIV assay system. To do so, we will fixed the concentration of VCR at 0.1 μg/ml in the combination usage of DCP (DCX) with VCR and detected the EC_{50} value of DCP(DCX).

The selected concentration of DCP (DCX) in chemosensitizing assay will be used in the synergistic anti-HIV assay combined with protease inhibitor saquinavir. In each independent assay, HIV-1 at 200 $TCID_{50}$ and various dilutions of test samples (eight dilutions, fourfold stepwise) are mixed in a total volume of 100 μL growth medium in 96-well black solid plates (Corning-Costar). In each independent assay, the samples include saquinavir alone, test compound alone, saquinavir + xx μM (to be determined from chemosensitizing assay mentioned above) test compound combination. A second set of samples was prepared identical to the first and were added to cells under identical conditions without virus (mock infection) for toxicity determination. In addition, verapamil and ritonavir (clinically used PI booster) were also assayed during each experiment as a positive drug control. On days 1 and 4 postinfection, spent media was removed from each well and replaced with fresh media. On day 6 postinfection, the assay was terminated and cultured supernatants were harvested for analysis of virus replication by p25 antigen capture. The

compound toxicity was performed according to CytoTox-GloTM cytotoxicity assay instructions for using product G9290, G9291, and G9292. (Promega)

Composite data from two or more independent experiments are analyzed using CalcuSyn (Biosoft, Cambridge, United Kingdom) to identify the characteristics of the combination effect.

7.3 References

1. Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, K. H. Anti-AIDS agents part 41: synthesis and anti-HIV activity of 3',4'-di-o-(-)-camphanoyl-(+)-cis-khellactone (DCK) lactam analogues. *Bioorg Med Chem Lett,* **2000,** 10, 1003-1005.

2. Cordon-Cardo, C.; O'Brien, J. P.; Casals, D.; Rittman-Grauer, L.; Biedler, J. L.; Melamed, M. R.; Bertino, J. R. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A,* **1989,** 86, 695-698.

3. Jette, L.; Pouliot, J. F.; Murphy, G. F.; Beliveau, R. Isoform I (mdr3) is the major form of P-glycoprotein expressed in mouse brain capillaries. Evidence for cross-reactivity of antibody C219 with an unrelated protein. *Biochem , J* **1995,** 305 (Pt 3), 761-766.

4. Jette, L.; Tetu, B.; Beliveau, R. High levels of P-glycoprotein detected in isolated brain capillaries. *Biochim Biophys Acta ,* **1993,** 1150, 147-154.

5. Ludescher, C.; Pall, G.; Irschick, E. U.; Gastl, G. Differential activity of P-glycoprotein in normal blood lymphocyte subsets. *Br J Haematol,* **1998,** 101, 722-727.

6. Fauci, A.; Bartlett, J. G.; Goosby, E. Early treatment of HIV-1 infection. *Lancet,* **1998,** 352, 1935; author reply 1936.

7. Perloff, M. D.; von Moltke, L. L.; Fahey, J. M.; Greenblatt, D. J. Induction of P-glycoprotein expression and activity by ritonavir in bovine brain microvessel endothelial cells. *J Pharm Pharmacol ,* **2007,** 59, 947-953.

8. Antonelli, G.; Turriziani, O.; Cianfriglia, M.; Riva, E.; Dong, G.; Fattorossi, A.; Dianzani, F. Resistance of HIV-1 to AZT might also involve the cellular expression of multidrug resistance P-glycoprotein. *AIDS Res Hum Retroviruses,* **1992,** 8, 1839-1844.

9. Ances, B. M.; Ellis, R. J. Dementia and neurocognitive disorders due to HIV-1 infection. *Semin Neurol ,* **2007,** 27, 86-92.

10. Lipniacki, A. Drug Resistance As the Primary Cause of Therapeutic Failures in HIV/AIDS. *HIV/AIDS rev ,* **2003,** 2, 2-7.

11. Shaik, N.; Giri, N.; Pan, G.; Elmquist, W. F. P-glycoprotein-mediated active efflux of the anti-HIV1 nucleoside abacavir limits cellular accumulation and brain distribution. *Drug Metab Dispos ,* **2007,** 35, 2076-2085.

12. Stormer, E.; von Moltke, L. L.; Perloff, M. D.; Greenblatt, D. J. Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. *Pharm Res,* **2002,** 19, 1038-1045.

13. Lee, C. G.; Gottesman, M. M.; Cardarelli, C. O.; Ramachandra, M.; Jeang, K. T.; Ambudkar, S. V.; Pastan, I.; Dey, S. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry,* **1998,** 37, 3594-3601.