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Antitumor agents 279. Structure-activity relationship and in vivo studies of novel 2-(furan-2-yl)naphthalen-1-ol (FNO) analogs as potent and selective anti-breast cancer agents

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Abstract

In our ongoing modification study of neo-tanshinlactone (**1**), we discovered 2-(furan-2-yl)naphthalen-1-ol (FNO) derivatives **3** and **4** as a new class of anti-tumor agents. To explore structure-activity relationships (SAR) of this scaffold, 18 new analogs, **6–12** and **14–24**, were designed and synthesized. The C11-esters **7** and **12** displayed broad anti-tumor activity (ED₅₀ 1.1–4.3 µg/mL against seven cancer cell lines), while C11-hydroxymethyl **14** showed unique selectivity against the SKBR-3 breast cancer cell line (ED₅₀ 0.73 µg/mL). Compounds **15** and **22** displayed potent and selective anti-breast tumor activity (ED₅₀ 1.7 and 0.85 µg/mL, respectively, against MDA-MB-231). The SAR results demonstrated that the substitutions from the ring-opened lactone ring C of **1** are critical to the anti-tumor potency as well as the apparent tumor-tissue type selectivity. Treatment with **3** in *Brca1*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice models significantly inhibited the proliferation of mammary epithelial cells and branching of mammary glands.

Keywords

2-(furan-2-yl)naphthalen-1-ol analogs; structure-activity relationships; anti-breast tumor agents

Neo-tanshinlactone (**1**), a natural product from *Salvia miltiorrhiza*, and its analog, 4-ethyl neo-tanshinlactone (**2**), are potent and selective anti-breast cancer agents (Figure 1).^{1–3} In our prior studies, they exhibited high tumor tissue-type as well as breast cancer cell line selectivity. The selective anti-breast tumor activity of **2** in mice models is consistent with the

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results in vitro.³ In our continuing study, we explored how the individual rings A–D in the molecule influence the in vitro anti-breast tumor activity. The results led to the discovery of a novel class of anti-breast cancer agents, 2-(furan-2-yl)naphthalen-1-ol (FNO) analogs (e.g., **3** and **4**) by opening ring-C.⁴ Our previous studies also explored the preliminary SAR and proved that the C8 and C11 substituents can greatly affect both potency and selectivity. FNO analog **3** showed significant potency (ED₅₀ 0.3 µg/mL) and selectivity against the ZR-7-51 (ER+, HER2+) cell line compared with other cancer cell lines tested, while **4** exhibited activity against all cancer cell lines tested.⁴ We wanted to use these promising results to design novel analogs with better pharmaceutical profiles and develop them as clinical trials candidates.

The initial studies showed that Et, H, and Me groups are preferred at the C4, C14, and C15 positions, respectively, of the FNO skeleton, and we retained this substitution pattern in our current study.⁴ However, we expanded the identities of the groups at the C8 and C11 positions (**3** and **4** contain hydroxyl/carboxylic acid and methyl ether/methyl ester, respectively). Some of the different combinations at these positions included ether/carboxylic acid, ether/ester, ether/amide, and ether/substituted methyl. We incorporated groups with different sizes and electrostatic effects to establish SAR and develop new lead compounds.

As shown in Scheme 1, carboxylic acids **5** and **6**, synthesized by the method reported before,⁴ were converted to esters **7**, **8**, and **12**, respectively, with thionyl chloride and the appropriate alcohols at room temperature.⁵ In addition, treatment of **5** with Lawesson's reagent led to methylthioate **9**.³ With methanamine to amide **10**, and with hydroxybenzotriazole (HOBt) to benzotriazole ester **11**. Meanwhile, known diol **13** was treated with iodomethane or iodoethane in the presence of Cs₂CO₃ to generate phenoxyethers **14** and **15**, respectively.⁵ The primary hydroxyl groups of **14** and **15** were alkylated with iodomethane, iodoethane, or 1,1,1-trifluoro-2-iodoethane in the presence of sodium hydride to obtain **17**, **18**, **20**, and **23**. Acetates **16**, **21**, and **24** were synthesized by acetylation of **13**–**15** with Ac₂O in pyridine. Benzylic bromination of **14** with triphenylphosphine, bromine, and imidazole afforded bromomethyl **19**. Ester **22** was obtained by reaction of **14** with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), 4-dimethylaminopyridine (DMAP), and 3-(diethylamino)propanoic acid hydrochloride.

All newly synthesized analogs **6**–**12** and **14**–**24**⁶ were tested for cytotoxic activity against a focused panel of human tumor cell lines according to previously published methods (Table 1).³ Cell lines included A549 (non small cell lung cancer), DU145 (prostate cancer cell line), KB (nasopharyngeal carcinoma) and KB-VIN (MDR KB subline selected using vincristine), MDA-MB-231 (estrogen receptor negative basal-like breast cancer), SK-BR-3 (estrogen receptor negative, HER2 over-expressing luminal-like breast cancer), and ZR-75-1 (estrogen receptor positive breast cancer, HER2 over-expressing luminal-like breast cancer).

Among analogs (**6**–**12**) with ether (OR¹) and ester or similar (R²) groups at C8 and C11, respectively, compound **12** (ethyl ether, methyl ester) showed comparable activity to **4** (methyl ether, methyl ester). The potencies were affected greatly by the R² functional group, with the following rank order: COOMe (**12**) ~ COOEt (**7**) > CSOMe (**9**) >> COOPr (**8**), CONHMe (**10**), COOH (**6**). Free carboxylic acid, larger ester, thioester, and amide groups (**6**, **8**, **9**, **10**) dramatically reduced or eliminated potency. The results also suggested that the combination of ether and carboxylic ester at C8 and C11, respectively, may lead to broad inhibition of cancer cell lines from different tissues (**4**, **7**, **9**, and **12**). Small ester groups, including COOMe (**4**, **12**) and COOEt (**7**), were preferred at C11.

Among analogs (**14–24**) with ether (OR³) and substituted methyl (CH₂R⁴) groups at C8 and C11, respectively, compound **14** with a C11 hydroxymethyl group (R⁴ = OH) displayed significant potency and unique selectivity, being more than nine-fold more active against the SKBR-3 breast cancer cell line (ED₅₀ 0.73 µg/mL) compared with other cancer cell lines tested (ED₅₀ 6.6 – >10 µg/mL). Interestingly, compound **15**, in which the C8 methyl ether (OR³ = OMe) of **14** is replaced by an ethyl ether (OR³ = OEt), showed high potency and selectivity against ZR-75-1 and MDA-MB-231 (ED₅₀ 1.4 and 1.7 µg/mL, respectively). These results indicate that the size of the R₃ group could change the apparent cell line selectivity. Compounds **15** and **22** (R₄ = OCOCH₂CH₂NEt₂; ED₅₀ 1.7 µg/mL, ZR-75-1; 0.85 µg/mL, MDA-MB-231) displayed comparable activity against all cell lines tested. Changing the R⁴ group in the C11 substituent to other groups (e.g., acetate, bromide, methoxy) generally led to lower potency. Although diacetate **16** (R⁴ = OR³ = OAc) was completely inactive, monoacetates **21** and **24** (R⁴ = OAc, R₂ = Me and Et, respectively) were active against ZR-75-1 (**21** and **24**) and MDA-MB-231 (**24**). The bromomethyl compound **19** (R⁴ = Br) was also selective against ZR-75-1, and **23** (R⁴ = OMe) showed moderate activity against MDA-MB-231. These results suggested that the identity of the R⁴ group might affect selectivity against ZR-75-1. Interestingly, **23** and **24** showed some growth inhibition against the P-glycoprotein over-expressing KB-VIN cell line, but no inhibition against the parent KB cell line. Further work will be needed to determine whether the apparent MDR-selectivity of **23** and **24** is significant.

Subsequently, we evaluated the anti-proliferation activity of **3**. *BRCA1* is the first cloned familial breast cancer susceptibility gene that is expressed in all cells.^{7,8} *BRCA1* mutations significantly increase breast and ovarian cancer risk in female carriers. Male *BRCA1* carriers also have increased breast cancer risk, but the risk factor is lower than that of *BRCA2* carriers.⁸ The *BRCA1* protein plays a critical role in DNA damage repair, cell cycle checkpoint control, and transcriptional regulation.^{9,10} *Brca1*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice show extensive mammary epithelial proliferation that can be effectively inhibited by the progesterone antagonist mifepristone (RU 486).¹¹ Thus, this mouse model can provide critical information on in vivo activity of novel experimental anti-breast tumor agents.

To assess the effects of **3** on mammary epithelial proliferation in vivo, we treated *Brca1*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice and wild-type mice with **3**.^{12–14} As previously reported, mammary glands from control vehicle-treated *Brca1*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice showed remarkable accumulation of side branches and extensive alveolar formation compared with similarly treated wild-type mice at three months of age (Figure 2A, a & c). Compared with vehicle-treated control, mammary gland branching points in *Brca1*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c and wild-type mice were significantly reduced, with a 70 ± % reduction in the former and a 29 ± % reduction in the latter (Figure 2A, b & c; Figure 2B).

To address the inhibitory effects of **3** on mammary epithelial cell proliferation, bromodeoxyuridine (BrdU) was used for labeling the S-phase. Drinking water containing BrdU (1 mg/mL) was supplied to mice during the last three days of treatment. BrdU-positive mammary epithelial cells were quantified and found to be about 3.5 fold higher in the vehicle-treated *Brca1*/*p53*-deficient mice than wild-type mice (Figure 3A, a & c; Figure 3B), indicating that the proliferation of mammary gland in *Brca1*/*p53*-deficient mice is 3.5 fold higher than wild-type. In **3**-treated wild-type mice, BrdU positive mammary epithelial cells were undetectable, while BrdU-positive cells were found occasionally in the extracellular matrix of the mammary gland (Figure 3A, b). These findings indicated that the inhibitory effects of **3** are likely specific to the mammary epithelial cells. Treatment of *Brca1*/*p53*-deficient mice with **3** dramatically reduced BrdU-positive mammary epithelial cells (83%) (Figure 3A, d; Figure 3B). This result implied that **3** inhibited the onset of the cell-cycle S-phase in both wild-type and *Brca1*/*p53*-deficient mammary epithelial cells.

In summary, we established the SAR of FNO analogs and developed lead compounds with high potency and selectivity. The results indicated that the C8 and C11 substituents, which are analogous to the opened ring-C, play a critical role in both potency and apparent tumor-tissue selectivity. Compounds **7** and **12** displayed broad activity spectrum against all cancer cell lines tested, while **15** and **22** showed more than four-fold greater potency against ZR-751 and MDA-MB-231 (ED₅₀ 1.7 and 0.85 µg/mL, respectively, against ZR-751), and **14** showed more than nine-fold greater potency against SKBR-3 (ED₅₀ 0.73 µg/mL). Based on this study, SAR results are as follows. (1) The combination of ether at C8 and ester at C11 may lead to broad inhibition of cancer cell lines from different tissues, and the combination of ether at C8 and carboxylic acid at C11 is not favored. (2) Small ester groups at C11 are preferred. (3) An acetoxymethyl or bromomethyl group at C11 might induce selectivity against ZR-75-1.

Proliferation of mammary epithelial cells is regulated by ovarian hormones.¹⁵ Several studies have indicated that *BRCA1* suppresses the hyperactivation of estrogen and progesterone receptors.¹⁶ Mutation in *BRCA1* leads to expansion of the mammary epithelial cells.¹⁷ The robust expansion of mammary epithelial cells in the *Brcal* mouse model of human breast cancer provides increased sensitivity and accuracy for identification and evaluation of compounds with clinically significant anti-proliferation activity in vivo. Although molecular targets of **3** are currently unknown, in vivo studies, including the branching of mammary glands and the prevention of mammary epithelial cells entering S-phase, showed that **3** has significant effects on preventing mammary epithelial cell proliferation in both wild-type and *Brcal/p53*-deficient mice.

Acknowledgments

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6. **Materials and Methods.** ¹H NMR spectra were measured on a 300 or 400 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was CDCl₃ unless indicated. Mass spectra were measured on a Shimadzu LC-MS2010 instrument. Thin-layer chromatography (TLC) and preparative TLC were performed on precoated silica gel GF plates purchased from Merck, Inc. Biotage Flash+ or Isco Companion systems were used for flash chromatography. Silica gel (200–400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc. and Fisher, Inc. Analogs **6–12** and **14–24** were prepared by the optimized methods described in our previous paper. Key compound **14** was >95% pure on the basis of HPLC conditions. Analytical data of target compounds are shown as follows. Compound **6**: ¹H NMR (300 MHz, CD₃COCD₃, ppm): δ 8.10 (d, 1H, *J* = 7.5 Hz, Ar-*H*), 7.89 (d, 1H, *J* = 8.7 Hz, Ar-*H*), 7.57 (m, 2H, Ar-*H*), 7.46 (m, 2H, Ar-*H* & OCH), 3.82 (q, 2H, *J* = 6.9 Hz, OCH₂CH₃), 3.13 (q, 2H, *J* = 7.5 Hz, CH₂CH₃), 2.25 (d, 3H, *J* = 1.2 Hz, CH₃), 1.36 (t, 3H, *J* = 7.2 Hz, CH₂CH₃), 1.25 (t, 3H, *J* = 7.2 Hz, OCH₂CH₃). MS *m/z* 323 (M⁺-1); Compound **7**: ¹H NMR (300 MHz, CDCl₃, ppm):

δ 8.10 (d, 1H, J = 8.1 Hz, Ar-*H*), 7.84 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.55 (d, 1H, J = 9.0 Hz, Ar-*H*), 7.46 (t, 1H, J = 8.4 Hz, Ar-*H*), 7.39 (d, 1H, J = 8.7 Hz, Ar-*H*), 7.35 (d, 1H, J = 0.9 Hz, OCH), 4.17 (q, 2H, J = 7.2 Hz, OCH₂CH₃), 3.70 (s, 3H, OCH₃), 3.12 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.26 (d, 3H, J = 1.2 Hz, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃), 1.07 (t, 3H, J = 7.2 Hz, OCH₂CH₃). MS m/z 339 (M^+ +1); Compound **8**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 8.09 (d, 1H, J = 8.0 Hz, Ar-*H*), 7.83 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.53 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.45 (t, 1H, J = 8.8 Hz, Ar-*H*), 7.38 (d, 1H, J = 6.4 Hz, Ar-*H*), 7.34 (d, 1H, J = 1.2 Hz, OCH), 4.07 (t, 2H, J = 6.8 Hz, OCH₂CH₂CH₃), 3.70 (s, 3H, OCH₃), 3.11 (q, 2H, J = 7.6 Hz, CH₂CH₃), 2.27 (d, 3H, J = 1.2 Hz, CH₃), 1.45 (h, 2H, J = 7.6 Hz, OCH₂CH₂CH₃), 1.38 (t, 3H, J = 7.2 Hz, CH₂CH₃), 0.65 (t, 3H, J = 7.2 Hz, OCH₂CH₂CH₃). MS m/z 353 (M^+ +1); Compound **9**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 8.08 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.83 (d, 1H, J = 9.2 Hz, Ar-*H*), 7.55 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.45 (t, 1H, J = 8.0 Hz, Ar-*H*), 7.38 (d, 1H, J = 6.8 Hz, Ar-*H*), 7.33 (d, 1H, J = 0.8 Hz, OCH), 3.96 (s, 3H, OCH₃), 3.66 (s, 3H, OCH₃), 3.11 (q, 2H, J = 6.8 Hz, CH₂CH₃), 2.28 (s, 3H, CH₃), 1.39 (t, 3H, J = 6.8 Hz, CH₂CH₃). MS m/z 341 (M^+ +1); Compound **10**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 8.09 (d, 1H, J = 8.2 Hz, Ar-*H*), 7.88 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.50 (m, 2H, Ar-*H*), 7.43 (t, 1H, J = 7.6 Hz, Ar-*H*), 7.32 (d, 1H, J = 1.2 Hz, OCH), 6.39 (brs, 1H, NH), 3.75 (s, 3H, OCH₃), 3.10 (q, 2H, J = 7.6 Hz, CH₂CH₃), 2.75 (d, 3H, J = 4.8 Hz, NCH₃), 2.49 (d, 3H, J = 1.2 Hz, CH₃), 1.39 (t, 3H, J = 7.6 Hz, CH₂CH₃). MS m/z 324 (M^+ +1); Compound **11**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 8.14 (d, 1H, J = 8.4 Hz, Ar-*H*), 8.00 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.92 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.70 (t, 1H, J = 7.2 Hz, Ar-*H*), 7.51 (m, 3H, Ar-*H*), 7.37 (m, 2H, Ar-*H* & OCH), 3.82 (s, 3H, OCH₃), 3.10 (q, 2H, J = 7.6 Hz, CH₂CH₃), 2.35 (d, 3H, J = 1.2 Hz, CH₃), 1.36 (t, 3H, J = 7.6 Hz, CH₂CH₃). MS m/z 428 (M^+ +1); Compound **12**: ¹H NMR (300 MHz, CDCl₃, ppm): δ 8.08 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.83 (d, 1H, J = 8.7 Hz, Ar-*H*), 7.53 (d, 1H, J = 9.3 Hz, Ar-*H*), 7.44 (t, 1H, J = 8.1 Hz, Ar-*H*), 7.37 (d, 1H, J = 6.3 Hz, Ar-*H*), 7.32 (d, 1H, J = 0.9 Hz, OCH), 3.76 (q, 2H, J = 7.2 Hz, OCH₂CH₃), 3.67 (s, 3H, OCH₃), 3.09 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.23 (d, 3H, J = 0.9 Hz, CH₃), 1.38 (t, 3H, J = 7.5 Hz, CH₂CH₃), 1.26 (t, 3H, J = 7.2 Hz, OCH₂CH₃). MS m/z 361 (M^+ +23); Compound **14**: ¹H NMR (300 MHz, CDCl₃, ppm): δ 8.09 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.90 (d, 1H, J = 9.0 Hz, Ar-*H*), 7.55 (d, 1H, J = 8.7 Hz, Ar-*H*), 7.49 (dd, 1H, J = 7.2, 8.1 Hz, Ar-*H*), 7.40 (d, 1H, J = 6.3 Hz, Ar-*H*), 7.37 (d, 1H, J = 1.2 Hz, OCH), 4.48 (d, 2H, J = 6.0 Hz, CH₂OH), 3.70 (s, 3H, OCH₃), 3.12 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.75 (t, 1H, J = 6.3 Hz, OH), 2.17 (d, 3H, J = 1.2 Hz, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃). HRMS Calcd for C₁₉H₂₁O₃ (M +H⁺): 297.1485, found: 297.1470; Compound **15**: ¹H NMR (400 MHz, CDCl₃): 8.09 (d, 1H, J = 8.3 Hz, Ar-*H*), 7.88 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.53 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.48 (dd, 1H, J = 8.4 and 7.0 Hz, Ar-*H*), 7.40 (d, 1H, J = 7.0 Hz, Ar-*H*), 7.38 (br s, 1H, Ar-*H*), 4.47 [s, 2H, CH₂OH], 3.85 (q, 2H, J = 7.1 Hz, OCH₂CH₃), 3.12 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.17 (s, 3H, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃), 1.29 (t, 3H, J = 7.1 Hz, OCH₂CH₃); Compound **16**: ¹H NMR (400 MHz, CDCl₃): 8.02 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.73 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.59 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.47 (dd, 1H, J = 8.4 and 7.0 Hz, Ar-*H*), 7.39 (d, 1H, J = 7.0 Hz, Ar-*H*), 7.33 (br s, 1H, Ar-*H*), 5.05 [s, 2H, CH₂OAc], 3.12 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.10 [s, 3H, OC(O)CH₃], 2.03 (s, 3H, CH₃), 2.36 [s, 3H, OC(O)CH₃], 2.09 [s, 3H, CH₂OC(O)CH₃], 2.08 (s, 3H, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃). MS m/z 389 (M^+ +Na); Compound **17**: ¹H NMR (300 MHz, CDCl₃, ppm): δ 8.13 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.85 (d, 1H, J = 9.0 Hz, Ar-*H*), 7.56 (d, 1H, J = 8.7 Hz, Ar-*H*), 7.47 (t, 1H, J = 7.2 Hz, Ar-*H*), 7.40 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.37 (s, 1H, OCH), 4.40 (s, 2H, CH₂OH), 3.71 (s, 3H, OCH₃), 3.32 (s, 3H, OCH₃), 3.11 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.14 (s, 3H, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃). MS m/z 311 (M^+ +1); Compound **18**: ¹H NMR (300 MHz, CDCl₃, ppm): δ 8.13 (d, 1H, J = 8.1 Hz, Ar-*H*), 7.85 (d, 1H, J = 9.0 Hz, Ar-*H*), 7.57 (d, 1H, J = 8.7 Hz, Ar-*H*), 7.47 (t, 1H, J = 8.4 Hz, Ar-*H*), 7.40 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.35 (d, 1H, J = 1.2 Hz, OCH), 4.44 (s, 2H, CH₂OH), 3.71 (s, 3H, OCH₃), 3.48 (q, 2H, J = 7.2 Hz, OCH₂CH₃), 3.12 (q, 2H, J = 7.5 Hz, CH₂CH₃), 2.15 (d, 3H, J = 1.2 Hz, CH₃), 1.39 (t, 3H, J = 7.5 Hz, CH₂CH₃), 1.20 (t, 3H, J = 7.2 Hz, OCH₂CH₃). MS m/z 325 (M^+ +1); Compound **19**: ¹H NMR (400 MHz, CDCl₃): 8.09 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.88 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.55 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.49 (dd, 1H, J = 8.4 and 7.2 Hz, Ar-*H*), 7.44-7.35 (m, 2H, Ar-*H*), 4.49 [s, 2H, CH₂Br], 3.70 (s, 3H, OCH₃), 3.11 (q, 2H, J = 7.4 Hz, CH₂CH₃), 2.17 (s, 3H, CH₃), 1.39 (t, 3H, J = 7.4 Hz, CH₂CH₃); Compound **20**: ¹H NMR (400 MHz, CDCl₃): 8.12 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.86 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.55 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.47 (dd, 1H, J = 8.4 and 7.2 Hz, Ar-*H*), 7.41-7.38 (m, 2H, Ar-*H*), 4.92 [s, 2H, CH₂OCH₂CF₃], 3.71 (s, 3H, OCH₃), 3.49 (t, 2H, J = 9.4 Hz, OCH₂CF₃), 3.11 (q, 2H, J = 7.4 Hz, CH₂CH₃), 2.17 (s, 3H, CH₃), 1.39 (t, 3H, J = 7.4 Hz, CH₂CH₃). MS m/z 402 (M^+ +Na);

Compound **21**: ^1H NMR (400 MHz, CDCl_3): 8.11 (d, 1H, $J = 8.4$ Hz, Ar-*H*), 7.86 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.54 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.46 (dd, 1H, $J = 8.4$ and 7.2 Hz, Ar-*H*), 7.40-7.36 (m, 2H, Ar-*H*), 5.10 [s, 2H, $\text{CH}_2\text{OC(O)-}$], 3.70 (s, 3H, OCH_3), 3.11 (q, 2H, $J = 7.4$ Hz, CH_2CH_3), 2.11 [s, 3H, C(O)CH_3], 2.04 (s, 3H, CH_3) 1.39 (t, 3H, $J = 7.4$ Hz, CH_2CH_3). MS m/z 339 ($\text{M}^+ - 1$); Compound **22**: ^1H NMR (400 MHz, CDCl_3): 8.10 (d, 1H, $J = 8.4$ Hz, Ar-*H*), 7.84 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.54 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.46 (dd, 1H, $J = 8.4$ and 7.2 Hz, Ar-*H*), 7.40-7.36 (m, 2H, Ar-*H*), 5.11 [s, 2H, $\text{CH}_2\text{OC(O)-}$], 3.70 (s, 3H, OCH_3), 3.10 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 2.74 (t, 2H, $J = 7.5$ Hz, $\text{CH}_2\text{CH}_2\text{NEt}_2$), 2.45 [q, 4H, $J = 7.2$ Hz, $\text{N(CH}_2\text{CH}_3)_2$], 2.42 [t, 2H, $J = 7.2$ Hz, $\text{C(O)-CH}_2\text{CH}_2\text{NEt}_2$], 2.11 (s, 3H, CH_3) 1.39 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 0.97 [t, 6H, $J = 7.2$ Hz, $\text{N(CH}_2\text{CH}_3)_2$]. MS m/z 424 ($\text{M}^+ - 1$); Compound **23**: ^1H NMR (400 MHz, CDCl_3): 8.13 (d, 1H, $J = 8.4$ Hz, Ar-*H*), 7.84 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.55 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.45 (dd, 1H, $J = 8.4$ and 7.0 Hz, Ar-*H*), 7.40-7.33 (m, 2H, Ar-*H*), 4.40 [s, 2H, CH_2OMe], 3.82 (q, 2H, $J = 7.0$ Hz, OCH_2CH_3), 3.30 (s, 3H, CH_2OCH_3), 3.11 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 2.14 (s, 3H, CH_3), 1.39 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 1.30 (t, 3H, $J = 7.0$ Hz, OCH_2CH_3); Compound **24**: ^1H NMR (400 MHz, CDCl_3): 8.13 (d, 1H, $J = 8.4$ Hz, Ar-*H*), 7.84 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.55 (d, 1H, $J = 8.8$ Hz, Ar-*H*), 7.44 (dd, 1H, $J = 8.4$ and 7.0 Hz, Ar-*H*), 7.40-7.35 (m, 2H, Ar-*H*), 5.10 [s, 2H, CH_2OAc], 3.82 (q, 2H, $J = 7.0$ Hz, OCH_2CH_3), 3.10 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 2.10 [s, 3H, OC(O)CH_3], 2.03 (s, 3H, CH_3), 1.39 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 1.30 (t, 3H, $J = 7.0$ Hz, OCH_2CH_3). MS m/z 353 ($\text{M}^+ - 1$).

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12. ***Brca1^{flp/tp}p53^{flp/tp}Cre Mutant Mice***. Generation of *Brca1^{flp/tp}p53^{flp/tp}Cre* and *p53^{flp/tp}Cre* mice has been described previously.^{11,13} The mice were in a C57BL/6 and 129/Sv, mixed background. All animal experiments were in accordance with guidelines of federal and Institutional Animal Care and Use Committee at the University of California, Irvine. **Treatment with 3**. Three-month-old mice were treated with 0.1 mg of **3** or vehicle daily for 11 days. Stock solution of **3** was 10 mg/mL in dimethylsulfoxide. A mixture of 10 μL of stock solution, 30 μL of 40% polyethylene glycol, and 60 μL of 0.9% NaCl solution was prepared at the time of treatment. Vehicle includes all solution except **3**. Vehicle or compound was administered i.p. every day for 11 days.
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14. **Histology and Immunohistochemistry**. The fourth pair glands were dissected and spread on a glass slide. After fixation with Carnoy's fixative for three h, the tissues were hydrated and stained in Carmine alum overnight as described (<http://mammary.nih.gov/tools/histological/Histology/index.html#a1>). Branching points in three random areas totaling approximately 2 mm² were counted. For histological section, tissues were fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) at 4 °C overnight followed by paraffin embedding. Paraffin sections were stained with hematoxylin and eosin and examined by light microscopy. Immunostaining was performed following the protocol described in the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA.) To retrieve the antigen, slides were heated for 20 min in 10 mM citrate buffer, pH 6.0, in a microwave oven. BrdU monoclonal antibody (GeneTex Inc. Irvine, CA) at 1:1000 dilution, and cyclin D1 polyclonal rat antibody (NeoMarkers/Thermo Fisher Scientific, Fremont, CA) at 1:500 dilution, respectively, were used for immunostaining.
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R = Me: Neo-tanshinlactone (**1**)
R = Et: 4-Ethyl neo-tanshinlactone (**2**)

3: R = H
4: R = Me

Figure 1.
Structures of neo-tanshinlactone (**1**), 4-ethyl neo-tanshinlactone (**2**), and FNO analogs **3** and **4**

Figure 2.

Treatment with **3** leads to decreased mammary ductal branching. Mammary gland whole mounts were prepared from wild-type and *Brca1^{f11/f11}p53^{f5&6/f5&6}Cre^c* mice following treatment with 0.1 mg of **3** daily for 11 days. (A) Mammary gland whole mounts of vehicle- (a & c) and **3**-treated (b & d) 3-month-old mice. (B) Number of branching points in wild-type (*wt*) and *Brca1^{f11/f11}p53^{f5&6/f5&6}Cre^c* mammary glands. The data represent average of branch points in five randomly selected areas $\pm$ SD. (* $P \leq 0.002$; ** $P \leq 0.0002$)

Figure 3.

Treatment with **3** leads to decreased mammary epithelial proliferation. BrdU-containing drinking water was provided during the last three days of **3** treatment. (A) Cells uptaking BrdU, indicative of DNA synthesis, were detected by immunostaining. Arrows indicate BrdU-positive cells. (B) Quantification of BrdU-positive cells in 15 mammary ducts. Average number of BrdU-positive cells in vehicle- and **3**-treated wild-type (*wt*) and *Brca1^{f11/f11}p53^{f5&6/f5&6}Cre^C* mice. Arrows indicate alveoli. Histogram shows the average number of BrdU labeled cells per duct $\pm$ SD (* $P \leq 0.002$; ** $P \leq 0.0001$). At least 15 mammary ducts/animal were evaluated (a minimum of three mice per genotype). Mammary glands from vector (a & c) or **3** (b & d) treated mice were removed and fixed with paraformaldehyde. Paraffin sections were labeled with antibody to cyclin D1. At least 15 mammary ducts/animal were evaluated.

Scheme 1.

Reactions and conditions: (a) NaOH, 18-crown-6, R₁I, CH₃CN, 90 °C; (b) SOCl₂, R₂OH, rt; (c) (i) SOCl₂, MeOH, rt; (ii) Lawesson's reagent, toluene, reflux, 5h; (d) HOBt, EDCI, CH₂Cl₂, (H₂NMe for **10**); (e) MeI or EtI, Cs₂CO₃, acetone, rt; (f) Ac₂O, Py, DMAP, 90 °C; (g) MeI or EtI, NaH, THF, rt, (CF₃CH₂I, DMF, 0 °C for **20**); (h) PPh₃, Br₂, imidazole, 0 °C to rt; (i) EDCI, DMAP, 3-(diethylamino)-propanoic acid hydrochloride, CH₂Cl₂, rt.

Table 1

Cytotoxicity data of 6–12 and 14–24 against human tumor cell line panel^a

Compd	KB	KB-vin	A549	DU145	SKBR-3	ZR-75-1	MDA-MB-231
3	9.1	7.0	10.6	8.7	1.0	0.3	>10
4	1.7	1.3	1.5	2.2	1.2	1.3	2.3
6	>10	>10	>10	>10	>10	>10	>10
7	2.2±0.3	1.8±0.003	3.8±0.3	2.2±0.1	2.5±0.2	3.9±0.2	4.3±0.3
8	>10	>10	>10	>10	>10	>10	>10
9	4.3±0.02	5.1±0.1	6.2±0.8	5.7±0.6	6.3±2.2	5.8±0.4	7.1±0.7
10	>10	>10	>10	>10	>10	>10	>10
11	>10	>10	>10	>10	>10	>10	>10
12	1.8±0.08	1.7±0.04	2.4±0.3	1.9±0.09	1.9±0.06	1.2±0.1	1.1±0.1
14	7.1±0.3	6.6±0.5	9.6±0.5	7.2±0.5	0.73±0.05	>10	>10
15	>10	6.4±1.7	>10	>10	8.8±0.7	1.4±0.1	1.7±0.1
16	>10	>10	>10	>10	>10	>10	>10
17	>10	8.8±1.6	>10	>10	>10	9.7±0.3	8.5±0.2
18	>10	>10	>10	>10	>10	9.1±0.4	9.0±0.1
19	>10	>10	>10	>10	>10	4.6±0.3	>10
20	6.6±0.3	6.0±0.5	>10	5.7±0.9	5.3±1.5	5.3±0.1	3.8±0.3
21	>10	>10	>10	>10	>10	3.5±0.3	4.3±0.1
22	>10	>10	>10	>10	>10	1.7±0.1	0.85±0.04
23	>10	5.9±0.5	>10	>10	>10	>10	4.9±0.4
24	>10	5.8±1.6	>10	>10	>10	4.9±0.3	>10

^a mean ED50± standard error (μg/mL), from three or more independent tests.