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Benzothieno[3,2-*b*]indole derivatives as potent selective estrogen receptor modulators

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Abstract—A series of estrogen receptor ligands based on benzothieno[3,2-*b*]indole were synthesized and their binding affinity for estrogen receptor subtypes (ERα and ERβ) and effects on mouse uterus and bone were evaluated. Some of these compounds showed strong binding affinity to ER and significantly increased the bone mineral density of ovariectomized mice.

Estrogen receptor and endogenous estrogens play important roles in the development and function of female reproductive system. At present, estrogen replacement therapy (ERT) is one of the most effective treatments for menopausal symptoms and postmenopausal osteoporosis arising from lowering levels of circulation estrogen.[1] Although ERT is effective, it is also associated with some serious side-effects. More recently, selective estrogen receptor modulators (SERMs) which fully antagonize the effects of estrogen on uterine and mammary tissues, while mimicking the effects of estrogen on the bone and cardiovascular system, have been investigated as a possible alternative to ERT.[2] Synthetic estrogen receptor ligands, such as tamoxifen and raloxifene, produce biological responses that can be either estrogenic or anti-estrogenic, depending upon the tissue in which their action is examined.[3] The SERMs presently used clinically have obvious advantages over the conventional ERT, but they retain some of the disadvantages as well. It is known that in the X-ray crystal structure of raloxifene bound to ERα, the basic side chain of the ligand occupied an orthogonal position relative to the benzothiophene core.[4] In an effort to constrain the side chain into its active conformation, we change the benzothiophene core to benzothiophene[3,2-*b*]indole (Fig. 1), which contains a tetracycle core-motif that simultaneously restricts rotation of the two phenolic groups so that the core is more coplanar and perhaps better mimics the steroidal ligand. In addition, attachment of the side chain can readily assume the axial position. This tetracycle core also contains a rigid stilbene moiety that has pharmaceutical relevance in many synthetic non-steroidal estrogen antagonists. This may lead to the discovery of compounds with potential biological activity in estrogen sensitive tissues.[5]

The amine-containing side chain of raloxifene is recognized to be critical in binding to the amino acid residue of the ER, and it also plays a role in determining the tissue specificity.[4] Therefore, it could be valuable to modify the side chain in order to understand the structure–activity relationship (SAR) of these compounds. In this article, we report the synthesis of benzothieno[3,2-*b*]indole derivatives and their biological activities.

Scheme 1 depicts the syntheses of the compounds. Alkylation of thiol **10** gave **11**. Compound **11** is suspended in tetrachloroethane and treated with PCl_3 at 90 °C for 3 h. After standing at room temperature overnight, the reaction mixture was added to a slurry of aluminum chloride in tetrachloroethane at 60 °C and kept at that temperature for 30 min. The ketone **12** thus formed was treated with phenylhydrazine or

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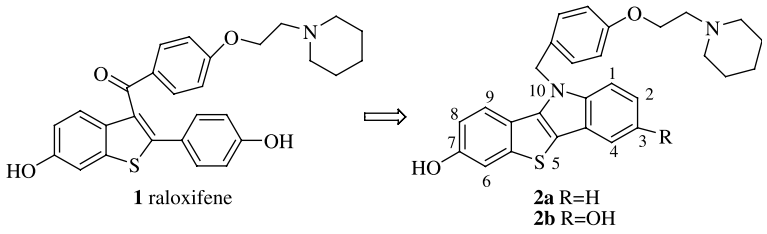
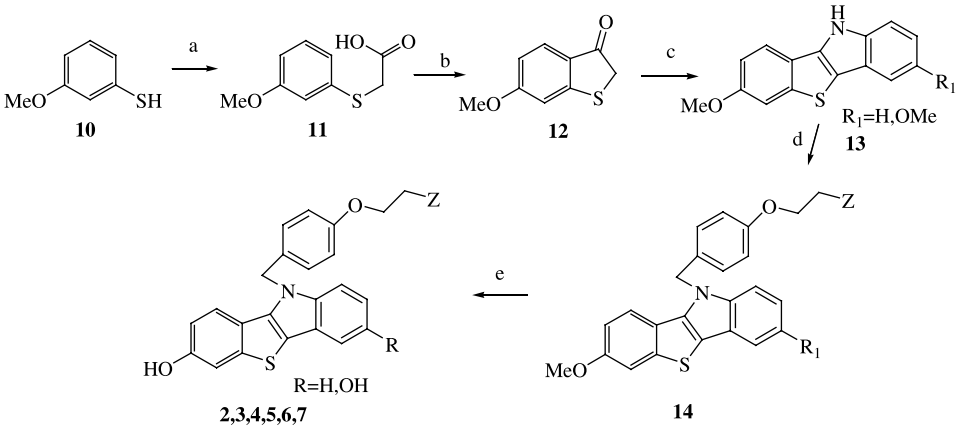


Figure 1. Structure of raloxifene and target compounds.

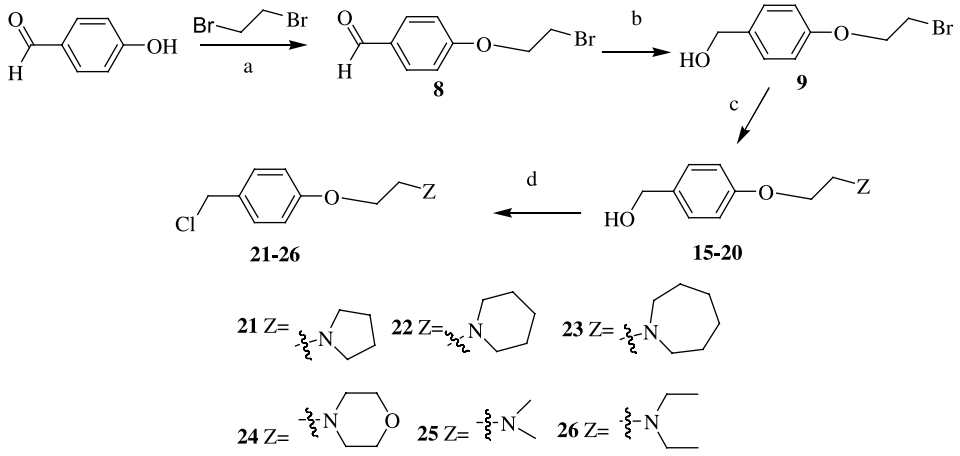


Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) $ClCH_2COOH$, 10% NaOH (aq), reflux, 2 h; (b) PCl_3, $CHCl_2CHCl_2$, $AlCl_3$, in two steps; (c) phenylhydrazine or *p*-methoxyphenylhydrazine, acetic acid, 80 °C; (d) aminoethoxybenzyl chloride, NaH, DMF, rt; (e) NaSEt, DMF, reflux.

p-methoxyphenylhydrazine in acetic acid to afford 7-methoxy-benzothiophene[3,2-*b*]indole and 3,7-dimethoxy-benzothiophene[3,2-*b*]indole, respectively, through the Fischer indole synthesis.[6] The indole nucleus and relevant aminoethoxybenzyl chloride were coupled in DMF in the presence of NaH, which after deprotection with NaSEt in dry DMF gave the target compounds.

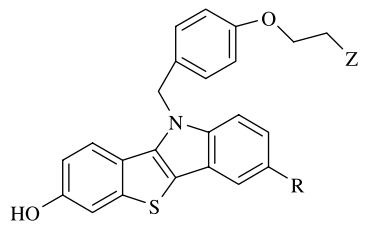
The synthesis of side chains is shown in Scheme 2. The coupling reaction between 4-hydroxybenzaldehyde and 1,2-dibromoethane afforded aldehyde **8**. Reduction with $NaBH_4$ in methanol at room temperature gave alcohol **9**, which was subsequently reacted with amines to give **15**–**20**. A solution of one compound of **15**–**20** in THF was cooled to 0 °C and gaseous HCl bubbled through until no more thickening was observed, then $SOCl_2$ was added and the mixture was heated at 50 °C until the mixture became clear. The reaction mixture was cooled to give the target compounds **21**–**26**, respectively.

The compounds were primarily tested for intrinsic activity in an ER binding assay with [^{3}H]17β-estradiol and full-length recombinant human ERα and ERβ proteins.[7]



Scheme 2. Synthesis of side chain. Reagents and conditions: (a) K_2CO_3, DMF, 120 °C; (b) $NaBH_4$, methanol; (c) amine, THF, reflux; (d) HCl, $SOCl_2$, THF in two steps.

Table 1. Binding affinities[a] and in vivo examination data[b]



Compounds	R	Z	Binding affinity (IC_{50} (nmol))		BMD (mg/cm^3)	Uterine weight assay	
			ERα	ERβ		Wet weight (mg)	Agonist (%)
Raloxifene			0.89 ± 0.02	11.6 ± 2.2	565 ± 21***	53.8 ± 7.3***	118.0
2a	H	Piperidine	39.0 ± 2.2	35.0 ± 3.0	436 ± 41*###	34.0 ± 4.3**###	37.7
2b	OH	Piperidine	2.84 ± 0.1	11.0 ± 0.2	548 ± 47***#	44.2 ± 4.9***#	79.0
3a	H	Pyrrolidine	27.2 ± 1.5	53.9 ± 8.0	505 ± 74*#	27.6 ± 3.0*###	11.7
3b	OH	Pyrrolidine	6.0 ± 0.7	20.2 ± 6.5	469 ± 36*###	30.7 ± 5.8*###	24.6
4a	H	Hexamethyleneimine	18.0 ± 7.76	16.6 ± 2.8	444 ± 15*#	28.5 ± 4.8*###	15.6
4b	OH	Hexamethyleneimine	3.34 ± 1.79	7.26 ± 0.4	534 ± 25***#	44.2 ± 4.8***#	79.3
5a	H	Dimethylamine	51.4 ± 3.62	45.6 ± 2.6	474 ± 73*##	28.2 ± 2.4*###	14.1
5b	OH	Dimethylamine	18.0 ± 3.37	31.0 ± 3.7	480 ± 33*###	26.2 ± 5.3*###	6.1
6a	H	Diethylamine	57.4 ± 10.6	25.0 ± 7.0	448 ± 31*###	34.9 ± 3.7***###	41.4
6b	OH	Diethylamine	31.2 ± 8.62	16.4 ± 7.1	479 ± 47*##	37.7 ± 8.1**###	52.9
7a	H	Morpholine	126.0 ± 60.5	215.0 ± 11.9	455 ± 56*###	27.0 ± 3.5*###	9.4
7b	OH	Morpholine	10.0 ± 0.7	21.4 ± 0.3	470 ± 28*###	26.3 ± 1.2*###	6.5
OVX + DW					434 ± 38	27.2 ± 4.7	
Sham + DW					569 ± 63***	152 ± 51.8***###	

[a] The compounds were evaluated for their binding ability to ERα and ERβ in competition with ^{3}H-estradiol. Data were analyzed using GraphPad Prism software. Each value represents the median observed in three independent experiments (±SD).

[b] Two months old ovariectomized Kun-Ming mice were treated (s.c.) with the test compounds at a dose of 4 μmol/kg q.d. for 4 weeks. The bone mineral density was determined by peripheral quantitative computed tomography (pQCT, Stratec, XCT Research SA, FRG). The uterine wet weights were measured on the day when mice were sacrificed [n = 5 per group; mean ± SD, $^*p$ >0.05, $^{**}p$ <0.05, $^{***}p$ <0.001 vs distilled water (DW); #p >0.05, ##p <0.05, ###p <0.01 vs raloxifene].

They were then examined for effects on stimulation of the bone density and uterine weight.[8] It is clear from the data shown in Table 1 that the 3-hydroxyl group is essential to the pharmacological properties of these compounds. Comparison of the binding activities of compounds **a** and **b** reveal 2- to 13-fold decrease in relative binding affinity when the 3-hydroxy group is replaced with a proton. Although the same phenomenon was not observed in the bone mineral density (BMD) measurement, the effects of compounds **2b** and **4b** on bone density are comparable with raloxifene (p >0.05), while the uterus effects are lower (p <0.05).

The importance of the amine-containing side chain with respect to estrogen antagonism observed in this study is similar to the effects reported in the raloxifene series.[9] It seems that the presence of piperidine base may enhance the pharmacological activities, as change of the base from piperidine to other cyclic or acyclic amine significantly reduced both the potency and selectivity except for Hexamethyleneimine.

Acknowledgements

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