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Structure–activity relationships and sub-type selectivity in an oxabicyclic estrogen receptor α/β agonist scaffold

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Abstract—An oxabicyclic template for estrogen receptor α and β agonists has been identified which can be tuned to provide moderate levels of selectivity for either receptor sub-type. Structure–activity relationships within this phenol-substituted oxabicyclo[3.3.1]nonene series are described. Select compounds from the present series showed activity in vivo after oral dosing in rodent models of uterine proliferation.

The estrogen receptor (ER) is a member of the nuclear hormone receptor superfamily of ligand-dependent transcription factors, which mediate a broad range of physiological processes in response to binding of the endogenous ligand 17β-estradiol (**1**) or exogenous ligands (Fig. 1).[1] Progress in medicinal chemistry programs focused on the ER has resulted in the identification of structurally diverse molecules with unique biological properties. A number of compounds, which act through the ER have been approved or are in late stages of clinical development for female osteoporosis, breast cancer, and as hormone replacement therapy.[2] Most notable among these is raloxifene (**2**), a selective estrogen receptor modulator (SERM). Significant advances have been made to date in elucidating the mechanisms by which SERM's achieve tissue selective action, which include the differential recruitment of key co-regulatory proteins to complete the transcriptional machinery in various cell and gene-specific contexts.[3]

The relatively recent identification of a second sub-type of the ER[4] may provide an additional opportunity for selective pharmacology through agents, which preferentially interact with ERβ. Although the pharmacology of ERβ is not well understood, its low level of expression in the uterus relative to ERα has generated interest in the development of selective modulators of ERβ with the potential for reduced risk of uterine stimulation for the treatment of vasomotor instability (hot flushes) and osteoporosis.[5] Recent reports[6] by workers at Wyeth demonstrate the utility of an ERβ selective agonist in animal models of chronic intestinal and joint inflammatory disease, suggesting a role for ERβ as a modulator of immune response.

Compounds of the general oxabicyclo[3.3.1]nonene structural class have been reported in the synthetic chemistry literature as products of the reactions of aromatic aldehydes and olefin-containing compounds in the acidic media of various clays.[7] These compounds had not been shown to possess significant biological activity, though unrelated bridged bicyclo[3.3.1]nonanes had been reported by Katzenellenbogen as an ER ligand scaffold.[8] A recent report from Bayer disclosed

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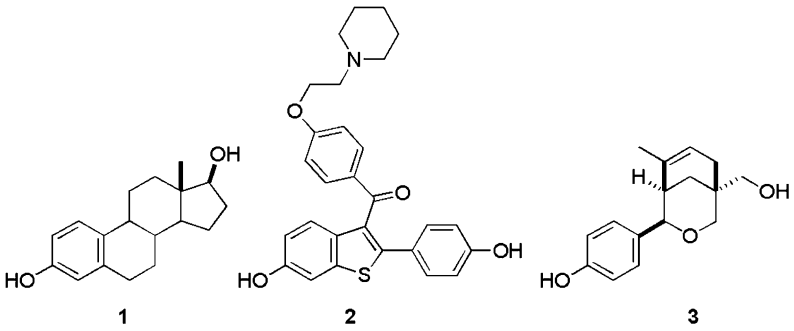
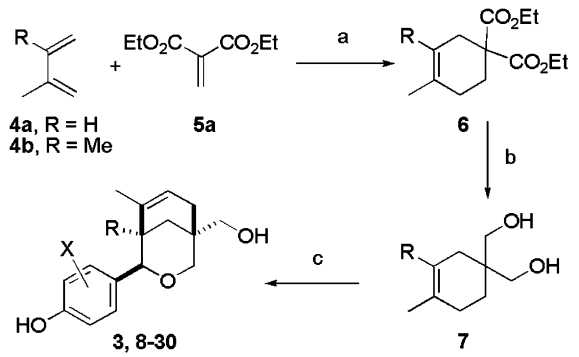


Figure 1. Estrogen receptor ligands.

compound **3** as a novel ER ligand, and further described structure–activity relationship (SAR) studies on the all-carbon isostere of this scaffold.[9] In the course of high-throughput screening efforts we independently identified compound **3** as a primary screening hit on ERα/β. Herein we describe the results of our investigations into the SAR in this oxabicyclic series with respect to both potency and ERα/β sub-type selectivity, and further describe the oral activity of compounds in this series in a standard rodent model of estrogenic action.

Compounds **3** and **8**–**30** of the present series were prepared by procedures similar to those reported in the chemical literature (Scheme 1).[7] Diels–Alder cycloaddition of isoprene or 2,3-dimethyl-1,3-butadiene (**4a**,**b**) and diethylmethylene malonate (**5a**) provided dicarboethoxycyclohexenes **6**, which were then reduced to the corresponding diols **7** with lithium aluminum hydride. *p*-Toluenesulfonic acid mediated cationic cyclization with various 4-hydroxybenzaldehyes gave the requisite oxabicyclo[3.3.1]nonene analogues in good to excellent yields as racemic mixtures of single diastereomers.[10] The *trans* relationship between the hydroxyaryl group and the hydroxymethylene group of the bicyclic scaffold was confirmed through ^{1}H NMR (including NOE) analysis. Compounds **3**, and **20**–**22** were subjected to chiral preparative HPLC separation to resolve these analogues into their respective pure enantiomers for the purposes of assessing absolute stereochemical contributions to biological activity.

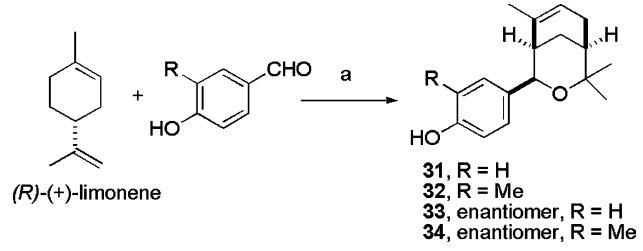


Scheme 1. Reagents and conditions: (a) toluene, reflux; (b) LAH, THF, −78 °C; (c) (4-HO)ArCHO, *p*-TsOH, 1,2-DCE, 50 °C.

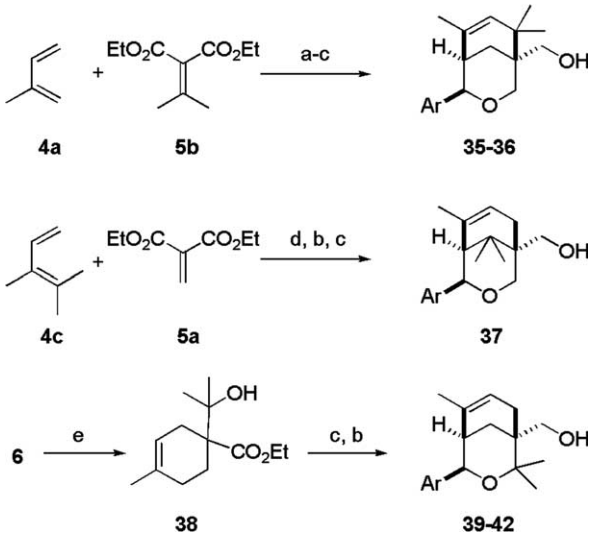
Optically pure analogues lacking a hydroxymethyl group at the bridgehead position were prepared starting with each respective pure isomer of limonene with gentle heating in the acidic environment afforded by Montmorillonite K10 clay to produce analogues **31**–**34** (Scheme 2). By analogy to the compounds derived from (*R*)-(+)-limonene (i.e., **31** and **32**), the dextrorotatory isomers **23**, **25**, **27**, **29** isolated by chiral HPLC were tentatively assigned the absolute stereochemistry as shown for **31** and **32**.

Geminal dimethyl substitutions were introduced at three separate positions on the oxabicyclo[3.3.1]nonene scaffold by variation of initial Diels–Alder reaction components, such as those leading to compounds **35**–**37**, or by Grignard addition to previously described diester **6** to afford the appropriate precursor **38** for acid-mediated cyclization with substituted benzaldehydes to afford analogues **39**–**42** (Scheme 3).

All compounds in the present series, along with the reference compound **1** were assayed for ERα and ERβ functional activity in cell-based transcriptional assays in COS-1 cells using methods previously reported (Table 1).[11] The initial lead **3** in the series showed moderate agonist activity on both ER sub-types. The addition of a fluoro substituent to either the 2 or 3 position of the aromatic ring lead to a significant enhancement in activity on both ERα and ERβ. Likewise, addition of a chloro substituent to the 2 position of the aromatic ring increased potency on both receptors, while introduction of a chlorine atom to the 3 position increased ERα potency while attenuating activity on ERβ. Other aryl substituents (Me, MeO, HO) tended to decrease potency on ERα/β. We were surprised to find that most of the ERα activity present in racemic mixture **3** resided in the dextrorotatory optical isomer, while both optical antipodes

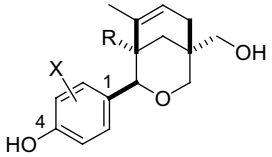


Scheme 2. Reagents: (a) montmorillonite K10 clay, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) $AlCl_3$, 1,2-DCE, 45 °C; (b) LAH, THF, −78 °C; (c) (4-HO)ArCHO, *p*-TsOH, 1,2-DCE, 50 °C; (d) toluene, reflux; (e) MeMgBr, PhH, rt.

Table 1. Estrogen receptor α and β agonist potencies of reference compound **1** and compounds **3** and **8**–**30** in transiently transfected COS-1 cells



Compound	Stereo	X	R	ERα EC_{50}[a,b] (nM)	ERβ EC_{50}[a,b] (nM)
1	—	—	—	2.4	9.7
3	Racemic	—	H	128	61
8	Racemic	3-Me	H	137	305
9	Racemic	3,5-Di-Me	H	743	2825
10	Racemic	3-F	H	4.6	25
11	Racemic	3-Cl	H	36	197
12	Racemic	2-Cl	H	11	5.3
13	Racemic	3-Br	H	336	485
14	Racemic	2-F	H	39	10
15	Racemic	2-Me	H	92	79
16	Racemic	2-OH	H	173	275
17	Racemic	3-OH	H	2411	3164
18	Racemic	3-MeO	H	1980	3050
19	Racemic	—	Me	203	39
20	Racemic	2-Cl	Me	43	67
21	Racemic	3-F	Me	68	6
22	Racemic	2-F	Me	50	12
23	(+)	—	H	35	51
24	(−)	—	H	835	38
25	(+)	2-Cl	H	138	46
26	(−)	2-Cl	H	18	6.1
27	(+)	3-F	H	2.6	40
28	(−)	3-F	H	254	57
29	(+)	2-F	H	21	180
30	(−)	2-F	H	19	3.1

[a] Values are means of three experiments.
[b] All compounds exhibited at least 75% intrinsic agonist activity.

were equiactive on ERβ (compare **23** and **24**). As a result, the levorotatory isomer **24** is a 22-fold selective agonist for the ERβ versus ERα. In general, it appears that the levorotatory isomers of these oxabicyclononene analogues are significantly more ERβ selective than their dextrorotatory counterparts (compare **27** with **28** and **29** with **30**). Addition of a methyl group to the bridgehead carbon as in analogues **19**–**22** also tended to improve ERβ selectivity relative to their des-methyl counterparts (e.g., **21** vs **10**).

Further optimizations in potency and/or sub-type selectivity were sought by the introduction of geminal dimethyl substitution at varying positions on the oxabicyclo[3.3.1]nonene skeleton. It was reasoned that a steric imposition proximal to a likely point of H-bonding contact within the ligand binding domain of the receptor (the hydroxymethyl substituent) might discern differences in the respective binding pockets (Table 2).

While addition of a *gem*-dimethyl substituent to the methylene bridge of **3** (e.g., **37**) led to a modest increase in ERα/β potencies, the regioisomeric *gem*-dimethyl analogue **39** displayed dramatically improved potency on both receptors. The contribution of the bridgehead hydroxymethyl group to activity of the oxabicyclononene analogues on ERα/β is best illustrated by comparing the activity of **39** with its des-hydroxymethyl counterparts **31/33**. Thus, **31/33** are 129-fold less active on ERα and 33-fold less active on ERβ than the corresponding bridgehead hydroxymethyl analogue **39**. The activity of **39** can be further enhanced by the addition of a 2 (or 3)-fluoro or 2-chloro substituent. The most potent analogues in this series are single digit nanomolar on both ER α and β (e.g., **39**–**42**) in the transcriptional assays.

Among the primary endocrinological actions of the native hormone estradiol (**1**) is the promotion of the growth and differentiation of female reproductive tissues. Therefore, the effects of ER agonists can be studied in rodent models of uterine proliferation in ovariectomized female rats.[12] Animals were treated once daily for 4 days by oral gavage with varying doses (0.1, 0.3,

Table 2. Estrogen receptor α and β agonist potencies of compounds **31**–**37** and **39**–**42** in transiently transfected COS-1 cells

Compound	Stereo	Ar	ERα EC_{50}[a,b] (nM)	ERβ EC_{50}[a,b] (nM)
31	(+)	4-OH-Ph	203	418
32	(+)	3-Me, 4-OH-Ph	143	318
33	(−)	4-OH-Ph	211	195
34	(−)	3-Me, 4-OH-Ph	218	318
35	Racemic	4-OH-Ph	141	183
36	Racemic	2-Cl, 4-OH-Ph	21	25
37	Racemic	4-OH-Ph	59	24
39	Racemic	4-OH-Ph	1.6	9.2
40	Racemic	2-Cl, 4-OH-Ph	1.0	6.0
41	Racemic	2-F, 4-OH-Ph	1.1	1.4
42	Racemic	3-F, 4-OH-Ph	0.6	7.2

[a] Values are means of three experiments.
[b] All compounds exhibited at least 75% intrinsic agonist activity.

Table 3. Effects of compound **23**, **24**, **26**, or estrone sulfate on uterine wet weight in ovariectomized female rats

Compound	Uterine weight ED_{50} ± SEM (mg/kg/day)	95% Confidence limits (mg/kg/day)
Estrone sulfate	0.16 ± 0.02	0.13–0.20
23	9.93 ± 2.00	6.69–5.67
24	>30	—
26	3.44 ± 0.88	2.09–5.67

1.0, 3.0, 10.0 mg/kg) of compound **23**, **24**, **26**, estrone sulfate (30 mg/kg) as a positive control, or vehicle (Table 3). Compounds **23** and **26** exhibited oral activity in this animal model, inducing a dose-dependent increase in uterine wet weight with ED_{50}'s of 9.93 and 3.44 mg/kg/day, respectively. In contrast, compound **24** (the optical antipode of **23**) was inactive at 30 mg/kg/day. Since **23** and **24** are approximately equiactive on ERβ but differ significantly in their activity on ERα, the uterotrophic effects of **23** and **26** are most likely due to the activation of ERα.

These results serve to validate the utility of the present template for further optimization to potentially yield ERβ selective pharmacological agents with physical properties suitable for oral dosing.

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10. A representative procedure for the preparation of compound **3** as outlined in Scheme 1 follows: (a) A 250 mL round-bottom flask was charged with diethyl methylenemalonate (17 g, 0.099 mol) and isoprene (12 g, 0.176 mol) and the mixture was heated neat at 100 °C for 3 h. The mixture was allowed to cool to rt, then concentrated. The residue was purified by silica gel chromatography (hexanes followed by 10% EtOAc/hexanes) to afford 17 g (72%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.34 (m, 1H), 4.15 (m, 4H), 2.51 (m, 2H), 2.12 (m, 2H), 1.98 (m, 2H), 1.62 (s, 3H), 1.23 (t, 6H, J = 7.3). (b) 1,1-Bis(hydroxymethyl)-4-methylcyclohex-3-ene. A solution of 4-methylcyclohex-3-ene-1,1-dicarboxylic acid diethyl ester (1.97 g, 8.21 mmol) in 50 mL of THF was cooled to −78 °C. Lithium aluminum hydride (0.494 g, 13.02 mmol) was then added in one portion. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was then poured into aqueous 1 M $NaHSO_4$ and extracted with EtOAc (2 ×). The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (hexanes followed by 25% EtOAc/hexanes) to afford 0.907 g (71%) of the title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.29 (br s, 1H), 3.61 (s, 4H), 2.21 (br s, 2H), 1.93 (m, 2H), 1.78 (m, 2H), 1.65 (s, 3H), 1.60 (t, 2H, J = 6.5). (c) 4-(4′-Hydroxyphenyl)-6-methyl-3-oxabicyclo[3.3.1]non-6-ene. To a solution of 1,1-bis(hydroxymethyl)-4-methylcyclohex-3-ene (104 mg, 0.66 mmol) in 9 mL of 1,2-dichloroethane was added *p*-toluenesulfonic acid monohydrate (39 mg, 0.21 mmol). The reaction mixture was heated to 50 °C, and 4-hydroxybenzaldehyde (82 mg, 0.67 mmol) was added. The reaction mixture was stirred for 5 h. The reaction mixture was allowed to cool to rt, poured into water, washed with brine, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography (hexanes followed by 20% EtOAc/hexanes) afforded 70 mg (40%) of the title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.17 (d, 2H, J = 8.5), 6.77 (d, 2H, J = 8.5), 5.56 (s, 1H), 4.73 (s, 1H), 4.50 (s, 1H), 3.96 (dd, 1H, J = 11.0, 2.5), 3.63 (d, 1H, J = 11.0), 3.40 (d, 2H, J = 4.0), 2.32 (d, 1H, J = 2.7), 2.21 and 2.08 (ABq, 2H, J_{AB} = 18.9), 1.82 (dd, 1H, J = 11.5, 3.0), 1.67 (ddd, 1H, J = 12.0, 3.0, 3.0), 1.01 (dd, 3H, J = 4.0, 2.5).
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