HETEROCYCLES, Vol. 67, No. 1, 2006, pp. 79 - 83. © The Japan Institute of Heterocyclic Chemistry
Received, 14th March, 2005, Accepted, 18th May, 2005, Published online, 20th May, 2005. COM-05-S(T)7

ESTROGEN RECEPTOR LIGANDS. PART 15: SYNTHESIS OF BENZOTHIOPYRAN-BASED SELECTIVE ESTROGEN RECEPTOR ALPHA MODULATORS (SERAM)

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Abstract – Benzothiopyran (**2**) was prepared and the bioactive (+)-**2** was found to exhibit a reduced affinity toward the estrogen receptors (ERα/β) when compared to the corresponding dihydrobenzoxathiin (+)-**1**.

In a previous communication,[1] we identified ERα subtype selective ligands or **S**elective **E**strogen **R**eceptor **A**lpha **M**odulators (SERAMs) that centered on the dihydrobenzoxathiin core structure.[2] This compound, as exemplified by **1**, exhibited low nanomolar binding affinity and sub-nanomolar functional activity, as well as *in vivo* efficacy for the suppression of estradiol-driven uterine proliferation, with minimal uterotropic activity. Subsequent expanded structure-activity relationship[3] eventually led to a potential developmental candidate.[4]

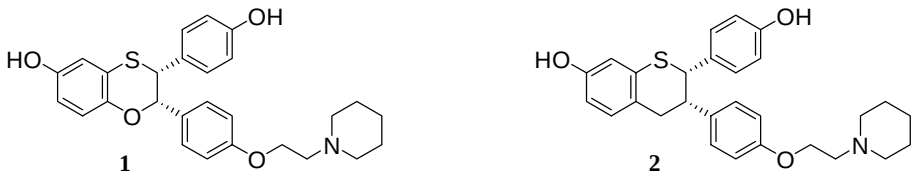


Figure 1

Although dihydrobenzoxathiin (**1**) had excellent potency and selectivity for ERα, it was judged to be unacceptably prone to oxidative metabolism, with subsequent formation of covalent protein adducts.[5] We initially identified that an iminium ion resulting from oxidation of the piperidine residue present in the side chain of **1** was a significant contributor to the formation of covalent adducts.[6] Meanwhile,

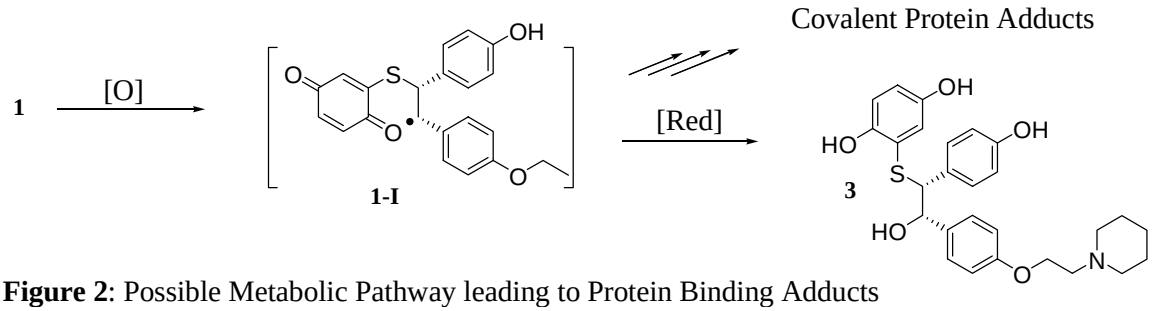
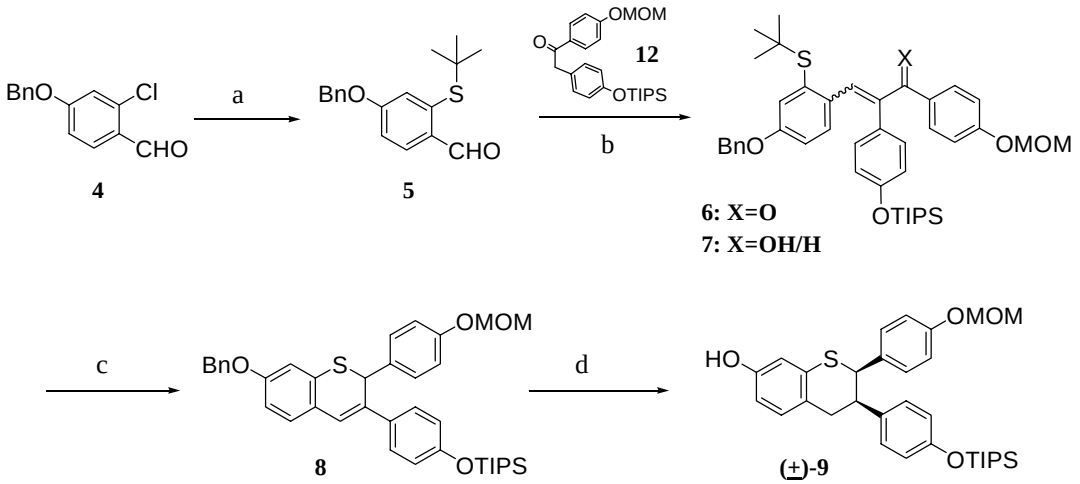


Figure 2: Possible Metabolic Pathway leading to Protein Binding Adducts

formation of a quinone intermediate (**1-I**), generated from a phenolic-radical fragmentation process embedded in the dihydrobenzoxathiin core, had also been postulated as being responsible for the covalent adducts with biological proteins.[5] Intuitively, the isolation of hydroquinone (**3**) from the *in vitro* incubation of **1** with human liver microsomes supported the plausibility of the above metabolic pathway (Figure 2).[7] Therefore, it seemed prudent to replace the oxygen in the core with a methylene unit, thus eliminating the possible formation of the quinone intermediate and thereby ameliorating any potential for cytochrome P-450 mediated activation. Herein, we report the synthesis, estrogen/anti-estrogen activity, and metabolic stability of target (**2**).

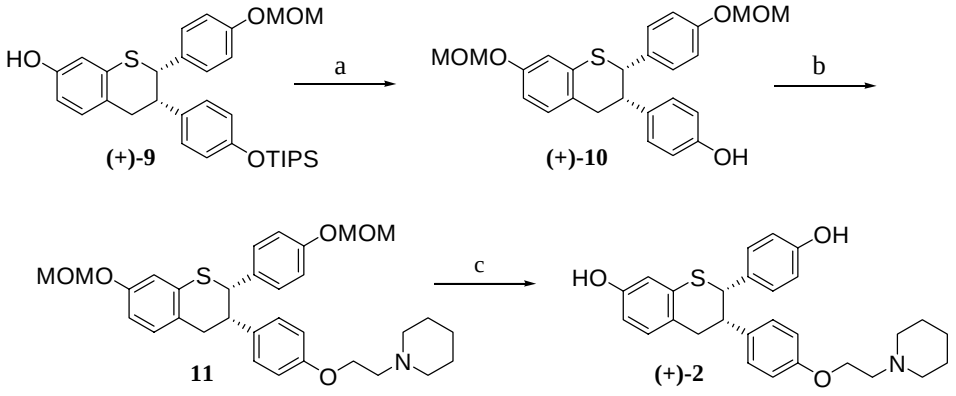


Scheme 1 *Reagents and Conditions*: (a) 1.5 equiv. *t*-Butylthiol, 1.8 equiv. NaH, Diglyme, 95°C, 15 h, 72% ; (b) Piperidine, AcOH, 110°C, 2 days, 50%; (c) i. $LiAlH_4$, 0°C ii. 5% TFA, CH_2Cl_2, -5°C, 10 min, 90% for two steps; (d) 1 equiv. Pd black, H_2, EtOH, rt, 2 days, 75%

The synthesis began with the known compound (**4**), which was easily synthesized from *m*-chlorophenol by a Reimer-Tiemann reaction in 60-70% yield.[8] Replacement of the chlorine atom with a *t*-butylthio group was initially problematic due, in part, to the lability of the benzyl group under the basic conditions.[9] However, the best conversion was realized by using a minimum amount of the reagents at lower temperature. The resulting *t*-butylthiobenzaldehyde (**5**) was condensed with a properly protected ketone (**12**) in the presence of piperidine/HOAc to give a mixture of (*E/Z*)-α,β-unsaturated ketones (**6**).[10] This

Figure 3

Knoevenagel reaction was very sluggish and reaction times of 2 days were necessary to ensure total consumption of the starting material. Selective 1,2-reduction of α,β-unsaturated ketones (**6**) with 1 equiv. $LiAlH_4$ in THF gave allylic alcohols (**7**), thus setting the stage for subsequent cyclization to the benzothiopyran.



Scheme 2 *Reagents and Conditions*: (a) i. 3 equiv. NaH, MOMCl, DMF, rt ii. TBAF, HOAc, THF, 80% for two steps; (b) 2-Chloroethylpiperidine, Cs_2CO_3, acetone, reflux; (c) 10% HCl, MeOH, reflux, 1 h, 81%

The allylic alchohols (**7**), without further purification, were then cyclized with TFA in dichloromethane to yield the 2,3-disubstituted 2*H*-1-benzothiopyran (**8**) in 90% yield.[11] An acid-mediated cyclization of the allylic carbocation **7-I** to **7-II** followed by loss of isobutylene was postulated as the reaction mechanism (Figure 3).[11] Hydrogenation of compound (**8**) with Pd black provided exclusively the racemic, *syn*-compound (**9**).[12] The *syn* relationship of the two phenyl groups was unambiguously established by the observed small coupling constant (J = 3.5 Hz, δ = 4.2 ppm, see ref. 2), between H_2-H_3. Subsequent chiral resolution of the racemic benzothiopyran (**9**) was realized by HPLC, using a Chiracel AD column and 30% IPA/hexane as the eluant, to provide the dextrorotatory enantiomer ((+)-**9**).[13]

With (+)-**9** now readily available, protection of the hydroxyl group in the A-ring, followed by deprotection of TIPS group produced (+)-**10** in quantitative yield (Scheme 2). The phenol ((+)-**10**) was next converted to the respective ether (**11**) by reaction with 2-piperidinoethyl chloride in the presence of cesium carbonate in acetone. Removal of the two MOM groups with dilute acid resulted in the final

compound ((+)-**2**) in 81% yield.[14] Similarly, in the racemic series, (**9**) was also converted to (**2**) by application of the same procedure.

It is clear from the data in Table 1 that the benzothiopyran (**2**) retained the ERα potency and the magnitude of receptor subtype selectivity (ERβ/ERα ratio) exhibited by the dihydrobenzoxathiin (**1**) in an *in vitro* ER binding assay. However, the replacement of the oxygen atom with a methylene unit altered the size of the ring, which presumably contributed to the reduction of the binding affinity (ERα for (+)-**1**= 0.8 nM and (+)-**2**= 3.1 nM).[15] This weaker potency paralleled the weaker estradiol antagonism observed in the immature rat uterine model (92% inhibition vs 76% inhibition).

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

	Binding affinity			Uterine Assay (*sc*)[c]
Compd	ERα	ERβ (α selectivity)		% inhib @ 1mpk / % control @ 1 mpk
(+)-**1**[b]	0.8	45	(56)	92/0.4
(±)-**1**[b]	3.0	143	(48)	77/5.0
(+)-**2**[d]	3.1	130	(43)	76/4.7
(±)-**2**	5.8	590	(101)	N.A.

[a]Utilizing full length recombinant human ERα/β proteins; IC_{50} (nM), n=>48-56, see ref 1. [b]Absolute stereochemistry was determined by X-Ray crystallography, see ref 1 [c]See ref 1 & 3; [d]The absolute stereochemistry of (+)-**2** was assigned based on analogy with (+)-**1**, see ref 13 and 14

As with the dihydrobenzoxathiins, only the single enantiomer ((+)-**2**) reproduced the activity exhibited by the racemate (**2**). In addition, the metabolism of the tritium-labelled ((+)-**2**) was investigated and, in spite of the modification, was found to have a similar level of covalent protein adducts as (+)-**1** in incubations with rat liver microsomes. Further results in this area will be reported in future publications from this laboratory.

ACKNOWLEDGEMENTS

We gratefully acknowledge Professor Barry M. Trost for helpful discussions during his scientific consultation. This paper is dedicated with our best wishes to Professor Barry M. Trost on the occasion of his 65th birthday.

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12. *Ca*. 3% yield of *trans* isomer was also formed. See also ref. 2.
13. The absolute stereochemistry of (+)-**9** was assigned based on analogy with (+)-**1**, and confirmed later by biological data, see also ref. 1 and Table 1. (+)-**10**: $[\alpha]_D$ +227.6^o (*c* 0.69, MeOH)
14. (+)-**2**: $[\alpha]_D$ +193.2^o (*c* 0.49, MeOH); ^{1}H NMR (500 MHz, Acetone-d_6) δ (ppm) 7.00 (d, *J*=8.3 Hz, 1H), 6.82 (d, *J*=8.4 Hz, 2H), 6.76 (d, *J*=8.5 Hz, 2H), 6.68 (d, *J*=8.7 Hz, 2H), 6.68 (d, hidden, 1H), 6.60 (d, *J*=8.5 Hz, 2H), 6.58 (dd, *J*=2.5 Hz, 1H), 4.49 (d, *J*=3.7 Hz, 1H), 4.10 (t, 2H), 3.60 (m, 1H), 3.05 (dd, *J*=10.5 and 16.25 Hz, 1H), 2.90 (dd, *J*=3.6 and 16.00 Hz, 1H), 2.80 (br t, 2H), 2.60 (br s, 4H), 1.60 (m, 4H), 1.50 (br m, 2H); MS *m/z* 462.0 (M^+)
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