Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3407–3410
Bioorganic & Medicinal Chemistry Letters

Lead identification of a potent benzopyranone selective estrogen receptor modulator

Jeffrey A. McKie,[a,*] Shripad S. Bhagwat,[a] Helen Brady,[c] Mary Doubleday,[a] Leah Gayo,[a] Mathew Hickman,[c] Ravi K. Jalluri,[a] Sak Khammungkhune,[a] Adam Kois,[a] Deborah Mortensen,[a] Normand Richard,[c] John Sapienza,[a] Graziella Shevlin,[a] Bernd Stein[b] and May Sutherland[b]

[a]*Medicinal Chemistry, Celgene, San Diego, CA 92121, USA*
[b]*Cellular and Molecular Pharmacology, Celgene, San Diego, CA 92121, USA*
[c]*Lead Discovery, Celgene, San Diego, CA 92121, USA*

Received 12 March 2002; accepted 27 April 2004

Abstract—Starting from a phenol screening hit (**6**), three series of benzopyranone selective estrogen receptor modulators (SERMs) have been designed, synthesized, and analyzed for both estrogen receptor alpha binding affinity and in vitro activity in two cell assays. The lead compound identified, SP500263 (**13**), was more potent than raloxifene and tamoxifen in a cell-based assay measuring inhibition of interleukin-6 release.

The hormone 17β-estradiol (E2) (**1**, Fig. 1) plays an important role in the physiology of both women and men and the effect of decreased plasma hormone concentrations in peri- and postmenopausal women has drawn significant attention.[1] To compensate for the reduced plasma E2 levels throughout menopause, estrogen replacement therapy (e.g., estrone, E2, estriol, and/or conjugated equine estrogens) or estrogen plus a progestin (also referred to as hormone replacement therapy) is typically prescribed. These protocols have proven effective in treating osteoporosis and hot flushes, while any beneficial role in cardiovascular and central nervous system health (Alzheimer's and cognition) is still under debate.[2] Estrogen replacement therapy results in side effects that include an increase in risk of uterine and breast cancer that is only partially overcome by the addition of a progestin to the treatment combination.[3] The increased risk of uterine and breast cancer associated with estrogen replacement therapy is believed to be due to the mitogenic action of E2 and E2 conjugates on these reproductive tissues.[4] Thus, estrogen and hormone

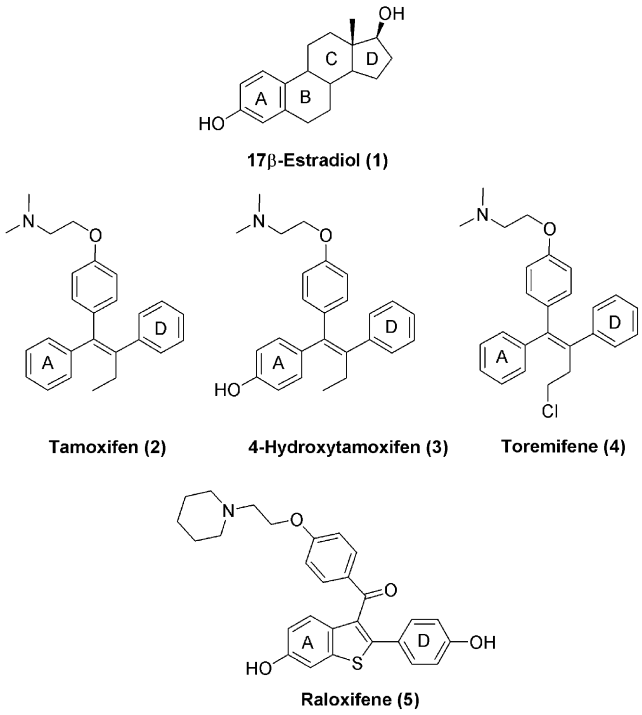


Figure 1. Structures of 17β-estradiol and marketed SERMs with corresponding A, B, C, and D rings of estradiol shown.

Keywords: Benzopyranone; Estrogen receptor modulator.
* Corresponding author. Tel.: +1-858-795-4781; fax: +1-858-795-4719; e-mail: jmckie@celgene.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.081

replacement therapies are viewed as having desirable as well as undesirable effects as therapeutic regimens. From this understanding, subsequent drug discovery programs have been initiated to develop potential nonsteroidal estrogen receptor ligands that retain the beneficial pharmacological effects of E2 and eliminate its undesirable properties.

Early drug discovery efforts focused on the design of small molecule nonsteroidal estrogen receptor ligands with antagonist properties against breast and other reproductive tissues.[5] Tamoxifen (**2**, Fig. 1) was the first marketed drug to be realized from these efforts and while this compound and its active metabolite, 4-hydroxytamoxifen (**3**, Fig. 1), are effective antiestrogens on estrogen receptor positive breast tissue, they subsequently were discovered to have undesirable estrogenic properties on the endometrium.[6] A second triphenylethylene compound, toremifene (**4**, Fig. 1), has also been approved for the treatment of breast cancer although it too was reported to have undesirable uterine stimulatory activity.[7] More recently, raloxifene (**5**, Fig. 1), a benzothiophene was marketed for the prevention and treatment of osteoporosis. This compound has been termed a selective estrogen receptor modulator (SERM) because of its estrogen agonist properties on bone and estrogen antagonist properties on the breast and endometrium. Unlike tamoxifen, raloxifene has not been associated with an increased risk of uterine cancer in women upon prolonged use.[8]

As part of our drug discovery effort toward identifying potential SERMs for the prevention and treatment of osteoporosis and breast cancer, we developed a unique cellular screen to measure the effect of small molecule estrogen receptor ligands at inhibiting release of the cytokine, interleukin-6 (IL-6), from an estrogen receptor negative osteosarcoma cell line (U2OS) transfected with ER-α.[9] The estrogen receptor-mediated effect on IL-6 release under our conditions was determined to proceed by a nonclassical mechanism. Since IL-6 is a cytokine that promotes bone resorption, proliferation of certain cancers, and other pathogenic processes,[10] inhibiting IL-6 release is predicted to be a measure of a potential drug candidate's ability to reduce both bone resorption and cellular proliferation.

We initiated screening of commercially available phenolic compounds in our primary assay to measure their ability at inhibiting IL-6 release in U2OS cells. Several analogues provided IC_{50} values of $<10\,\mu M$ and the benzopyranone analogue, **6**, proved to be of particular interest with an IC_{50} of 300 nM (Fig. 2).

Although **6** displayed desirable estrogen receptor agonist activity in our IL-6 assay by blocking cytokine release, it also had undesirable agonist properties in the MCF-7 proliferation assay that manifest in an increase in cellular proliferation. A review of journal and patent literature indicated that other benzopyranone molecules had been reported to have ER-modulating properties, however, the disclosed compounds did not incorporate the functional groups that we believed necessary for ideal SERM activity.[11] Published extensive structure–activity studies on SERMs provided key information on structural requirements necessary for selective antagonist properties. Incorporation of the side-chain amine in SERMs such as tamoxifen and raloxifene has been shown to be critical for providing selective tissue antagonist properties. Furthermore, crystal structure data of bound agonists and antagonists has facilitated the design of a useful model for correlating compound structure with agonist or antagonist receptor conformation.[12]

With this information in hand, a set of benzopyranone analogues (**8**–**19**) was designed based on the structures of 4-hydroxytamoxifen (**3**) and raloxifene (**5**). In the series, we proposed to initially fix the D-ring substituent (3-position of the benzopyranone core) as a phenyl group and attach a variable length piperidinylalkyl chain (SERM side chain) from the benzopyranone 4-phenoxy (**8**–**12**), 3-phenoxy (**16**–**19**) or 4-benzyloxy (**13**–**15**) substituent (Fig. 3).

The synthetic route that provided the desired products most efficiently is shown in Scheme 1. Commercially available 3-methoxyphenol was acylated under Fries reaction conditions using $POCl_3$ and $ZnCl_2$ in

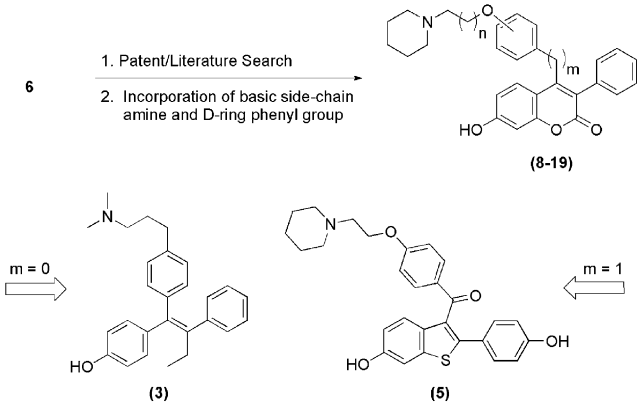


Figure 3. Design of analogues of screening hit **6** to incorporate tamoxifen and raloxifene structural features.

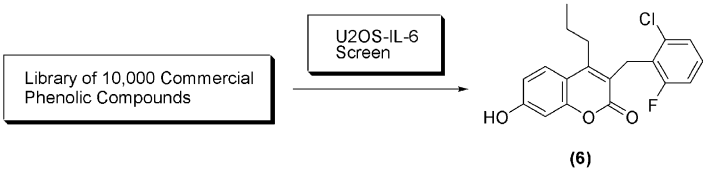
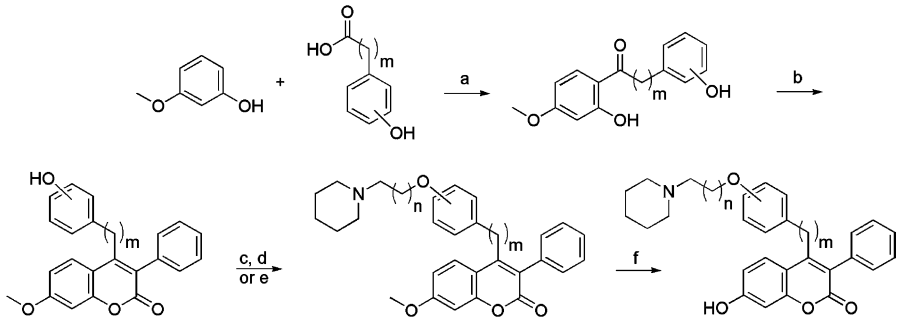


Figure 2. High-throughput screen of commercial phenols to identify screening hit **6.**



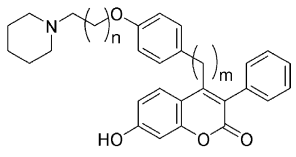
Scheme 1. Reagents and conditions: (a) $POCl_3$, $ZnCl_2$, 1,2-dichloroethane 70 °C, 4 h; or $BF_3{\cdot}OEt_2$, chlorobenzene, 70 °C, 2 h, yield = 40–60%; (b) benzoic acid or phenylacetic acid, carbonyl diimidazole, K_2CO_3, DMAP, DMF, 85 °C, 4 h, yield = 60–70%; (c) 1,ω-dibromoalkane, K_2CO_3, acetone, reflux, 12 h, yield = 80–90%; (d) piperidine, THF, 60 °C, 5 h, yield = 85%; (e) 2-chloroethylpiperidine hydrochloride, K_2CO_3, DMF, 5 h, 80 °C, yield = 75%; (f) 30% HBr in acetic acid, 90 °C, 8 h, yield = 60% or ethanethiol, $AlBr_3$, CH_2Cl_2, room temperature, 2 h, yield = 75%.

dichloroethane or $BF_3{\cdot}OEt_2$ in chlorobenzene at 70 °C. The major product was the desired benzophenone or benzoacetophenone indicated, although the unwanted Fries-reaction regioisomer (*para* to the phenol group of 3-methoxyphenol) was also detected in the reaction mixture. The second reaction step was a benzopyranone core formation reaction that was accomplished by modification of a procedure reported by Rao and Srimannarayana.[13] Introduction of the side-chain amine functional group was successfully completed by either a one-step ($n = 2$) or two-step process ($n = 3, 4, 5, 6$). The one-step process involved alkylation of the phenol, **7**, with chloroethylpiperidine hydrochloride by heating a mixture of these two reagents and K_2CO_3 in DMF. The two-step procedure progressed by alkylation of phenol, **7**, with 1,ω-dibromoalkane and subsequent displacement of the terminal bromide with piperidine. The final products (**8**–**15**) were prepared through demethylation of the resulting A-ring methylethers by heating in 30% HBr–acetic acid. Alternatively, the demethylation could be accomplished at room temperature with ethanethiol and $AlBr_3$ in CH_2Cl_2.

As indicated in Table 1, the tamoxifen mimetics (**8**–**12**) proved to be potent estrogen receptor ligands, but significantly less active than desired in our in vitro IL-6 and MCF-7 assays. In comparing this series to tamoxifen and raloxifene, the most potent benzopyranone (**10**) was approximately 5–30-fold less active in our cellular assays than the SERM standards. While extending the side-chain amine improved in vitro activity up to eightfold for $n = 3$ (**10**) compared to $n = 2$ (**9**), additional chain extension resulted in decreased activity (**11**–**12**). The ER-α relative binding affinity (relative to E2) for the series ranged from 488%. The compound from this series that displayed the weakest ER receptor affinity, **8**, was also the least potent in the cellular assays, however, there was not a direct correlation between receptor binding affinity and cellular functional activity. Although the cellular data was promising, we were confident that optimal positioning of the side-chain substituent within the receptor binding pocket would provide us with more potent analogues.

The raloxifene mimetics (**13**–**15**) provided an approximately two-order of magnitude improvement in in vitro potency in our IL-6 assay as well as an improvement in antiproliferative activity in the MCF-7 assay. The receptor binding affinity for the series once again provided evidence that receptor binding affinity and cellular functional activity do not correlate directly. Previous reports have also supported the hypothesis that the resulting conformation of the estrogen receptor–ligand complex can be more important than the affinity of the ligand for the receptor in determining ligand agonist or antagonist potency.[14] The additional atom spacer provided by the benzylic carbon atom resulted in optimum positioning of the molecules within the receptor.[15] Compound **13** was significantly more potent in the IL-6 assay than either raloxifene or tamoxifen, while the MCF-7 activity was similar to raloxifene. Extension of the side chain (**14**–**15**) resulted in a decrease in activity. Despite this significant loss of activity, the 4-benzyl series of benzopyranones (**13**–**15**) was surprisingly more active than the 4-phenyl series of analogues (**8**–**12**).

Table 1. In vitro MCF-7 and IL-6 assay data for compounds **8**–**15**, raloxifene (**5**), and 4-hydroxytamoxifen (**3**)



(8-15)

Compd	*n*	*m*	U2OS (ER-α) IL-6,[a] IC_{50} (nM)[b]	MCF-7[a] IC_{50} (nM)[b]	RBA,[a,c] ER-α
3	na	na	1.9	24	48
5	na	na	3.1	4.9	35
8	1	0	505	425	4
9	2	0	355	335	21
10	3	0	69	134	29
11	4	0	114	335	79
12	5	0	145	720	88
13	*1*	*1*	*0.38*	*7.7*	*85*
14	2	1	4.4	18	90
15	3	1	5.0	13	139

[a] See Ref. 9 for a description of the assay.
[b] Values are an average of at least two experiments with standard errors below 15%.
[c] Relative binding affinity (RBA, where RBA of E2 = 100).

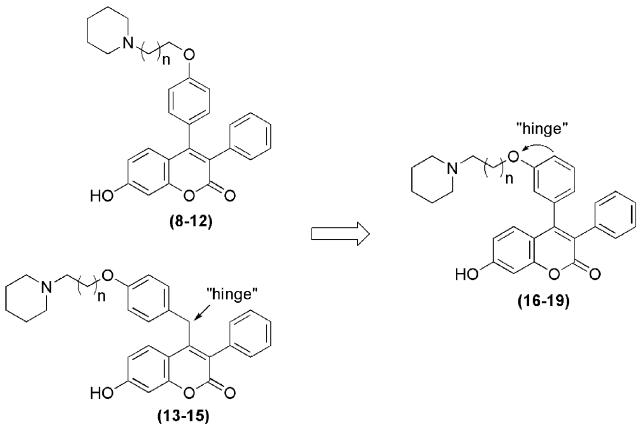


Figure 4. Modification of the benzopyranone 4-phenyl group to incorporate the critical hinge feature identified from the benzypyranone 4-benzyl series.

In overlaying the structures of the two series of compounds, the 4-phenyl and 4-benzyl analogues, we concluded that positioning of the amine side chain was critical for selective estrogen receptor activity. The hinge created by the methylene spacer in the benzyl series clearly provided ideal positioning of the basic amine side chain. Based on this observation we elected to incorporate this effective feature into the 4-phenyl series by moving the basic amine side chain to the *meta* position of the benzopyranone 4-phenyl ring as exemplified in compounds **16**–**19** in Figure 4.

Moving the basic amine side chain from the phenyl group 4-position to the 3-position in the 4-phenyl benzopyranone series provided a significant improvement in U2OS/IL-6 as well as MCF-7 antiproliferative activity. In directly comparing the 2 and 3 atom spacers of the amine side-chain spacer from these two series, compounds **16** versus **8** and **17** versus **9**, a 40- and >200-fold improvement in U2OS/IL-6 activity was realized. These results provided evidence that this manipulation of the side chain was effective in mimicking the hinge substituent found in the benzopyranone 4-benzyl series. The observation that extending the side-chain length improved receptor binding affinity but failed to provide a corresponding improvement in functional activity in our assays was consistent with results from the other two series (Table 2).

Table 2. In vitro MCF-7 and IL-6 assay data for compounds **13**, and **16**–**18** incorporating the hinge feature into the 4-phenyl series

Compd	*n*	*m*	U2OS (ER-α) IL-6,[a] IC_{50} (nM)[b]	MCF-7[a] IC_{50} (nM)[b]	RBA,[a,c] ER-α
13	*1*	*1*	*0.38*	*7.7*	*85*
16	1	0	12	85	20
17	2	0	1.5	9.5	45
18	3	0	3.2	12	52
19	4	0	11	15	105

[a] See Ref. 9 for a description of the assay.
[b] Values are an average of at least two experiments with standard errors below 15%.
[c] Relative binding affinity (RBA, where RBA of E2 = 100).

In summary, we have designed and synthesized three series of benzopyranone selective estrogen receptor modulators based on a phenol screening hit. Analogue synthesis resulted in the development of a clear SAR for each series and the potential for improvement in tissue selective activity and target potency. Future research will be focused on optimizing the in vivo activity of our current lead structure SP500263 (**13**).

References and notes

1. Simpson, E. R.; Davis, S. R. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 2214.
2. (a) WHI Steering Committee *JAMA* **2004**, *291*, 1701; (b) Herrington, D. M. *Ann. Int. Med.* **1999**, *131*, 463.
3. Stanford, J. L.; Weiss, N. S.; Voigt, L. F.; Daling, J. R.; Habel, L. A.; Rossing, M. A. *JAMA* **1995**, *274*, 137.
4. De Lignieres, B. *Maturitas* **1996**, *23*, 31.
5. Lerner, L. J.; Holthaus, J. F.; Thompson, C. R. *Endocrinology* **1958**, *63*, 295.
6. Killackey, M. A.; Hakes, T. B.; Pierce, V. K. *Cancer Treat. Rep.* **1985**, *69*, 237.
7. Maenpaa, J.; Holli, K.; Pasanen, T. *Eur. J. Cancer* **2000**, *36*, S61.
8. Cummings, S. R.; Eckert, S.; Krueger, K. A.; Grady, D.; Powles, T. J.; Cauley, J. A.; Norton, L.; Nickelsen, T.; Bjarnason, N. H.; Morrow, M.; Lippman, M. E.; Black, D.; Glusman, J. E.; Costa, A.; Jordan, V. C. *JAMA* **1999**, *316*, 2189.
9. (a) Stein, B.; Yang, M. X. *Mol. Cell. Biol.* **1995**, *10*, 4971; (b) Brady, H.; Desai, S.; Gayo-Fung, L.; Khammungkhune, S.; McKie, J. A.; O'Leary, E.; Pascasio, L.; Kung-Sutherland, M.; Anderson, D. W.; Bhagwat, S. S.; Stein, B. *Cancer Res.* **2002**, *62*, 1439.
10. (a) Stein, B.; Kung-Sutherland, M. S. *Drug Discovery Today* **1998**, *3*, 202; (b) Simpson, R. J.; Hammacher, A.; Smith, D. K.; Matthews, J. M.; Ward, L. D. *Protein Sci.* **1997**, *6*, 929; (c) Yudkin, J. S.; Kumari, M.; Humphries, S. E.; Mahamed-Ali, V. *Atherosclerosis* **2000**, *148*, 209; (d) Ershler, W. B.; Keller, E. T. *Annu. Rev. Med.* **2000**, *51*, 245.
11. (a) Lednicer, D.; Lyster, S. C.; Duncan, D. W. *J. Med. Chem.* **1965**, *8*, 725; (b) Wani, M. C.; Rector, D. H.; Christensen, H. D.; Kimmel, G. L.; Cook, C. E. *J. Med. Chem.* **1978**, *18*, 982; (c) Pollard, I.; Martin, L. *Steroids* **1968**, *11*, 897.
12. (a) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. *Nature* **1997**, *389*, 753; (b) Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cullinan, G. J.; Glasebrook, A. L.; Jones, C. D.; Matsumoto, K.; Palkowitz, A. D.; Sato, M.; Teermine, J. D.; Winter, M. A.; Yang, N. N.; Dodge, J. A. *Proc. Natl. Acad. Sci.* **1997**, *94*, 14105.
13. Rao, P. P.; Srimannarayana, G. *Synthesis* **1981**, 887.
14. Wijayaratne, A. L.; Nagel, S. C.; Paige, L. A.; Christensen, D. J.; Norris, J. D.; Fowlkes, D. M.; McDonnell, D. P. *Endocrinology* **1999**, *140*, 5828.
15. Weatherman, R. V.; Carroll, D. C.; Scanlan, T. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3129.