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Benzothiophene and naphthalene derived constrained SERMs

Owen B. Wallace,* Henry U. Bryant, Pamela K. Shetler, Mary D. Adrian and Andrew G. Geiser

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

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Abstract—For selective estrogen receptor modulators (SERMs), the orientation of the basic side chain relative to the SERM core has a significant impact on function. The synthesis and biological evaluation of two series of SERMs are disclosed, where the ligand side chain is constrained to adopt a defined orientation. Compounds where the side chain is forced into the plane of the SERM core have a different profile compared to those compounds where the side chain is pseudo-orthogonal, particularly with regard to antagonism of estradiol action on an Ishikawa uterine cell line.

The estrogen receptor (ER) is a ligand activated transcription factor, which plays a critical role in female reproductive function, and influences skeletal, cardiovascular and CNS health. There are two known subtypes, ERα and ERβ, which are expressionally and functionally unique. Selective estrogen receptor modulators (SERMs) are compounds that may mimic the effects of estrogen in some tissues, while antagonizing the effects of estrogen in others.[1] While the precise reason for this tissue selectivity is not fully understood, it is clear that the conformation of the receptor and its ability to interact with cofactors is critically important. Moreover, subtle changes of ligand structure can have a dramatic impact on receptor conformation.

Of significant importance for a ligand's ability to act as a SERM is the nature of the basic side chain and its orientation relative to the ligand backbone. It is known that in the X-ray crystal structure of raloxifene bound to ERα, the basic side chain of the ligand occupies an orthogonal position relative to the benzothiophene core.[2] In an effort to constrain the side chain into its presumed active conformation, Grese et al. prepared a series of benzopyran derivatives, some of which displayed an excellent in vitro and in vivo profile.[3] Other recent examples of constrained SERMs come from the labs of Katzenellenbogen.[4,5] The design, synthesis, and biological activity of two series of conformationally restricted benzanthracene derivatives is herein reported—one series based on a benzothiophene scaffold and the other based on a naphthalene core.

Benzothiophene analogs were prepared from *N*,*N*-dimethylamino derivative **1** (Scheme 1), which has previously been shown to be a useful electrophile for the synthesis of raloxifene analogs.[6] Addition of a benzyl Grignard reagent to **1** resulted in benzothiophene **2**. Reduction with LAH in THF at room temperature afforded alcohol **3**. Treatment with TFA yielded the cyclized product, resulting from trapping of the resulting carbonium ion with the pendant aryl ring at the C2 position. Deprotection of the phenol (BBr_3, CH_2Cl_2) then yielded the desired products. A more expeditious route to the cyclized product was developed wherein alcohol **3** was simply treated with BBr_3 to affect cyclization with concomitant deprotection in a single pot.[7] Interestingly, when the cyclization reaction was attempted with the *para*-methoxy derivative (**3**, R = 4-OMe), the fully aromatic analog **7** was isolated as the minor product component together with **6** in a 1:8 ratio.[8] The two products could be readily separated by reverse phase HPLC before testing in the in vitro assays. The *meta*-methoxy alcohol intermediate (**3**, R = 3-OMe) on treatment with BBr_3 afforded a ~2.9:1 mixture of the 3- and 5-phenols (**5** and **8**), which again could be readily separated by reverse phase HPLC.

Naphthalene analogs were prepared from naphthalene triflate **9**.[9] Using Knochel's protocol, triflate **9** was coupled with a benzyl zinc reagent in the presence of

* Corresponding author. Fax: +1 317 433 0715; e-mail: owen.wallace@lilly.com

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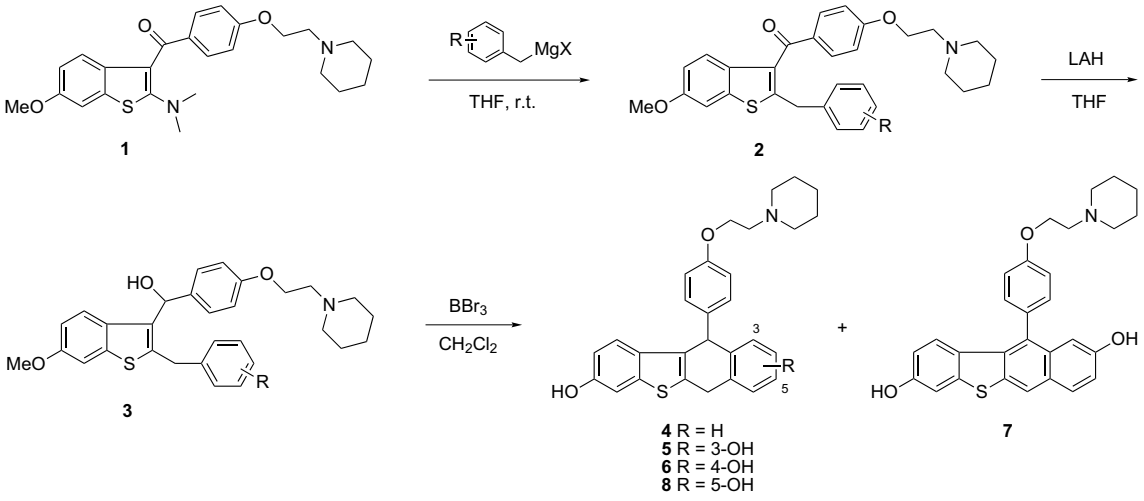
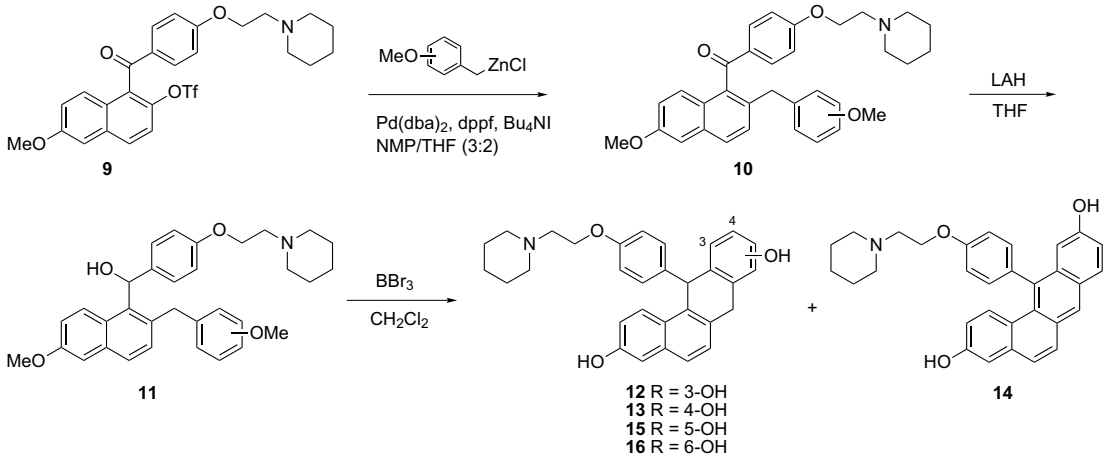


Scheme 1. Synthesis of benzothiophene constrained SERMs.

$Pd(dba)_2$, dppf, and Bu_4NI in a mixture of NMP and THF to afford naphthalene **10**.[10] Reduction with LAH in THF at room temperature afforded alcohol **11**, which was then subjected to BBr_3 in CH_2Cl_2 at room temperature to yield the desired dihydrobenzanthracenes. Once again, the *para*-methoxyphenyl derivative **11** (R = 4-OMe) gave a ~2:1 mixture of the desired product **13** along with the aromatized benzo[*a*]anthracene derivative **14**, which were separable by HPLC. The *meta*-methoxy analog **11** (R = 3-OMe) afforded the 3- and 5-phenols **12** and **15**, respectively, in a ~1:3 ratio, which were also separable (Scheme 2).

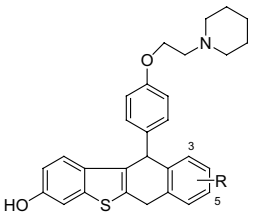
Compounds were first tested in a radioligand binding assay[11] and were then examined for functional activity in breast cancer (MCF-7) and endometrial adenocarcinoma (Ishikawa) cell lines (Table 1). Briefly, antagonism of estrogen action in MCF-7 cells was assayed via inhibition of cell proliferation by 10 pM of 17β-estradiol, and is reported as an IC_{50} value and percent efficacy. Agonist activity (EC_{50} and percent efficacy) of the compounds in Ishikawa cells was determined by quantitating alkaline phosphatase induction by the compound alone. Antagonist activity (IC_{50} and percent efficacy) was determined by inhibition of alkaline phosphatase induction by the compound in the presence of 1 nM 17β-estradiol.[12] 4-Hydroxytamoxifen (4-OHT) was used as a standard in each assay.[13] Compounds with a desirable SERM profile are those with potent binding to the estrogen receptors and are potent and efficacious antagonists of MCF-7 cell proliferation. In addition, in order to minimize undesirable uterine effects, compounds should display weak Ishikawa agonism and potent and efficacious Ishikawa antagonism.

In the benzothiophene series, the parent compound **4** (R = H) has reasonable binding affinity to both ERα and ERβ (K_i = 2.4 and 5.3 nM, respectively), and antagonizes the effect of estrogen in the MCF-7 cell line (IC_{50} 17 nM). However, the compound displays a mixed agonist/antagonist profile in the Ishikawa cell line, and is a considerably more potent agonist than antagonist (IC_{50} 3.5 vs 710 nM). Introduction of a hydroxyl at C3 (**5**) neither improves binding affinity nor functional antagonism. In fact, the compound is a weaker antagonist in MCF-7 cells (IC_{50} 42 nM) and is a more effica-

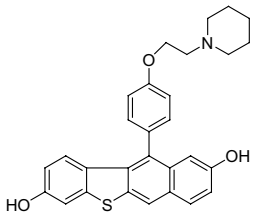


Scheme 2. Synthesis of naphthalene constrained SERMs.

Table 1. Binding and functional data



4 R = H **5** R = 3-OH
6 R = 4-OH **8** R = 5-OH



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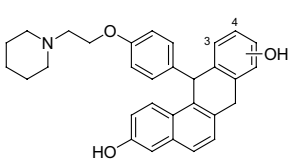
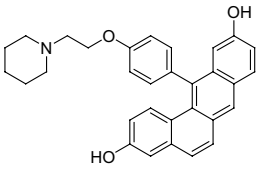


12 R = 3-OH **13** R = 4-OH
15 R = 5-OH **15a** R = 5-OH (*ent* 1)
15b R = 5-OH (*ent* 2) **16** R = 6-OH



14

	Binding		MCF-7		Ishikawa		Ishikawa	
	K_i ERα (nM)	K_i ERβ (nM)	IC_{50} (nM)[a]	Efficacy (%)[a]	EC_{50} (nM)[b]	Efficacy (%)[b]	IC_{50} (nM)[c]	Efficacy (%)[c]
4-OHT	0.5	0.5	2.6	79	0.8	140	510	45
4	2.4	5.3	17	72	3.5	77	710	66
5	2.6	14	42	66	3.1	130	680	67
6	0.5	0.9	0.9	80	0.9	66	53	84
7	0.4	0.3	1.0	77	1.6	170	690	63
8	0.7	1.3	0.7	74	0.4	100	45	70
12	7.7	40	68	72	28	77	580	75
13	1.0	3.2	6.6	79	2.6	75	130	82
14	0.8	0.5	57	69	10	130	820	59
15	0.8	4.2	1.9	79	0.8	27	31	91
15a	1.1	5.5	23	65	3.1	56	160	71
15b	0.7	4.4	2.4	70	0.5	19	28	92
16	1.1	5.4	2.9	85	0.7	120	50	84

[a] IC_{50} and % efficacy data are quoted for MCF-7 assay in antagonist mode.
[b] EC_{50} and % efficacy data are for Ishikawa assay in agonist mode.
[c] IC_{50} and % efficacy are for Ishikawa assay in antagonist mode.

cious agonist in Ishikawa uterine cells compared to the unsubstituted parent (**4**). A significant increase in binding affinity is seen with the 4-hydroxyl derivative (**6**), and this analog also displays a much improved functional profile. The binding affinity of **6** is approximately 5-fold greater than **4** and the compound now displays sub-nM inhibition of estradiol action on MCF-7 cells with good efficacy (80%). The compound is also ~10-fold more potent in the Ishikawa antagonist assay, but still displays relatively potent agonism in this cell line (EC_{50} 0.9 nM). The importance of the relative orientation of the side chain is clearly seen by comparing **6** to it aromatic analog **7** where the side chain is unable to adopt an orthogonal relationship to the benzothiophene core. Although **7** binds with similar high affinity to both ERα and ERβ and is a potent MCF-7 antagonist (IC_{50} 1 nM), the functional profile in uterine adenocarcinoma cells is markedly different. Benzothiophene **7** is a weaker Ishikawa antagonist (IC_{50} 690 nM) than analog **6** (IC_{50} 53 nM), and is also a more efficacious agonist (170% efficacy). The 5-hydroxyl analog **8** displays a very similar profile to the 4-hydroxyl analog **6** in terms of binding, MCF7 antagonism and Ishikawa antagonism, but is a more potent and efficacious agonist in the uterine cell line (EC_{50} 0.4 nM, 100% efficacy).

In the naphthalene-based series, the 3-hydroxyl derivative (**12**) again displays relatively weak binding, MCF-7 and Ishikawa antagonism, and its agonist potency is somewhat weaker than its benzothiophene counterpart **5**. The 4-hydroxyl analog (**13**) has an improved binding and functional profile relative to analog **12**. It is a significantly more potent antagonist in both breast (IC_{50} 6.6 nM) and uterine (IC_{50} 130 nM) cell lines. The fully aromatic benzanthracene derivative **14** displays potent binding but weaker functional antagonism relative to **13**, again demonstrating the importance of the side chain orientation. Benzanthracene **14** is approximately 8-fold less potent in the MCF-7 assay, and 6-fold less potent in the Ishikawa antagonist assay compared to **13**. Interestingly, introduction of aromaticity into the naphthalene core has a larger detrimental effect on MCF-7 antagonism relative to the benzothiophene core, where **7** maintained potent inhibition. Introduction of the hydroxyl at the 5-position improves the functional profile further. Naphthalene **15** is now a potent and efficacious MCF-7 antagonist (IC_{50} 1.9 nM, 79% efficacy) and Ishikawa antagonist (IC_{50} 31 nM, 91% efficacy). More significantly, its efficacy as an agonist in Ishikawa cells (27% efficacy) is considerably less than any of the other analogs tested, whether from the benzothiophene or naphthalene scaffold. The favorable profile of **15** then prompted the separation and testing of the enantiomers. The distomer, **15a**, has a similar binding profile but a weaker antagonist profile compared to the racemate. The compound is also a more efficacious agonist in the Ishikawa assay (56% efficacy). The eutomer, **15b**, has an almost identical binding and functional profile as the racemate (**15**).

Compound **15b** was tested for its ability to block the effects of estradiol (E2) on the uterus in a three day immature rat assay.[14] The compound was administered PO at 0.01, 0.1, and 1 mg/kg and showed little inhibition even

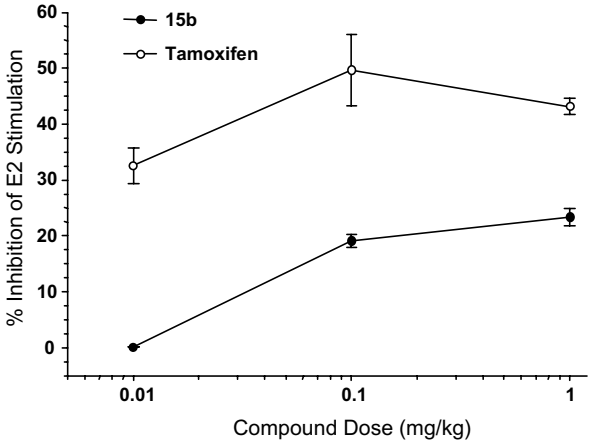


Figure 1. Inhibition of estradiol-induced uterine stimulation in a three day immature rat assay.

at the highest dose (Fig. 1).[15] In fact, compound **15b** is much less efficacious at inhibiting estradiol-induced stimulation of the uterus than the known partial agonist, tamoxifen. The reason for the lack of in vivo efficacy of **15b** was not investigated, but may be due to poor inherent ADME properties of the compound. For example, a possible explanation is that **15b** may undergo oxidation in vivo to benzanthracene **14**, which is approximately 30-fold less potent as an antagonist in the Ishikawa uterine cell line.

In summary, we have prepared a new series of constrained benzothiophene- and naphthalene-based SERMs. In both series, the spatial relationship between the side chain and ligand core has a significant impact on functional activity. Compounds where the side chain is forced into the same plane as the backbone display significantly weaker antagonist effects (most notably in the Ishikawa cell line) compared to the related structures where the side chain is pseudo-orthogonal to the core. This is presumably due to induction of differential receptor conformations by the ligand, which in turn leads to differential recruitment of coactivators and corepressors.

Acknowledgements

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7. On occasion during quenching, bromination *ortho* to phenol was observed as a side reaction. This could be eliminated by quenching the BBr_3 reaction with MeOH and 2-methy-2-butene as a bromine scavenger, followed by immediate purification on an SCX column.
8. The antiestrogenic effects of benzanthracenes in vivo has been described. See: Morreal, C. E.; Sinha, D. K.; Schneider, S. L.; Bronstein, R. E.; Dawidzik, J. *J. Med. Chem.* **1982**, *25*, 323; The interaction of benzanthracene diols with the estrogen receptor has also been described: Anstead, G. M.; Kym, P. R.; Albert, B. *Steroids* **1995**, *60*, 383.
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12. Ishikawa human endometrial tumor cells were assayed in DMEM/F-12 (3:1) (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, phenol red-free, Gibco BRL) supplemented with 5% dextran coated charcoal stripped fetal bovine serum (DCC-FBS) (Hyclone, Logen, UT). The assay was run in both agonist (compound alone) and antagonist (compound plus 1 nM E2 (β-estradiol, Sigma, St. Louis, MO)) modes. Cells were incubated with compound for 48 h, then fresh assay medium plus compound were added and incubated for an additional 72 h. The assay was quenched by removing media and rinsing plates twice in PBS. Assay plates were dried for 5 min and frozen at −70 °C for at least 1 h. Plates were removed, allowed to warm to room temperature, and then 100 μL of 1-Step™ PNPP (Pierce Chemical Company, Rockford, IL) was added. After a 20-min incubation, plates were read on a spectophotometer at 405 nm to quantitate alkaline phosphatase. For the agonist mode, a percentage increase over control for each compound was calculated. The data were fitted to a linear interpolation to derive IC_{50} values for the antagonist mode and a percentage efficacy was calculated that blocks the E2 (1 nM) stimulus. For the agonist mode, a % efficacy for each compound was calculated compared to control and an EC_{50} calculated.
13. Unless otherwise stated, compounds were tested as racemates.
14. Three week old Sprague Dawley female rats were orally treated with E2 (0.1 mg/kg) and 1.0, 0.1, and 0.01 mg/kg SERM for 3 days, six rats per group. Test compounds were dissolved in 20% β-hydroxycyclodextrin and administered PO 15 min prior to E2. Animals were weighed, then euthanized (by carbon dioxide asphyxiation), and the uteri were collected and weighed. The percentage inhibition of the estrogen-induced response was calculated as: $100 \times (UWR_{estrogen} - UWR_{test\ compound})/(UWR_{estrogen} - UWR_{control})$ where UWR = uterine weight/body weight ratio.
15. In Figure 1, error bars for compound **15b** are intra-assay SE ($n = 6$ animals per group). Error bars for Tamoxifen are inter-assay SE (three assays, $n = 6$ animals per group).