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NOVEL NONSTEROIDAL SELECTIVE ESTROGEN RECEPTOR MODULATORS. CARBON AND HETEROATOM REPLACEMENT OF OXYGEN IN THE ETHOXYPIPERIDINE REGION OF RALOXIFENE

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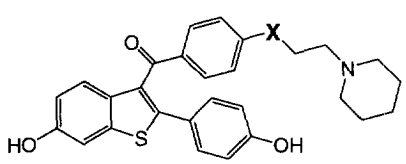
Abstract: Compounds were synthesized where oxygen in the ethoxypiperidine region of raloxifene is replaced with carbon, sulfur, or nitrogen linkages. Thia- and aza-substituted compounds were prepared by novel methodology. The compounds were evaluated in vitro as selective estrogen receptor modulators (SERMs). Calculations suggested the compounds exhibit an ER-α binding affinity/conformational energy relationship.

Raloxifene (**1**) is a selective estrogen receptor modulator (SERM) currently undergoing clinical evaluation for the treatment of osteoporosis.[1] It has recently gained approval in both the United States and Europe for the prevention of osteoporosis.[1c] SERMs represent a class of compounds that are tissue-selective in their agonism or antagonism of estrogen receptors,[2] and hold great potential as post-menopausal therapeutics, inasmuch as estrogen deficiency has been implicated in a wide range of post-menopausal disorders, including osteoporosis, depression and schizophrenia, cardiovascular disease and Alzheimer's disease.[3] The synthesis and biological evaluation of numerous compounds have been undertaken, and additional highly potent compounds have been identified.[4] Our interest in extending the structure–activity relationship (SAR) centered around the replacement of oxygen in the ethoxypiperidine region of raloxifene with sulfur-, nitrogen-, and carbon-based linkages. The synthesis and biological activity of these compounds is reported here.



1, raloxifene, X = O
5a, X = S
5b, X = NH
5c, X = NCH_3
5d, X = SO_2
5e, X = CH_2

Chemistry

The successful use of the aryl fluoride **3** (from benzothiophene **2** and *p*-fluorobenzoyl chloride)[5] in nucleophilic aromatic substitution (S_NAr) reactions with 2-dialkylamino-1-hydroxyethyl alkoxides under basic conditions[6] led to application of that novel methodology to the synthesis of the corresponding thia and aza compounds **5a** and **5b** (Scheme 1). 1-Piperidineethanethiol (from thiourea and β-chloroethylpiperidine hydrochloride) was treated with NaH and reacted with **3** to afford protected thia analogue **4a** in 98% yield.

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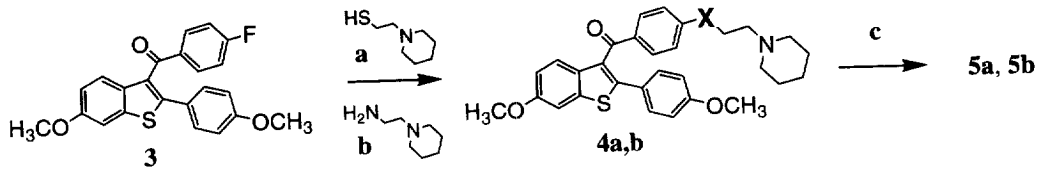
Scheme 1

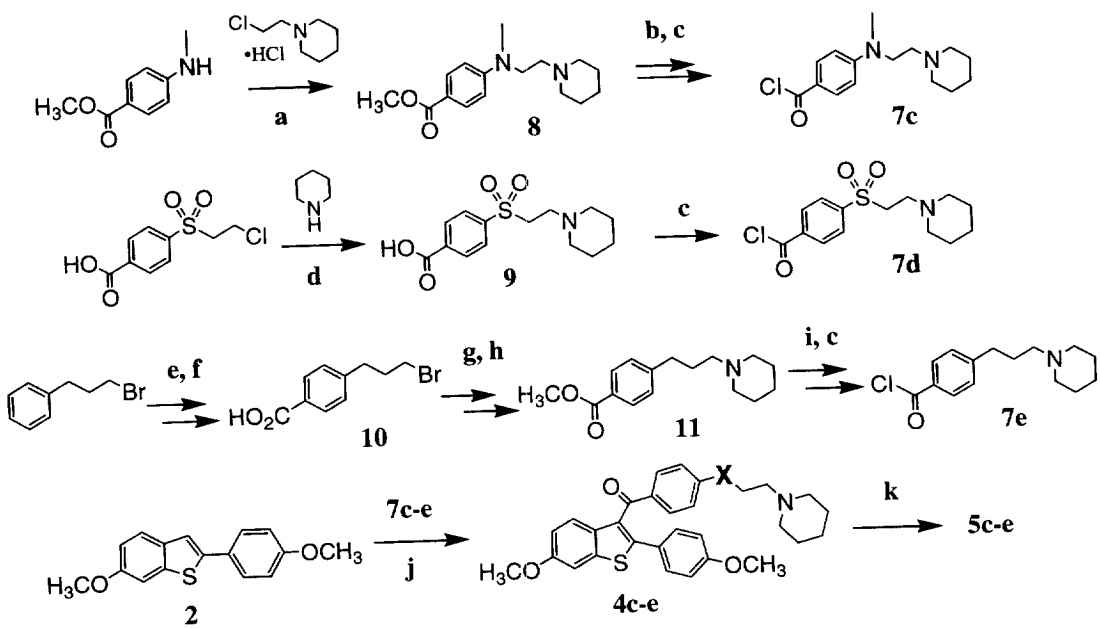


Scheme 1: (a) NaH, DMF, 98%; (b) 37% KF/Al_2O_3, DMSO, 120 °C, 18-C-6, 74%; (c) BBr_3, 1,2-DCE, 5 °C, 29% (**5a**), 74% (**5b**).

While the approach did not work with the corresponding aminoethylpiperidine, reaction of that material with **3** under 37% KF/Al_2O_3 conditions[7] delivered the amino-substituted analogue **4b** in good yield (72%). Deprotection of the methyl ethers afforded the desired species **5a** (29%) and **5b** (74%).

N-Methylamino, sulfonyl, and methylene-based compounds were prepared via Lewis acid-mediated acylations of 2-arylbenzothiophene **2** with the corresponding *p*-substituted benzoyl chlorides **7** (Scheme 2). Thus methyl 4-(*N*-methyl)aminobenzoate was alkylated with β-chloroethylpiperidine hydrochloride (46%) to give ester **8** and the ester converted to the acid chloride **7c** via saponification and reaction with thionyl chloride/DMF.

Scheme 2



Scheme 2: (a) NaH, THF, reflux, 2d, 46%; (b) NaOH, 2 h; (c) $SOCl_2$; (d) DMF, 120 °C, 1 h, 58%; (e) AcCl, CS_2, 5 °C, 88%; (f) Br_2, NaOH, 5 °C, 2 h, 68%; (g) MeOH, H^+, reflux, 5 h, 97%; (h) Piperidine, KI, DMF, rt, 4 h, 95%; (i) NaOH, rt, 12 h, 70%; (j) $AlCl_3$, 1,2-DCE, 5-23 °C, 3-12 h, (**8**, 36%), (**9**, 68%), (**11**, 65%); (k) BBr_3 (**5c**, 38%), BCl_3 (**5d**, 68%), $AlCl_3$, PrSH (**5e**, 88%).

Direct acylation of **2** with **7c** provided protected species **4c** (35% from **8**). Acid chloride **7d** was prepared by reaction of piperidine with *p*-carboxyphenyl-2-chloroethyl sulfone[8] (58%) followed by acid chloride formation. Acylation of **2** with **7d** delivered **4d** (43% from **9**). Deprotection gave **5c** (38%) and **5d** (68%).

Preparation of the methylene-containing species **5e** was more involved. Substituted benzoyl chloride **7e** was prepared in six steps. Aromatic acetylation of 3-phenyl-1-bromopropane (88%) followed by bromoform reaction afforded carboxylic acid **10** (68%). Acid **10** was then esterified (97%) and treated with piperidine (95%) to deliver ester **11**. Saponification under acid conditions (70%), followed by acid chloride formation as before delivered **7e**. The acid chloride was reacted with **2** (65% over two steps) to afford **4e**. Demethylation (88%) provided **5e**.

Biology

Estrogen binding affinities were determined through competitive displacement of ^{3}[H]-17β-estradiol in MCF-7 cell lysates, and are reported as relative binding affinities (RBA, 17β-estradiol RBA = 1).[4c] Inhibition of estrogen-stimulated MCF-7 cell proliferation was employed to assess estrogen antagonism; values are reported as the concentration required for 50% inhibition of proliferation stimulated by 10^{-11} M 17β-estradiol (IC_{50}).[4c] Data are compiled in the Table.[9]

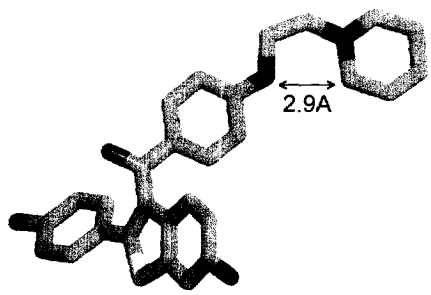
Table. Estrogen Receptor Affinity and Antiproliferative Data for SERMs[10]

Compound	ER RBA	Inhibition of MCF-7 Proliferation (nM)
1	0.34	0.2
4d	0.003	>1000
4e	< 0.002	1000
5a	0.15	0.9
5b	0.26	2
5c	0.19	10
5d	0.084	30
5e	0.29	10

As anticipated,[4c] the protected compounds **4d** and **4e** exhibited poor relative binding affinity and were essentially inactive in vitro as estrogen antagonists. By contrast, the entire series of compounds **5** were potent antiproliferatives (30–0.9 nM), and with the exception of **5d**, exhibited reasonable relative binding affinity, approaching raloxifene in the case of **5e**. Although the relative binding affinity of the series is lower by 1.2-4 fold and the antagonist activity is reduced 5- to 15- fold relative to raloxifene, these compounds appear to maintain the desired in vitro SERM profile[2] of strong antiproliferative activity combined with reasonable relative binding affinity for the estrogen receptor. Further testing in vivo will be required to confirm this.

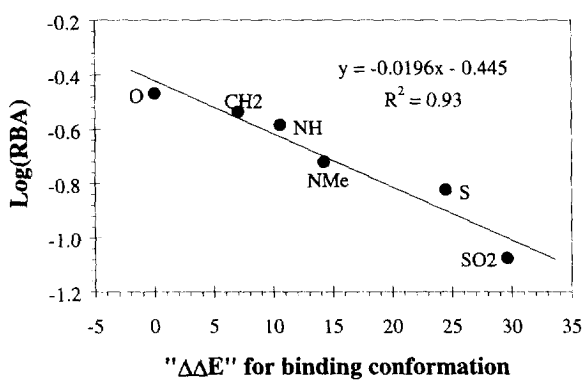
Examination of the recently released ER-α•raloxifene X-ray crystal structure revealed that the ethoxy tether is bound in a hydrophobic pocket of the receptor and that a specific hydrogen bond is not formed between the ether oxygen and the receptor.[11] Furthermore, the binding pocket for the ethoxypiperidine moiety appeared to be too small, causing a highly constricted conformation for this group, resulting in a van der Waals contact between the ether oxygen and the alpha methylene of the piperidine ring (Figure 1).

Figure 1. Raloxifene Binding Conformation from the Estrogen Receptor-α•Raloxifene Crystal Structure



To explore the possibility that tight packing of the side chain linking the piperidine ring might explain the relative binding affinities of the compounds described herein, molecular mechanics techniques were used to examine the energy penalty associated with forcing the compounds in series **5** into the receptor-bound raloxifene conformation. The molecular mechanics energy for the minimum energy conformation for each compound was subtracted from the energy calculated for the compound forced into the ER binding raloxifene conformation.[12] The ΔE's were then referenced to raloxifene and the resulting "ΔΔE's" for the binding conformations were then correlated with the log(RBA) values for the series as shown in Figure 2.

Figure 2. Correlation of Log(RBA) with Estimated Conformational Energy



The correlation indicated that >90% ($P < 0.002$) of the observed dispersion in the Log(RBA) values for this series could be accounted for by the conformational energy. Examination of the molecular mechanics simulations identified two features which appeared to account for most of the $\Delta\Delta E$ values. The first was the lack of sufficient room for substituted groups (NMe and SO_2) because of the close approach of the piperidine Cα. The second feature was the longer bond lengths associated with the S and SO_2 groups resulting in an overall chain lengthening of 0.2–0.3 Å. Overall, these results indicated that although the oxygen atom did not participate in a specific polar interaction with the ERα, it did contribute to the overall length and packing of the side chain when bound by the receptor. Small isosteres of the oxygen, such as CH_2 and NH, were well tolerated, whereas larger or longer isosteres such as NMe, S or SO_2 were less well tolerated by the receptor.

In summary, access to novel SERMs containing alternative heteroatom and methylene groups, replacing oxygen in the ethoxypiperidine region of raloxifene, has provided a series of compounds exhibiting both significant ER affinity and potent MCF-7 antiproliferative activity. Computer modeling and comparison of these compounds with ER-α•raloxifene suggested variation in conformational energy as a satisfactory explanation of the binding results obtained.

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