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SYNTHESIS AND BIOLOGICAL ACTIVITY OF *TRANS*-2,3-DIHYDRORALOXIFENE

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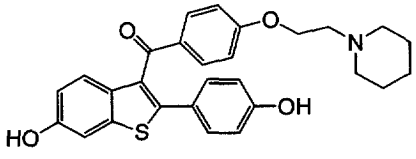
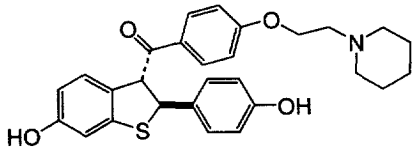
Abstract: The synthesis and biological evaluation of *trans*-2,3-dihydroraloxifene, **2**, is described. The synthesis proceeds in 8 steps in 20% overall yield. Relative *trans* 2,3-stereochemistry is definitively established in ester **6**, which is converted to the title compound via derivatization, Grignard addition, and deprotection. Evaluation in vitro shows the compound to be a potent selective estrogen receptor modulator (SERM).

Raloxifene (**1**), a 3-aroyl-2-arylbenzothiophene, is a selective estrogen receptor modulator (SERM) marketed for the prevention of osteoporosis, and currently under clinical evaluation for the treatment of osteoporosis.[1] Raloxifene and other SERMs function as tissue-selective estrogen agonists, producing estrogenic effects in serum lipids and bone, while functioning as estrogen antagonists in uterine and mammary tissue.[2] The potential of this class of compounds to treat a range of diseases in the postmenopausal female population has generated considerable interest in the scientific and lay press. It has also given rise to extensive structure–activity relationship (SAR) studies in the hopes of identifying additional potent SERMs.[3]



raloxifene, **1**

The SAR studies undertaken to date have largely employed the benzothiophene nucleus as the core structure for synthetic elaboration. Compounds derived from the corresponding dihydrobenzothiophene nucleus would offer potential for further extension of the SAR, in addition to affording a stereochemical probe of receptor sites not provided by the benzothiophenes. Consequently, the synthesis of the 2,3-dihydro version of raloxifene, dihydroraloxifene, (**2**), was undertaken, and the compound's in vitro biological activity compared against other species to ascertain whether this novel class of compounds held promise as an additional source of SERMs.



2

The synthesis of **2** was envisioned as arising from reaction of an aryl nucleophile at the carbonyl of a suitably substituted 2-aryl-3-carbomethoxy-2,3-dihydrobenzothiophene. Access to the latter compound could potentially be gained via methodology first reported by Conley and Heindel,[4a] and would require the previously unknown 6-methoxythianapthen-2-one (**3**) and *p*-anisaldehyde as condensation partners to deliver the oxygen ring substitution pattern of the desired compound. Compound **3**[3a] was accessed (Scheme) via acid hydrolysis of

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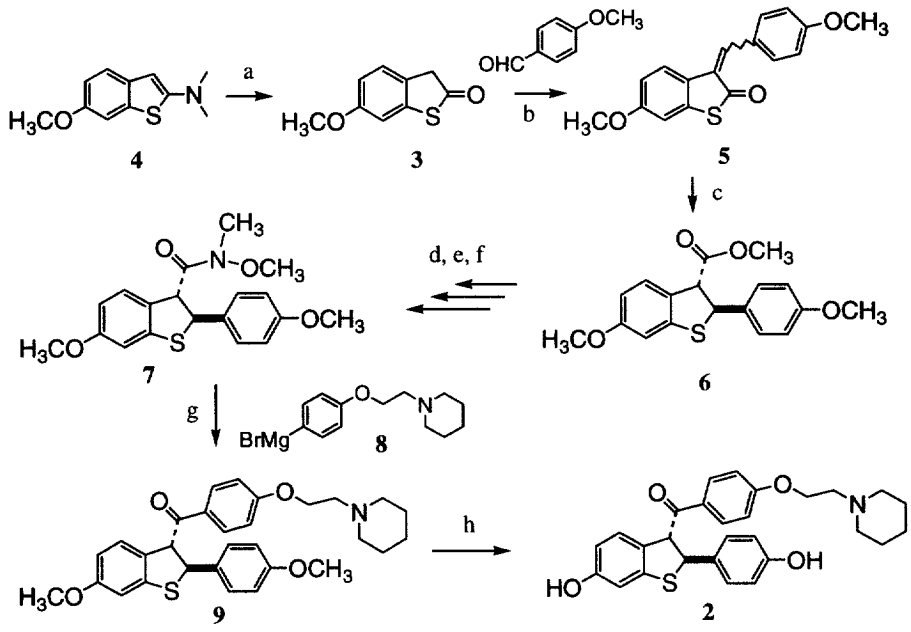
2-(dimethylamino)-6-methoxybenzothiophene (**4**), itself a material employed in the synthesis of a number of SERMs,[3c] as well as raloxifene itself,[5] and obtained by a two-step literature process.[6]

Scheme

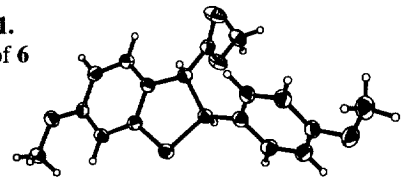
Synthesis of *trans*-2,3-Dihydroraloxifene 2



Scheme: (a) 1:1 THF:HCl (1 N), reflux, 4 h, 80%; (b) Piperidine, methanol, 5 °C, 16 h, 75%; (c) Piperidine, methanol, reflux, 3 h, 71%; (d) Methanol, NaOH (1 N), 5 °C, 1 h, 88%; (e) $SOCl_2$, CH_2Cl_2, DMF; (f) Pyridine, $HNCH_3(OCH_3)HCl$, rt, 16 h, 66%; (g) THF, 5 °C, 3 h, 80%; (h), CH_2Cl_2, $AlCl_3$, PrSH, rt, 3 h, 99%.

Condensation of **3** and *p*-anisaldehyde afforded benzylidene thiolactone **5** as a mixture of *E* and *Z* isomers. Subjection of this mixture to piperidine in methanol at reflux afforded the 2-aryl-3-carbomethoxy-2,3-dihydrobenzothiophene **6** via benzylidene thiolactone rearrangement[4a] in 71% crystallized yield. While relative stereochemistry across the 2,3 bond of dihydrobenzothiophenes obtained by this method had been assigned by Conley and Heindel as *cis*,[4a] the equilibrium nature of the reaction and the large coupling constant observed (8.1 Hz) led to speculation that the *trans* isomer was in fact the main product. Examination of the crystallization mother liquors by ^1H NMR revealed a minor isomer exhibiting approximately the same 2H-3H coupling constant as that of the main product; NOESY showed 2H-3H through-space coupling in the minor isomer, but not in product **6**, further supporting our case. Ultimately, single crystal X-ray analysis of **6** (Figure 1) confirmed the *trans* assignment, in contrast to the assignment in the original work. Compound **6** was transformed into the corresponding *N*-

Figure 1. ORTEP of **6**



methoxy-*N*-methyl (Weinreb)[7] amide **7** via a three-step sequence of ester saponification, acid chloride formation and reaction with *N*-methoxy-*N*-methylamine in an overall yield of 58%.

With **7** in hand, the stage was set for introduction of the 3-aroyl moiety. Grignard reagent **8**[3a] reacted with amide **7** smoothly to afford the protected dihydroraloxifene **9** in 80% yield as a solid after chromatography. and recrystallization (heptane/dichloromethane). Preservation of the *trans* relative stereochemistry through ester saponification and Grignard addition was confirmed via X-ray analysis of **9**. Although disorder of the ethylpiperidinyl region of **9** prevented adequate refinement of the complete molecule, the dihydrobenzothiophene substructure was well refined, and conclusively showed preservation of the *trans* relative stereochemistry (Figure 2).[8] Final deprotection of **9** with $AlCl_3$/PrSH[9] followed by chromatography delivered racemic *trans*-dihydroraloxifene **2** as a yellow foam (99%). The eight-step synthesis was accomplished with an overall yield of 20% from **4**.

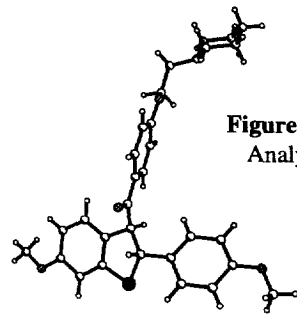


Figure 2. Partial Analysis of **9**

The in vitro biological activity of **2** was evaluated using established methods.[10] Inhibition of MCF-7 cell proliferation stimulated by 17-β-estradiol (10^{-11} M) was employed to measure estrogen antagonism, while estrogen receptor binding affinity of **2** was measured by competitive displacement of [^{3}H]-17-β-estradiol from MCF-7 cell lysates. Dihydroraloxifene was found to be a potent estrogen antagonist in the proliferation assay (IC_{50}: 0.52 ± 0.13 nM), comparing favorably with raloxifene (0.34 ± 0.06 nM), tamoxifen (904 ± 206 nM)[11] and the recently reported compound **10**, the most potent benzothiophene-based SERM identified to date (0.05 ± 0.02 nM).[3b] A graph of the concentration/inhibition curve for these compounds can be seen in Figure 3. In the

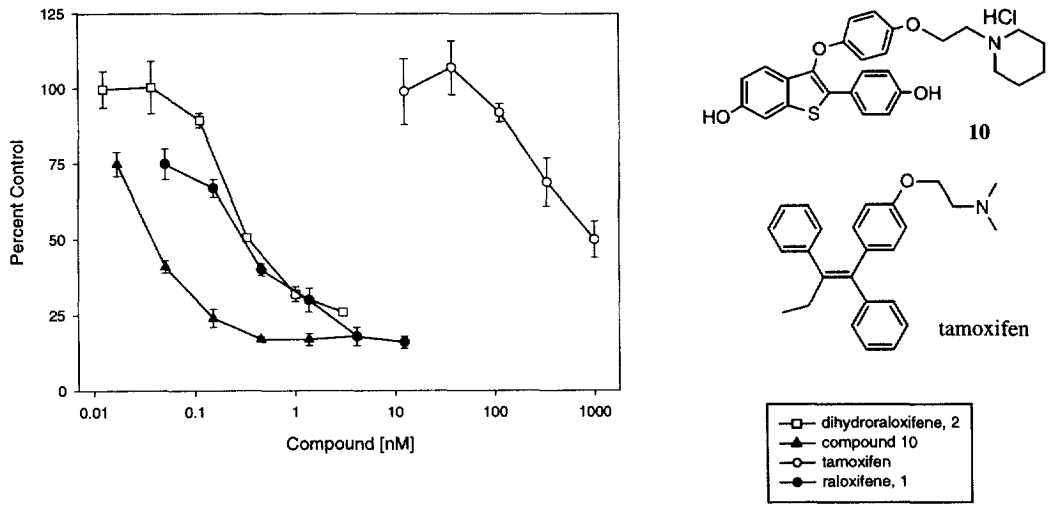


Figure 3. MCF-7 Proliferation Inhibition by Selected SERMs

estrogen receptor binding assay, **2** showed a relative binding affinity (RBA) of 0.20, (estrogen = 1.0) again comparing favorably with raloxifene (0.34)[3a] and other SERMs (0.01–0.4).[3b–e] As can be seen from Figure 3, the subnanomolar estrogen antagonism exhibited by **2** rivaled that of raloxifene, placing it at the very high end of compounds identified to date. When this antagonism was combined with the observed relative binding affinity, the in vitro SERM profile of dihydroraloxifene became evident.

An eight-step total synthesis of *trans*-2,3-dihydroraloxifene, **2**, has been achieved, representing the first access into dihydrobenzothiophene-based compounds possessing a SERM profile. The definitive establishment of *trans* stereochemistry stands in contrast with that previously assigned for species obtained by this synthesis method.[4a] The compound's strong in vitro estrogen antagonism and moderate relative binding affinity suggest it to be a potent SERM, and indicates that use of the dihydrobenzothiophene scaffold for exploration of SAR merits further consideration.

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