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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1505–1507
Bioorganic & Medicinal Chemistry Letters

Benzothiophenes containing a piperazine side chain as selective ligands for the estrogen receptor α and their bioactivities in vivo

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Received 20 June 2004; revised 21 December 2004; accepted 22 December 2004
Available online 22 January 2005

Abstract—The synthesis of benzothiophenes containing a piperazine side chain and their binding affinities for estrogen receptors are described. These compounds bearing piperazine side chains were identified to be high-affinity ligands with high selectivity for ER α subtype. They were also potent agonists in bone tissue.

Due to the side effects of estrogen replacement therapy (ERT), selective estrogen receptor modulators (SERMs) which potentially antagonize the proliferative effects of estrogen on uterine and mammary tissue, while mimicking the effects of estrogen on the bone and cardiovascular system, provide an advantageous alternative approach.[1] Raloxifene, the represented SERM, has been marketed as Evista® for the prevention and treatment of osteoprosis in postmenopausal women in Europe and the United States. Extensive structure activity relationship (SAR) studies on benzothiophene-based SERMs have been undertaken,[2] and the basic side chain (BSC) of raloxifene is reported to be crucial for its antagonist activity.[3] In order to enhance the selectivity between the ER α and ER β of raloxifene while maintaining its unique pharmacological profile, our studies was centered on modification of the aminoethoxy side chain, the most widely used BSC. It is hoped that compounds containing rigid piperazine side chain can potentially impact the selectivity for estrogen receptor subtype in comparison with those with flexible basic side chain. While our program was completed, there appeared one report about a class of SERMs containing the piperazine side chain.[4] Herein, we also report the results of our studies on a novel class of selective ligands for the ER α (Fig. 1).

The benzothiophenes were synthesized using standard chemical methods, starting from 2-(4-methoxy-phenyl)-6-methoxy-benzothiophene **3**.(Scheme 1).[5] Compound **4** was synthesized by reacting 4-fluoro-benzoyl chloride with benzothiophene **3** under general Friedel–Crafts acylation conditions,[2c] then compound **4** was reacted with 1-benzylpiperazine to afford compound **5** via nucleophilic aromatic substitution (S_NAr). The previous described conditions with poisonous KF/Al_2O_3, DMSO, and harmful 18-C-6 for S_NAr substitution in the case of aza nucleophiles were not employed.[2c,6] In this case *N*-methyl-2-pyrrolidone was used as solvent in the presence of excess benzylpiperazine. Debenzylation of compound **5** afforded the common intermediate **6** under transfer hydrogenation condition ($Pd/C–HCOONH_4$).[7] The compounds **7** were obtained via acylation or alkylation of the key intermediate **6**, and then treatment of **7** with $AlCl_3$/EtSH or BBr_3 afforded the desired compounds **2** with free phenol. When R is benzoyl (substituted benzoyl) or benzyl (substituted benzyl), benzyl halide or benzoyl chloride, triethylamine was used as the base. When R is alkyl or cycloalkyl, alkyl halide (chloride/bromide) did not work well while alkyl methanesulfonate or toluenesulfonate were used as alkylation reagent with potassium carbonate as the base, reaction proceeded smoothly and the yield is 70–85%.

Keywords: Benzothiophenes; Piperazine; High selectivity; Estrogen receptor.
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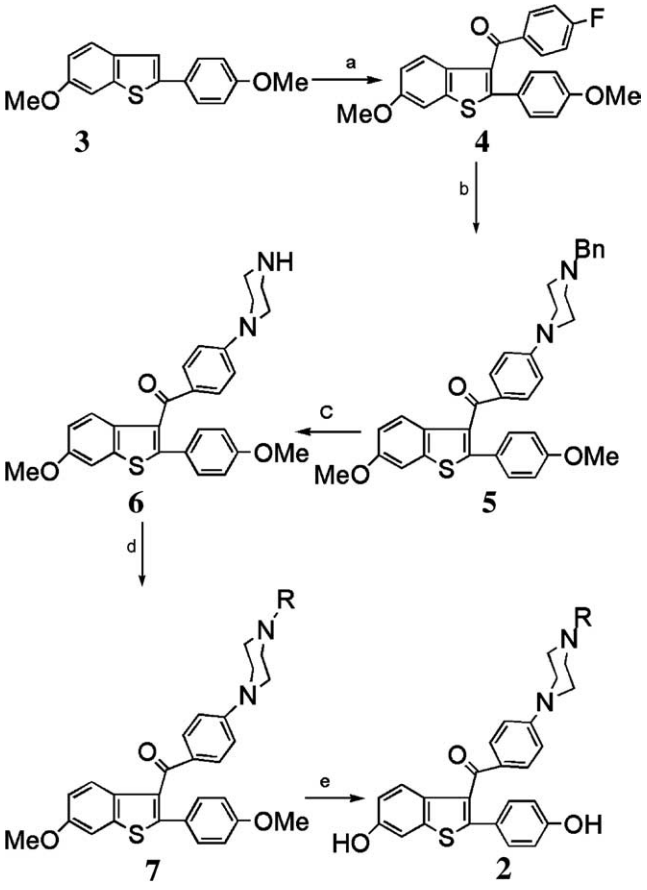
doi:10.1016/j.bmcl.2004.12.074



Scheme 1. Synthesis of compounds **2a**–**t**. Reagents and conditions: (a) *p*-fluoro-benzoyl chloride, $AlCl_3$, CH_2Cl_2, 5–10 °C; (b) benzylpiperazine, NMP, 140 °C, 20 h; (c) MeOH, Pd/C–$HCOONH_4$, reflux, 1.5 h; (d) benzyl halide/benzoyl chloride, THF, TEA or alkyl methanesulfonate, THF, K_2CO_3, reflux; (e) $AlCl_3$/EtSH or BBr_3, CH_2Cl_2.

Compounds **2a**–**t** were evaluated for their ability to compete with [^{3}H] 17β-estradiol for binding to both

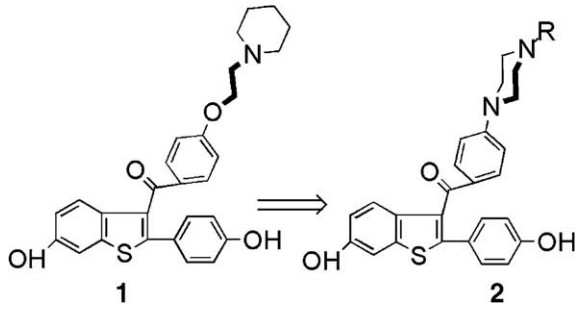


Figure 1. Designed compounds derived from raloxifene.

ER α and ER β.[8] The results are listed in Table 1. As expected, the benzothiophenes containing piperazines exhibit efficient binding to human estrogen receptors. Most of the compounds displayed high binding affinity for both ER α and ER β that is comparable to raloxifene even with bulky substitutents on piperazines. Several compounds such as **2e**,**j**,**n**, and **q** turned out to be potent estrogen receptor ligands. Compounds **2j**,**n**,**q** are even more potent than raloxifene. In addition, the piperazine basic side chain not only affect the binding affinity, but also impact on the selectivity of SERMs. **2j**,**n** have modest selectivity for ER α (up to 40×), and **2q** showed highest selectivity for ER α (up to 120×). These results demonstrated that the piperazine basic side chain can bind reasonably to the ER in the binding pocket and the steric bulk could also be well tolerated.

According to the binding affinities, we selected three compounds **2e**,**n**,**q** to test their effects on bone and uterus of immature mice.[9] The biological data showed that all three compounds can prevent the lose of bone mineral and reduce the stimulation to uterus, and the effect of **2q**[10] on bone mineral is very close to that of raloxifene. It also reduced the stimulation to mice uterus distinctly compared to raloxifene. We also found that bulky

Table 1. Estrogen receptor binding affinities (IC_{50}) for compounds

Compd	R	ER α (IC_{50}, nM)[a]	ER β (IC_{50}, nM)[a]	Selectivity [β]/[α]
1	—	0.73	18.90	25.9
2a	Benzyl	0.97	5.53	5.7
2b	Benzoyl	0.86	25.29	29.4
2c	Furan-2-carbonyl	2.65	20.09	7.6
2d	*p*-Toluoyl	2.74	4.94	1.8
2e	3-Chlorobenzoyl	0.46	3.70	8.0
2f	4-Chlorobenzoyl	0.78	4.02	5.2
2g	3-Hydroxybenzoyl	1.39	19.77	14.2
2h	2-Hydroxybenzoyl	3.05	7.92	2.6
2i	*o*-Toluoyl	3.94	17.67	4.5
2j	*m*-Toluoyl	0.27	12.47	46.2
2k	2-Chlorobenzoyl	1.72	3.21	1.9
2l	Isonicotinoyl	14.46	93.57	6.5
2m	4-Nitrobenzoyl	2.15	1.27	0.6
2n	4-Chlorobenzyl	0.22	9.07	41
2o	2-Nitrobenzoyl	3.58	12.90	3.6
2p	4-Nitrobenzyl	0.43	14.22	33
2q	Isopropyl	0.28	33.92	121
2r	Cyclopentyl	1.08	18.15	17
2s	4,6-Dimethyl-pyrimidin-2-yl	2.12	66.52	31
2t	*n*-Propyl	1.43	29.37	20

[a] Values are means of at least three experiments.

Table 2. Biological data in vivo

Compd	Bone mineral density (mg/cm^3)	Uterine weight assay (mg)
2e	$432.1 \pm 10.6^{**\#\#\#}$	$29.3 \pm 1.3^{*\#\#\#}$
2n	$482.5 \pm 37.5^{***\#}$	$30.5 \pm 2.3^{*\#}$
2q	$540.8 \pm 77.6^{***\#}$	$38.8 \pm 9.8^{*\#\#}$
Raloxifene	$549.1 \pm 75.2^{***}$	$52.0 \pm 6.0^{***}$
OVX + DW	386.2 ± 24.8	28.9 ± 1.1
Sham + DW	$592.0 \pm 51.9^{***\#}$	$203.4 \pm 37.6^{***\#\#\#}$

Kun-Ming female mice of two months old which were ovariectomized were treated (sc) with test compounds for 4 weeks at 4 μM. The bone mineral density was determined by peripheral quantitative computed tomography (pQCT, Stratec XCT-Research SA). The uterine weights were determined on the day the mice were killed ($n = 5$).

$^{*}P > 0.05$.

$^{**}P < 0.05$.

$^{***}P < 0.01$ versus distilled water (DW).

$^{\#}p > 0.05$.

$^{\#\#}p < 0.05$.

$^{\#\#\#}p < 0.01$ versus raloxifene.

substituents on piperazines displayed lower agonist property in bone and higher antagonist property in uterine tissue than smaller substituents (Table 2).

In summary, the piperazine basic side chain was found to be functionally equipotent to the aminoethoxy group in benzothiophenes SERMs. Of all the compounds tested, **2q** was found to have highest binding affinity and selectivity for ER α. It is also a potent agonist in bone tissue and an antagonist in uterus. It's expected that these findings could provide a valuable hit in developing new SERMs.

Acknowledgement

This work was financially supported in part by the National Natural Science Foundation of China (20302009).

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10. Physical data of **2q:** ^{1}H NMR (400 MHz, acetone-d_6) δ 1.02 (6H, d, $J = 6.3$), 2.60 (4H, t, $J = 5.0$), 2.69 (1H, m), 3.33 (4H, t, $J = 5.0$), 6.74 (2H, q, $J = 1.9$, $J = 6.6$), 6.83 (2H, d, $J = 9.0$), 6.90 (1H, dd, $J = 2.2$, $J = 8.8$), 7.26–7.30 (3H, m), 7.35 (1H, d, $J = 2.2$), 7.63 (2H, q, $J = 1.9$, $J = 7.1$). Mp 158–160 °C. MS(EI):472(M^+). Anal. Calcd for $C_{28}H_{28}N_2O_3S{\cdot}2H_2O$: C, 66.12; H, 6.34; N, 5.51. Found: C, 66.19; H, 6.06; N, 5.39.