Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1281–1284

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of Hexahydrochrysene and Tetrahydrobenzofluorene Ligands for the Estrogen Receptor

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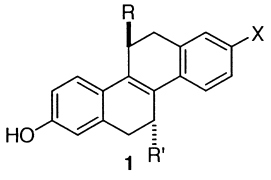
Received 23 January 2001; accepted 12 March 2001

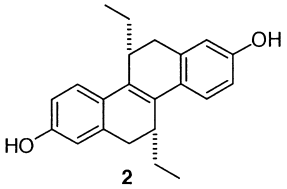
Abstract—To prepare novel estrogen receptor (ER) ligands, we have developed a facile approach to substituted hexahydrochrysene and tetrahydrobenzo[*a*]fluorene systems. Substituents, including basic side chains, were added to these systems, and their binding affinity to ERα and ERβ, and in some cases their transcriptional activity were evaluated.

Selective estrogen receptor modulators (SERMs)[1] are estrogens that show a distinct pattern of tissue selective action, possibly based on their selective binding to the two ER subtypes, ERα and ERβ,[2] or their ability to induce distinct receptor conformations.[3] We have studied certain chrysene derivatives as part of an effort to understand how ligand structural features determine ER subtype selectivity and impart SERM-like profiles. The 5,6,11,12-tetrahydrochrysenes (THCs), first developed as fluorescent ER ligands (**1**),[4] proved to be particularly interesting, because the *cis*-isomers, such as **2**, display remarkable ER subtype efficacy selectivity, being agonists on ERα and antagonists on ERβ.[5]



1

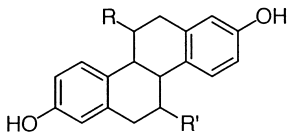
X = OH, $COCH_3$, $COOCH_3$



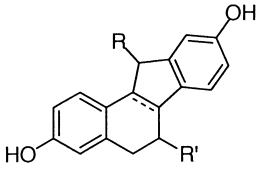
2

(R,R)-Diethyl THC

To examine these systems further, we now describe the preparation of saturated analogues of the THCs, the hexahydrochrysenes (HHC, **3**), as well as saturated analogues of the related benzo[*a*]fluorene core, namely tetrahydrobenzo[*a*]fluorenes (THBF, **5**). (The dihydrobenzo[*a*]fluorenes (DHBF, **4**) proved to be extremely unstable, and were not studied.) We also introduced a piperidinyl-ethoxyphenyl substituent, referred as a basic side chain (BSC), into both the HHCs and TBHF. Despite their overall similarity, these two ligand series have considerably different shapes, resulting in a different topological display of substituents around the rigid ligand core. This has a significant effect on the ER binding of the HHCs and THBFs.



HHC (3)



DHBF (4) and **THBF (5)**

Synthesis of Ketones 8ab, Key Intermediates for the HHC and THBF Series

The tetracyclic ketones **8a** and **8b** proved to be versatile intermediates for the synthesis of substituted HHCs and THBFs, respectively. They were readily prepared through their acyclic ketone precursors, **7a** and **7b** (Scheme 1). A Shapiro reaction on tosylhydrazone **6** generated a vinyllithium intermediate that was trapped with tributyltin chloride.[6] Stille coupling[7] of the resulting vinyl stannane with *m*-methoxyphenylacetyl chloride or *m*-anisoyl chloride gave the acyclic ketones **7a** and **7b**, respectively, in good yield. These were cyclized with methanesulfonic acid (MsOH) to give the desired cyclic ketones **8a** and **8b**, as well as minor amounts of

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00189-5

the isomeric products derived from attack *ortho* to the methoxy group (**9**). In the HHC series, ketone **8b** also provided the spiro compound **10**.

Under all of the cyclization conditions we examined, the major ketone products **8ab** in both the HHC and THBF series had a *cis*-ring junction. This was confirmed by X-ray crystallography for the THBF derivative, and was ascertained by ^{1}H NMR in the HHC case. Furthermore, in the latter case, reduction, deoxygenation and demethylation[8] of ketone **8b** (Scheme 2) yielded material that was identical to the known *cis*-hexahydrochrysene diol **12**.[9]

Synthesis of Substituted Tetrahydrobenzo[*a*]fluorene Systems

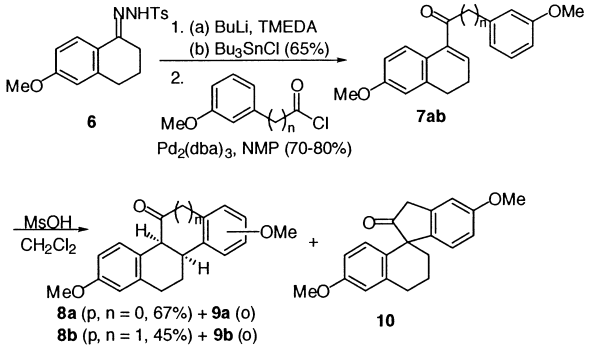
Ketone **8a** was deprotected to the bisphenol **13** and also converted to the THBF-2,8-diol **14** by deoxygenation and deprotection. Addition of EtMgBr to ketone **8a** gave **15** as a single epimer of undetermined configuration. After deoxygenation, a 2:1 mixture of diastereoisomers (**16ab**) was obtained, which could be separated after deprotection (**17ab**) (Scheme 3). We were unable to assign the **17ab** stereoisomers by ^{1}H NMR analysis.

To add a basic side-chain substituent, ketone **8a** was treated with the Grignard reagent **18** to afford **19**, which was deoxygenated by treatment with Et_3SiH and $BF_3{\cdot}Et_2O$ followed by the addition of TFA. Monophenol **20** was obtained after selective removal of the TBS protecting group (Scheme 4).

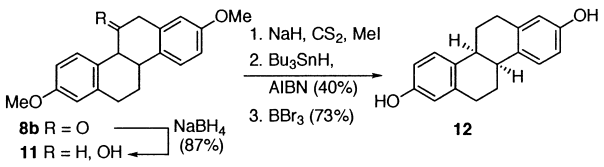
We could not assign the relative configuration of **20** by ^{1}H NMR; however, X-ray analysis established the *cis*–*cis* configuration, in which all of the protons are on the convex face of the molecule. The phenyl substituent in this THBF system adopts an orientation that is almost in the plane of the benzocyclopentane bicyclic portion, whereas the *cis*-fused benzocyclohexane ring portion is disposed in nearly a perpendicular arrangement with respect to the benzocyclopentane. The basic side-chain substituted THBF **21** was synthesized by coupling of **20** with piperidine ethanol and removal of the methoxy-protecting groups.[10]

Synthesis of Substituted Hexahydrochrysene Systems

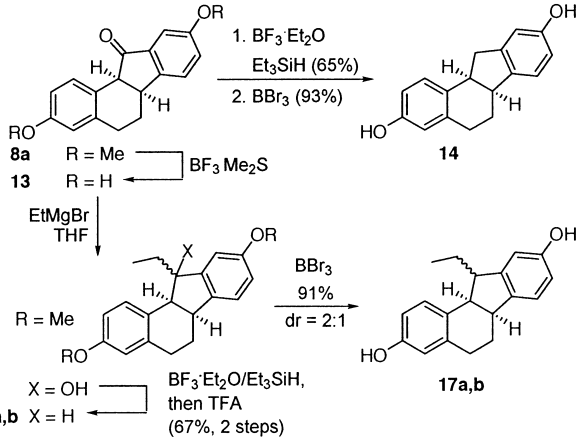
Attempts to add Grignard reagent **18** or its cerium analogue to ketone **8b** gave only fully aromatized products. However, Suzuki coupling[11] of boronic acid **23** with the mixture of enol triflates **22**, derived from ketone **8b**, gave regioisomers **24** (69%) and **25** (18%) (Scheme 5). While reduction of **25** gave a mixture of isomeric products, reduction of **24** with Et_3SiH/TFA and $BF_3{\cdot}Et_2O$ gave primarily the saturated system **26**. The TBS group, partially removed during this reduction, was fully cleaved by treating the crude reaction product with TBAF. The basic side chain was added to



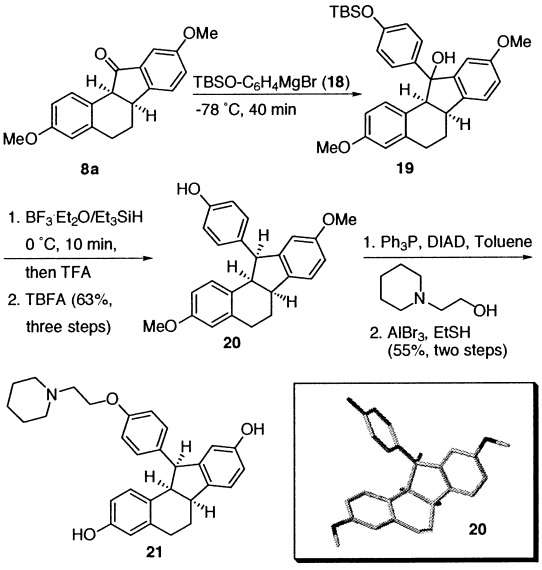
Scheme 1.



Scheme 2.



Scheme 3.



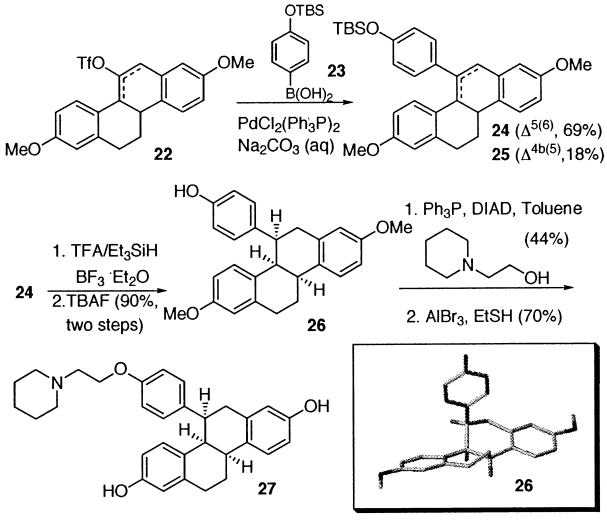
Scheme 4.

the free phenol **26** by standard methods,[10] and final deprotection was accomplished using $AlBr_3$/EtSH to afford **27**.

The *cis–cis* configuration, initially assigned from ^1H NMR coupling constants, was confirmed by X-ray crystallography. The conformation of this HHC system is quite different from that of the THBF system **21** described above: the ring with the phenyl substituent adopts a half chair conformation, placing the phenyl substituent in an axial-like position where it avoids nonbonding interaction with the other aryl groups, and the rings of the decalin system are more nearly coplanar than they were in the THBF one.

The relative binding affinity (RBA, $E_2 = 100\%$) of the compounds prepared above was determined in a competitive radiometric binding assay, using purified, full-length human ERα and ERβ (PanVera).[12] The results of these assays are summarized in Table 1, which also includes RBA values on some related compounds whose preparation, by other routes, is described elsewhere.[13] A few of the most interesting compounds were also tested in cell-based reporter gene transcription assays.[14]

Apart from the compounds bearing a BSC, **21** and **27**, all of the derivatives have some selectivity for binding to ERβ. Both *cis* and *trans* unsubstituted HHCs have higher affinity than their unsubstituted THC parent (**1**, R = R′ = H, X = OH), though their ERβ affinity selectivity is dramatically different. A diethyl HHC (**3**, R = R′ = Et), of which only the *trans–cis–trans* isomer was available,[13] binds much less well than its THC parent (**2**), but has the highest ERβ affinity selectivity of all the compounds we have investigated here. In the THBF series, the ketone **13** has very low affinity, but the unsubstituted system **14** and especially the two ethyl epimers **17ab** (of unassigned configuration) are very high affinity ligands, having ERβ affinities substantially greater than that of estradiol, with one epimer (**17b**) having significant ERβ affinity selectivity. The moderate ERβ binding selectivity of these compounds is reminiscent of a number of compounds we have described in the tetrahydrochrysene series,[5] but very different from the high ERα binding selectivities of a number of triaryl pyrazoles we have investigated.[15]



Scheme 5.

The HHC and THBF analogues having the piperidinylethoxyphenyl basic side-chain substituent, **21** and **27**, present an interesting contrast. The THBF analogue **21** has much higher affinity than the HHC analogue **27**, and both have a significant ERα affinity selectivity. It is not easy to account for these affinity and selectivity differences, because they are probably related to differences in how the substituted and unsubstituted compounds interact with various residues in the ligand-binding pockets of ERα and ERβ.

We assayed the transcriptional activity of three ligands (lacking the BSC) that showed both good affinity and had higher ERβ binding affinity selectivity, namely **12**, *trans*-HHC[13] and the *cis*-THBF **14**; none of them showed a significant preferential potency for either ER subtype (data not shown). The results of transcription activation assays with the two BSC compounds are shown in Figure 1. The HHC-derivative **27** (Fig. 1A) acts as a mixed agonist–antagonist through ERα and as an antagonist through ERβ. By contrast, the THBF derivative **21** behaves as an antagonist on both receptors (Fig. 1B). The higher antagonistic character of the THBF compound **21** could be related to the conformation of the BSC relative to the two ring systems. As one might expect, changes in this orientation could preclude or favor interactions between the basic side chain and the key residues in the receptor (e.g., ER-Asp354).[16,17]

We have developed effective synthetic approaches to the hexahydrochrysene (HHC) system, long known as estrogen receptor ligands, and to the related members of tetrahydrobenzo[*a*]fluorene (THBF) system. Our approach enables the addition of an alkyl or phenyl substituent (the latter of which was adorned with a basic side chain), and it produced compounds having predominantly the *cis* configuration at the internal ring fusion. Some of the unsubstituted or ethyl-substituted systems showed moderate ERβ binding affinity selectivity, but had essentially no potency and/or efficacy selectivity in cell-based assays. The HHC and THBF systems substituted with a basic side chain were antagonists on

Table 1. ERα and ERβ binding affinities and selectivities

Compound		Relative binding affinity (RBA %)[a]		
		ERα	ERβ	β/α
1	R,R′ = H; X = OH[5]	3.0	6.5	2
2	di-Et-THC[5]	23	144	6
12	*cis*-HHC	11	71	6.7
	trans-HHC[13]	14	130	8.9
3	R,R′ = Et (t,c,t)[13]	1.0	16	16
13	THBF ketone	0.53	2.0	3.8
14	THBF	8.7	63	7.2
17a	Et-THBF	150	300	2.0
17b	Et-THBF	48	280	5.9
21	BSC[b]-THBF	38	10	0.26
27	BSC[b]-HHC	1.1	0.06	0.06

[a]Relative binding affinity (RBA) values, where estradiol = 100%.
[b]BSC = basic side chain.

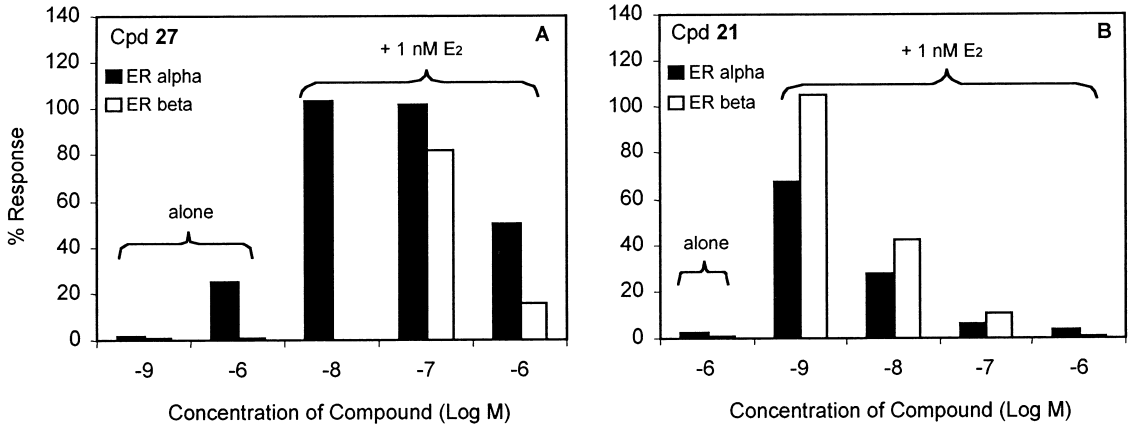


Figure 1. Transcriptional activity of **27** (left) and **21** (right) through ERα and ERβ in human endometrial carcinoma (HEC-1) cells. All values are relative to the response to 1 nM estradiol (100%). For methods, see refs 5 and 14.

both ERα and ERβ, with the THBF derivative displaying a more complete antagonism and higher potency than the HHC derivative.

A synthesis of the THBF derivative **21** has recently been described in a patent, together with an indication of its utility for the treatment of osteoporosis and hyperlipidemia.[18] The THBF used in that report was a mixture of isomers, and the relative configuration of the components of the mixture was not specified. The interesting biological profile of this THBF derivative, together with the antagonist character that we have observed in our THBF and HHC basic side-chain derivatives, suggests that it would be promising to study further the individual diastereomers of these systems, because the geometric relationship between the BSC substituent and the ligand core can be varied dramatically.

Acknowledgements

This work was supported through research grants from the NIH (5R37 DK15556 and CA18119) and instrumentation grants from the NIH and NSF. We are grateful to Kathryn Carlson, Jun Sun and Benita Katzenellenbogen for biological assays.

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