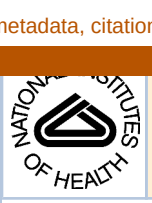

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Antitumor Agents. 272. Structure–Activity Relationships and In Vivo Selective Anti-Breast Cancer Activity of Novel Neo-tanshinlactone Analogs

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Abstract

Neo-tanshinlactone (**1**) and its previously reported analogs, such as **2**, are potent and selective in vitro anti-breast cancer agents. The synthetic pathway to **2** was optimized from seven to five steps, with a better overall yield. Structure–activity relationships studies on these compounds revealed some key molecular determinants for this family of anti-breast agents. Several derivatives (**19-21** and **24**) exerted potent and selective anti-breast cancer activity with IC_{50} values of 0.3, 0.2, 0.1 and 0.1 μg/mL, respectively, against the ZR-75-1 cell lines. Compound **24** was two- to three-fold more potent than **1** against SK-BR-3 and ZR-75-1. Importantly, **21** exhibited high selectivity; it was 23 times more active against ZR-75-1 than MCF-7. Compound **20** had an approximately 12-fold ratio of SK-BR-3/MCF-7 selectivity. In addition, analog **2** showed potent activity against a ZR-75-1 xenograft model, but not PC-3 and MDA-MB-231 xenografts, as well as high selectivity against breast cancer cell line compared with normal breast tissue-derived cell lines. Further development of lead compounds **19-21** and **24** as clinical trial candidates is warranted.

Introduction

Historically, natural products have been the most significant source of drugs and drug leads, which have led to numerous clinically used medicines.[1-4] Accordingly, our group is interested

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Supporting Information Available: HPLC analysis and statistical analysis for final compounds and preliminary results of kinases inhibition assay for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

in the discovery and development of novel anticancer drugs from natural plants. Drug discovery from medicinal plants has played an important role in the treatment of cancer, and about 74% of anticancer compounds are either natural products or natural product-derived.[5] Worldwide, over ten million new cases of cancer (all types, excluding non-melanoma skin) and over six million deaths were estimated to occur in the year 2000.[6] More than 1 million women are diagnosed with breast cancer every year, accounting for 10% of all new cancers and 23% of all female cancer cases.[7] The disease accounts for 40,000 deaths each year in the United States alone.[8] Tamoxifen (TAM, Figure 1) is the most widely used selective estrogen receptor modulator (SERM) for the treatment of breast cancer.[9] Other drugs, including cyclophosphamide, doxorubicin (adriamycin), and paclitaxel (taxol), are also recommended to be used in combination in early breast cancer.[8] Although the death rate from breast cancer has declined significantly because of earlier detection and more effective treatments, toxic side effects, low tumor selectivity, and multidrug resistance with cancer chemotherapy still prompt the development of novel potent anti-breast cancer agents.[10]

Tanshen, the rhizome of *Salvia miltiorrhiza* Bunge, is used primarily in traditional Chinese medicine (TCM) for the treatment of coronary heart diseases, inflammatory diseases, and chronic hepatitis. Many biologically active constituents, including neo-tanshinlactone, tanshinone I, and tanshinone IIA, which have been studied extensively as anticancer agents, were first isolated from the roots of *Salvia miltiorrhiza*.[11] Neo-tanshinlactone (**1**) (Figure 1), a minor component isolated from an EtOH extract of *S. miltiorrhiza*, showed significant selective in vitro anti-breast cancer activity as compared to TAM. Specifically, it was 10-fold more potent and 20-fold more selective than TAM against ER+ and HER2++ breast cancer cells.[12] Compound **2** (Figure 1), a congener of **1**, was about twice as active against MCF-7 and SK-BR-3 cell lines as **1**.[13] Compound **2** was effective against approximately 40% of human breast cancer cell lines and ineffective against other cell lines tested (total 29 cell lines, unpublished data). Furthermore, preliminary in vivo evaluation in mice models suggested that **2** is a potent and selective anti-breast cancer agent. In both BRCA1/p53 and wild-type mice treated with **2**, mammary gland side branching was dramatically reduced (unpublished data). In addition, **2** significantly suppressed several important protein kinases including CK2α1, ABL, and AKT1 (Table 3 in supporting information). Mechanism of action studies are ongoing and will be reported in due course.

Preliminary structure-activity relationships (SAR) showed that a methylated furan ring-D and the C-4 substituent in ring A are critical for anti-breast cancer activity.[13, 14] These promising results encouraged us to continue the modification of this series to develop novel anticancer drug candidates. To increase the chemical availability, we also optimized the synthetic pathway. In this paper, we describe further modifications of the A-, B-, C- and D-rings, as well as biological evaluation of newly synthesized analogs against several human cancer cell lines, including MCF-7 (estrogen receptor positive, luminal-like breast cancer), SK-BR-3 (estrogen receptor negative, HER2 over-expressing luminal-like breast cancer), ZR-75-1 (estrogen receptor positive, HER2 over-expressing luminal-like breast cancer), MDA MB-231 (estrogen receptor negative, basal cell-like breast cancer), A549 (human lung cancer), DU145 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-vin (vincristine-resistant KB subline).

Design

Our general goals in drug design are to optimize the synthesis of active analogs, systematically explore SAR, and develop new more potent lead compounds. Thus, our first goal in this study was to optimize the synthetic route to **2**. We aimed to reduce the number of steps and increase yields. The optimized synthetic route would then be applied to synthesize new analogs. Secondly, synthetic modifications of **1** were considered, because the resulting fundamental chemical and physical changes may affect molecular shapes, bond angles, and partition

coefficients. Different substituents can have different hydrophobic interactions, sizes, and electrostatic effects that can influence interaction of a ligand with its target receptors. For our **1**-analogs, we reported previously that a C-4 substituent in ring-A is critical for anti-breast cancer activity.[13] Thus, compounds **19-27** with substituents of different sizes and electrostatic properties were designed to find optimal groups at this position. In the B-ring, we changed the phenyl ring to a pyridinone ring in **32** and **33** to explore a ring system effect. The strategy of bioisoteric replacement can be a powerful and highly productive tool in analog design. Based on this concept, the oxygens in ring-C were changed to sulfur and nitrogen (**38-39** and **40-42**). Compounds with different substituents on the furan D-ring (**44-53**) were also designed. Moreover, the degree of saturation (number of double bonds) can change the orientation of a molecule and affect its in vitro activity and selectivity. Consequently, we designed **54** and **55** to have a more saturated dihydrofuran ring-D. Finally, the furan D-ring was changed to a substituted phenyl ring in **58** to examine the ring system effect and interaction volume.

Chemistry

Synthesis of analog **2** was achieved in five steps and an overall yield of 18%, compared with seven steps and 3% yield reported before[12,13] (Scheme 1). A Grignard reaction of **4** in the presence of zinc chloride gave **5** in an improved yield of 85%.[15] Addition of zinc chloride increased the yield more than 25%. Analog **6** was obtained in one step, by oxidation of **5** with Pd/C, rather than the prior two steps (hydrochloric acid and Pd/C).[13] Demethylation of **6** with boron tribromide gave naphthol **7**. Treatment of **7** with polyphosphoric acid (PPA) in the presence of 85% P_2O_5 and malonic acid produced **13** in 53% yield. Phosphorus pentoxide was used to remove water from the reaction system, and its use increased the yield and reproducibility. Finally, analog **2** was obtained via a tandem alkylation/intramolecular Aldol reaction with an optimized procedure (70% yield),[14] which increased the yield in this step by around 20%. This overall optimized synthetic route can be applied to synthesize new analogues and produce **2** in large scale for animal testing.

Using the optimized synthetic pathway, target compounds **19-23** were prepared from various substituted 1-naphthols (**8-12**), as shown in Scheme 1. Treating naphthols **8-12** with malonic acid in the presence of PPA (85%P_2O_5) provided benzochromenones **14-18**, which were converted to target compounds **19-23** under the same conditions as for synthesis of **2**.[16] Compound **24** was obtained by treatment of **2** with N-bromosuccinimide (NBS) and dibenzoyl peroxide.[17] Removal of the methyl group of **21** with boron tribromide afforded **25**, which was esterified with acetic anhydride and alkylated with 2-chloro-*N*,*N*-dimethylethanamine under basic conditions to give **26** and **27**, respectively.

B-ring modification was achieved through a two-step reaction sequence. Commercially available substituted anilines **28** and **29** were reacted with diethyl malonate at 220 °C for 8 h to give intermediates **30** and **31** (Scheme 2). The desired compounds **32** and **33** were obtained through the tandem alkylation/intramolecular Aldol reaction described above and shown in Scheme 1. Target compounds **38** and **39**, which are bio-isosteres of **3**, were obtained by using the same two synthetic steps shown in Scheme 1 for **2** from naphthol **7**, except that the starting materials were naphthalene-1-thiol **34** and naphthalen-1-amine **35** (Scheme 3). Compounds **3** and **2** were converted to thiolactones **40** and **41**, respectively, using Lawesson's reagent.[18] Compound **42** was obtained by treatment of **41** with sodium acetate and hydroxylamine hydrochloride.[19]

Target compounds **45-53** with various substituents on the D-ring were synthesized with the same tandem alkylation/intramolecular Aldol reaction using different bromoketones (Scheme 4). Reduction of **3** and **2** with palladium acetate, triethyl amine, and formic acid[20] afforded

54 and **55**, respectively. Compound **58** was obtained from **56** by using esterification[21] and Heck reactions.[22]

Results and Discussion

Together with **1** and previously reported analogs **2** and **3**, the newly synthesized analogs (**19-27**, **32-33**, **37-38**, **40-42**, **45-53**, **54-55**, and **58**) were evaluated for in vitro anti-breast cancer activity against two human tumor cell lines: MCF-7 (ER+) and SK-BR-3 (HER2+). Compounds that had ED_{50} values less than 4 μg/mL were also examined against ZR-75-1 (ER +, HER2+) and MDAMB-231 (ER−) breast cancer cell lines (Table 1).

Initially, we investigated the effects of substitutions around the skeleton of **1** by comparing **1**-**3** with **19-27**. The compounds displayed different degrees of activity and selectivity toward the four breast cancer cell lines.

Against the MCF-7 cell line, small alkyl groups were favored relative to other groups at C-4 on ring-A. Analog **2**, which has a C-4 ethyl group, was the most potent compound among those tested against MCF-7. Its ED_{50} (0.2 μg/mL) was slightly better than that (0.6 μg/mL) of **1**, which has a C-4 methyl group. The rank order of potency for all C-4 substituted analogs was **2** (Et) > **1** (Me) > **20** (Pr) = **19** (i-Pr) > **21** (OMe) > **3** (H) > **23** (F) > **27** (dimethylamino) > **26** (OAc) > **25** (OH) > **22** (OEt). The substituents on the furan (ring-D) double bond were also investigated. A methyl group (**2**) was better than either an ethyl (**47**) or methoxyphenyl (**53**) group at the R_2 position. However, at the R_3 position, a methyl group was distinctly disfavored (**48-51**). These results indicated that the optimal combination on ring-D was methyl at R_2 and hydrogen at R_3.

Most compounds were equipotent or more potent against SK-BR-3 compared with MCF-7 cells. Compounds **2** and **1** were even more potent against SK-BR-3, with ED_{50} values of 0.1 and 0.2 μg/mL, respectively. However, **20** and **24** (4-bromoethyl) were also equipotent to **2**, and **19** was equipotent to **1** against this cell line. Compounds **3**, **23**, **19**, and **22** showed good but lower activity (ED_{50} 1.0, 1.1, 2.0, 2.5 μg/mL, respectively), while **25**, **26**, and **27** were even less potent. These results indicate that the size, orientation, and electronic effect of groups at C-4 are important to the activity. Perhaps even more importantly, certain substituents could greatly affect the SK-BR-3/MCF-7 selectivity. Compounds **20-22** had approximately tenfold ratios of SK-BR-3/MCF-7 selectivity. For the D-ring analogs (**45-53**), most showed similar activity against SKBR-3 and MCF-7. An exception was **51**, which showed moderate activity against SK-BR-3 (ED_{50} 2.1 μg/mL), but was completely inactive against MCF-7.

To further explore the selectivity, compounds with ED_{50} values less than 4 μg/mL, were further examined against two additional breast cancer cell lines, ZR-75-1 (ER+, HER2+) and MDA-MB-231 (ER−). Most compounds had similar potency against the SK-BR-3 and ZR-75-1 cell lines. However, **19** had a tenfold ratio of ZR-75-1/SK-BR-3 selectivity, while **20** had a threefold ratio. Importantly, **21** showed a 23-fold ratio of ZR-75-1/MCF-7 selectivity. All tested compounds were not active against the MDA-MB-231 cell line, which confirmed that these novel analogs were highly selective.

As indicated by ZR-75-1/SK-BR-3 selectivity ratios, we observed that some compounds (e.g., **20** and **21**) were more sensitive to cell lines over-expressing only HER2 (SK-BR-3 and ZR-75-1), while others (e.g., **19**) were more sensitive to cell lines over-expressing both HER2 and ER (ZR-75-1). These results will facilitate our studies on the mechanism(s) of action. Because ring-A is critical to activity and selectivity, we will further explore C1-C4 positions in the future. We also investigated analogs involving skeletal modifications in ring-B, -C, or –D. Compounds **32-33** contain a pyridinone rather than phenyl ring-B and were much less active than **3**. Bioisosteric modifications of either lactone oxygen to nitrogen or sulfur in ring-

C led to dramatically decreased or abolished anti-breast cancer activity (**38**-**39**, **40**-**42**). The results demonstrated that the lactone ring is an important feature to the activity. Compounds **54** and **55** with a dihydrofuran ring-D showed decreased activity compared with **3** and **2**, respectively. Compound **58** with a substituted phenyl rather than furan D-ring was inactive. These results, together with our previously reported data, indicated that an unsaturated furan is favored for ring-D. Studies on different ring systems, such as **59** and **60**, are also ongoing (Figure 2).

To further investigate human tumor-tissue-type selectivity, compounds with ED_{50} values less than 4 μg/mL against breast cancer cell lines were tested against four different human cancer cell lines, A549 (lung), DU145 (prostate), KB (nasopharnygeal) and KB-vin (its vincristine-resistant subline) using **2** as a positive control (Table 2). All compounds, except **22**, were not active against these four tumor cell lines. These results demonstrated that our novel analogs were extremely selective for breast cancer cell lines.

Compound **2** was tested independently against cell lines derived from normal breast tissue (MCF10A and 184A1) versus SK-BR-3 as a positive breast cancer cell line control, and results are shown in Figure 3. The interpolated ED_{50} values are 0.1, 4.4, and 2.7 μg/mL against SK-BR-3, 184A1 and MCF10A cells, respectively, showing that **2** is selective for a sub-set of breast cancer-derived cell lines and is significantly less active against normal breast-derived tissue.

We have examined the anti-cancer activity of compound **2** in several xenograft models, such as PC-3 (androgen-independent human prostate carcinoma cells), MDA-MB-231 (estrogen receptor negative basal-like breast cancer cells), and ZR-75-1 (estrogen receptor positive HER2 over-expressing breast cancer cells). Compound **2** was administered intraperitoneally (i.p.) in a 4% benzyl alcohol/6% cremophor/90% D5W solution, and was given at 10 mg/kg every other day to endpoint (qod to end). A positive reference group received paclitaxel i.p. at 20 mg/kg every fourth day for 5 doses (q4d × 5). A control group received vehicle i.p. on a qod to end schedule. As shown in Figure 4, the treatment of SCID mice with **2** resulted in inhibition of estrogen-positive ZR-75-1 tumor xenograft growth. There was significant reduction in growth of estrogen-positive breast tumors in **2**-treated animals as compared with the control group. Treatment results were presented as percent tumor growth delay (%TGD), which is the percent increase in the mean time to endpoint (TTE) for drug-treated versus control mice. Logrank tests determine significance of the differences between TTE values for compound **2**-treated and control mice, at $P \leq 0.05$. In ZR-75-1 xenograft model, the mean TTE for the control group was 15.1 days. Paclitaxel produced a mean TTE of 35.0 days, corresponding to a %TGD of 132. Compound **2** at 10 mg/kg produced a mean TTE of 29.5 days, corresponding to a %TGD of 95 ($p = 0.0067$, logrank). Of the xenografts studied, treatment with **2** only suppressed estrogen-dependent breast cancer, but had no effect on PC-3 (androgen-independent human prostate carcinoma cells) or MDA-MB-231 (estrogen receptor negative basal-like breast cancer cells). These findings suggest that compound **2** may be selectively used to inhibit the growth of hormone-dependent breast cancers, particularly re-growth of residual tumor in postmenopausal breast cancer survivors receiving estrogen and progesterone replacement therapy.

Conclusions

In summary, a highly efficient synthesis of **2** was accomplished with fewer steps and higher overall yield than those previously reported. This synthetic pathway was used to prepare new analogs. The SAR study led to the following observations. (1) C-4 position is critical for both potency and selectivity. The order of potency against SK-BR-3 was ethyl = 2-bromoethyl = propyl > methyl = methoxy > fluoro = hydrogen > isopropyl > ethoxy > dimethylamino >

acetate > hydroxyl. Analogs with 4-isopropyl, -propyl and -methoxy groups showed high selectivity against different breast cancer cell lines. (2) The order of potency at the C-17 position was methyl > ethyl > hydrogen, while the order of potency at the C-16 position was hydrogen > methyl. (3) Pyridinone ring is not favored for ring-B. (4) Lactone ring-C is essential for activity; analogs with thiolactone and lactam rings were inactive or less active. (5) Ring-D is preferably an unsaturated furan ring. Based upon all results, a mechanism of action study is in progress. Due to their high selectivity and potency, **19-21** and **24** are novel promising anti-breast cancer candidates. In addition, analog **2** showed potent activity against a ZR-75-1 xenograft model, but not PC-3 and MDA-MB-231 xenografts. Resistance to endocrine therapy is an important issue in the management of HER2 over-expressing estrogen receptor-positive breast cancer.[23] Studies will continue to further establish the suitability of neo-tanshinlactone analogs as clinical trials candidates for treating breast cancer.

Experimental Section

Materials and Methods

Melting points were measured with a Fisher Johns melting apparatus without correction. ^{1}H 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. 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. Intermediates **7-18** and **36-37** for target compounds **19-23** and **38-39** were prepared by the optimized methods described in our previous and this paper.[13] Intermediates **30-31** were prepared by the reported method.[24, 25] All final compounds are >95% pure on the basis of two HPLC conditions.

Cell Growth Inhibition Assay

All stock cultures are grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates with compounds added from DMSO-diluted stock. The plates were incubated for an additional 72 h after attachment and drug addition, and the assay was terminated by 10% TCA. Then, 0.4% SRB dye in 1% HOAc was added to stain the cells for 10 min. Unbound dye was removed by repeated washing with 1% HOAc and the plates were air dried. Bound stain was subsequently solved with 10 mM trizma base, and the absorbance read at 515 nm. Growth inhibition of 50% (ED_{50}) was calculated as the drug concentration that caused a 50% reduction in the net protein increase in control cells during the drug incubation. The mean ED_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations. Variation between replicates was no more than 5% of the mean. The following human tumor cell lines were used in the assay: A549 (non-small cell lung cancer), MCF-7 (estrogen receptor positive luminal-like breast cancer), MDA MB-231 (estrogen receptor negative basal-like breast cancer), SK-BR-3 (estrogen receptor negative, HER2 over-expressing luminal-like breast cancer), ZR-75-1 (estrogen receptor positive breast cancer, HER2 over-expressing luminal-like breast cancer), KB (nasopharyngeal carcinoma), KB-vin (vincristine-resistant KB subline). All cell lines were obtained from the Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD). Cells propagated in RPMI-1640 supplemented with 10% FBS, Penicillin-100 IU/mL, Streptomycin-1μg/mL, Amphotericin B-0.25μg/mL, and were cultured at 37°C in a humidified atmosphere of 95% air/5% CO_2.

Optimized synthetic procedure to analog 2—To a solution of EtMgCl (3.0 *M* in diethyl ether, 26 mL, 78 mmol), ZnCl2 (798 mg, 6 mmol) was added at rt under argon atmosphere.

This mixture was stirred at rt for 1 h. Then, the solution was cooled to 0 °C, and 5-methoxy-1-tetralone **4** (10.56 g, 60 mmol) was added at 0 °C. The mixture was stirred overnight. The reaction mixture was quenched by saturated aqueous NH_4Cl, extracted with EtOAc, and dried over Na_2SO_4. The organic phase was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography (eluent: hexane/EtOAc), to give the desired product **5** (10.5g, 85% yield). 10% Pd/C (7.18 g. 33.24 mmol) was added to a solution of **5** (7.18g) in triglyme (15 mL), and the mixture was heated to reflux for 3 days to afford **6** (3.71 g, 60% yield). To a solution of **6** (3.5 g, 18.8 mmol) in anhydrous CH_2Cl_2 (20 mL) was added a solution of boron tribromide in CH_2Cl_2 (1.0 M, 56.5 mL, 56.5 mmol) dropwise at 0 ° C. The mixture was stirred overnight at rt to give the desired naphthol intermediate **7** (3.07 g, yield 95%) after silica gel chromatography. A mixture of **7** (2.14 g, 12.47 mmol), malonic acid (1.43 g, 13.72 mmol), and PPA (85%P_2O_5, 20 g) was heated at 75 °C for 3 h. After cooling, ice-water was added to the black residue. The mixture was filtered, and the solid dissolved in MeOH. The organic layer was concentrated and purified with flash chromatography, eluting with CH_2Cl_2:MeOH=10:1, to yield **13** as a yellow solid (1.52g, 53% yield). To a solution of **13** (440 mg, 1.83 mmol) in toluene (55 mL) was added a mixture of HOAc (549 mg, 9.15 mmol) and NH_4OAc (704 mg, 9.15 mmol) in EtOH (16 mL) and chloroacetone (842 mg, 9.15 mmol). The mixture was stirred for 30 min at rt, and then heated to 60 °C for 30 min. Subsequently, it was refluxed for 24 h. After cooling, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to give **2** (356 mg, 70% yield).

Synthesis of neo-tanshinlactone analogs 19-23, 32-33, 38-39, and 45-53—To a solution of **13-18**, or **30-31**, or **36-37**, or **43-44** (0.20 mmol) in toluene (8 mL) was added a mixture of HOAc (59 mg, 1.0 mmol) and NH_4OAc (75 mg, 1.0 mmol) in EtOH (2 mL) and chloroacetone (90 mg, 1.0 mmol) or 3-bromobutan-2-one, 2-bromopropanal, 1-bromobutan-2-one, 2-bromo-1-(4-methoxyphenyl)ethanone. The mixture was stirred for 30 min at rt, and then heated to 60 °C for 30 min. Subsequently, it was refluxed for 24 h. After cooling, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (hexane/EtOAc) to give a white solid.

6-Isopropyl-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (19)—65% yield; mp 155-157 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.42 (t, *J* = 6.6 Hz, 6H, $(CH_3)_2$), 2.41 (d, *J* = 1.2 Hz, 3H, CH_3), 3.76 (h, *J* = 6.9 Hz, 1H, $CH(CH_3)_2$), 7.44 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.56-7.64 (m, 2H, aromatic), 7.87 (d, *J* = 9.0 Hz, 1H, aromatic), 8.05 (d, *J* = 9.0 Hz, 1H, aromatic), 8.51 (d, *J* = 7.8 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 293.1178, found: 293.1168.

1-Methyl-6-propyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (20)—59% yield; mp 141-143 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.04 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2CH_3$), 1.78 (m, 2H, $CH_2CH_2CH_3$), 2.40 (d, *J* = 1.2 Hz, 3H, CH_3), 3.06 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 7.42-7.46 (m, 2H, aromatic & OCH), 7.55 (t, *J* = 8.4 Hz, 1H, aromatic), 7.83 (d, *J* = 9.0 Hz, 1H, aromatic), 7.94 (d, *J* = 9.0 Hz, 1H, aromatic), 8.49 (d, *J* = 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 293.1178, found: 293.1175.

6-Methoxy-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (21)—29% yield; mp 225-227 °C ; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.40 (s, 3H, CH_3), 4.02 (s, 3H, OCH_3), 6.95 (d, *J* = 7.8 Hz, 1H, aromatic), 7.42 (s, 1H, OC*H*), 7.54 (t, *J* = 7.8 Hz, 1H, aromatic), 7.78 (d, *J* = 9.0 Hz, 1H, aromatic), 8.15 (d, *J* = 9.0 Hz, 2H, aromatic); HRMS for (M^++H): calcd. 281.0814, found: 281.0816.

6-Ethoxy-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (22)—28% yield; mp 201-203 °C ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.56 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.39 (s, 3H, CH_3), 4.20 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 6.91 (d, *J* = 7.8 Hz, 1H, aromatic), 7.40 (s, 1H, OC*H*), 7.50 (t, *J* = 8.4 Hz, 1H, aromatic), 7.75 (d, *J* = 9.0 Hz, 1H, aromatic), 8.11 (d, *J* = 8.4 Hz, 1H, aromatic), 8.16 (d, *J* = 9.0 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 295.0970, found: 295.0970.

6-Fluoro-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (23)—20% yield; mp 215-217 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.42 (d, *J* = 1.8 Hz, 3H, CH_3), 7.27-7.33 (m, 1H, aromatic), 7.48 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.55-7.62 (m, 1H, aromatic), 7.93 (d, *J* = 9.0 Hz, 1H, aromatic), 8.03 (d, *J* = 9.0 Hz, 1H), 8.40 (d, *J* = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 269.0614, found: 269.0616.

5-Aza-*N*-methyl-1-methyl-4*H*-benzo[*h*]furo[3,2,*c*]chromene-4,11-dione (32)—50% yield; mp 265-267 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.38 (d, *J* = 1.5 Hz, 3H, CH_3), 3.82 (s, 3H, NCH_3), 7.39 (t, *J* = 8.1 Hz, 1H, aromatic), 7.46 (d, *J* = 8.4 Hz, 1H, aromatic), 7.54 (d, *J* = 1.5 Hz, 1H, OC*H*), 7.68-7.74 (m, 1H, aromatic), 8.36 (dd, *J* = 1.2, 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 282.0766, found: 282.0757.

5-Aza-*N*-propyl-1-methyl-4*H*-benzo[*h*]furo[3,2,*c*]chromene-4,11-dione (33)—55% yield; mp 238-240 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.08 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.83 (h, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.38 (d, *J* = 0.9 Hz, 3H, CH_3), 4.35 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 7.37 (t, *J* = 7.8 Hz, 1H, aromatic), 7.43 (d, *J* = 8.7 Hz, 1H, aromatic), 7.53 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.66-7.72 (m, 1H, aromatic), 8.35 (dd, *J* = 1.8, 7.8 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 310.1079, found: 310.1068.

1-Methyl-11*H*-benzo[*h*]furo[3,2-*c*]thiochromen-11-one (38)—8% yield; mp 137-139 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.42 (d, *J* = 1.5 Hz, 3H, CH_3), 7.44 (q, *J* = 1.5 Hz, 1H, OC*H*), 7.61-7.64 (m, 2H, aromatic), 7.85 (d, *J* = 8.4 Hz, 1H, aromatic), 7.88-7.92 (m, 1H, aromatic), 8.15 (d, *J* = 8.7 Hz, 1H, aromatic), 8.21-8.22 (m ,1H, aromatic); HRMS for (M^++H): calcd. 267.0480, found: 267.0473.

1-Methylbenzo[*h*]furo[3,2-*c*]quinolin-11(10*H*)-one (39)—10% yield; mp 135-137 ° C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.53 (d, *J* = 1.5 Hz, 3H, CH_3), 7.47 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.61-7.71 (m, 3H, aromatic), 7.93 (d, *J* = 8.4 Hz, 1H, aromatic), 7.98 (d, *J* = 8.7 Hz, 1H, aromatic), 8.45 (d, *J* = 8.1 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 250.0868, found: 250.0855.

1-Ethyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (45)—64% yield; mp 151-153 ° C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.32 (t, *J* = 7.5 Hz, 3H, CH_2CH_3), 2.78 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 7.32 (s, 1H, OC*H*), 7.52-7.61 (m, 3H, aromatic), 7.66 (d, *J* = 8.4 Hz, 1H, aromatic), 7.76 (d, *J* = 8.7 Hz, 1H, aromatic), 8.43 (d, *J* = 7.5 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 265.0865, found: 265.0865.

1-Ethyl-6-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (46)—65% yield; mp 183-185 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.33 (q, *J* = 7.5 Hz, 3H, CH_2CH_3), 2.65 (s, 3H, CH_3), 2.81 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 7.37 (s, 2H, aromatic & OC*H*), 7.45 (t, *J* = 8.1 Hz, 1H, aromatic), 7.71 (d, *J* = 8.7 Hz, 1H, aromatic), 7.78 (d, *J* = 9.0 Hz, 1H, aromatic), 8.36 (d, *J* = 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 279.1021, found: 279.1017.

1,6-Diethyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (47)—75% yield; mp 101-103 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.33 (q, *J* = 7.8 Hz, 6H, CH_3), 2.83 (q, *J* = 7.5 Hz,

2H, C*H*$_2$CH$_3$), 3.04 (q, *J* = 7.8 Hz, 2H, C*H*$_2$CH$_3$), 7.35-7.40 (m, 2H, aromatic & OC*H*), 7.48 (t, *J* = 7.5 Hz, 1H, aromatic), 7.70 (d, *J* = 9.3 Hz, 1H, aromatic), 7.83 (d, *J* = 8.7 Hz, 1H, aromatic), 8.37 (d, *J* = 8.1 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 293.1178, found: 293.1169.

1,2-Dimethyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (48)—15% yield; mp 103-105 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 2.32 (d, *J* = 0.9 Hz, 3H, OCC*H*$_3$), 2.42 (d, *J* = 0.9 Hz, 3H, C*H*$_3$), 7.59-7.65 (m, 2H, aromatic), 7.72 (d, *J* = 8.7 Hz, 1H, aromatic), 7.82 (d, *J* = 8.7 Hz, 1H, aromatic), 7.85-7.88 (m, 1H, aromatic), 8.58 (d, *J* = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 265.0865, found: 265.0860.

6-Ethyl-1,2-dimethyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (49)—29% yield; mp 141-143 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.38 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 2.29 (s, 3H, OCC*H*$_3$), 2.39(s, 3H, C*H*$_3$), 3.09 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 7.42 (d, *J* = 6.9 Hz, 1H, aromatic), 7.50-7.55 (m, 1H, aromatic), 7.75-7.80 (m, 1H, aromatic), 7.88-7.91 (m, 1H, aromatic), 8.44 (d, *J* = 8.1 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 293.1178, found: 293.1172.

2-Methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (50)—12% yield; mp 229-231 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 2.53 (s, 3H, C*H*$_3$), 6.63 (s, 1H, OCC*H*), 7.61-7.65 (m, 2H, aromatic), 7.75 (d, *J* = 8.7 Hz, 1H, aromatic), 7.83-7.90 (m, 2H, aromatic), 8.59 (d, *J* = 7.5 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 251.0708, found: 251.0703.

6-Ethyl-2-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (51)—2% yield; mp 175-177 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.41 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 2.54 (s, 3H, C*H*$_3$), 3.15 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 6.64 (s, 1H, OCC*H*), 7.48 (d, *J* = 7.2 Hz, 1H, aromatic), 7.58 (t, *J* = 7.2 Hz, 1H, aromatic), 7.88 (d, *J* = 9.0 Hz, 1H, aromatic), 8.00 (d, *J* = 9.0 Hz, 1H, aromatic), 8.50 (d, *J* = 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 279.1021, found: 279.1017.

1-(4-Methoxyphenyl)-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (52)—20% yield; mp 173-175 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 3.88 (s, 3H, C*H*$_3$), 7.02 (d, *J* = 9.0 Hz, 2H, aromatic), 7.64-7.68 (m, 2H, aromatic & OC*H*), 7.59-7.80 (m, 4H, aromatic), 7.90-7.94 (m, 2H, aromatic), 8.62-8.65 (m, 1H, aromatic); HRMS for (M^++H): calcd. 343.0970, found: 343.0975.

6-Ethyl-1-(4-methoxyphenyl)-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (53)—34% yield; mp 185-187 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.39 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 3.11 (q, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_3$), 3.86 (s, 3H, OC*H*$_3$), 6.85-7.01 (m, 2H, aromatic), 7.46 (d, *J* = 6.6 Hz, 1H, aromatic), 7.56 (t, *J* = 7.8 Hz, 1H, aromatic), 7.72-7.77 (m, 3H, aromatic & OC*H*), 7.86 (d, *J* = 8.7 Hz, 1H, aromatic), 7.95 (d, *J* = 8.7 Hz, 1H, aromatic), 8.47 (d, *J* = 8.1 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 371.1283, found: 371.1291.

6-(1-Bromoethyl)-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (24)—To a solution of **2** (27 mg, 0.1 mmol) in CCl$_4$ (3 mL) was added N-bromosuccinimide (18 mg, 0.1 mmol) and dibenzoyl peroxide (2 mg). The reaction mixture was stirred and heated at reflux for 9 h. After the mixture was cooled in an ice bath, the solid was removed by filtration and washed with CCl$_4$. Concentration and silica gel flash column chromatography (hexane-EtOAc, 8:1) gave **24** (18 mg, 52%) as a white solid.

52% yield; mp 173-175 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 2.29 (d, *J* = 6.9 Hz, 3H, CHBrC*H*$_3$), 2.42 (d, *J* = 1.2 Hz, 3H, C*H*$_3$), 5.97 (q, *J* = 7.5 Hz, 1H, C*H*CH$_3$), 7.47 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.65 (t, *J* = 7.8 Hz, 1H, aromatic), 7.88 (d, *J* = 6.9 Hz, 1H, aromatic), 8.00 (d,

J = 9.0 Hz, 1H, aromatic), 8.15 (d, *J* = 8.7 Hz, 1H, aromatic), 8.65 (d, *J* = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 357.0126, found: 357.0120.

6-Hydroxy-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (25)—To a solution of **21** (32 mg, 0.114 mmol) in DCM (3 ml) was added BBr_3 (1.12 mL, 1.12 mmol) dropwise at 0 °C. The reaction mixture was stirred and warmed to rt for 12 h. Water was added to quench the reaction. The solution was extracted with $CHCl_3$. The organic layer was concentrated purified with flash chromatography, eluting with DCM-MeOH, 15:1, to give **25**.

52% yield; ^{1}H NMR (300 MHz, DMSO, ppm): δ 2.30 (s, 3H, C*H*$_3$), 7.06 (d, *J* = 8.7 Hz, 1H, aromatic), 7.53 (t, *J* = 8.4 Hz, 1H, aromatic), 7.82-7.86 (m, 2H, aromatic), 7.97 (d, *J* = 1.2 Hz, 1H, OC*H*), 8.11 (d, *J* = 9.3 Hz, 1H, aromatic), 10.58 (s, 1H, O*H*); HRMS for (M^+-H): calcd. 265.0501, found: 265.0505.

1-Methyl-11-oxo-11*H*-benzo[*h*]furo[3,2-*c*]chromen-6-yl acetate (26)—Compound **25** (0.1 mmol) was dissolved in acetic anhydride under argon. Triethylamine (0.14 mL, 1.0 mmol) was added to the solution. After stirring overnight at 60 °C, the solution was washed with water and extracted with DCM, and dried ($MgSO_4$). Removal of solvent under reduced pressure yielded a white solid, which was purified by column chromatography, eluting with EtOAc–hexane (1:4).

43% yield; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.41 (s, 3H, C*H*$_3$), 2.50 (s, 3H, COC*H*$_3$), 7.39 (d, *J* = 6.9 Hz, 1H, aromatic), 7.46 (s, 1H, OC*H*), 7.65 (t, *J* = 8.1 Hz, 1H, aromatic), 7.80 (d, *J* = 8.7 Hz, 1H, aromatic), 7.90 (d, *J* = 8.7 Hz, 1H, aromatic), 8.52 (d, *J* = 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 309.0763, found: 309.0762.

6-(2-(Dimethylamino)ethoxy)-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one (27)—Compound **25** (0.1 mmol) was dissolved in acetone under argon. K_2CO_3 (235 mg, 1.7 mmol) was added to the solution. After stirring for 10 min, 2-chloro-N,N-dimethylethylamine hydrochloride (30 mg, 0.2 mmol) was added to the mixture. After refluxing for 10 h, the mixture was filtrated and concentrated. The residue was purified by column chromatography, eluting with EtOAc–hexane (1:2).

9% yield; mp 209-211 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.41 (s, 3H, C*H*$_3$), 2.44 (s, 6H, N(C*H*$_3$)$_2$), 2.96 (t, *J* = 5.1 Hz, 2H, OCH$_2$C*H*$_2$), 4.31 (t, *J* = 5.1 Hz, 2H, OC*H*$_2$CH$_2$), 6.98 (d, *J* = 7.5 Hz, 1H, aromatic), 7.45 (s, 1H, OC*H*), 7.55 (t, *J* = 8.1 Hz, 1H, aromatic), 7.83 (d, *J* = 8.7 Hz, 1H, aromatic), 8.20 (dd, *J* = 7.2, 8.7 Hz, 2H, aromatic); HRMS for (M^++H): calcd. 338.1392, found: 338.1389.

Synthesis of neo-tanshinlactone analogs 40-41—A mixture of compound **3** or **2** (0.1 mmol) and Lawesson's reagent (81 mg, 0.2 mmol) in dry toluene (5 mL) was heated to reflux for 7 h. After cooling, toluene was removed in vacuo, and the red residue was dissolved in EtOAc and partitioned with H_2O. The organic phase was separated and dried over $MgSO_4$. Removal of solvent in vacuo afforded an oily residue, which was purified by column chromatography (EtOAc-hexane) to give a yellow solid.

1-Methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromene-11-thione (40)—90% yield; mp 267-269 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.53 (s, 3H, C*H*$_3$), 7.46 (s, 1H, OC*H*), 7.67-7.69 (m, 2H, aromatic), 7.79-7.92 (m, 3H, aromatic), 8.75 (d, *J* = 7.2 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 267.0480, found: 267.0471.

6-Ethyl-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromene-11-thione (41)—84% yield; mp 189-191 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.38 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 2.49

(d, J = 1.2 Hz, 3H, CH_3), 3.09 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 7.40 (d, J = 1.5 Hz, 1H, OCH), 7.46 (d, J = 7.2 Hz, 1H, aromatic), 7.56 (t, J = 7.2, 8.1 Hz, 1H, aromatic), 7.78 (d, J = 9.0 Hz, 1H, aromatic), 7.95 (d, J = 9.0 Hz, 1H, aromatic), 8.55 (d, J = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 295.0793, found: 295.0779.

6-Ethyl-1-methyl-11*H*-benzo[*h*]furo[3,2-*c*]chromen-11-one oxime (42)—A mixture of **41** (22 mg. 0.075 mmol), hydroxylamine hydrochloride (10.4 mg, 0.15 mmol), sodium acetate (12 mg, 0.15 mmol), and MeOH (5 mL) was refluxed overnight and then filtered. The filtrate was concentrated under reduced pressure to give an oil. Purification by the column chromatography (EtOAc–hexane) on silica gel gave **42** as a white in 87% yield.

89% yield; mp 211-213 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.39 (t, J = 7.2 Hz, 3H, CH$_2$CH_3), 2.28 (d, J = 0.9 Hz, 3H, CH_3), 3.11 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 7.18 (s, 1H, OH), 7.33 (d, J = 1.2 Hz, 1H, OCH), 7.40 (d, J = 6.9 Hz, 1H, aromatic), 7.52 (dd, J = 7.2, 8.4 Hz, 1H, aromatic), 7.71 (d, J = 9.0 Hz, 1H, aromatic), 7.86 (d, J = 9.0 Hz, 1H, aromatic), 8.40 (d, J = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 294.1130, found: 294.1118.

Synthesis of neo-tanshinlactone analogs 54-55—Compound **3** or **2** (0.2 mmol) was dissolved in acetone at 40 °C under argon. Pd/C (81 mg, 10%), triethylamine (0.33 mL, 2.40 mmol) and formic acid (0.075 mL, 2.00 mmol) were added to the solution. After stirring overnight, TLC showed some substrate remained unreacted. The solution was filtered through Celite and solvent removed in vacuo to yield a dark oil. The residue was dissolved in DCM before washing with saturated aqueous sodium bicarbonate (5 mL), aqueous citric acid (5 mL, 10% v/v), water (5 mL) and brine (5 mL), and then dried (MgSO$_4$). Removal of solvent under reduced pressure yielded a white solid, which was purified by column chromatography, eluting with EtOAc–hexane (1:4).

1-Methyl-1*H*-benzo[*h*]furo[3,2-*c*]chromen-11(2*H*)-one (54)—30% recovered yield; mp 143-145 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.46 (d, J = 6.9 Hz, 3H, CHCH_3), 3.68-3.77 (m, 1H, CHCH$_3$), 4.47 (q, J = 6.3 Hz, 1H, CH_2), 5.00 (t, J = 6.6 Hz, 1H, CH_2), 7.62-7.71 (m, 4H, aromatic), 7.87-7.90 (m, 1H, aromatic), 8.59-8.62 (m, 1H, aromatic); HRMS for (M^++H): calcd. 253.0865, found: 253.0856.

6-Ethyl-1-methyl-1*H*-benzo[*h*]furo[3,2-*c*]chromen-11(2*H*)-one (55)—56% recovered yield; mp 83-85 °C; ^{1}H NMR (300 MHz, CDCl$_3$, ppm): δ 1.39 (t, J = 7.8 Hz, 3H, CH$_2$CH_3), 1.46 (d, J = 7.5 Hz, 3H, CHCH$_3$), 3.13 (q, J = 7.5 Hz, 2H, CH_2CH$_3$), 3.66-3.78 (m, 1H, CHCH$_3$), 4.47 (q, J = 6.0 Hz, 1H, CH_2), 5.00 (t, J = 6.6 Hz, 1H, CH_2), 7.50-7.60 (m, 2H, aromatic), 7.64 (d, J = 9.3 Hz, 1H, aromatic), 7.91 (d, J = 9.3 Hz, 1H, aromatic), 8.49 (d, J = 8.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd. 281.1178, found: 281.1163.

Naphthalen-1-yl 2-bromo-4-methylbenzoate (57)—Thionyl chloride (0.17ml, 2.40 mmol) was added to 2-bromo-4-methylbenzoic acid (430 mg, 2 mmol) in DCM (3 mL) and DMF (0.1 mL), and the mixture was refluxed under nitrogen atmosphere for 1 h. After cooling to rt, it was concentrated in vacuo to give the title compound as a pale yellow solid, which was used directly in the next step.

Naphthalen-1-ol (288 mg, 2.00 mmol) was dissolved in THF (5 mL), then DMAP (5 mg) and ethyldiisopropylamine (0.36 mL, 2.05 mmol) were added, and the mixture was cooled to 0 ° C for 10 min. Freshly prepared 2-bromo-4-methylbenzoyl chloride in dry THF (10 mL) was added to the mixture via cannula, and the resulting mixture was stirred at 25 °C for 2 h, diluted with diethyl ether (150 mL), and quenched by the addition of water (15 mL). The organic layer was washed with HCl and NaHCO$_3$ and then dried (Na$_2$SO$_4$) and concentrated in vacuo. The

residue was purified with flash chromatography, eluting with hexane: EtOAc=10:1, to give **57**.

96% yield; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.41 (s, 3H, C*H*$_3$), 7.27 (dd, *J* = 0.9, 8.1 Hz, 1H, aromatic), 7.40 (dd, *J* = 1.2, 7.5 Hz, 1H, aromatic), 7.48-7.52 (m, 3H, aromatic), 7.60 (d, *J* = 0.9 Hz, 1H, aromatic), 7.77 (d, *J* = 8.1 Hz, 1H, aromatic), 7.87-7.90 (m, 1H, aromatic), 7.96-7.99 (m, 1H, aromatic), 8.13 (d, *J* = 8.1 Hz, 1H, aromatic).

9-Methyl-6*H*-dibenzo[*c,h*]chromen-6-one (58)—A mixture of **57** (68 mg, 0.2 mmol), $PdCl(OAc)_2$ (4.5 mg, 0.02 mmol), PPh_3 (10.5 mg, 0.04 mmol), and NaOAc (32.8 mg, 0.4 mmol) was dissolved in dry dimethylacetamide (10 mL), and the solution was degassed and then heated to 150 °C for 3 h. On cooling to rt, the solution was diluted with diethyl ether (50 mL) and washed with HCl, and the ethereal extracts were dried over Na_2SO_4. The solution was filtered, the filtrate condensed in vacuo, and the resulting oil purified by flash chromatography (hexane:EtOAc 4:1) to give the title compound as a white solid.

65% yield; mp 193-195 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.55 (s, 3H, C*H*$_3$), 7.35-7.38 (m, 1H, aromatic), 7.57-7.63 (m, 2H, aromatic), 7.71 (d, *J* = 9.0 Hz, 1H, aromatic), 7.82-7.85 (m, 1H, aromatic), 7.91 (s, 1H, aromatic), 8.00 (d, *J* = 9.3 Hz, 1H, aromatic), 8.30 (d, *J* = 7.8 Hz, 1H, aromatic), 8.53-8.56 (m, 1H, aromatic); HRMS for (M^++H): calcd. 261.0916, found: 261.0909.

Methodology of MTT assay[26]—The MTT assay was used to access the in vitro anticancer activity of **2** against two normal breast cancer cell lines 184A1 and MCF10A (CRL-10317) purchased from ATCC (Rockville, MD) and using SK-BR-3 as a positive control. Cells were seeded into 96 well plates at a density of 5000 cells per well in the recommended growth medium. The drug was dissolved in DMSO. The drug was added into wells after overnight incubation. After 72 h of incubation at 37 °C in 5% CO_2, 20 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyl tetrazolium bromide] reagent was added to each well and incubated for two h. The amount of formazon product was measured at an OD of 570 nM using a plate-reader.

Antitumor Activity in Vivo

Male (for PC-3) and female (for MDA-MB-231, and ZR-75-1) SCID mice (NTUH Animal Facility) were 5 weeks old, and had a body weight (BW) range of 20-24 g, on D1 of the study. The animals were fed ad libitum water (reverse osmosis, 1 ppm Cl) and PicoLab Rodent Diet 20 Modified and Irradiated Lab Diet® consisting of 20.0% crude protein, 9.9% crude fat, and 4.7% crude fiber. The mice were housed at National Taiwan University Laboratory Animal Center, NTUMC, on a 12-hour light cycle at 21–23 °C and 60–85% humidity. Nude-athymic mice were maintained in accordance with the Institutional Animal Care and Use Committee procedures and guidelines. All human cancer cells were maintained in RPMI 1640 medium containing 100 units/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B, and 25 μg/mL gentamicin. The medium was supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine. The cells were cultured in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO2 and 95% air. All human cancer cells used for implantation were harvested during log phase growth and resuspended in phosphate-buffered saline at 5×10^7 cells/mL. Each mouse was injected s.c. in the right flank with 1×10^7 cells (0.2 mL cell suspension). Tumors were monitored twice weekly and then daily as their volumes approached 80-150 mm^3. Tumor size, in mm^3, was calculated from: Tumor Volume = $w^2 \times l / 2$ where *w* = width and *l* = length in mm of the tumor. Tumor weight can be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume. Treatment efficacy was determined from tumor growth delay (TGD), which is defined as the

increase in the mean TTE (the time to endpoint) for a treatment group compared to the control group:

$$TGD = T - C,$$

expressed in days, or as a percentage of the mean TTE of the control group:

$$\%TGD = \frac{T - C}{C} \times 100$$

where T = mean TTE for a treatment group, C = mean TTE for control Group 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant CA-17625 from the National Cancer Institute, awarded to K.H. Lee.

Abbreviations

TAM	tamoxifen
SERM	selective estrogen receptor modulator
TCM	traditional Chinese medicine
SAR	structure-activity relationship
PPA	polyphosphoric acid
TGD	tumor growth delay
TTE	time to endpoint

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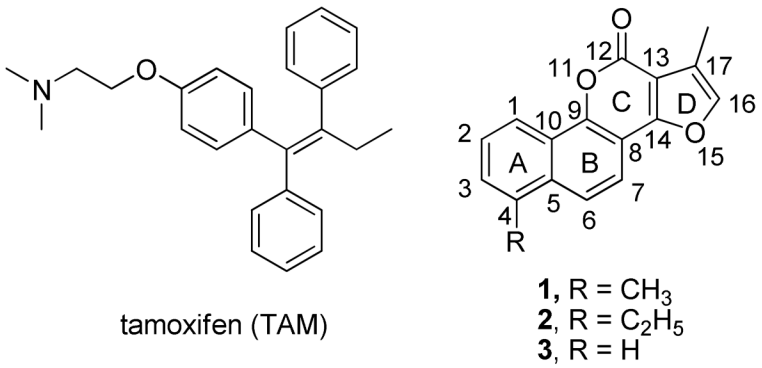
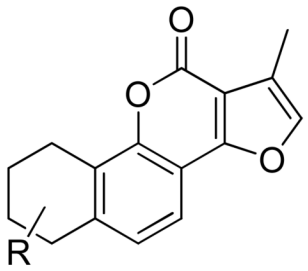


Figure 1.
Structures of tamoxifen, neo-tanshinlactone (**1**) and its analogs **2-3**



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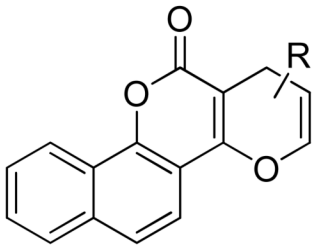

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Figure 2.
Structures designed for future study

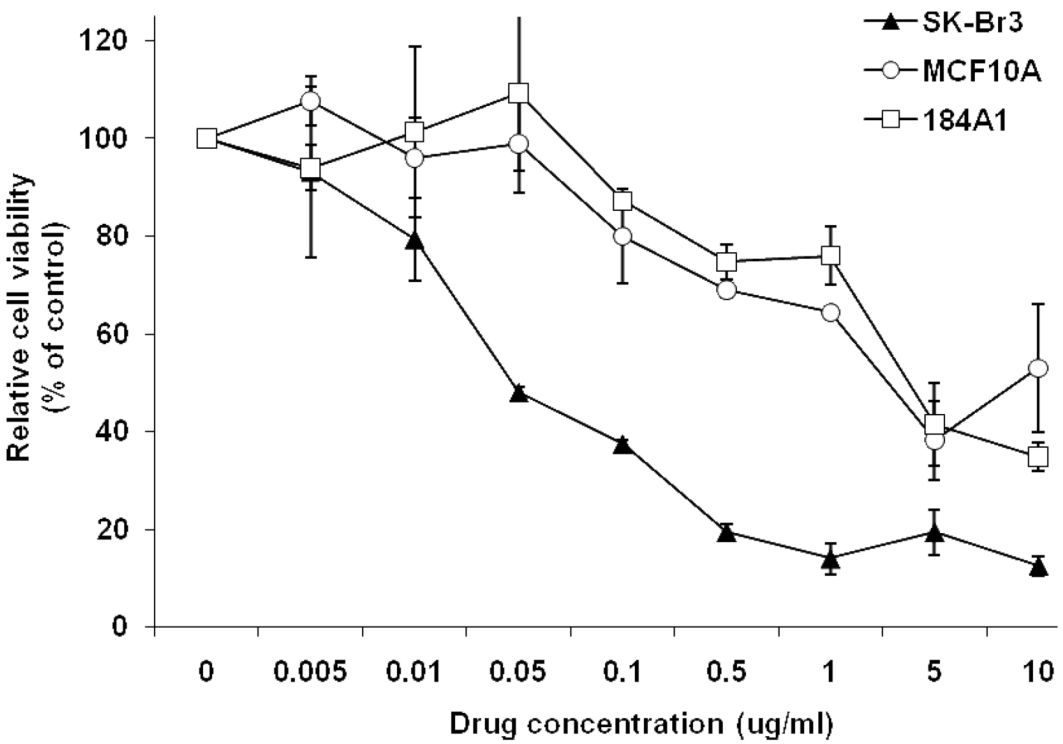


Figure 3.

Selective in vitro anticancer activity of **2** against SK-BR-3 breast cancer versus normal breast tissue-derived cell lines (MCF10A and 184A1). Legend: Cell line description, source and activity determination using the MTT-dye assay are described in the experimental section. Graphical data are the mean and standard deviation of values obtained from replicates in a single experiment.

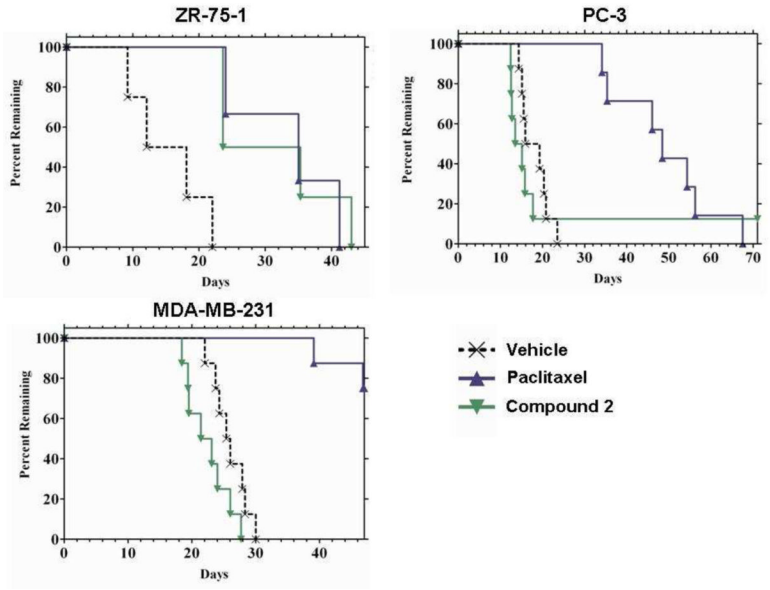
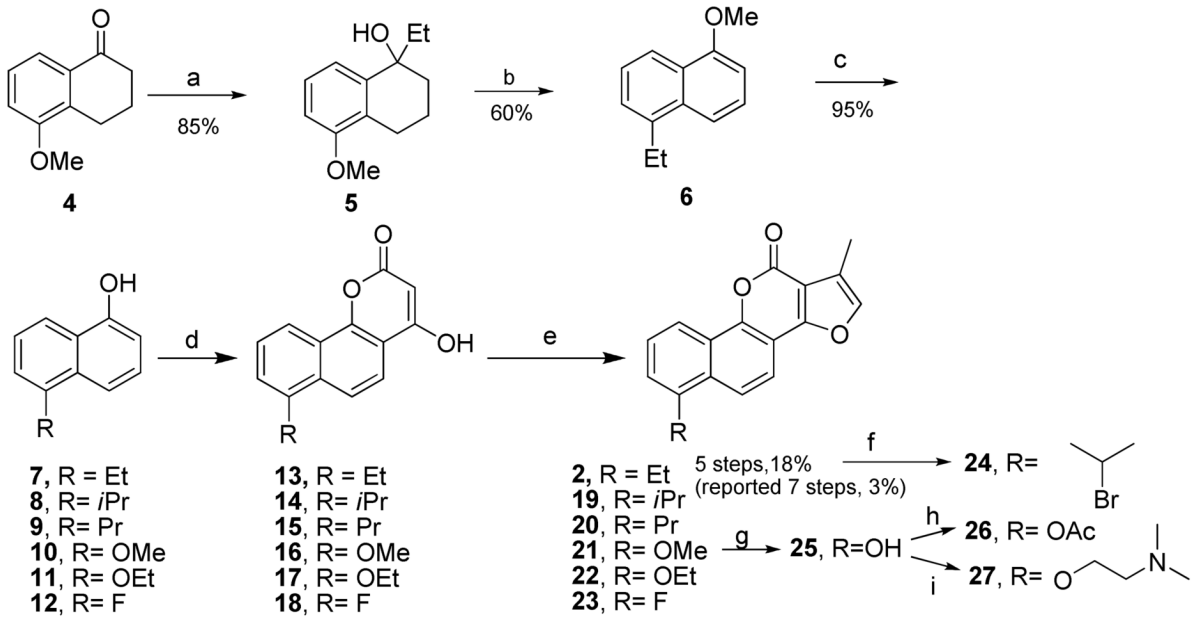


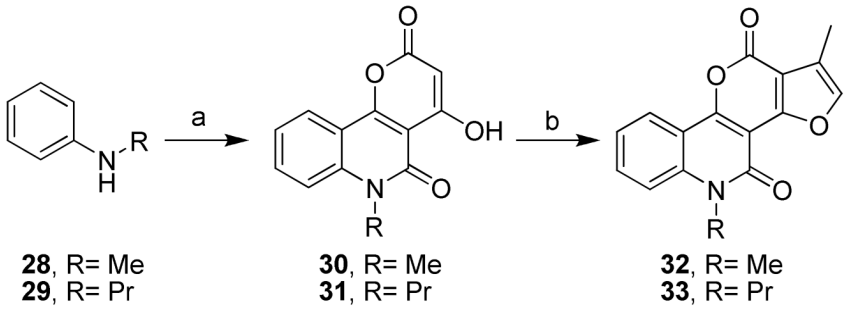
Figure 4. Anticancer activity of 2

The efficacy of **2** on the growth of human PC-3, MDA-MB-231, and ZR-75-1 xenografts in SCID mice. Treatment with compound **2** selectively abrogated the hormone-dependent breast cancer. Tumor growth is presented as the mean tumor volume (mm^3) ± SE. Tumor volume was determined by caliper measurements and was calculated as the product of 1/2 × length × $width^2$. Each value represents the mean of at least five animals.



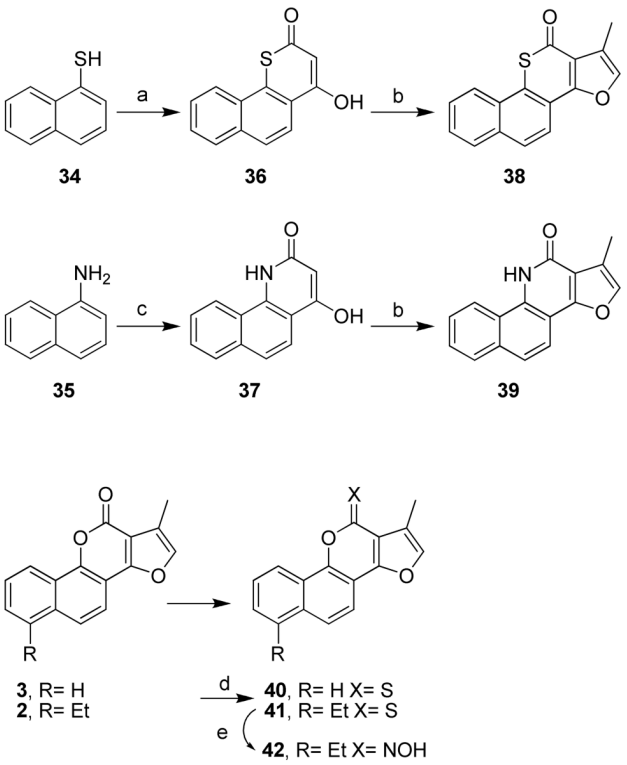
Scheme 1.

Reagents and conditions: (a) EtMgBr, $ZnCl_2$, THF, rt; (b) Pd/C, triglyme, reflux; (c) BBr_3, CH_2Cl_2; (d) malonic acid, PPA (85% P_2O_5), 75 °C, 3 h; (e) chloroacetone, HOAc/NH_4OAc, toluene/EtOH, reflux, 24 h; (f) NBS, dibenzoyl peroxide, toluene, reflux; (g) BBr_3, CH_2Cl_2, reflux, 3 h; (h) Ac_2O, Et_3N, 10 h; (i) 2-chloro-N,N-dimethylethanamine, K_2CO_3, acetone, 12h.



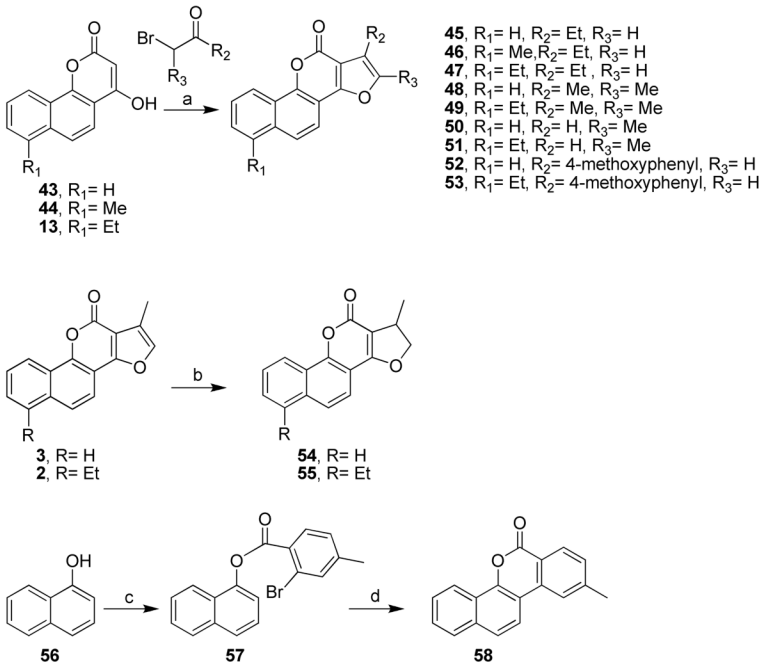
Scheme 2.

Reagents and conditions: (a) diethyl malonate, 220°C, 8h; (b) chloroacetone, HOAc/NH_4OAc, toluene/EtOH, reflux, 24 h.



Scheme 3.

Reagents and conditions: (a) malonic acid, PPA (85% P_2O_5), 75 °C, 3 h; (b) chloroacetone, HOAc/NH_4OAc, toluene, EtOH, reflux, 24 h; (c) diethyl malonate, PPA (85% P_2O_5), 170 °C, 2h; (d) Lawesson's reagent, toluene, reflux, 5h; (e) NH_2OH HCl, NaOAc, MeOH, reflux, 12 h.



Scheme 4.

Reagents and conditions: (a) $HOAc/NH_4OAc$, toluene, EtOH, reflux, 24 h; (b) Et_3N, formic acid, Pd/C, acetone, 12 h; (c) 2-bromo-4-methylbenzoyl chloride, DMAP, DIEA, THF, rt, 12h; (d) $Pd(OAc)_2$, PPh_3, NaOAc, DMF, reflux, 3h.

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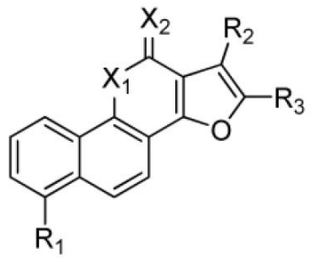
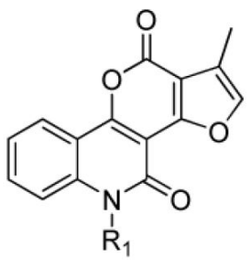
Table 1

Cytotoxicity of Compounds against Tumor Cell Lines[a]



1-3, 19-27, 38-39 40-42, 45-53



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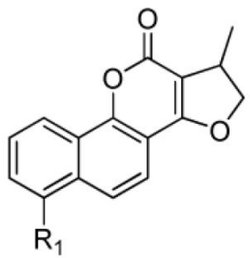

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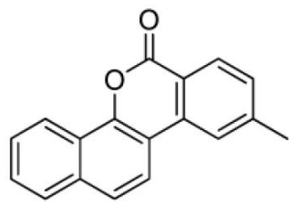

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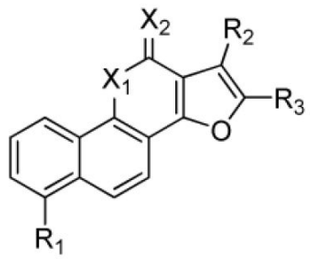
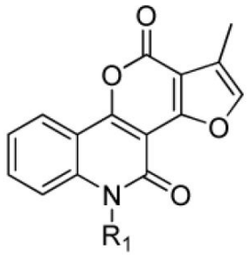
Compd	R1	R2	R3	X1	X2	MCF-7	SK-BR-3	ZR-75-1	MDA-MB-231
1	Me	Me	H	O	O	0.60	0.20	0.30	10
2	Et	Me	H	O	O	0.20	0.10	0.10	8.8
3	H	Me	H	O	O	4.0	1.0	4.0	10.3
19	*i*Pr	Me	H	O	O	1.4	2.0	0.2	>10
20	Pr	Me	H	O	O	1.2	0.10	0.30	>10
21	OMe	Me	H	O	O	2.3	0.20	0.10	6.4
22	OEt	Me	H	O	O	>20	2.5	1.9	9.8
23	F	Me	H	O	O	4.5	1.1	0.80	>10
24	1-bromoethyl	Me	H	O	O	NT	0.10	0.10	14
25	OH	Me	H	O	O	15.0	5.0	NT	NT
26	OAc	Me	H	O	O	6.0	5.7	NT	NT
27	DAE	Me	H	O	O	5.0	5.8	NT	NT
32	Me	--	--	--	--	11	12	NT	NT
33	Pr	--	--	--	--	>20	15	NT	NT
38	H	Me	H	S	O	>20	12	NT	NT
39	H	Me	H	N	O	NT	4.4	NT	NT
40	H	Me	H	O	S	>20	>20	NT	NT
41	Et	Me	H	O	S	>20	16	NT	NT



1-3, 19-27, 38-39 40-42, 45-53



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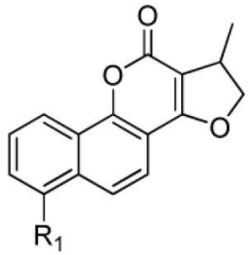

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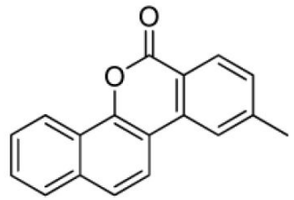

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Compd	R1	R2	R3	X1	X2	MCF-7	SK-BR-3	ZR-75-1	MDA-MB-231
42	Et	Me	H	O	NOH	>20	19	NT	NT
45	H	Et	H	O	O	2.5	1.8	2.3	>10
46	Me	Et	H	O	O	7.5	11	NT	NT
47	Et	Et	H	O	O	1.3	1.5	0.60	>10
48	H	Me	Me	O	O	>20	6.9	NT	NT
49	Et	Me	Me	O	O	8.0	9.8	NT	NT
50	H	H	Me	O	O	>20	12	NT	NT
51	Et	H	Me	O	O	>20	2.1	2.2	9.6
52	H	PMP	H	O	O	NT	5.8	NT	NT
53	Et	PMP	H	O	O	>20	>20	NT	NT
54	H	--	--	--	--	NT	14	NT	NT
55	Et	--	--	--	--	NT	5.1	NT	NT
58	--	--	--	--	--	NT	>20	NT	NT

[a] mean ED_{50} (μg/mL), from 2 or more independent tests (standard error is listed in supporting information); NT: not tested; PMP: 4-methoxyphenyl, DAE: 2-dimethylamino)ethoxy.

Table 2

Cytotoxicity of Compounds against Tumor Cell Lines[a]

Compd	A549	DU145	KB	KBvin
2	11	16	13	13
19	11	11	11	7.3
20	12	15	12	11
21	10	14	13	12
22	3.5	4.7	3.7	5.3
23	14	18	13	15
24	>20	18	13	>20
45	8.2	8.7	7.5	6.6
47	18	16	12	15
51	12	14	14	13

[a] mean ED_{50} (μg/mL), from 2 or more independent tests (standard error is listed in supporting information).