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Androstenediol analogs as ER-β-selective SERMs

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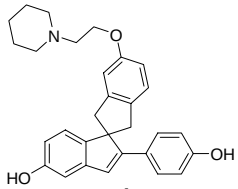
Abstract—A series of 19-substituted androstenediol derivatives was prepared. Some of the novel analogs were surprisingly potent and selective ligands for ER-β.

The clinical significance of the selective estrogen receptor modulators (SERMs)[1] generated substantial interest in the discovery of new SERMs.[2] Reports of a second estrogen receptor subtype[3] prompted interest in the development of receptor subtype-selective SERMs, and both ER-α[4] and ER-β[5] selective SERMs have since been described in the literature. Although these efforts have focused on non-steroid structures, several steroidal SERMs have also been reported.[6] We have previously reported the discovery of spiroindenes (e.g., **1**)[7] as non-subtype-selective SERMs and of dihydrobenzoxathiins (e.g., **2**) as ER-α selective SERMs.[5b,c,d]



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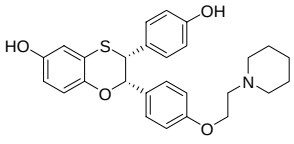
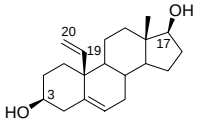


2

Naturally, we were also interested in discovering ER-β-selective ligands. High-throughput screening[8a] of the Merck sample collection resulted in the discovery of 19-methylene-androstenediol (**3**) as a potent and highly selective ligand for ER-β.[8b] An old patent[6d] describes **3** as having anabolic-androgenic and anti-estrogen activities (and other activities) but provides no binding data and does not discuss estrogen receptor subtype selectivity.



3

The excellent binding affinity and ER-β selectivity of **3** (Table 1) prompted us to initiate a medicinal chemistry program to explore the SAR of this remarkable lead. We decided to focus initially on substitution of the 19,20 olefin. A series of olefin-substituted analogs was prepared and evaluated as summarized below (Schemes 1–6).[9] Wittig olefination of aldehyde **29**[10] followed by deacylation afforded the C-20-substituted olefin analogs **4**–**14** in low to moderate yields (Scheme 1). As expected, the Wittig reaction afforded a mixture of olefin isomers from which the cis and trans isomers of the product could be isolated by careful chromatography. Interestingly, the outcome of Wittig olefination was dependent on the nature of the protecting groups at C-3 and C-17. Substitution of TBDMS or THP for the acetate-protecting groups of **29** resulted in significant changes in yield and cis:trans product ratio in many cases. Despite numerous attempts, we were unable to prepare analogs

Keywords: SERMs; SERAMs; Estrogen; Androstenediol.
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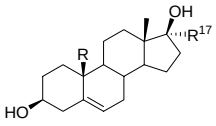
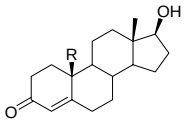
Table 1. Biodata

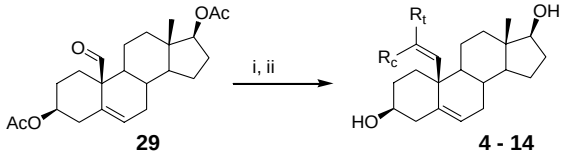


3-26



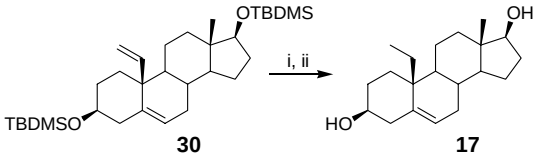
27-28

Compound	R	R^{17}	ER binding (IC_{50}, nM)[12]			ER transactivation (EC_{50}, nM)[13]			AR (IC_{50}, nM)[14]	
			hERα	hERβ	α/β	α	β	α/β	AR	AR/ERβ
3	$CH{=}CH_2$	H	2236	11	212	982	4.1	246	33	3
4	*cis*-CH=CHF	H	4280	25	171	622	3.8	164	11	0.4
5	*trans*-CH=CHF	H	4165	22	188	324	8.7	37	85	4
6	*cis*-CH=CHCl	H	6690	83	80	>1000	91	>11	172	2
7	*trans*-CH=CHCl	H	5600	122	46	603	68	9	1436	12
8	*cis*-CH=CHBr	H	>10,000	212	47	>1000	96	>10	20	0.1
9	*trans*-CH=CHBr	H	3984	210	19	>1000	67	15	120	0.6
10	*cis*-CH=CHOMe	H	3349	303	11	3237	188	17	1402	5
11	*trans*-CH=CHOMe	H	>10,000	1299	8	583	827	0.7	4617	4
12	*cis*-CH=CHMe	H	1956	16	126	344	21	16	718	45
13	*trans*-CH=CHMe	H	1164	96	12	47	39	1	843	9
14	*cis*-CH=CHEt	H	3042	361	8	719	252	3	3767	10
15	CH_2CHO	H	>10,00	1619	>6	>1000	>1000	1	>5000	—
16	$CH_2C{=}CH_2$	H	1738	128	14	>1000	148	7	1768	12
17	CH_2CH_3	H	2329	16	144	—	—	—	88	6
18	$CH_2CH_2CH_3$	H	5155	132	39	—	—	—	1769	14
19	C≡CH	H	425	17	25	160	8	20	1761	104
20	$C{\equiv}CCH_3$	H	8419	381	22	1000	171	6	3733	10
21	$CH_2C{\equiv}CH$	H	4643	286	16	984	153	6	1717	6
22	CH_3	H	210	10	21	26	6	4	212	21
23	CH_2OH	H	6324	1754	4	>1000	641	>1	>5000	>2.9
24	CHO	H	>10,000	230	>43	844	78	11	>5000	>22
25	$CH{=}CH_2$	Me	1053	25	42	494	31	16	169	7
26	$CH{=}CH_2$	C≡CH	147	14	10	129	24	5	287	20
27	CH_3	H	>10,000	>10,000	1	—	—	—	2.7	<0.0002
28	$CH{=}CH_2$	H	>10,000	782	>12	>1000	379	>2.6	7.9	0.01
17β-estradiol	—	—	1.4	1.2	1.2	0.75	2.1	2.8	19.1	16



29 4 - 14

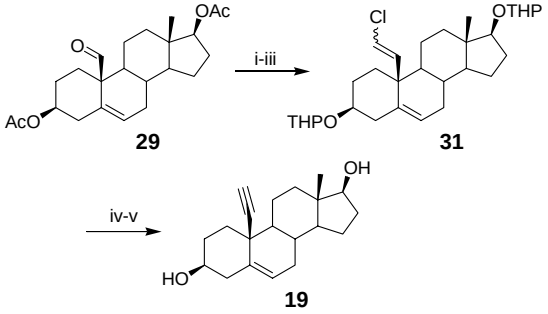
Scheme 1. Reagents: (i) $RtRcC{=}PPh_3$; (ii) MeOH, KOH.



30 17

Scheme 2. Reagents: (i) H_2 (50 psi), 5% Rh/C, EtOAc, EtOH; (ii) TBAF, THF.

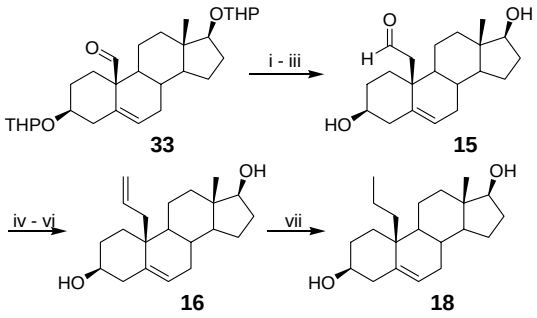
of **3** with substitution at C-19. For example, attempts to prepare the 19-methyl-substituted analog of **3** by olefination of the methyl ketone analog of aldehyde **29** failed due to the complete unreactivity of the ketone to both the Wittig and Tebbe methylenation reagents, presumably due to steric hindrance at C-19. Alternative approaches were also unsuccessful.



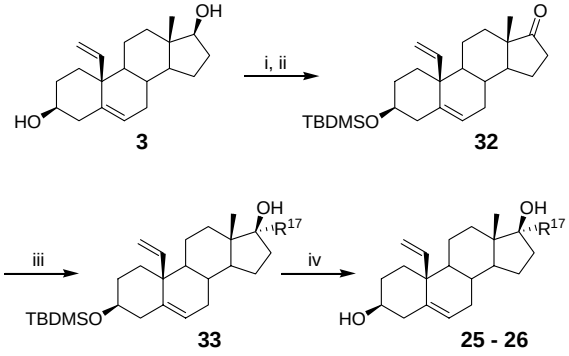
29 31 19

Scheme 3. Reagents: (i) Ph_3PCH_2Cl Cl, *n*BuLi; (ii) KOH, MeOH, (iii) DHP, pyridinium tosylate, 90% from **29**; (iv) *n*BuLi, 56%; (v) pyridinium tosylate, MeOH, 78%.

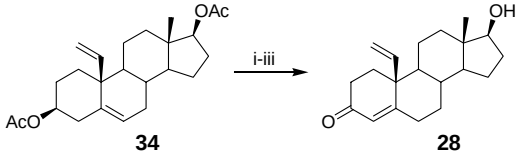
To determine whether a double bond at C-19,20 was the optimum degree of unsaturation, we prepared the corresponding saturated analog **17** and the acetylene **19**. For comparison, we also evaluated the parent compound androstenediol (**22**).[11] The ethyl analog **17** was prepared by hydrogenation of **30** (Scheme 2). This poorly reproducible reaction proved to be somewhat more difficult than anticipated, probably due to the hindered nature of the C-19,20 olefin.



Scheme 4. Reagents: (i) $MeOC{=}PPh_3$; (ii) MeOH, pyridinium tosylate; (iii) HCl; (iv) DHP, pyridinium tosylate; (v) $H_2C{=}PPh_3$; (vi) MeOH, pyridinium tosylate; (vii) H_2 (50 psi), 5% Rh/C, EtOAc, EtOH.



Scheme 5. Reagents: (i) TBDMS-Cl, imidazole, DMF, 60%; (ii) TPAP, NMO, CH_2Cl_2; (iii) $R^{17}MgCl$ (R^{17}=Me) or $R^{17}Li$ (R^{17}=CCH), THF; (iv) TBAF, THF.



Scheme 6. Reagents: (i) NaOH, MeOH; (ii) H_2CrO_4; (iii) NaOH, MeOH.

The synthesis of acetylene **19** began with conversion of aldehyde **29** to a mixture of cis and trans chlorides **31**. Reaction of **31** with *n*-butyllithium and deprotection afforded acetylene **19** in modest overall yield (Scheme 3). Modification of this route (reaction of the acetylene anion intermediate with iodomethane) afforded the methyl acetylene **20**.

The extended analog **16** was prepared by olefination of extended aldehyde **15** which, in turn, was prepared by olefination of **33**, the THP-protected analog of **29**. Hydrogenation of **16** afforded the propyl analog **18** (Scheme 4). The extended acetylene **21** was prepared by subjecting the protected analog of extended aldehyde **15** to the sequence outlined in Scheme 3 for the synthesis of acetylene **19**.

We also prepared the 17-methyl (**25**) and 17-ethynyl (**26**) analogs of **3** by reaction of ketone **32** (prepared from **3** by selective silylation of the 3-hydroxyl followed by oxidation of the 17-hydroxyl to the ketone) with either MeMgBr or lithium TMS-acetylide followed by deprotection to afford **25** or **26** (Scheme 5).

The 3-*keto*-delta-4 analog **28** was prepared by selective deacetylation of **34**, an intermediate in the preparation of **3**, followed by oxidation of the C-3 hydroxyl and deprotection (Scheme 6).

The compounds were evaluated first in an ER ligand binding assay (Table 1).[12] All were ER-β selective although the degree of selectivity varied considerably. The olefin-substituted analogs **4**-**14** were generally less effective than **3** as ER-β ligands although the fluoro analogs **4** and **5**, and the *cis*-methyl analog **12** were comparable to **3** in selectivity and binding affinity. *cis*-substitution at C-20 was clearly preferred and smaller substituents were better than larger ones. Interestingly, substitution at C-17 (compounds **25** and **26**) had little effect on ER-β binding but substantially improved binding to ER-α (especially with 17-ethynyl substitution) resulting in lower ER-β selectivity. Both the saturated derivative **17** and the acetylene analog **19** retained the ER-β binding affinity of **3**. However, the acetylene was a surprisingly better ligand for ERα so **19** was less selective for ER-β than either **3** or **17**. The extended olefin was clearly less effective than **3** and the same trend was also observed in the saturated (**18** vs **17**) and acetylene (**20** and **21** vs **19**) cases. Androstenediol (**22**) was an excellent ligand for ER-β but was also a good ligand for ER-α and so was much less selective than **3**. The oxygenated analogs **23** and **24** were much weaker ligands.

The compounds were also evaluated in a cell-based transactivation assay.[13] With the exception of the *cis*-fluoro analog **4**, the olefin-substituted analogs **4**–**14** were somewhat less active than **3** in this assay and were less selective as well. Once again, *cis*-substitution at C-20 was preferred. In general, ER-β selectivity was lower in this assay compared to the binding assay. The 17-substituted analogs **25** and **26** had good ER-β potency but were less selective than **3**.

One of our concerns when we initiated this project was the possibility of off-target activity mediated by binding to other steroid receptors, particularly the androgen receptor (AR). The lead compound **3** was a 33 nM ligand for AR but had no significant binding (IC_{50} > 3000 nM) to either the glucocorticoid receptor (GR) or the progesterone receptor (PR). New compounds were therefore routinely evaluated in an AR binding assay.[14] The 20-*cis*-methyl analog **12** had the best AR/ERβ binding ratio of the olefin-substituted analogs **4**–**14**, and was perhaps the most interesting compound overall, with a 15-fold improvement in selectivity for ERβ over AR (relative to 3) and only a minimal loss in selectivity for ERβ over ERα. Overall, the acetylene **19** had the best AR/ERβ selectivity. However, selectivity for ER-β over ER-α was somewhat less for this compound. As expected, testosterone (**27**) and the testosterone analog **28** were excellent ligands for the androgen receptor.

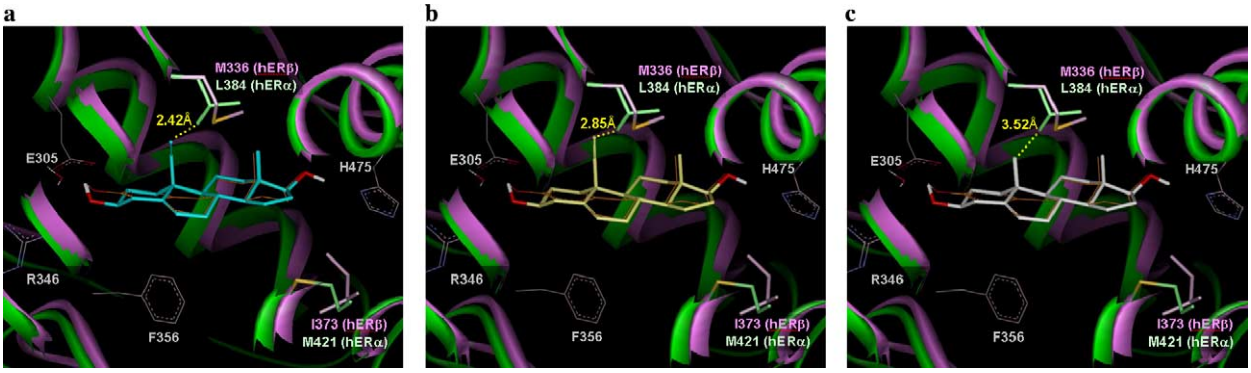


Figure 1. Superposition of estradiol (orange) in the context of hER-α (green) with (a) olefin **3** (cyan) (manually docked into a homology model of ER-β; proposed binding mode for **3** was later confirmed crystallographically); (b) acetylene analog **19** (yellow); and (c) androstenediol **22** (white) in the context of hER-β (purple) complexed with compound **3**. Unless otherwise indicated, residue numbering is that of hER-β. See Ref. 15 for modeling details.

Molecular modeling[15] of compounds **3**, **17**, **19**, and **22** provides a rationale for the ER-β selectivity observed with **3** and **17** (Fig. 1). The terminal carbon of the vinyl group for **3** is approximately 2.4 Å away from Leu384 in ER-α, which is about 1.0 Å closer than the distance to the corresponding Met336 in ER-β. Similarly, for the saturated analog **17**, the terminal carbon of the ethyl group is about 2.3 Å away from Leu384 in ER-α, which is about 1.0 Å closer than the distance to the corresponding Met336 in ER-β (not shown in Fig. 1). The proposed negative steric interaction with Leu384 in hER-α is consistent with the SAR for this series of compounds for which ER-β selectivity is gained through a decrease in ER-α affinity. Compounds **19** and **22**, by comparison, have smaller substituents which are further removed from Leu384 (2.9 and 3.5 Å, respectively) and would be expected to have less of a negative interaction with ER-α. Both lose ER-β selectivity relative to **3** and **17** through an increase in ER-α affinity.

In conclusion, the androstenediol analogs reported herein exhibit a range of binding affinities and selectivities for the estrogen receptors (up to ~200-fold selective for ER-β over ER-α). Additional studies will be reported in future communications.

Acknowledgments

The authors thank Susanne Miranda and Joe Laquidara for the large-scale preparation of **29**.

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12. The 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. Compounds were evaluated in duplicate in a single assay. In our experience, this assay provides IC_{50} values that are reproducible to within a factor of 2–3.
13. This assay was run at MRL-CIBE in Spain using the procedure described by Barkhem et al: Barkhem, T.; Carlsson, B.; Nilsson, Y.; Enmark, E.; Gustafsson, J.; Nilsson, S. *Mol. Pharmacol.* **1998**, *54*, 105.
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15. (a) Models of **3** using crystallographically determined estradiol cocrystallized with hERα(pdb:1ERE) were built and docked into a homology model of hERβ. The proposed binding mode was later confirmed crystallographically (Fitzgerald et al., manuscript in preparation). Models **17**, **19**, and **22** were based on the crystallographic coordinates of **3** as cocrystallized with hERβ. Energy minimization for all of the models within the context of the hERβ receptor (**3** cocrystallized) was accomplished by rigidly fixing all residues, except for side chains which fell within 5 Å of the modeled ligand which were allowed to minimize in conjunction with the ligand. All minimizations were conducted using the MMFFs forcefield[15b] with a distance dependent dielectric model of 2*r*; (b) Halgren, T. A. *J. Comp. Chem.* **1999**, *20*, 730.