PROGRESS TOWARDS THE DESIGN AND SYNTHESIS OF NOVEL ANTI-TUMOR NEO-TANSHINLACTONE ANALOGUES

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A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Division of Chemical Biology and Medicinal Chemistry in the School of Pharmacy.

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ABSTRACT

Joshua D. Brattlie: Progress Towards the Design and Synthesis of Novel Anti-tumor Neo-tanshinlactone Analogues
(Under the direction of Kenan Distinguished Professor Kuo-Hsiung Lee)

The goals of this research are to design and synthesize novel neo-tanshinlactone analogues utilizing bioisosterism and molecular modification, and to evaluate their bioactivity as anti-breast cancer agents.

It has been shown that neo-tanshinlactone (**1**) and several of its analogues retain potent and selective *in vitro* anti- breast cancer activity. Previous research on neo-tanshinlactone focused on designing a more efficient synthetic route to synthesize 4-ethyl neo-tanshinlactone (**2**). This improved route was utilized to design and synthesize new compounds to elucidate structure-activity relationships (SAR) of **1**. Previously designed novel analogues of **1** also revolved around the systematic breakdown of its individual rings.

In this continuing study, we designed three target scaffolds (**A**, **B**, and **C**), and three target compounds (**13**, **14**, and **15**), based on **1** and **2** to study the effects of bioisosterism and ring size on bioactivity. Each scaffold systematically replaces a specific, and different, oxygen atom with a nitrogen atom. We have successfully synthesized two (**13** and **14)** target compounds, and progress has been made towards the synthesis of the third (**15**). Compound **13**, in which the naphthalene ring system of neo-tanshinlactone has been replaced by an indole ring system, has been tested for cytotoxicity against seven cancer cell lines and has exhibited a decrease in potency and selectivity in comparison to **1** and **2**.

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Ar	Argon
ACN	Acetonitrile
$AlCl_3$	Aluminum chloride
BBr_3	Boron tribromide
BCl_3	Boron trichloride
C	Celsius
d	Doublet
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DMAP	4-(*N,N*-Dimethylamino)pyridine
DME	1,2-Dimethoxyethane
DMSO	Dimethyl sulfoxide
EtI	Iodoethane
EtMgBr	Ethylmagnesium bromide
EtOAc	Ethyl acetate
g	Grams
h	Hour
HCl	Hydrochloric acid
H_2O	Water
K_2CO_3	Potassium carbonate
KI	Potassium iodide

KOH	Potassium hydroxide
m	Multiplet
M	Molar
$MgSO_4$	Magnesium sulfate
min	Minutes
mL	Milliliters
mmol	Millimole
NaH	Sodium hydride
NH_4Cl	Ammonium chloride
NH_4OH	Ammonium hydroxide
NMR	Nuclear magnetic resonance
Pd/C	Palladium on activated charcoal
$Pd(OH)_2/C$	Palladium hydroxide on activated charcoal
$POCl_3$	Phosphorous oxychloride
PPTS	Pyridinium-*p*-toluenesulfonate
q	Quintet
rt	Room temperature
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Triglyme	Triethylene glycol dimethyl ether
$ZnCl_2$	Zinc chloride

CHAPTER 1

BREAST CANCER: OVERVIEW, TREATMENTS, AND CURRENT THERAPEUTIC AGENTS

1.1 Introduction

Breast cancer is a form of cancer that develops in the cells of the breast.[1] Among women in the United States, breast cancer is the leading form of cancer and, after lung cancer, is the second leading cause of cancer death in women.[1] Breast cancer is a worldwide problem; the World Health Organization estimated that in 2011, 508,000 women died worldwide due to breast cancer.[2] It is also estimated that more than 230,000 new cases of invasive breast cancer, over 60,000 new cases of carcinoma in situ of the breast, and 40,000 deaths will occur from breast cancer in 2014.[1] While breast cancer is more prevalent in women, men are still susceptible. The National Cancer Institute estimates that in 2014, over 2,300 new cases of male breast cancer will occur causing an estimate 430 deaths.[3]

1.2 Risk Factors

Risk factors are described as being something that affects your chances of getting a disease. Some risk factors are determined by lifestyle such as the use of tobacco or alcohol, while other risk factors cannot be changed. Several risk factors have been shown to be associated with the prevalence of breast cancer.[1,4] Some risk factors include:

Gender

With less than 1% of breast cancer cases being attributed to males, being of the female gender is a significant risk factor.[1,5]

Age

As women increase in age, their chances of acquiring breast cancer increase. The age at which women start menstruating and start menopause also affect the risk of breast cancer. Women who start menstruating earlier and start menopause later in life have shown an increased risk of developing breast cancer.[1,4,6]

Race

Caucasian women tend to be at a higher risk of acquiring breast cancer than African-American women, although African-American women have a higher risk of death from breast cancer.[1]

Family history and genetic factors

A family history of breast cancer is a relevant risk factor for acquiring breast cancer. Around 5-10% of all breast cancer cases and between 25-40% of cases in which the patient is under the age of 35 have been associated with hereditary factors. Mutations in *BRCA1* and *BRCA2*, which are known as breast cancer type 1 and 2 susceptible genes, respectively, have been found in 30-40% of familial breast cancer cases. While there is an increased risk with these mutations, their presence does not account for all familial breast cancer cases, nor does it mean that someone with a mutation in one, or both, of these genes will definitely acquire breast cancer.[4,7]

Previous occurrence of breast cancer

Women who have had previous occurrences of breast cancer or other breast diseases, such as atypical epithelial hyperplasia, have an increased risk of acquiring the cancer in the other breast or another area within the same breast.[1,4,6]

Hormone therapy

The use of combined hormone therapy, in which postmenopausal women take estrogen and progesterone, has been associated with an increased risk of breast cancer. Discontinuation of the therapy seems to lower the risk to levels seen before the hormone therapy. While, an increased risk in breast cancer has been associated with the use of these hormones, the risk is not as great in comparison to women in which menopause was delayed for the same amount of time.[1,4,6]

Lifestyle choices

Certain lifestyle choices have also been associated with an increased risk in breast cancer. Some of these include: consuming alcohol and tobacco products, not bearing children, not breastfeeding, and allowing oneself to become obese or overweight.[1,4,6]

1.3 Treatments and Currently Used Agents

Because several treatment methods are available to patients to combat breast cancer, treatment should be discussed thoroughly with a physician. The choice of treatment will most likely be affected by a number of factors including the stage of the disease, patient and physician preferences, and characteristics of the patient.[1,8]

Surgery

In the case of breast cancer, surgery focuses on removing as much of the cancerous tissue as possible. One form of breast cancer surgery is breast-conserving surgery (BCS), also known as lumpectomy. BCS is usually done in situations where the cancer is localized to a small area of the breast. The surgery is also used as a diagnostic tool to determine whether the cancer has spread to the axillary lymph nodes. Radiation therapy is commonly used in combination with BCS. Another form of surgery is mastectomy, which removes the entire breast. Mastectomies are often done when the cancer is not localized to a small area of the breast or as a prophylactic treatment to ensure that the cancer does not metastasize. A radical mastectomy can also be performed in which the axillary lymph nodes are removed along with the breast tissue in cases where the cancer has spread to the lymph nodes.[1,6,9]

Radiation therapy

Radiation therapy is generally used in combination with surgery or chemotherapy. In combination with surgery, radiation therapy can be used before, in attempt to shrink the size of the tumor to allow for a better outcome for surgery, or after, to ensure than any remaining cancer cells are eradicated from the breast tissue or surrounding area. Radiation therapy consists of treating the affected area with high-energy rays or particles to kill cancer cells.[1,6]

Chemotherapy

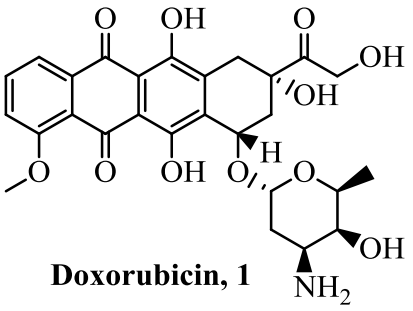
Chemotherapy consists of using drugs that kill breast cancer cells. These drugs tend to target highly proliferating cells, so side effects are common as the drugs can target highly proliferating normal cells as well as the highly proliferating cancer cells. Chemotherapy is usually done as a combination therapy, either pre-surgery in attempt to shrink the size of the

tumor or post-surgery in an attempt to rid the body of any remaining cancer cells, and also in combination with hormone therapy. The use of multiple chemotherapy drugs in a combination therapy can also be highly effective in breast cancer treatment.[1] Common chemotherapeutic agents include: 1) doxorubicin (Fig. 1-1, **1**) and epirubicin (Fig 1-1, **2**), topoisomerase II inhibitors which act by intercalating DNA, and halting replication[10]; 2) paclitaxel (Fig 1-1, **3**) and docetaxel (Fig 1-1, **4**), microtubule inhibitors which disrupt the normal cell division[11]; 3) 5-fluorouracil (Fig 1-1, **5**), a thymidylate synthase inhibitor which prevents the production of thymidine necessary for DNA replication[12]; 4) cyclophosphamide (Fig 1-1, **6**), a DNA alkylating agent which causes inter-strand and intra-strand DNA linkages leading to apoptosis.[12]

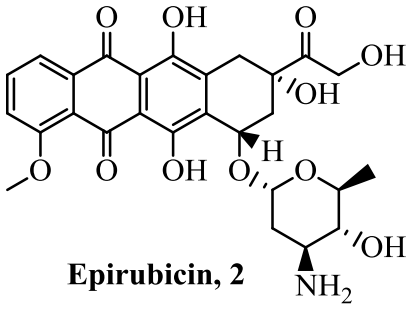
Hormone therapy

Hormone therapy is useful when the cancer cells are positive for estrogen receptors or progesterone receptors. Estrogen is able to promote the growth of breast cancer cells via these hormone receptors and is the major hormone involved in the development of breast cancer. The goal of hormone therapy is to block the actions of estrogen at these receptors and prevent estrogen-mediated cancer growth. Tamoxifen (Fig 1-1, **7**) has been widely used for hormone therapy of breast cancer. Tamoxifen is thought to produce its antitumor activity by competitively inhibiting estrogen from acting on hormone receptors. Anastrazole (Fig 1-1, **8**) represents another class of hormone therapy known as aromatase inhibitors. Anastrazole acts to reduce estrogen levels by blocking the actions of aromatase, the enzyme responsible for the generation of estrogens in postmenopausal women.[1,13,14,15]

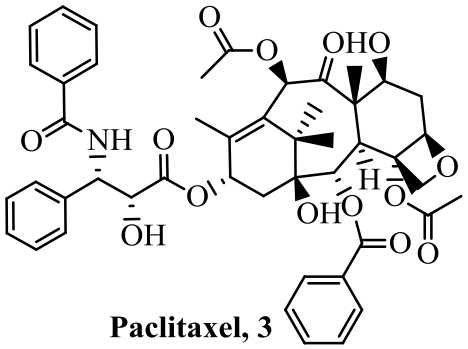
Figure 1-1. Common chemotherapeutic and hormone therapy agents



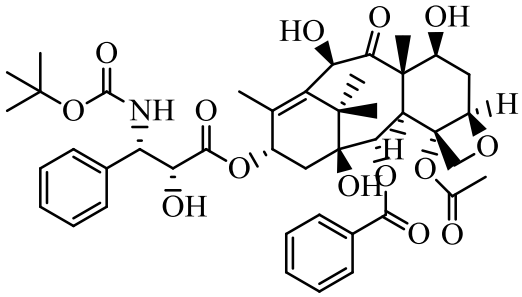
Doxorubicin, 1



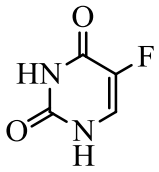
Epirubicin, 2



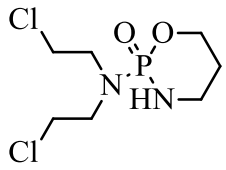
Paclitaxel, 3



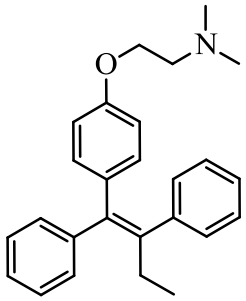
Docetaxel, 4



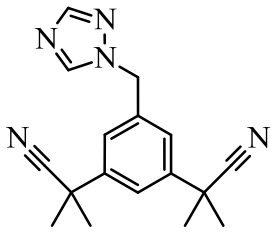
5-Fluorouracil, 5



Cyclophosphamide, 6



Tamoxifen, 7



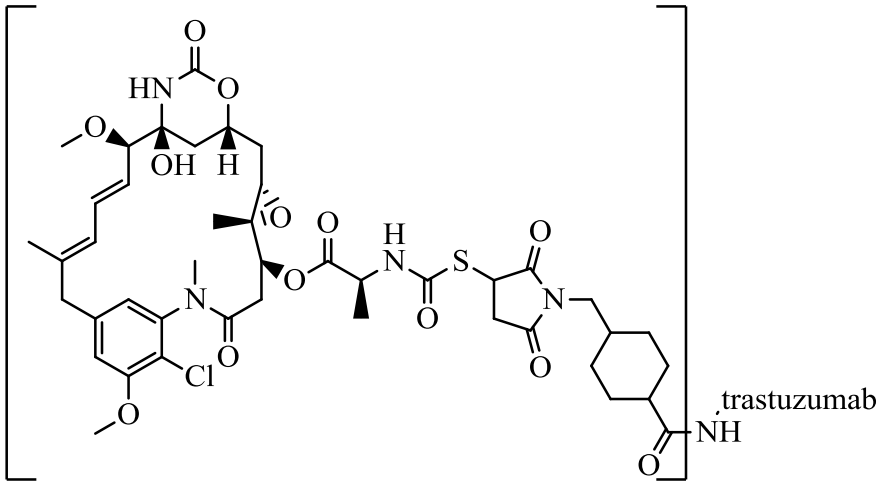
Anastrazole, 8

Targeted therapy

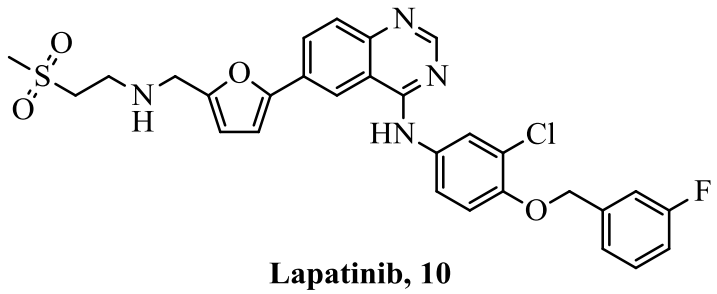
The identification of the molecular targets EGFR(epidermal growth factor receptor) and the related HER-2 (human epidermal growth factor receptor 2) associated with breast cancer has allowed for the development of more targeted therapies. HER2 is overexpressed in around 30% of early-stage and invasive breast cancer cases.[14,16,17] A significant correlation has been found between the overexpression of HER-2 and the reduced survival rates and increased recurrence rates in breast cancer patients.[14,16,17] Researchers have taken advantage of this discovery to develop agents that target HER2. Common targeted therapy agents include: 1) trastuzumab (Herceptin), an anti-HER2 monoclonal antibody, which uses a humanized mouse monoclonal antibody that targets HER2 on the surface of tumor cells causing internalization and subsequent cell cycle inhibition; 2) pertuzumab, another humanized monoclonal antibody which is often used in combination with trastuzumab as an HER2 dimerization inhibitor, preventing the dimerization of HER2 with other HER receptors; 3) ado-trastuzumab emtansine (Fig 1-2, **9**), an anti-HER2 antibody drug conjugate in which the antibody portion of the drug targets HER2, and once binding occurs the conjugate is internalized and subsequent degradation leads to release of the chemotherapeutic drug inside the tumor cell; and 4) lapatinib (Fig 1-2, **10**), a dual EGFR/HER2 inhibitor, which acts via inhibition of the intracellular kinase domains.[14,16,17] Figure 1-3 displays the various actions of targeted therapy agents on EGFR and HER receptors. While targeted therapy has been proven to be an effective strategy to treat breast cancer, some limitations are still present. Trastuzumab, for example, is effective when the patient is tested positive for HER2 receptor overexpression. Since HER2-overexpression is only present in a third of breast cancer cases there remains a large population of patients who are unable to benefit from this therapy. Also, resistance remains a limitation to targeted therapy. Overexpression of EGFR

can negate the effects of trastuzumab on HER2-overexpressing patients as well as shedding of the HER2 surface receptor protein.[18]

Figure 1-2. Common targeted therapy agents

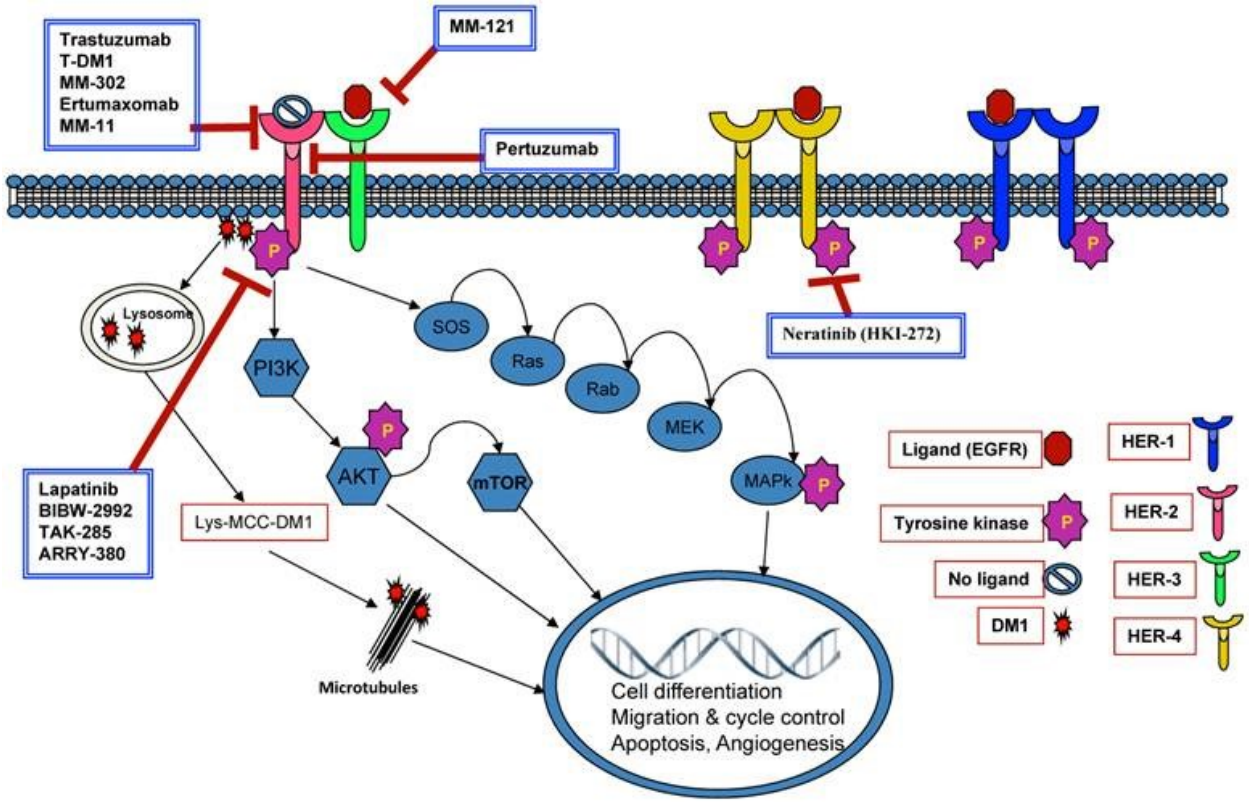


Ado-trastuzumab emanstine, 9



Lapatinib, 10

Figure 1-3. Therapies targeting EGFR and HER pathways[19]



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CHAPTER 2

DESIGN AND PROGRESS TOWARD THE SYNTHESIS OF NOVEL NEO-TANSHINLACTONE ANALOGUES

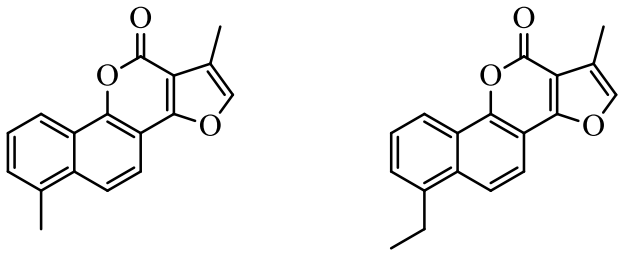
2.1 Introduction

Natural products have been used extensively throughout history as remedies for various ailments. In the past, these remedies would have included crude forms of the drug such as teas, tinctures or powders. Modern technology has now allowed us to isolate the bioactive constituents from natural sources to use as drugs, or drug leads.[1,2] Natural products offer immense structural variety and chemical diversity that are unique and would not likely be discovered by the traditional medicinal chemistry approaches.[3,4] Early efforts in drug discovery from natural products led to drugs such as digoxin, morphine, codeine, aspirin, penicillin, and quinine.[2,5] The use of natural products has had a huge impact on modern drug discovery. Between 1984 and 2004, around 50% of drugs introduced to the market were in some way derived from natural products. This percentage increased to 78 in the case of antibacterial agents.[1] Accordingly, it is no surprise that over 60% of all currently used anticancer agents are derived from natural products.[6] For these reasons, our group has maintained interest in the discovery of novel anticancer agents from natural sources.

Salvia miltiorrhiza, has been of particular interest to our group over the past decade. The rhizome of *Salvia miltiorrhiza* Bunge, also known as Tanshen in traditional Chinese medicine (TCM), has been used extensively throughout Chinese history for a variety of ailments. Coronary heart diseases such as angina pectoris and myocardial infarction, along with disorders of the

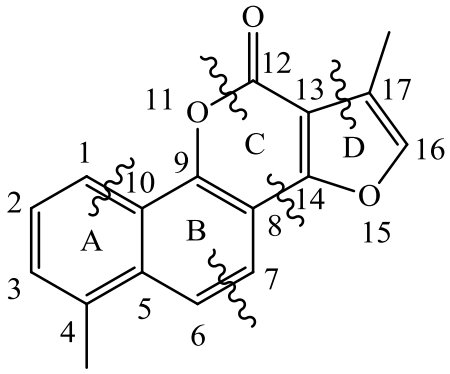
blood vessels such as atherosclerosis and blood clotting abnormalities are just a few of the ailments treated with Tanshen.[7,8] Extensive work has been done to determine the bioactive constituents of Tanshen, and more than 70 compounds have been isolated. These compounds have been divided into two groups, water-soluble and lipophilic. In 2004, Dr. Xihong Wang isolated neo-tanshinlactone (NTL, **11**) (Figure 2-1) from an ethanol extract of *Salvia miltiorrhiza*. After evaluating **11** *in vitro*, it was found that **11** expressed significant anti-breast cancer activity in comparison to Tamoxifen. Neo-tanshinlactone exhibited a 10-fold increase in potency and was 20-fold more selective than Tamoxifen when tested against ER+ and HER++ breast cancer cells.[9] Further studies done by Dr. Wang led to the development of 4-ethyl neo-tanshinlactone (ENTL, **12**) (Figure 2-1), which displayed increased potency in comparison to **11** against breast cancer cell lines MCF-7 and SK-BR-3.[10] Research on **11** and **12** was continued by Dr. Yizhou Dong, from our group, who designed numerous analogues of **11** to elucidate structure-activity relationship (SAR) correlations for this compound. Dr. Dong focused his studies on optimization of the synthesis of **12** and the systematic breakdown of the ring structures of **11**, as depicted by the wavy lines in Figure 2-2. In general, Dr. Dong found several important SAR correlations: 1) the C-4 position was important for anti-breast cancer activity and selectivity with ethyl being the most effective along with 2-bromoethyl and propyl; 2) the C-17 and C-16 positions were also important for activity with a methyl group at C-17 and a hydrogen atom at C-16 being the most favorable combination; 3) the lactone ring-C was essential for activity; 4) unsaturated rings were preferable over their saturated counterparts for rings A and D, an aromatic moiety for ring-A and a furan moiety for ring-D being the most preferable (Figure 2-2).[11,12] Due to these findings, we felt that continued modification of **11** was warranted to design novel analogues and develop new lead compounds.

Figure 2-1. Neo-tanshinlactone and its analogue 4-ethyl neo-tanshinlactone



NTL, 11 **ENTL, 12**

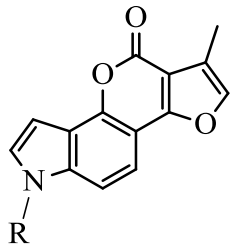
Figure 2-2. Systematic breakdown of NTL used to elucidate SAR



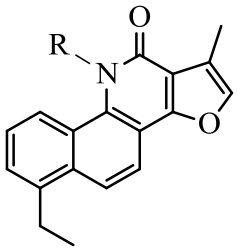
2.2 Design

The goal of the design of analogues **13**, **14**, and **15**, and more generally the scaffolds **A**, **B**, and **C** was to utilize bioisosterism to systematically replace specific oxygens of **11** with nitrogens (Figure 2-3). In replacing these oxygens with nitrogens, we can observe how changes in hydrophobicity and increased numbers of hydrogen bonding atoms affect the cytotoxicity of the new compounds against cancer cell lines. Also, the replacement of an oxygen atom with a nitrogen atom in the lactone ring and furan ring of scaffolds **B** and **C**, respectively, will allow for the attachment of substituents to further explore the SAR of neo-tanshinlactone.

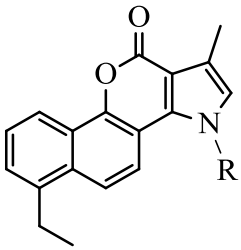
Figure 2-3. Design of scaffolds A, B, and C along with corresponding analogues 13, 14, and 15



Scaffold A
13, R= Et



Scaffold B
14, R= H



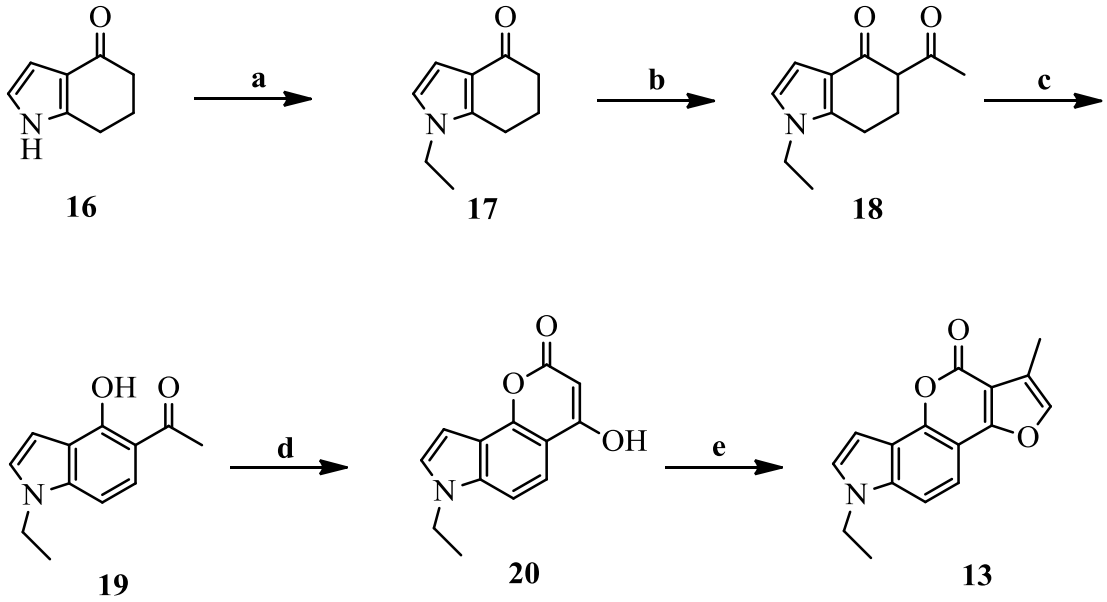
Scaffold C
15, R= H

2.3 Chemistry

2.3.1 Chemistry of 1-ethyl-6-methylfuro[2',3':4,5]pyrano[2,3-e]indol-5(1H)-one (13)

The synthesis of analogue **13** was achieved in five steps with an overall yield of 16% (Scheme 3-1). Sodium hydride was first used, along with the alkyl halide (iodoethane), to achieve *N*-alkylation of **16**.[13] Sodium hydride was again used to generate the enolate form of **16**, allowing for the coupling with EtOAc to give **17**.[14] Dehydrogenation of **18** using DDQ afforded **19**.[14] Next, sodium hydride was used, along with an acylating agent (diethyl carbonate), to produce **20** via acylation and internal ring cyclization.[15,16] The last step utilized a tandem alkylation/intramolecular Aldol reaction to produce **13**.[12]

Scheme 2-1. Synthetic pathway to analogue 13



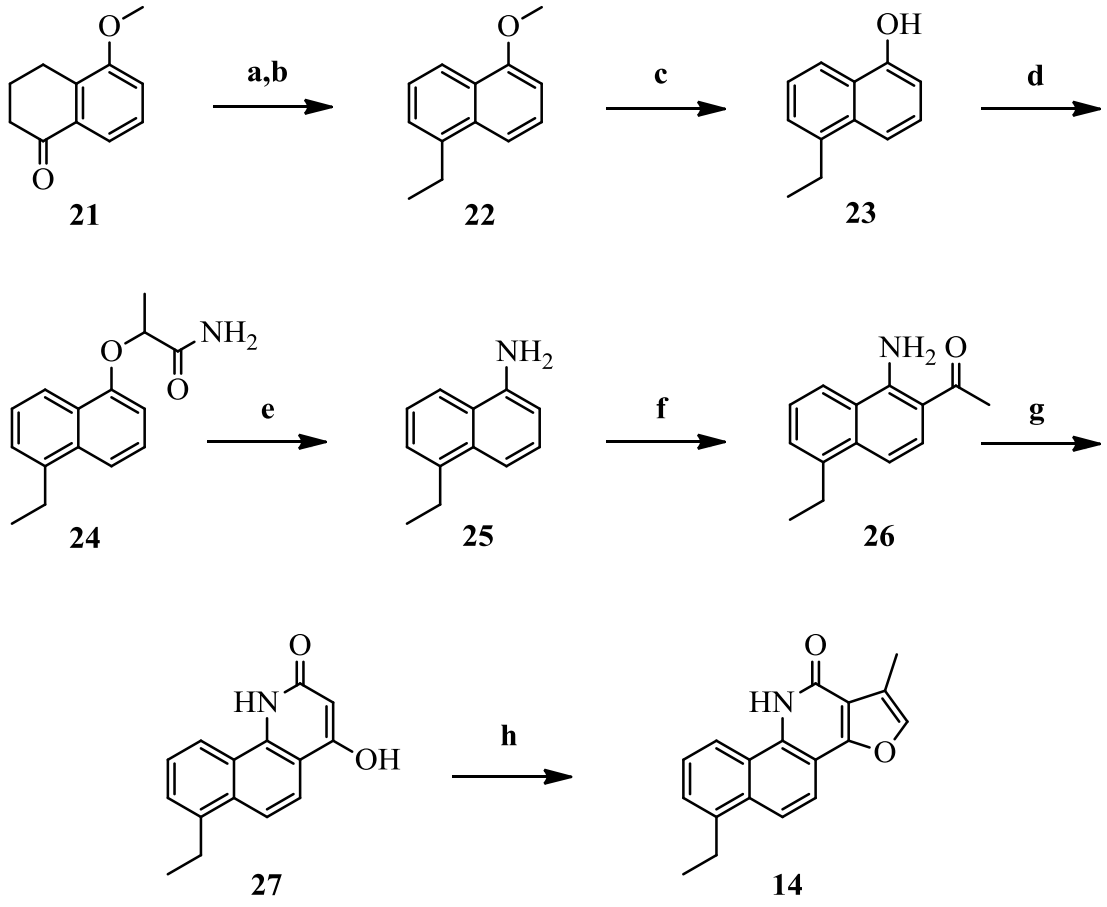
Reagents and conditions: (a) NaH, EtI, THF 0°C → rt, Ar, overnight; (b) NaH, EtOAc, DME, 0°C → reflux, Ar, 3 h; (c) DDQ, 1,4-dioxane, relux, 2 h; (d) NaH, diethyl carbonate, toluene, 100°C, 5 h; (e) chloroacetone, AcOH, ammonium acetate, toluene, EtOH, rt →60°C → reflux, 5 h.

2.3.2 Chemistry of 5-ethyl-1-methylbenzo[h]furo[3,2-c]quinolin-11(10H)-one (14)

The synthesis of analogue **14** was achieved in eight steps with a yield of 2% through the first five steps (Scheme 2-2). The overall yield was not determined because only an analytical sample of the final product was isolated for NMR and MS analysis. The first three steps have been used in the synthesis of previously synthesized neo-tanshinlactone analogues. These steps include the use of a Grignard reaction with zinc chloride as a catalyst, and subsequent oxidation with the use of Pd/C to produce **22** from **21**.[12] Boron tribromide was then used to demethylate **22**

to produce the naphthol **23**.[12] The aryloxyamide **24** was produced by treating the naphthol with ethyl 2-bromopropionate to generate the naphthalene ester, followed by production of the amide **24** via amination of the ethyl ester.[17] The naphthylamine **25** was generated from the aryloxyamide using potassium hydroxide via cleavage of the aryl C-O bond and subsequent cleavage of the amide C-N bond.[17] Friedel-Craft's acylation of the naphthylamine with boron trichloride, aluminum chloride, and acetonitrile generated **26**.[18] From **26**, the previous method of acylation and internal cyclization gave **27**, followed by tandem alkylation/intramolecular Aldol reactions were generate **14**.[12]

Scheme 2-2. Synthetic pathway to analogue 14



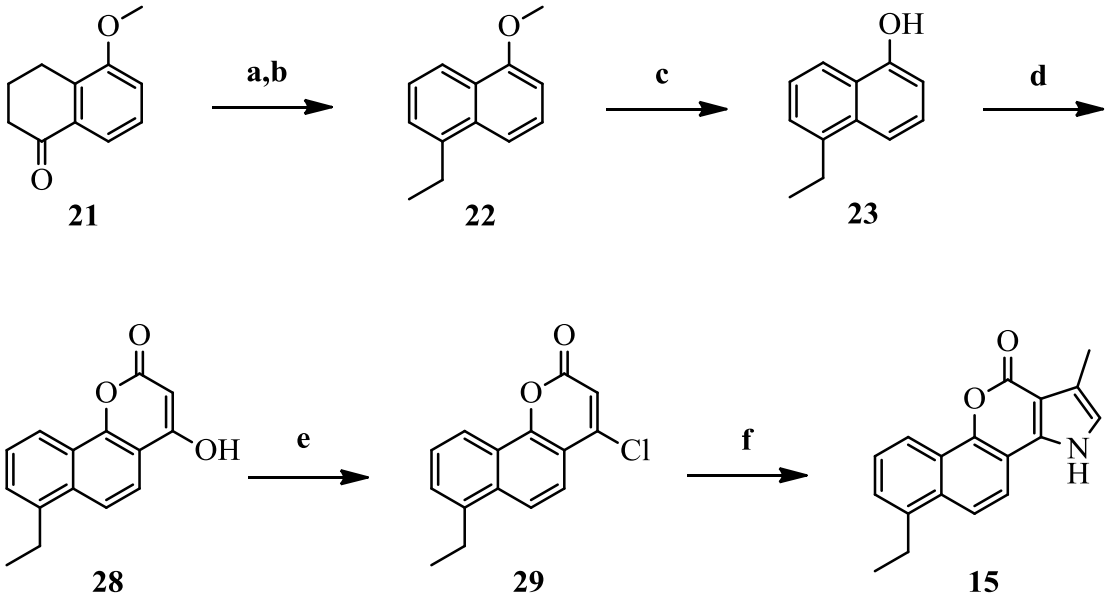
Reagents and conditions: (a) EtMgBr, $ZnCl_2$, THF, rt → 0°C → rt, Ar, overnight; (b) Pd/C (10%), Triglyme, 250°C, 2 days; (c) BBr_3, DCM, 0°C → reflux, Ar, 3 h; (d) (i) ethyl 2-bromopropionate, K_2CO_3, KI, acetone, reflux, 12 h; (ii) NH_4OH (28-30%), EtOH, rt, 18 h; (e) KOH, DMSO, 140°C, 12 h; (f) BCl_3, $AlCl_3$, ACN, DME, 0°C → reflux, Ar, 24 h; (g) NaH, diethyl carbonate, toluene, 100°C, Ar, 5 h; (h) chloroacetone, AcOH, ammonium acetate, EtOH, toluene, rt → 60°C → reflux, Ar, 24 h.

2.3.3 Chemistry of 6-ethyl-1-methylbenzo[7,8]chromeno[4,3-b]pyrrol-11(3H)-one (15)

Progress has been made toward the synthesis of **15** (Scheme 2-3), generating the two reagents necessary to perform the final step of the total synthesis. The intermediate, 4-chloro-7-ethyl-2H-benzo[h]chromen-2-one (**29**) and reagent (2-methyl-1,3-dioxolan-2-yl)methanamine, have been synthesized with an overall yield of 4% and 14%, respectively. For compound **29**, previously described methods produced the naphthol intermediate **23**.[12] Meldrum's acid (the cyclic form of diethyl malonate) was used to generate the monoester of **23** under solvent-free conditions.[19,20] Eaton's reagent (7.7% phosphorous pentoxide solution in methanesulfonic acid) was then used to promote a Friedel-Crafts acylation, producing **28**.[19,20] Substitution of the hydroxyl group for a chlorine atom using phosphorous oxychloride and triethylamine afforded compound **29**.[21] (2-methyl-1,3-dioxolan-2-yl)methanamine was synthesized in four steps (Scheme 2-4) using 1-amino-2-propanol, **30**, as the starting material. The amine was first protected using benzyl chloroformate in the presence of 4-DMAP and triethylamine to generate **31**.[22] Next, the hydroxyl group in **31** was oxidized to the ketone group, in **32**, using the Swern oxidation[23], followed by the use of ethylene glycol and pyridinium *p*-toluenesulfonate (PPTS) to produce the acetal group, in **33**.[22] Finally, the amine was deprotected by removing the

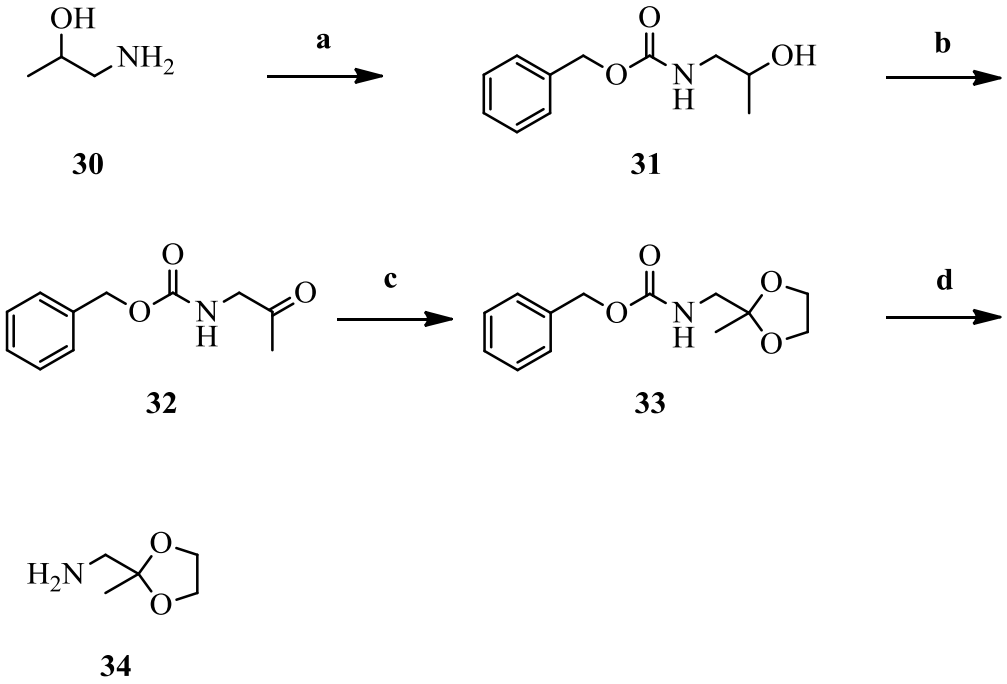
carboxybenzyl group via hydrogenation using $Pd(OH)_2/C$ under H_2 atmosphere to afford the target compound (2-methyl-1,3-dioxolan-2-yl)methanamine, (**34**).[22] The next step, which has yet to be completed, would be to react compounds **29** and **34** in the presence of triethylamine, followed by an acetic acid/H_2O work up to afford the final compound **15**.[24]

Scheme 2-3. Proposed synthetic pathway to analogue 15



Reagents and conditions: (a) EtMgBr, $ZnCl_2$, THF, rt → 0°C → rt, Ar, overnight; (b) Pd/C (10%), Triglyme, 250°C, 2 days; (c) BBr_3, DCM, 0°C → reflux, Ar, 3 h; (d) (i) Meldrum's acid, 90°C, 5 h; (ii) Eaton's reagent, 70°, 1.5 h; (e) $POCl_3$, triethylamine, relux, 1 h; (f) (2-methyl-1,3-dioxolan-2-yl)methanamine, triethylamine, EtOH, reflux, 24 h.

Scheme 2-4. Synthetic pathway to (2-methyl-1,3-dioxolan-2-yl)methanamine reagent 34



Reagents and conditions: (a) Benzyl chloroformate, DMAP, triethylamine, DMSO, 50°C, Ar, 5 h; (b) oxalyl chloride, DMSO, triethylamine, DCM, -78°C → rt, 1.3 h; (c) PPTS, ethylene glycol, benzene, reflux, 4 h; (d) $Pd(OH)_2/C$ (20%), EtOAc, H_2, 50 psi, 5 h.

2.4 Results and Conclusions

Analogue **13** was evaluated *in vitro* against seven different cancer cell lines: SK-BR-3 (ER/PgR-negative, HER2-overexpressing breast cancer); MCF-7 (ER-postive, PgR±, HER2-negative breast cancer); ZR-75-1 (ER/PgR/HER2-positive breast cancer); MDA-MB-231 (triple-negative (ER,PgR, and erbB2(HER2) negative) breast cancer); A549 (lung adenocarcinoma); KB (originally isolated as the epidermoid carcinoma of the nasopharynx, but now clarified as HeLa –

cervical cancer); and KB-VIN (multidrug-resistant KB cell line). Paclitaxel, a clinically used anti-breast cancer agent was used for comparison. Compound **13** (JDB5-54 in Figure 2-1) showed no significant cytotoxicity with the average IC_{50} being greater than 34 μM for the three non-breast cancer cell lines and the triple negative breast cancer cell lines (Table 2-1). Compound **13** exhibited a decreased potency in comparison to NTL and ENTL, and the selectivity towards breast cancer has also diminished. In conclusion, we have successfully synthesized two novel neo-tanshinlactone analogues, **13** and **14**, and have made progress toward the synthesis of a third novel analogue, **15**.

Table 2-1. Cytotoxicity assay results for analogue 13

Cytotoxicity IC50
SRB assay

Sample		SK-BR-3	MCF-7	ZR-75-1	MDA-MB-231	A549	KB	KB-VIN
		Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD
JDB 5-54	μM	15.68 ± 12.60	25.87 ± 0.50	22.14 ± 4.97	37.32 ± 7.57	38.00 ± 3.63	36.86 ± 2.02	34.82 ± 2.42
NTL (JDB2-36)	μM	10.36 ± 2.98	9.63 ± 1.05	8.10 ± 1.17	(>40)	(>40)	(>40)	(>40)
ENT (JDB2-10)	μM	12.83	2.27	0.770	(>40)	(>40)	(>40)	(>40)
Paclitaxel	μM	0.179	0.0119	0.162	0.00425 ± 0.002	0.0006 ± 0.0009	0.0011 ± 0.0007	1.754 ± 0.273

SK-BR-3, ER-negative, PgR-negative, HER2-overexpressed breast cancer; **MCF-7**, ER-positive, PgR±, HER2-negative breast cancer; **ZR-75-1**, ER-positive, PgR-negative, HER2-overexpressed breast cancer; **MDA-MB-231**, Triple-negative (ER-nega/PgR-nega/erbB2 (HER2)-nega) breast cancer **A549**, Lung adenocarcinoma;; **KB**, originally isolated as the epidermoid carcinoma of the nasopharynx (ATCC stock cell line KB is recently clarified as the **HeLa) KB-VIN**, Multidrug-resistant KB cell line.

2.5 Future Studies

Continued research will be done on scaffolds **A**, **B**, and **C**. Focus will be on completing the synthesis of analogue **15** and linking new moieties to the pyrrole nitrogen and to further explore the SAR of neo-tanshinlactone. Analogue **14** will also be explored in more depth; for example, new moieties that are known to interact with progesterone receptors will be added to

the nitrogen of the lactam ring which replaced one lactone oxygen in neo-tanshinlactone. Further studies will also be done to increase hydrophobicity, in hopes to increase membrane permeability.

2.6 Experimental Section

2.6.1 Chemistry

Materials and Methods. ^{1}H NMR spectra were measured on a Varian 400 MHz VNMRS spectrometer using TMS as an internal standard. The solvent used was either $CDCl_3$ or $(CD_3)_2SO$, as indicated in the detailed procedure. Mass spectra were measured on a Shimadzu LCMS-2010 instrument. TLC analysis was performed on Sorbent Technologies aluminum backed silica XG TLC plates and preparatory TLC was performed on ANALTECH Uniplate Silica Gel GF 1500 micron plates. Flash chromatography was performed on either Teledyne Isco CombiFlashR_f or Biotage Isolera Prime instruments using Teledyne Isco RediSepR_f normal phase columns and 230-400 mesh silica gel from Fisher Chemical. Chemicals were obtained from Aldrich, Fisher, TCI America, or Acros Organics.

Synthesis of 1-ethyl-6-methylfuro[2',3':4,5]pyrano[2,3-e]indol-5(1H)-one (13).

1-Ethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one, 17. To cooled solution of 1,5,6,7-tetrahydro-4*H*-indol-4-one (1.35 g, 10 mmol) in THF (30 mL) at 0 °C was added sodium hydride (1.00 g, 25 mmol) under argon atmosphere. The mixture was stirred at 0 °C for 30 min, and then iodoethane (1.2 mL, 15 mmol) was added. The remaining mixture was allowed to warm to rt and stirred overnight. After the reaction was completed, as determined by TLC analysis, excess sodium hydride was quenched with the addition of H_2O (30 mL) and the mixture was poured into a separatory funnel. Ethyl acetate (EtOAc, 30 mL x 3) was used to extract the organic layer. The

organic layer was then dried over magnesium sulfate ($MgSO_4$), filtered, concentrated *in vacuo,* and purified via flash chromatography over silica gel, eluting with EtOAc:hexane (1:4), to give **17** as a pale yellow viscous oil. 1.46 g (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 6.59-6.58 (1H, d, J = 4 Hz, aromatic), 6.56-6.55 (1H, d, J = 4 Hz, aromatic), 3.90-3.84 (2H, q, J = 8 Hz, CH_2), 2.76-2.72 (2H, t, J = 8 Hz, CH_2), 2.49-2.45 (2H, t, J = 8 Hz, CH_2), 2.19-2.15 (2H, m, CH_2), 1.41-1.39 (3H, t, J = 4 Hz, CH_3)

5-Acetyl-1-ethyl-4-oxo-4,5,6,7-tetrahydroindole, 18. To a stirred solution of sodium hydride (5.40 g, 135 mmol) in dry 1,2-dimethoxyethane (30 mL) was added 1-ethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (1.47 g, 9 mmol) in 25 mL dry 1,2-dimethoxyethane at 0 °C under argon atmosphere. The reaction mixture was stirred for 30 min and EtOAc (4.4 mL, 45 mmol) was added dropwise. The reaction mixture was then heated to reflux for 3 h. After completion of the reaction as determined by TLC analysis, ice cold NH_4Cl was added dropwise to quench the reaction and the mixture was then poured into a separatory funnel containing NH_4Cl. The organic layer was extracted with EtOAc (3 x 50 mL), washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel, eluting with EtOAc:hexane (1:4) to give a light yellow viscous oil. 756 mg (41% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 6.59-6.58 (1H, d, J = 4 Hz, aromatic), 6.54-6.53 (1H, d, J = 4 Hz, aromatic), 3.87-3.81 (2H, q, J = 8 Hz, CH_2), 3.52-3.50 (1H, t, J = 8 Hz, CH), 3.04-2.97 (1H, m, CH_2), 2.70-2.63 (1H, m, CH_2), 2.58-2.51 (2H, m, CH_2), 2.29 (1H, s, CH_3), 2.24-2.19 (1H, m, CH_2), 1.38-1.36 (3H, t, J = 4, CH_2). MS: *m/z* (M+2) 207.

5-Acetyl-4-hydroxy-1-ethyl-indole, 19. To dry 1,4-dioxane (20 mL) was added 5-acetyl-1-ethyl-4-oxo-4,5,6,7-tetrahydroindole (756 mg, 3.68 mmol) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 1.00 g, 4.42 mmol). The mixture was refluxed for 2 h after which the

mixture was cooled in an ice bath, filtered through celite, and washed with 1,4-dioxane. The filtrate was then evaporated under reduced pressure. The remaining crude product was purified via flash chromatography over silica gel, eluting with EtOAc:hexane (1:5) to give a pale yellow solid. 481 mg (64%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 13.63 (1H, s, O*H*), 7.51-7.49 (1H, d, *J* = 8 Hz, aromatic), 7.35-7.25 (1H, d, *J* = 4 Hz, aromatic), 6.84-6.82 (1H, d, *J* = 8 Hz, aromatic), 6.79-6.78 (1H, d, *J* = 4 Hz, aromatic), 4.18-4.12 (2H, q, *J* = 8 Hz, C*H*$_2$), 2.64 (3H, s, C*H*$_3$), 1.49-1.45 (3H, t, *J* = 8 Hz, C*H*$_3$).

7-Ethyl-4-hydroxypyrano[2,3-e]indol-2(7H)-one, 20. To a stirred suspension of sodium hydride (60% dispersion in mineral oil, 473 mg, 11.83 mmol) in anhydrous toluene (15 mL) was added dropwise 5-acetyl-4-hydroxy-1-ethyl-indole (481 mg, 2.37 mmol) in 10 mL anhydrous toluene under argon atmosphere. Diethyl carbonate (0.43 mL, 3.56 mmol) in 5 mL anhydrous toluene was added dropwise to the reaction vessel and the mixture was heated to 100 °C for 5 h. The solvent was removed under reduced pressure and the remaining residue was treated with H_2O (25 mL). The solution was acidified to a pH of 1-2 with 1M HCl causing a precipitate to form. The precipitate was filtered and washed several times with H_2O. The resulting crude product was used in the next step without further purification. Pale yellow solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$, ppm): δ 12.19 (1H, s, O*H*), 7.56-7.54 (1H, d, *J* = 8 Hz, aromatic), 7.53-7.52 (1H, d, *J* = 4 Hz, aromatic), 7.49-7.47 (1H, d, *J* = 8 Hz, aromatic), 6.72-6.71 (1H, d, *J* = 4 Hz, aromatic), 5.49 (1H, s, C*H*), 4.31-4.25 (2H, q, *J* = 8 Hz, C*H*$_2$), 1.40-1.36 (3H, t, *J* = 8 Hz, C*H*$_3$).

1-Ethyl-6-methylfuro[2',3':4,5]pyrano[2,3-e]indol-5(1H)-one (13). To a room temperature solution of 1-ethyl-6-methylfuro[2',3':4,5]pyrano[2,3-e]indol-5(1H)-one (100 mg, 0.44 mmol) in toluene (20 mL) was added a mixture of AcOH (0.13 mL, 2.18 mmol) and ammonium acetate (168 mg, 2.18 mmol) in EtOH (5 mL) under argon atmosphere.

Chloroacetone (0.174 mL, 2.18 mmol) was then added and the mixture was stirred at rt for 30 min, heated to 60°C for 30 min, followed by heating to reflux for 5 h. When no further transformation occurred as determined by TLC analysis, the reaction mixture was cooled to rt, diluted with H_2O, and the organic layer was extracted with EtOAc (3 x 30 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The remaining crude product was purified via flash chromatography over silica gel, eluting with EtOAc:hexane (1:6). 83 mg (71% yield). Pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.64-7.62 (1H, d, J = 8 Hz, aromatic), 7.34 (1H, s, aromatic), 7.31-7.29 (1H, d, J = 8 Hz, aromatic), 7.18-7.17 (1H, d, J = 4 Hz, aromatic), 6.95-6.94 (1H, d, J = 4 Hz, aromatic), 4.26-4.20 (2H, q, J = 8 Hz, CH_2), 2.39 (3H, s, CH_3), 1.53-1.49 (3H, t, J = 8 Hz, CH_3). MS: *m/z* 268.

Synthesis of 5-ethyl-1-naphthol intermediate 23.

1-Ethyl-5-methoxynaphthalene, 22. To a solution of ethylmagnesium bromide (1.0 M in THF, 85.13 mL, 85.13 mmol) was added zinc (II) chloride (0.5 M in THF, 11.36 mL, 5.68 mmol) at rt under argon atmosphere. The solution was stirred at rt for 1 h then cooled to 0°C at which point 5-methoxy-1-tetralone (10 g, 56.75 mmol) in 80 mL THF was added via syringe. The ice bath was removed and the mixture was allowed to warm to rt and stirred overnight. NH_4Cl was used to quench the reaction mixture and the organic layer was extracted with EtOAc (3 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography over silica gel was used to collect compound **21** using an EtOAc-hexane gradient. 7.60 g (65% yield). To a solution of **21** (7.59 g, 36.79 mmol) in 30 mL triglyme was added Pd/C (10% by weight, 7.59 g) and the mixture was refluxed for 2 d.

Once cooled, the mixture was filtered through celite, washed with EtOAc (3 x 50 mL), poured into a separatory funnel, extracted with EtOAc (3 x 50 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:9) to afford 1-ethyl-5-methoxynaphthalene. 4.13 g (60% yield). Light yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.16-8.14 (1H, d, *J* = 8 Hz, aromatic), 7.64-7.62 (1H, d, *J* = 8 Hz, aromatic), 7.43-7.42 (1H, d, *J* = 4 Hz, aromatic), 7.41-7.39 (1H, t, *J* = 4 Hz, aromatic), 7.38-7.34 (1H, t, *J* = 8 Hz, aromatic), 6.83-6.82 (1H, d, *J* = 4 Hz, aromatic), 4.00 (1H, s, OC*H*$_3$), 3.12-3.06 (2H, q, *J* = 8 Hz, C*H*$_2$), 1.39-1.35 (3H, t, *J* = 8 Hz, C*H*$_3$). MS: *m/z* 186.

5-Ethyl-1-naphthol, 23. To a stirred solution of 1-ethyl-5-methoxynaphthalene (4.12 g, 22.1 mmol) in 25 mL anhydrous DCM at 0°C was added boron tribromide (1.0 M in DCM, 66 mL, 66 mmol) dropwise. After removing the ice bath, the reaction mixture was allowed to warm to rt and heated to reflux for 3 h. After completion of the reaction as determined by TLC analysis, the reaction mixture was cooled to rt. H_2O was carefully added dropwise to quench remaining boron tribromide, poured into a separatory funnel containing H_2O, extracted with DCM (2 x 75 mL), dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to afford the crude product. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:4). 2.99 g (79% yield). Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.07-8.05 (1H, d, *J* = 8 Hz, aromatic), 7.65-7.63 (1H, d, *J* = 8 Hz, aromatic), 7.44-7.40 (1H, t, *J* = 8 Hz, aromatic), 7.36-7.35 (1H, d, *J* = 4 Hz, aromatic), 7.35-7.31 (1H, t, *J* = 8 Hz, aromatic), 6.82-6.81 (1H, d, *J* = 4 Hz, aromatic), 5.22 (1H, b, O*H*), 3.12-3.06 (2H, q, *J* = 8 Hz, C*H*$_2$), 1.39-1.35 (3H, t, *J* = 8 Hz, C*H*$_3$). MS: *m/z* 172.

Synthesis of 5-ethyl-1-methylbenzo[h]furo[3,2-c]quinolin-11(10H)-one (14).

2-((5-Ethylnaphthalen-1-yl)oxy)propanamide, 24. A mixture of 5-ethyl-1-naphthol (1.10 g, 6.39 mmol), ethyl 2-bromopropionate (1.24 mL, 9.58 mmol), anhydrous K_2CO_3 (1.76 g, 12.77 mmol), and KI (106 mg, 0.64 mmol) was refluxed in dry acetone (30 mL) for 12 h. The mixture was cooled to rt and the solvent was removed under reduced pressure. H_2O was added to the remaining residue, extracted three times with DCM, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Aqueous NH_4OH (28-30% NH_4 in H_2O, 15 mL) was added to the residue and the mixture was dissolved in 15 mL of EtOH. This mixture was stirred at rt for 18 h, then the solvent was removed under reduced pressure, and the remaining residue was purified by flash chromatography over silica gel, eluting with an EtOAc-hexane gradient to afford the desired aryloxyamide **24**. 500 mg (32% yield). Pale pink solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.16-8.14 (1H, d, *J* = 8 Hz, aromatic), 7.72-7.70 (1H, d, *J* = 8 Hz, aromatic), 7.46-7.42 (1H, t, *J* = 8 Hz, aromatic), 7.41-7.37 (1H, t, *J* = 8 Hz, aromatic), 7.39-7.37 (1H, d, *J* = 8 Hz, aromatic), 6.84-6.82 (1H, d, *J* = 8 Hz, aromatic), 6.43 (2H, b, N*H*$_2$), 4.90-4.86 (1H, q, *J* = 8 Hz, C*H*), 3.12-3.08 (2H, q, *J* = 8 Hz, C*H*$_2$), 1.73-1.71 (3H, d, *J* = 8 Hz, C*H*$_3$), 1.39-1.35 (3H, t, *J* = 8 Hz, C*H*$_3$). MS:*m/z* 244.

5-Ethylnaphthalen-1-amine, 25. A mixture of the prepared aryloxyamide **24** (1.27 g, 5.22 mmol) and KOH (586 mg, 10.44 mmol) in 25 mL dry DMSO was heated to 140°C for 12 h. The mixture was cooled to rt, diluted with saturated brine, and extracted with DCM. The combined organic layers were washed with saturated brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography was done over silica gel, using an EtOAc-hexane gradient. 200 mg (22% yield). Pale pink solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.71-7.69 (1H, d, *J* = 8 Hz, aromatic), 7.54-7.52 (1H, d, *J* = 8 Hz, aromatic), 7.40-7.36 (1H, t, *J*

= 8 Hz, aromatic), 7.36-7.29 (2H, m, aromatic), 6.80-6.78 (1H, d, *J* = 8 Hz, aromatic), 4.13 (2H, b, CH_2), 3.12-3.08 (2H, q, *J* = 8 Hz, CH_2), 1.39-1.35 (3H, t, *J* = 8 Hz, CH_3).

1-(1-Amino-5-ethylnaphthalen-2-yl)ethanone, 26. To a solution of boron trichloride (1.0M solution in DCM, 1.3 mL, 1.3 mmol) was added a solution of 5-ethylnaphthalen-1-amine (200 mg, 1.18 mmol) in 10 mL of 1,2-dichloroethane at 0°C under inert atmosphere. Anhydrous aluminum chloride (173 mg, 1.3 mmol) and anhydrous acetonitrile (0.062 mL, 1.18 mmol) were then added and the mixture was refluxed for 24 h. The solution was then cooled to 0°C, 2N HCl was added, and the mixture was stirred at 80°C for 30 min. Then, the mixture was extracted with DCM, washed with 1N NaOH and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude product. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:6). 210 mg (84% yield). Pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.81-7.79 (1H, d, *J* = 8 Hz, aromatic), 7.75-7.72 (1H, d, *J* = 12 Hz, aromatic), 7.58 (2H, b, NH_2), 7.44-7.38 (2H, m, aromatic), 7.27-7.26 (1H, d, *J* = 4 Hz, aromatic), 3.08-3.02 (2H, q, *J* = 8 Hz, CH_2), 2.67 (3H, s, CH_3), 1.38-1.34 (3H, t, *J* = 8 Hz, CH_3).

5-Ethyl-1-methylbenzo[h]furo[3,2-c]quinolin-11(10H)-one, 27. To a stirred suspension of sodium hydride (60% dispersion in mineral oil, 197 mg, 4.92 mmol) in anhydrous toluene (5 mL) was added dropwise 1-(1-amino-5-ethylnaphthalen-2-yl)ethanone (210 mg , 0.98 mmol) in 3 mL anhydrous toluene under argon atmostphere. Diethyl carbonate (0.18 mL, 1.48 mmol) in 2 mL anhydrous toluene was added dropwise to the reaction vessel and the mixture was heated to 100 °C for 5 h. The solvent was removed under reduced pressure and the remaining residue was treated with H_2O (25 mL). The solution was acidified to a pH of 1-2 with 1M HCl, causing a precipitate to form. The precipitate was filtered and washed several times with H_2O. To a room temperature solution of the crude hydroxylactam intermediate (80 mg, 0.34 mmol) in toulene (10

mL) was added a mixture of AcOH (0.096 mL, 1.68 mmol) and ammonium acetate (129 mg, 1.68 mmol) in EtOH (2 mL) under argon atmosphere. Chloroacetone (0.134 mL, 1.68 mmol) was then added and the mixture was stirred at rt for 30 min, heated to 60°C for 30 min, followed by heating to reflux for 24 h. When no further transformation occurred as determined by TLC analysis, the reaction mixture was cooled to rt, diluted with H_2O, and the organic layer was extracted with EtOAc (3 x 30 mL). The remaining brown residue was rinsed with EtOAc leaving a light yellow solid, which was dissolved in a mixture of $CHCl_3$ and MeOH and subjected to preparatory TLC analysis using a EtOAc:hexane (1:1) solvent mixture to separate the final product from the starting material. The band corresponding to the final product was scraped from the TLC plate and subjected to flash chromatography without a column, eluting with $CHCl_3$:MeOH (4:1) to provide a sample for NMR analysis. Pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ10.06 (1H, s, N*H*), 8.21-8.19 (1H, d, *J* = 8 Hz, aromatic), 7.94-7.92 (1H, d, *J* = 8 Hz, aromatic), 7.63-7.59 (1H, t, *J* = 8 Hz, aromatic), 7.51-7.49 (1H, d, *J* = 8 Hz, aromatic), 7.46 (1H, s, aromatic), 3.19-3.13 (2H, q, *J* = 8 Hz, C*H*$_2$), 2.51 (3H, s, C*H*$_3$), 1.43-1.39 (3H, t, *J* = 8 Hz, C*H*$_3$). MS: *m/z* (M+1) 278.

Synthesis of (2-methyl-1,3-dioxolan-2-yl)methanamine (34).

Benzyl 2-oxopropylcarbamate, 32. Benzyl chloroformate (25.70 mL, 180 mmol) was added dropwise under argon atmosphere to a mixture of 1-amino-propanol (3.12 mL, 40 mmol), 4-(dimethylamino)pyridine (978 mg, 8 mmol), and triethylamine (24 mL, 172 mmol) in dry DMSO (50 mL). The mixture was stirred for 5 h at 50°C, cooled to rt, quenched with aqueous saturated sodium bicarbonate, and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:5). The combined

fractions were concentrated under reduced pressure to afford the intermediate benzyl 2-hydroxypropylcarbamate, **31**. DMSO (3.57 mL50.21 mmol) was added to a solution of oxalyl chloride (3.28 mL, 37.65 mmol) in 40 mL DCM at -78°C under argon atmosphere. This mixture was stirred for 20 min after which a solution of benzyl 2-hydroxypropylcarbamate in 20 mL was added and the mixture was stirred for a further 1 h. Triethylamine (10.66 mL) was then added to the reaction and the mixture was allowed to warm to rt, followed by a wash with 1M HCl and aqueous saturated sodium bicarbonate, successively. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:2). 1.40 g (17% yield). Clear oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.36-7.31 (5H, m, aromatic), 5.46 (1H, b, N*H*), 5.12 (2H, s, CH_2), 4.1 (2H, s, CH_2), 2.19 (3H, s, CH_3). MS: *m/z* (M+1) 208.

Benzyl (2-methyl-1,3-dioxolan-2-yl)methylcarbamate, 33. A mixture of benzyl 2-oxopropylcarbamate (1.40 g, 6.75 mmol), pyridinium *p*-toluenesulfonate (71 mg, 0.28 mmol), and ethylene glycol (1.21 mL, 21.61 mmol) in dry benzene (20 mL) was refluxed for 4 h. H_2O was added to quench the reaction and the organic layer was washed with H_2O, saturated aqueous sodium bicarbonate, and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:3). 1.62 g (95% yield). Clear oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.36-7.31 (5H, m, aromatic), 5.11 (2H, s, CH_2), 4.97 (1H, b, N*H*), 3.99-3.88 (4H, m, (CH_2)$_2$), 3.36-3.35 (2H, d, *J* = 8Hz, CH_2), 1.33 (3H, s, CH_3). MS: *m/z* (M+1) 252.

(2-Methyl-1,3-dioxolan-2-yl)methanamine, 34. Benzyl (2-methyl-1,3-dioxolan-2-yl)methylcarbamate (1.62 g, 6.44 mmol), $Pd(OH)_2$/C (20% loading on activated charcoal, 1.69 g), and EtOAc (40 mL) were added to a Parr shaker hydrogenation apparatus and shaken under

H_2 atmosphere at 50 psi. The mixture was filtered through celite and the filtrate was evaporated under reduced pressure and dried in the air overnight to afford (2-methyl-1,3-dioxolan-2-yl)methanamine. 655 mg (87% yield). Pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 4.01-3.97 (4H, m, (CH_2)$_2$), 2.76 (2H, s, CH_2), 1.48 (2H, s, NH_2), 1.30 (3H, s, CH_3). MS: *m/z* 117.

Synthesis of 6-ethyl-1-methylbenzo[7,8]chromeno[4,3-b]pyrrol-11(3H)-one (15).

7-Ethyl-4-hydroxy-2H-benzo[h]chromen-2-one, 28. A mixture of 5-ethyl-1-naphthol (1.34 g, 7.75 mmol) and Meldrum's acid (1.17 g, 8.14 mmol) was heated to 90°C for 5 h. After cooling to rt, the mixture was then diluted with saturated aqueous sodium bicarbonate and extracted with EtOAc. The aqueous layer was acidified to a pH of 1-2 using 12M HCl and extracted several times with DCM. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford the malonic acid ester intermediate. Eaton's reagent (13 mL) was then added and the mixture was heated to 70°C for 1.5 h. After the reaction mixture cooled to rt, H_2O was added while stirring, the precipitate was filtered off and washed several times with H_2O, dried in the air overnight and recrystallized from EtOH. 610 mg (33% yield). Light yellow solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$, ppm): δ 12.61 (1H, s, OH), 8.26-8.24 (1H, d, *J* = 8 Hz, aromatic), 8.01-7.98 (1H, d, *J* = 12 Hz, aromatic), 7.88-7.84 (1H, t, *J* = 8 Hz, aromatic), 7.66-7.58 (2H, m, aromatic), 5.69 (1H, s, C*H*), 3.15-3.09 (2H, q, *J* = 8 Hz, CH_2), 1.34-1.30 (3H, t, *J* = 8 Hz, CH_3). MS: *m/z* 240.

4-Chloro-7-ethyl-2H-benzo[h]chromen-2-one, 29. A mixture of 7-ethyl-4-hydroxy-2H-benzo[h]chromen-2-one (360 mg, 1.5 mmol), triethylamine (0.21 mL, 1.5 mmol), and 5 mL phosphorous oxychloride was heated to reflux for 1 h. The mixture was cooled to rt, poured into ice water and extracted with DCM. The organic layer was dried over $MgSO_4$, filtered and

concentrated under reduced pressure. Flash chromatography was done over silica gel, eluting with EtOAc:hexane (1:9). 160 mg (41% yield). Light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.47-8.45 (1H, d, *J* = 8 Hz, aromatic), 7.99-7.97 (1H, d, *J* = 12 Hz, aromatic), 7.88-7.86 (1H, d, *J* = 8 Hz, aromatic), 7.62-7.54 (2H, m, aromatic), 6.69 (1H, s, C*H*), 3.17-3.11 (2H, q, *J* = 8 Hz, CH_2), 1.41-1.37 (3H, t, *J* = 8 Hz, CH_3). MS: *m/z* 259.

2.6.2 Cytotoxicity Assay

The following human tumor cell lines were used in the assay: A549 (lung carcinoma), KB (originally isolated from epidermoid carcinoma of the nasopharynx), KB-VIN (VIN-resistant KB subline showing MDR phenotype by overexpressing P-gp), MCF-7 (estrogen receptor (ER)-positive, HER2-negative breast cancer), SK-BR-3 (ER-negative, progesterone receptor (PgR)-negative, HER2-overexpressing breast cancer), ZR-75-1 (ER-positive, HER2-overexpressing breast cancer) . All cell lines were obtained either from the Lineberger Comprehensive Cancer Center (UNC-CH) or from the ATCC (Manassas, VA), except KB-VIN, which was a generous gift of Professor Y.-C. Cheng (Yale University). All cell lines were maintained in RPMI-1640 medium containing 2 mM l-glutamine and 25 mM HEPES (HyClone), supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 100 µg/mL streptomycin, 100 IU/mL penicillin, and 0.25 µg/mL amphotericin B (Cellgro) at 37 °C with 5% CO_2 in air. KB-VIN cell line was maintained in the presence of 100 nM VIN. Freshly prepared cell suspensions were seeded with compounds in 96-well microtiter plates at densities of 4,000-7,500 cells per well for A549, MDA-MB-231, KB and KB-VIN. 10,000-12,500 cells per well were used for MCF-7, SK-BR-3 and ZR-75-1. Under the assay conditions, the highest concentration of DMSO in the cultures (0.1% v/v) was without effect on cell growth. The living cells were fixed in 10% trichloroacetic

acid after 72 h, and then stained with 0.04% sulforhodamine B (SRB). Then, the protein-bound SRB was solubilized with 10 mM Tris base, and absorbance of SRB was measured at 515 nm using a microplate reader (ELx800, BioTek) operated by Gen5 software (BioTek). The mean IC_{50} is the concentration of agent that reduced cell growth by 50% compared with vehicle (DMSO) control under the experimental conditions and is the average from at least three independent experiments with duplicate samples.

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