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Antitumor agents 270. Novel substituted 6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one derivatives as potent and highly selective anti-breast cancer agents

Yizhou Dong [a], Qian Shi [a,*], Kyoko Nakagawa-Goto [a], Pei-Chi Wu [a], Susan L. Morris-Natschke [a], Arnold Brossi [a], Kenneth F. Bastow [b,*], Jing-Yu Lang [c], Mien-Chie Hung [c,d], Kuo-Hsiung Lee [a,*]

[a] *Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7568, United States*
[b] *Division of Medicinal Chemistry and Natural Products, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7568, United States*
[c] *Department of Molecular and Cellular Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, United States*
[d] *Center for Molecular Medicine and Graduate Institute of Cancer Biology, China Medical University and Hospital, Taichung, Taiwan 404, China*

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ABSTRACT

6-Phenyl-4*H*-furo[3,2-*c*]pyran-4-one derivatives based on neo-tashinlactone (**1**) were synthesized and evaluated as novel anti-breast cancer agents. Compounds **10–13**, **23**, **25**, and **27** showed potent inhibition against the SK-BR-3 breast cancer cell line. Importantly, **25** and **27** showed the highest cancer cell line selectivity, being approximately 100–250-fold more potent against SK-BR-3 (ED_{50} 0.28 and 0.44 μM, respectively) compared with other cancer cell lines tested. In addition, **25** displayed low cytotoxicity against normal breast cell lines 184A1 and MCF10A. Compounds **25** and **27** merit further investigation in our continuing program to generate and develop selective anti-breast cancer agents.

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1. Introduction

Breast cancer is the most common cancer among women.[1–3] According to the American Cancer Society, the disease accounts for more than one quarter of cancers diagnosed in US women. In 2007, it was estimated that 18,000 new cases of invasive breast cancer would be diagnosed in women, as well as an estimated 60,000 additional cases of in situ breast cancer.[4]

Most clinically used anticancer drugs cause general toxicity to proliferating cells, which can severely limit the therapeutic value of these drugs.[5] Thus, much effort has been made to increase tissue, cell, and target selectivity for chemotherapy.[6–8] However, although new cytotoxic agents with unique mechanisms of action have been developed continuously, many of them have not been therapeutically useful due to low tumor selectivity.[5] These facts prompted us to design and develop novel potent and selective anti-breast cancer agents.

Natural products have been the most important source of new medicinal leads.[9–11] However, the structural complexity of natural products, such as intricate ring systems and numerous chiral centers, may lead to limited supplies and hamper mechanism of action studies and clinical development.[12] For this reason, structural simplification of natural products is a powerful and highly productive tool for lead development and analog design.[13] A well-known example is the simplification of morphine, which led to the clinically used medicines levophanol and meperidine.[14] Neo-tanshinlactone (**1**) is a steroid-like tetracyclic natural product originally isolated from the traditional Chinese medicine Tanshen (Fig. 1). Compound **1** and its first generation analog **2** with various substituents around the molecular scaffold were totally synthesized and previously studied for biological activity. Compound **1** was reported as a highly selective inhibitor of the growth of breast cancer cell lines SK-BR-3 (HER2 over-expressing breast cancer) and ZR-75-1 (estrogen receptor positive breast cancer).[2,15] Although previous studies provided much information about the structure–activity relationships, several questions remained unanswered: how does the skeletal planarity affect activity and selectivity, how do each of the four individual rings contribute to activity, and how will the activity and selectivity change by simplification of the tetracyclic molecule of **1**. We used chemical and biological strategies to investigate structurally simplified **1**-analogs to answer these questions.

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; SAR, structure–activity relationship; TAM, tamoxifen; DIEA, diisopropylethylamine; DMAP, 4-(dimethyl amino) pyridine; LDA, lithium diisopropylamide; TMEDA, tetramethylethylenediamine.

* Corresponding authors. Tel.: +1 919 843 6325 (Q.S.); +1 919 966 7633 (K.F.B.); +1 919 962 0066; fax: +919 966 3893 (K.H.L.).
E-mail addresses: qshi1@email.unc.edu (Q. Shi), ken_bastow@unc.edu (K.F. Bastow), khlee@unc.edu (K.-H. Lee).

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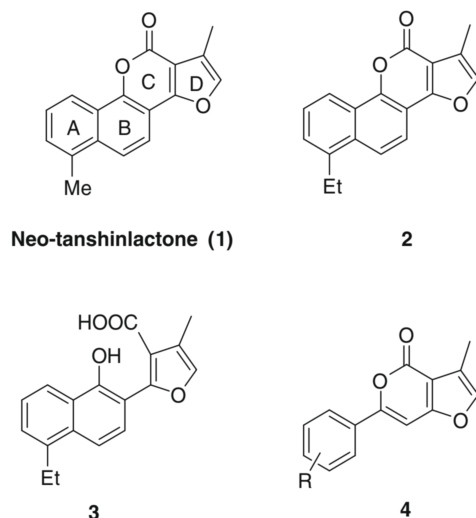


Figure 1. Structures of neo-tanshinlactone (**1**), a first generation neo-tanshinlactone analog **2**, a second generation optimized analog **3**, and a newly designed scaffold **4**.

In our prior paper, we reported a study on how the individual A, C, and D rings influence in vitro anti-breast cancer activity.[16,17] The results revealed that 2-(furan-2-yl)-naphthalen-1-ol derivatives (e.g., **3**), in which ring-C of **1** is missing, are a new class of potent and selective anti-breast cancer agents. These results encouraged us to further simplify the scaffold of **1**. Herein, we report a new chemical entity, substituted 6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one derivatives (**4**), the synthesis of these **4**-analogs, and their cytotoxic activity against a human tumor cell line panel.

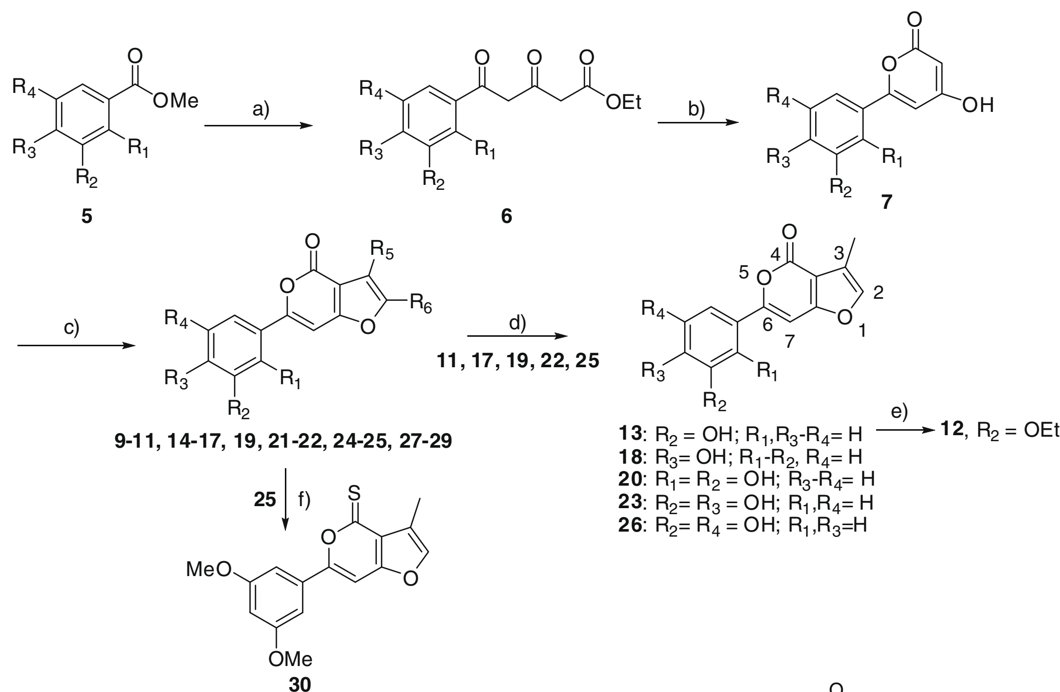
2. Chemistry

All target compounds **9–30** were synthesized through a three to five-step sequence (Scheme 1). Various substituted esters **5** were reacted with a dianion intermediate generated from ethyl acetoacetate with LDA and TMEDA to give triketoesters **6** as tautomeric mixtures. Pyrones **7** were prepared by heating **6** in a 170 °C oil bath under reduced pressure. The resulting yellow solid was isolated by vacuum filtration, and the compound used directly in the next step.[18] Target compounds **9–11**, **14–17**, **19**, **21–22**, **24–25**, and **27–29** were obtained via a tandem alkylation/ intramolecular Aldol reaction of **7**.[17,19] Removal of the methyl group in **11**, **17**, **19**, **22**, and **25** by BBr_3 gave **13**, **18**, **20**, **23**, and **26**. Compound **12** was obtained by treatment of **13** with iodoethane under basic conditions (Scheme 1). Compound **25** was reacted with Lawesson's reagent to afford **30**.[20]

3. Results and discussion

Together with **1,** the newly synthesized 6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one analogs **9**–**30** were evaluated for in vitro anti-breast cancer activity against the SK-BR-3 human tumor cell line. Results from **9**–**27** (Table 1) showed that different substituents around the phenyl ring were critical to the potency and selectivity. Modifications in the furopyranone ring system were also explored with **28**–**30** (Table 1). Selected active compounds with ED_{50} values less than 10 μM against SK-BR-3 were further examined against ZR-7-51, MDA-MB-231 (estrogen receptor negative breast cancer), A549 (human lung cancer), DU145 (prostate cancer), KB (nasopharyngeal carcinoma), and KB-vin (vincristine-resistant KB subline) cancer cell lines (Table 2).

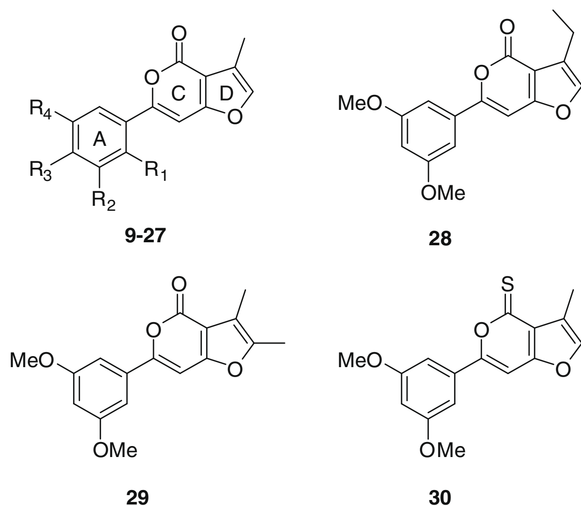
Structurally, both **1** and **10** have a methyl substituent at corresponding positions on their phenyl rings. Thus, the two compounds



Conditions: a) Ethyl acetoacetate, LDA, TMDA, then HOAc; b) 170 °C, 5 mmHg; c) $R_5C(O)CH(Cl)R_6$, HOAc, NH_4OAc; d) BBr_3, CH_2Cl_2; e) EtI, K_2CO_3, acetone; f) Lawesson's reagent
R_1, R_2, R_3, R_4 are found in Table 1; for **9**-**27** R_5=Me, R_6=H; for **28** R_5=Et, R_6=H; for **29** R_5=R_6=Me

Scheme 1.

Table 1
Cytotoxicity of **9–27** against SK-BR-3 tumor cell line[a]



Compd	R_1	R_2	R_3	R_4	SK-BR-3
1	—	—	—	—	0.95 ± 0.01
9	H	H	H	H	15.49 ± 0.60
10	H	Me	H	H	1.50 ± 0.04
11	H	OMe	H	H	2.58 ± 0.15
12	H	OEt	H	H	0.67 ± 0.03
13	H	OH	H	H	1.61 ± 0.09
14	H	F	H	H	20.49 ± 1.50
15	H	H	F	H	19.26 ± 0.65
16	H	H	Me	H	>83.3
17	H	H	OMe	H	>78.1
18	H	H	OH	H	35.95 ± 1.50
19	OMe	OMe	H	H	65.03 ± 2.50
20	OH	OH	H	H	34.11 ± 1.15
21	H	Me	Me	H	14.57 ± 0.63
22	H	OMe	OMe	H	>69.9
23	H	OH	OH	H	0.47 ± 0.01
24	H	Me	H	Me	22.44 ± 0.95
25	H	OMe	H	OMe	0.28 ± 0.01
26	H	OH	H	OH	38.37 ± 0.95
27	H	OMe	OMe	OMe	0.44 ± 0.01
28	—	—	—	—	55.57 ± 1.65
29	—	—	—	—	>66.7
30	—	—	—	—	>66.2

[a] Mean ED_{50}+SEM (μM), from 2 or more independent tests.

are identical, except that **10** has no ring-B. Interestingly, **10** showed potent activity with an ED_{50} value of 1.50 μM, which is slightly less active than **1**. The unsubstituted analog **9** was less potent than either **1** or **10**. Addition of methyl (**10**), methoxy (**11**), ethoxy (**12**), and hydroxyl (**13**) at the R_2-position of the phenyl ring increased activity against the SK-BR-3 cell line, compared with **9**. The rank order of potency of the five compounds was **12** > **13** > **11** > **10** > **9**. Compound **12**, with a R_2-ethoxyphenyl ring, displayed slightly greater activity (ED_{50} 0.67 μM) than **1**. In contrast, fluorine at the R_2-position (**14**), as well as R_3-position (**15**), led to somewhat decreased potency compared with the unsubstituted analog **9**. Addition of methyl, methoxy, or hydroxyl at the phenyl R_3-position (**16–18**) reduced potency significantly. Compounds **19–26** and **27** are di- and tri-substituted derivatives, respectively, with one substituent always present at the phenyl R_2-position. Neither R_1, R_2-disubstituted compounds (**19**, **20**) showed significant activity, leading us to speculate that a substituent in the R_1-position may have a steric effect on the orientation of the lactone ring and reduce the ligand–receptor interaction. Analogs with the same substituent at both the R_2- and R_3-positions showed increased potency relative to the corresponding R_3-mono-substituted analog (**16** vs **21**, **17** vs **22**, **18** vs **23**). Thus, alkyl, alkoxy, and hydroxy groups are favored at R_2-position, while they are not favored at the R_3-position. Comparison of **25** with **24** and **26** indicated that a methoxy group is favored at R_4-position, while methyl and hydroxy groups are not. Furthermore, the R_2, R_3, R_4-trimethoxy (**27**) and R_2, R_4-dimethoxy (**25**) analogs showed dramatically enhanced potency compared with the R_2-methoxy compound (**11**), while the R_2, R_3-dimethoxy (**22**) and R_1, R_2-dimethoxy (**19**) analogs showed decreased potency. In fact, the R_2, R_4-dimethoxy analog **25** (ED_{50} 0.28 μM) was the most active analog among the 19 substituted phenyl A-ring analogs (**9**–**27**). It was also approximately threefold more potent than **1**.

We also investigated the cytotoxic activity of **28–30**, which have a modified ring-C or -D, as shown in Table 1. Insertion of an ethyl (**28**) or two methyl (**29**) groups rather than a single methyl group on the furan, as well as bioisosteric replacement of sulfur (thiolactone **30**) for oxygen in the lactone carbonyl led to greatly reduced or no anti-breast cancer activity (Table 1). More SAR studies of ring-C and -D are in progress and will be reported in a future publication.

To examine the human tumor-tissue-type selectivity, active compounds **10–13**, **23**, **25**, and **27** were tested against a limited but diverse panel of human cancer cell lines, using **1** as a positive control (Table 2). Compounds **10–13** and **23** displayed similar inhibition of the ZR-75-1 and SK-BR-3 cell lines. Interestingly, **25** and **27** showed very weak activity against ZR-75-1. Except for **23**, all lead compounds had weak activity or no activity against MDA-MB-231 breast cancer or the remaining four cancer cell lines tested, which suggested high tumor-tissue-type selectivity. Furthermore, **23** showed only moderate inhibition. Importantly, **25** and **27** showed unique selectivity against the SK-BR-3 breast cancer cell line (HER2+), with approximately 100–250-fold differences compared with the other cancer cell lines tested. The unique selectivity of these novel lead compounds could be exploited to develop novel anti-breast cancer trials candidates and explore the mechanism(s) of action.

Compound **25** 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

Table 2
Cytotoxicity of selected compounds against tumor cell line panel[a]

Compd	ZR-75-1	MDA-MB-231	A549	DU145	KB	KBvin
1	0.9 ± 0.02	37.9 ± 1.4	54.2 ± 3.4	58.3 ± 2.0	>37.9	>75.8
10	6.3 ± 0.4	>83.3	53.8 ± 0.8	24.6 ± 1.5	43.8 ± 1.3	40.0 ± 1.1
11	5.5 ± 0.2	>39.1	58.2 ± 1.7	>78.1	>78.1	71.1 ± 2.1
12	2.2 ± 0.06	>37.0	57.8 ± 3.7	74.1 ± 5.6	64.4 ± 3.0	>74.1
13	0.9 ± 0.03	>41.3	68.6 ± 2.9	82.6 ± 3.4	65.3 ± 0.9	60.3 ± 2.9
23	1.2 ± 0.07	22.9 ± 0.65	19.4 ± 0.71	25.6 ± 1.2	20.2 ± 0.5	23.3 ± 0.39
25	30.8 ± 1.7	>69.9	>69.9	65.4 ± 3.1	>69.9	>69.9
27	29.1 ± 0.8	>31.6	63.3 ± 2.7	>63.3	>63.3	>63.3

[a] Mean ED_{50}+SEM (μM), from 2 or more independent tests.

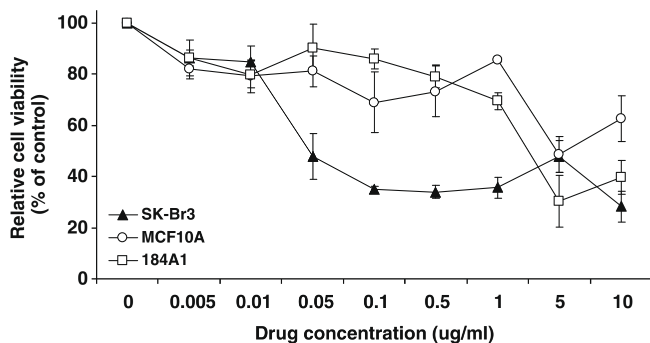


Figure 2. Selective in vitro anticancer activity of **25** against SK-BR-3 breast cancer versus normal breast tissue-derived cell lines (MCF10A and 184A1). Legend: Cell line description, source and activity determination using the MTT-dye assay are described in Section 5. Graphical data are the mean and standard deviation of values obtained from replicates in a single experiment.

are shown in Figure 2. The interpolated ED_{50} values were 1.0, 16.8 and >35.0 μM against SK-BR-3, 184A1, and MCF10A cells, respectively, showing that **25** is selective for a sub-set of breast cancer-derived cell lines and is significantly less active against normal breast-derived tissue.

4. Conclusions

In conclusion, this study discovered a novel class of promising anti-breast cancer agents, substituted 6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one derivatives. The ED_{50} values of the two most potent analogs (**25** and **27**) against SK-BR-3 were 0.28 and 0.44 μM, respectively. More importantly, **25** and **27** showed extremely high cancer cell line selectivity, being approximately 100–250-fold more potent against SK-BR-3 compared with six additional tested cancer cell lines. Furthermore, **25** displayed much greater potency against the SK-BR-3 breast cancer cell line compared with normal breast cell lines 184A1 and MCF10A. Preliminary SAR studies led to the following observations. (1) R_2-Methyl, methoxy, ethoxy, and hydroxy groups, but not a R_2-fluoro group, could increase potency. (2) Among disubstituted phenyl compounds, R_1-, R_3-, or R_4-methyl groups, R_1- or R_3-methoxy groups, and R_4-hydroxy groups decreased potency; while a R_3-hydroxy or R_4-methoxy group increased potency. (3) Current modifications in ring-C and -D were not preferred. The SAR profile established from the current study is different from that with the neo-tanshinlactone series, which is a four-ring system. Thus, skeletal planarity is not indispensable for the entire molecule, though it may be important to some extent. Focused studies will continue to develop promising novel analogs as clinical trials candidates for anti-breast cancer treatment.

5. Experimental section

5.1. Materials and methods

Melting points were measured with a Fisher Johns melting apparatus without correction. ^1H NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. 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. Preparation of intermediates **6** and **7** were reported by Douglas, etc.[17]

5.2. 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}) is calculated as the drug concentration, which caused a 50% reduction in the net protein increase 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 with SEM from at least two 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, ZR-75-1 (estrogen receptor positive breast cancer), MDA MB-231 (estrogen receptor negative breast cancer), SK-BR-3 (HER2 over-expressing 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 (1 μg/mL), and amphotericin B (0.25 μg/mL), and were cultured at 37 °C in a humidified atmosphere of 95% air/5% CO_2.

5.2.1. General preparation of9–11, 14–17, 19, 21–22, 24–25, 27–29

To a solution of **7** (1.04 mmol) in toluene (9 mL) was added a mixture of HOAc (0.30 ml, 5.20 mmol) and NH_4OAc (400 mg, 5.20 mmol) in EtOH (3 mL) and chloroacetone (0.42 mL, 5.20 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 in vacuo. The residue was purified by column chromatography to give a solid.

5.2.2. 3-Methyl-6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one (9)

50% Yield; mp 105–107 °C; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 1.2 Hz, 3H, C*H*$_3$), 7.00 (s, 1H, OC*H*), 7.28–7.29 (m, 1H, C7-*H*), 7.43–7.46 (m, 3H, aromatic), 7.83–7.87 (m, 2H, aromatic); HRMS for (M^++H): calcd 227.0708, found: 227.0696.

5.2.3. 3-Methyl-6-*m*-tolyl-4*H*-furo[3,2-*c*]pyran-4-one (10)

52% Yield; mp 135–137 °C; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 1.2 Hz, 3H, C*H*$_3$), 2.42 (s, 3H, C*H*$_3$), 6.99 (s, 1H, C7-*H*), 7.23–7.36 (m, 3H, aromatic), 7.62 (d, *J* = 7.2 Hz, 1H, aromatic), 7.69 (d, *J* = 1.5 Hz, 1H, OC*H*); HRMS for (M^++H): calcd 241.0865, found: 241.0851.

5.2.4. 6-(3-Methoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (11)

44% Yield; mp 119–121 °C; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.5 Hz, 3H, C*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 6.96–7.00 (m, 2H), 7.29–7.44 (m, 4H); ^{13}C NMR (300 MHz, CDCl3, ppm): δ 8.52, 55.47, 93.64, 109.91, 110.68, 116.36, 117.82, 119.55, 129.91, 133.07, 140.76, 157.71, 159.39, 160.01, 161.93; HRMS for (M^++H): calcd 257.0814, found: 257.0800.

5.2.5. 6-(3-Fluorophenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (14)

34% Yield; mp 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.5 Hz, 3H, CH_3), 7.01 (s, 1H, C7–*H*), 7.10–7.17 (m, 1H, aromatic), 7.31 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.39–7.46 (m, 1H, aromatic), 7.54–7.58 (m, 1H, aromatic), 7.61–7.65 (m, 1H, aromatic); HRMS for (M^++H): calcd 245.0614, found: 245.0603.

5.2.6. 6-(4-Fluorophenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (15)

52% Yield; mp 175–177 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.5 Hz, 3H, CH_3), 6.94 (s, 1H, C7–*H*), 7.12–7.18 (m, 2H, aromatic), 7.29 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.82–7.87 (m, 2H, aromatic); HRMS for (M^++H): calcd 245.0614, found: 245.0603.

5.2.7. 3-Methyl-6-*p*-tolyl-4*H*-furo[3,2-*c*]pyran-4-one (16)

62% Yield; mp 153–155 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 1.2 Hz, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.95 (s, 1H, C7-*H*), 7.24–7.27 (m, 3H, aromatic & OC*H*), 7.74 (d, *J* = 8.1 Hz, 2H, aromatic); HRMS for ($[M+H]^+$): calcd 241.0865, found: 241.0848.

5.2.8. 6-(4-Methoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (17)

60% Yield; mp 146–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 1.5 Hz, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.88 (s, 1H, C7–*H*), 6.97 (d, *J* = 9.0 Hz, 2H, aromatic), 7.26 (d, *J* = 1.5 Hz, 1H, OC*H*), 7.80 (d, *J* = 9.0 Hz, 2H, aromatic); HRMS for ($[M+H]^+$): calcd 257.0808, found: 257.0816.

5.2.9. 6-(2,3-Dimethoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (19)

40% Yield; mp 111–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.2 Hz, 3H, CH_3), 3.87 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.00 (dd, *J* = 1.2, 8.1 Hz, 1H, aromatic), 7.16 (t, *J* = 8.1 Hz, 1H, aromatic), 7.34 (q, *J* = 1.2 Hz, 1H, OC*H*), 7.48 (s, 1H, C7–*H*), 7.54 (dd, *J* = 1.2, 8.1 Hz, 1H, aromatic); HRMS for (M^++H): calcd 287.0919, found: 287.0906.

5.2.10. 6-(3,4-Dimethylphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (21)

67% Yield; mp 182–184 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.30 (s, 3H, CH_3) 2.32 (s, 3H, CH_3), 2.33 (d, *J* = 1.5 Hz, 3H, CH_3), 6.95 (s, 1H, C7–*H*), 7.20 (d, *J* = 8.1 Hz, 1H, aromatic), 7.26 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.57 (d, *J* = 8.1 Hz, 1H, aromatic), 7.64 (s, 1H, aromatic); HRMS for ($[M+H]^+$): calcd 255.1016, found: 255.1010.

5.2.11. 6-(3,4-Dimethoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (22)

83% Yield; mp 154–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 1.5 Hz, 3H, CH_3), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.90 (s, 1H, C7–*H*), 6.92 (d, *J* = 8.7 Hz, 1H, aromatic), 7.27 (t, *J* = 1.5 Hz, 1H, OC*H*), 7.34 (d, *J* = 2.1 Hz, 1H, aromatic), 7.43 (dd, *J* = 2.1, 8.4 Hz, 1H, aromatic); HRMS for (M^++H): calcd 287.0919, found: 287.0900.

5.2.12. 6-(3,5-Dimethylphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (24)

30% Yield; mp 171–173 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.33 (d, *J* = 0.9 Hz, 3H, CH_3), 2.37 (s, 6H, CH_3), 6.98 (s, 1H, C7–*H*), 7.07 (s, 1H, aromatic), 7.28 (d, *J* = 0.9 Hz, 1H, OC*H*), 7.48 (s, 2H, aromatic); HRMS for (M^++H): calcd 255.1021, found: 255.1010.

5.2.13. 6-(3,5-Dimethoxyphenyl)-3-methyl-4*H*-furo [3,2-*c*]pyran-4-one (25)

38%, Yield; mp 153–155 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.5 Hz, 3H, CH_3), 3.86 (s, 6H, OCH_3), 6.54 (t, *J* = 2.1 Hz, 1H, C7–*H*), 6.98 (s, 1H, aromatic), 6.99 (d, *J* = 2.7 Hz, 2H, aromatic), 7.30 (d, *J* = 1.5 Hz, 1H, OC*H*); HRMS for (M^++H): calcd 287.0919, found: 287.0898.

5.2.14. 3-Methyl-6-(3,4,5-trimethoxyphenyl)-4*H*-furo[3,2-*c*]pyran-4-one (27)

40% Yield; mp 201–203 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.34 (d, *J* = 1.5 Hz, 3H, CH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 6H, OCH_3), 6.94 (s, 1H, C7–*H*), 7.06 (s, 2H, aromatic), 7.29 (d, *J* = 1.2 Hz, 1H, OC*H*); HRMS for (M^++H): calcd 317.1025, found: 317.1037.

5.2.15. 6-(3,5-Dimethoxyphenyl)-3-ethyl-4*H*-furo[3,2-*c*]pyran-4-one (28)

51% Yield; mp 131–133 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.31 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.77 (q, *J* = 7.5 Hz, 2H, C$H_2$$CH_3$), 3.86 (s, 6H, OC$H_3$), 6.54 (t, *J* = 2.1 Hz, 1H, C7–*H*), 6.98 (d, *J* = 2.1 Hz, 3H, aromatic), 7.30 (t, *J* = 1.2 Hz, 1H, OC*H*); HRMS for (M^++H): calcd 301.1076, found: 301.1057.

5.2.16. 6-(3,5-Dimethoxyphenyl)-2,3-dimethyl-4*H*-furo[3,2-*c*]pyran-4-one (29)

12% Yield; mp 163–165 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.25 (d, *J* = 0.6 Hz, 3H, CH_3), 2.33 (d, *J* = 0.6 Hz, 3H, CH_3), 3.86 (s, 6H, OCH_3), 6.53 (t, *J* = 2.1 Hz, 1H, aromatic), 6.93 (s, 1H, OC*H*), 6.97 (d, *J* = 2.1 Hz, 2H, aromatic); HRMS for ($[M+H]^+$): calcd 301.1071, found: 301.1067.

5.3. General preparation of 13, 20, 23, and 26

To a solution of **13, 20, 23,** or **26** (0.2 mmol) in DCM (3 ml) was added BBr_3 (0.6 ml, 0.6 mmol) dropwise at 0 °C. The reaction mixture was stirred overnight. Water was added to quench the reaction. The solution was extracted with $CHCl_3$ and concentrated. The residue was purified by column chromatography to give a white solid.

5.3.1. 6-(3-Hydroxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (13)

78% Yield; mp 225–227 °C; ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.29 (d, *J* = 1.5 Hz, 3H, CH_3), 6.86–6.90 (m, 1H, C7–*H*), 7.26–7.31 (m, 3H, aromatic & OC*H*), 7.35–7.39 (m, 1H, aromatic), 7.52 (dd, *J* = 1.2, 2.7 Hz, 1H, aromatic); HRMS for (M^++H): calcd 243.0657, found: 243.0659.

5.3.2. 6-(4-Hydroxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (18)

80% Yield; mp 258–260 °C; ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.27 (d, *J* = 1.5 Hz, 3H, CH_3), 6.86 (d, *J* = 9.0 Hz, 2H, aromatic) 7.18 (s, 1H, C7–*H*), 7.46 (d, *J* = 1.2 Hz, 1H, OC*H*), 7.75 (d, *J* = 9.3 Hz, 2H, aromatic); HRMS for ($[M+H]^+$): calcd 243.0657, found: 243.0641.

5.3.3. 6-(2,3-Dihydroxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (20)

66% Yield; mp 239–241 °C; ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.28 (d, *J* = 0.9 Hz, 3H, CH_3), 6.76 (t, *J* = 1.5 Hz, 1H, aromatic), 6.86 (dd, *J* = 1.5, 1.8 Hz, 1H, aromatic), 7.38 (dd, *J* = 1.2, 1.5 Hz, 1H, aromatic), 7.48 (q, *J* = 1.2 Hz, 1H, OC*H*), 7.75 (s, 1H, C7–*H*); HRMS for (M^++H): calcd 259.0606, found: 259.0602.

5.3.4. 6-(3,4-Dihydroxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (23)

60% Yield; mp 259–261 °C; ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.69 (d, *J* = 1.5 Hz, 3H, CH_3), 6.83 (d, *J* = 7.8 Hz, 3H, C7–*H*), 7.12 (s, 1H, aromatic), 7.26–7.31 (m, 2H, aromatic), 7.46 (dd, J = 1.2 Hz, 1H, OCH); HRMS for (M^+–H): calcd 257.0450, found: 257.0464.

5.3.5. 6-(3,5-Dihydroxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (26)

70% Yield; mp >300 °C; ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.86 (d, *J* = 1.2 Hz, 3H, CH_3), 6.35 (t, *J* = 2.1 Hz, 1H, aromatic), 6.80 (d, *J* = 2.1 Hz, 2H, aromatic), 7.21 (s, 1H, C7–*H*), 7.51 (d, *J* = 1.2 Hz, 1H, OC*H*); HRMS for ($[M+H]^+$): calcd 259.0601, found: 259.0594.

5.3.6. 6-(3-Ethoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-one (12)

To a mixture of **13** (212 mg, 1.00 mmol), K_2CO_3 (300 mg, 2.17 mmol) in acetone (8 mL) was added iodoethane (0.4 mL, 5.00 mmol). The mixture was stirred for 12 h. The mixture was concentrated and 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 to give a white solid.

35% Yield; mp 128–130 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.45 (t, *J* = 6.9 Hz, 3H, CH_2CH_3), 2.33 (d, *J* = 1.5 Hz, 3H, CH_3), 4.10 (d, *J* = 7.2 Hz, 2H, CH_2CH_3), 6.95–6.96 (m, 1H, aromatic), 6.98 (s, 1H, C7–*H*), 7.29 (d, *J* = 1.5 Hz, 1H, OC*H*), 7.32–7.43 (m, 3H, aromatic); HRMS for ($[M+H]^+$): calcd 271.0965, found: 271.0962.

5.3.7. 6-(3,5-Dimethoxyphenyl)-3-methyl-4*H*-furo[3,2-*c*]pyran-4-thione (30)

A mixture of **25** (0.1 mmol) and Lawesson's reagent (81 mg, 0.2 mmol) in dry toluene (5 mL) was heated to reflux for 12 h. Toluene was removed and the red residue was dissolved in EtOAc and partitioned with H_2O. The organic phase was separated and dried over Na_2SO_4. Removal of solvent in vacuo afforded an oily, residue which was purified by column chromatography resulting in a yellow solid.

60% Yield; mp 147–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.46 (d, *J* = 1.5 Hz, 3H, CH_3), 3.87 (s, 6H, OCH_3), 6.57 (t, *J* = 2.1 Hz, 1H, C7–*H*), 7.02 (s, 1H, aromatic), 7.03 (s, 1H, aromatic), 7.17 (s, 1H, aromatic), 7.32 (d, *J* = 1.5 Hz, 1H, OC*H*); HRMS for (M^++H): calcd 303.0691, found: 303.0702.

5.4. Methodology of MTT assay

The MTT assay was used to independently test the potent activity of **25** against SK-BR-3 and to investigate antitumor selectivity versus activity against two normal breast cancer cell lines: 184A1 and MCF10A (CRL-10317) purchased from ATCC (Rockville, MD). 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 incubation continued for 2 h. The amount of formazan product was measured at an OD of 570 nM using a plate-reader.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.11.049.

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