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Estrogen receptor ligands. Part 11: Synthesis and activity of isochromans and isothiochromans

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Abstract—The ring oxygen and sulfur analogs of lasofoxifene, **1a** and **1b**, were synthesized in an attempt to impart ERα selectivity, as found in the closely related dihydrobenzoxathiin compound **I**, recently discovered in these laboratories. The resulting isochroman and isothiochroman compounds were found to exhibit equipotent binding affinities to the ER isoforms and were less active in the inhibition of estradiol-triggered uterine growth when compared to **I** and lasofoxifene.

Endogenous estrogen interacts with the estrogen receptors, the nuclear hormone receptors that mediate effects on bone mineral density and regulate the female reproductive, cardiovascular, and central nervous systems. The selective estrogen receptor modulators (SERMs)[1] are compounds with mixed agonist/antagonist properties, exemplified by the clinically approved tamoxifen,[2] for the treatment of breast cancer, and raloxifene,[3] for the treatment and prevention of osteoporosis. Since the discovery of the estrogen receptor subtype β (ERβ),[4] the search for subtype selective SERMs, in particular ERα selective SERMs or SERAMs (selective estrogen receptor alpha modulators),[5] has attracted attention in an attempt to achieve an advantage over nonsubtype selective SERMs. We have previously reported on a new series of dihydrobenzoxathiin-based SERAMs, exemplified by **I** (Fig. 1), and we postulated that the interaction between the bulky sulfur in the ring and the two discriminating residues in the binding pocket of the two receptor isoforms (Leu_{384} for ERα, Met_{354} for ERβ) was responsible for the observed selectivity.[5b] In support of the hypothesis, replacement of the sulfur atom by the smaller carbon atom resulted in a complete loss of α-selectivity.

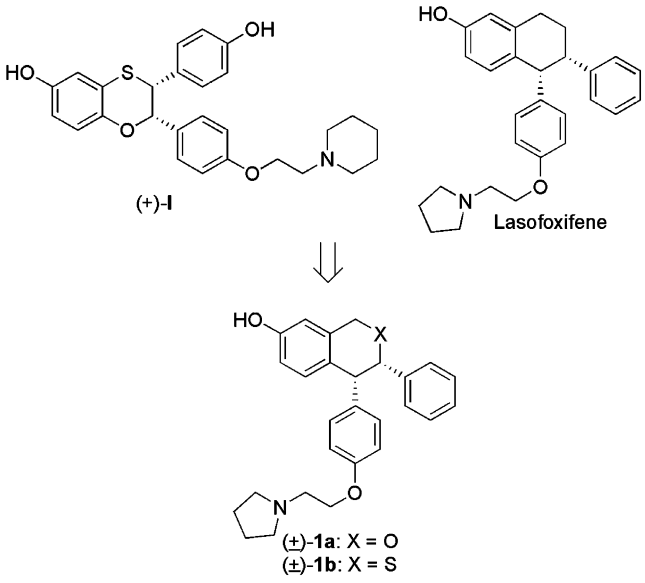

Figure 1.

Lasofoxifene[6] is a potent, nonsubtype selective SERM based on a tetrahydronaphthalene core structure developed by Pfizer, which has advanced to late stage clinical trials for osteoporosis. Herein, we report our effort to apply the rationalization for the selectivity in the dihydrobenzoxathiin series to the tetrahydronaphthalene

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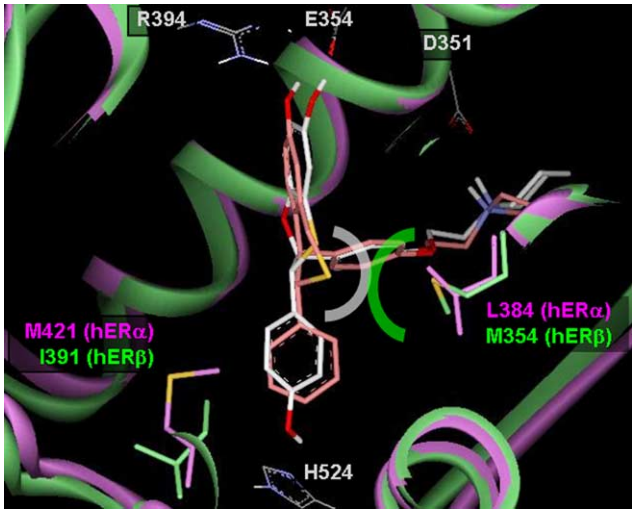
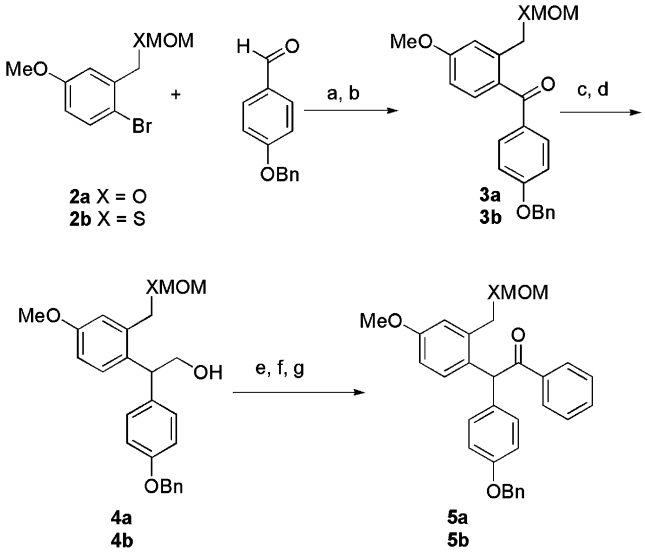


Figure 2. Molecular model of hERα selective **I** (white) versus model of **1b** (pink). HERα is depicted in purple and hERβ in green. Residue numbering is hERα unless otherwise indicated. Arcs represent desired clash between S and Met354 (hERβ) sidechain to achieve selectivity.

skeleton by conversion into isochroman **1a** and isothiochroman **1b**. Based on visual inspection of the molecular models[7] (shown in Fig. 2), it was hoped that either oxygen or sulfur substitution in the ring might offer the same discrimination between the receptor subtypes as is seen in compound **I**.

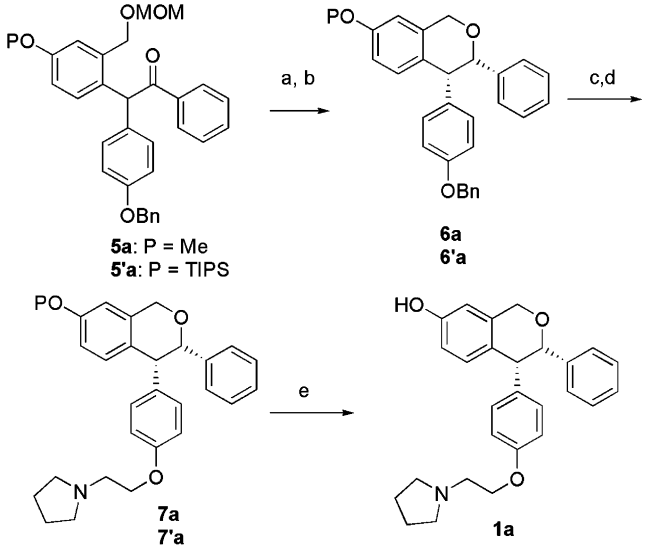
The method reported by Kim et al.[8] was utilized for the construction of the *cis*-diaryl iso- and isothiochromans. Therefore, we started to build ketones **5a** and **5b** as precursors for the cyclization–dehydrative–reduction process (Scheme 1). Lithiation of the aryl bromides **2a** and **2b**[9] with *n*-butyl lithium followed by reaction with 4-benzyloxy benzaldehyde provided the corresponding benzyl alcohols. In turn, TPAP/NMO oxidation afforded ketones **3a** and **3b**, which upon treatment with Tebbe's reagent and subsequent hydroboration yielded alcohols **4a** and **4b**. Then **4a** and **4b** were converted to ketones **5a** and **5b** by sequential oxidation with Dess–Martin reagent, addition of phenyl lithium and re-oxidation.



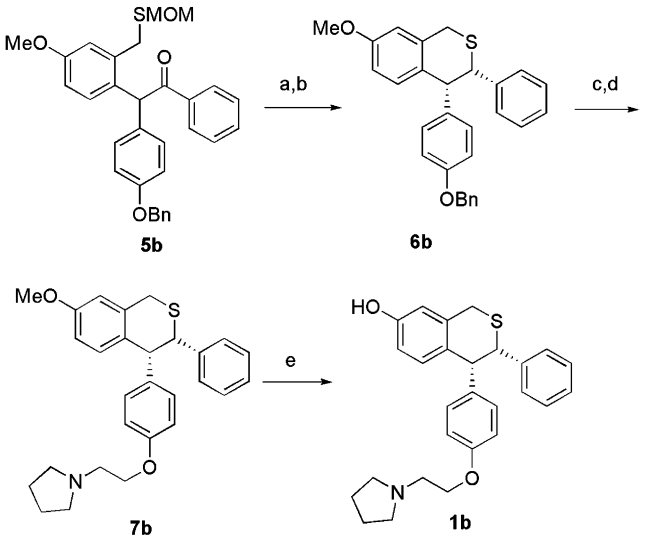
Scheme 1. Reagents and conditions: (a) *n*-butyl lithium, THF, −78 °C, 85%. (b) TPAP, NMO, MS 4 Å, CH_2Cl_2, 95%. (c) Tebbe reagent, THF, 85%. (d) BH_3, THF, then H_2O_2, NaOH, 90%. (e) Dess–Martin Periodinane, pyridine, CH_2Cl_2, 96%. (f) Phenyl lithium, THF, 60 %. (g) Dess–Martin periodinane, pyridine, CH_2Cl_2, 85%.

The cyclization conditions to form the iso- and isothiochroman rings were a little different. The preparation of the isochroman **1a** is depicted in Scheme 2. The ketone **5a** was treated with TFA in methylene chloride to remove the MOM protection, followed by addition of triethylsilane to affect the dehydrative reduction to form exclusively the *cis*-isochroman **6a**. The *cis*-configuration was confirmed by ^{1}H NMR as judged by the presence of a small coupling constant ($J = 2.5$ Hz @ $\delta = 5.03$ and 4.02 ppm) between the C-3 and C-4 protons of the pyran ring. After removal of the benzyl protecting group by catalytic hydrogenation, the pyrrolidinoethyloxy side chain was installed utilizing a Mitsunobu reaction to form **7a**. Unfortunately, no suitable method was found to convert the methylether to the requisite phenol, as all conditions concomitantly destroyed the pyran ring.[10] Instead, TIPS was used as the protection for the phenol from the beginning of the synthesis leading to **7′a**, where the TIPS was readily removed by TBAF to provide **1a**.[11]

In contrast to the reaction of **5a**, treatment of **5b** with TFA quickly led to the analogous isothiocoumarin ring containing a tetrasubstituted double bond, which resisted reduction by either triethyl silane or catalytic hydrogenation. Hence, as shown in Scheme 3, the MOM protection in **5b** was readily removed by exposure to silver nitrate to generate the thiol. Subsequent treatment with triethyl silane and boron trifluoride–diethyl etherate successfully formed only the *cis*-isothiochroman **6b**. After debenzylation with boron tribromide at



Scheme 2. Synthesis of **1a**. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) Et_3SiH, 60%. (c) H_2, Pd black, EtOH, 98%. (d) N-(2-hydroxyethyl) pyrrolidine, PPh_3, DIAD, THF, 75%. (e) TBAF, THF, 80%.



Scheme 3. Synthesis of **1b**. Reagents and conditions: (a) $AgNO_3$, EtOH, 80%. (b) Et_3SiH, CH_2Cl_2, $BF_3{\cdot}Et_2O$, 0 °C, 60%. (c) BBr_3, CH_2Cl_2, −78 °C, 80%. (d) N-(2-hydroxyethyl)pyrrolidine, PPh_3, DIAD, THF, 75%. (e) BBr_3, CH_2Cl_2, −10 °C, 55%.

Table 1. Binding affinities (IC_{50}) and in vivo data for compounds **7a**,**b**, and **1a**,**b**

Compds[a]	Binding affinity[13]	MCF-7[14]	Uterine weight[15]
	IC_{50} (nM) human ERα/ERβ (fold selective for ERα)	Inhibition IC_{50} (nM)	% Inhibition/% control (antagonism/ agonism)
7a	39/315 (8)	57	ND
7b	13/110 (8)	164	ND
1a	2.4/3.6 (2)	0.15	24/43
1b	0.6/2.1 (4)	0.25	47/49
(+) **I**	0.8/45 (56)	3.0	99/9
Lasofoxifene	1.3/1.8 (1.4)[b]	0.10[c]	74/26
Estradiol	1.3/1.1		—/100

[a] The isochromans and isothiochromans were a pair of racemic compounds characterized by spectroscopy.
[b] Data cited from Ref. 16 is 0.5/1.2 (2.4).
[c] Data cited from Ref. 6a is 0.05.

−78 °C,[12] the pyrrolidinoethyloxy side chain was appended to form **3b**. Again in contrast to isochroman **7a**, treatment of **7b** with boron tribromide at −10 °C successfully removed the methyl protection on the phenol without degrading the isothiochroman ring to provide **1b**.[11]

As indicated in Table 1, four compounds were evaluated in the estrogen receptor binding assay, the MCF-7 cell proliferation assay and the immature rat uterine weight assay. Consistent with prior observations, methylethers **7a**,**b** exhibited much lower binding affinity than the phenolic counterparts **1a** and **1b**, the latter of which rivaled the potency of lasofoxifene. While in agreement with the calculated energetics,[7] the lack of desired selectivity suggests that even small differences in the position of the sulfur in compound **I** and **1b** relative to the receptor residues (depicted in Fig. 2) can have a significant impact on selectivity. It is interesting to contrast these results with those reported[16] for the corresponding substituted nitrogen (tetrahydro-isoquinoline) derivatives lacking the C-3 phenyl group, in which a modest preference for ERβ was observed amongst substantially less potent ligands. In spite of the potent binding affinity and potent antagonism of the estradiol dependent growth of MCF-7 cells exhibited by both **1a**[17] and **1b**, the uterine weight assay response contrasted that reported,[6c] and confirmed in our assay, for lasofoxifene. Both **1a** and **1b** exhibited substantially less activity in the inhibition of the estradiol-triggered uterine growth (antagonism) while effecting significantly more uterine growth (agonism).

In conclusion, we have synthesized the iso- and isothiochroman analogs related to lasofoxifene and demonstrated that the compounds had very high potency in both an estrogen receptor binding assay and an MCF-7 inhibition assay. In addition, we demonstrated that in spite of these similarities to lasofoxifene, the effects on immature rat uterine tissue were strikingly different. The conversion of lasofoxifene into an SERAM using the rationalization for selectivity postulated in the dihydrobenzoxathiin series was unsuccessful.

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7. In work to be published elsewhere, the interaction energies for lasofoxifene, **1a** and **1b** with the two receptor subtypes fall within 1 kcal/mol of each other compared within each subtypes. Despite the visual similarity of placement of the S in compound **I** and compound **1b**, these results suggest that the selectivity profile of **1a** and **1b** should be similar to that of lasofoxifene.
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9. Compound **2a** was prepared from commercially available methyl 2-bromo-5-methoxybenzoate in two steps, while **2b** was synthesized from 2-bromo-5-methoxytoluene in three steps.
10. Several conditions were tried: (a) BBr_3, CH_2Cl_2, −78 to −20 °C; (b) $AlCl_3$, 3 h, 0 °C; (c) LiI, Collidine, reflux, 10 h. All of the conditions led to the degradation of the benzopyran ring.
11. Owing to the lack of selectivity in binding to the estrogen receptor isoforms described in Table 1, the chiral separation of **1a** and **1b** was not undertaken.
12. The use of catalytic hydrogenation with a palladium catalyst to remove the benzyl protecting group was unsuccessful, presumably owing to the poisoning of the catalyst by sulfur.
13. The single IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN basic flashplates using tritiated estradiol and full length recombinant human ERα and ERβ proteins with a 3 h incubation time. This assay provides IC_{50} values that are reproducible to within a factor of 2–3.
14. In the MCF-7 proliferation assay, estrogen depleted MCF-7 cells were plated into 96-well cell culture plates at a density of 1000 cells/well. To determine the antagonist activity of a compound, the test compounds and 3 pmol estradiol were applied to the cells on days 1 and 4. The assay was terminated between days 8 and 10 and the cellular protein content/well used to determine the IC_{50}.
15. Twenty-day old intact Sprague–Dawley rats were treated (sc) with the tested compounds for 3 days at 1 mpk. The anti-estrogenic activity of compounds was determined by co-administration of the compound with a subcutaneous injection of 17-β-estradiol one hour after compound at 4 μg/kg dose and reported as % inhibition. The estrogenic activity (partial agonism) of the compounds was determined by administering the test compound without estradiol and reported as % control.
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