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Androstene-3,5-dienes as ER-β selective SERMs

Timothy A. Blizzard,[a,*] Candido Gude,[a] Jerry D. Morgan, II,[a] Wanda Chan,[a] Elizabeth T. Birzin,[a] Marina Mojena,[b] Consuelo Tudela,[b] Fang Chen,[c] Kristin Knecht,[c] Qin Su,[c] Bryan Kraker,[a] Ralph T. Mosley,[a] Mark A. Holmes,[a] Susan P. Rohrer[a] and Milton L. Hammond[a]

[a]*Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Merck Research Laboratories-CIBE, Madrid, Spain*
[c]*Merck Research Laboratories, West Point, PA 19486, USA*

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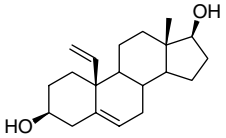
Abstract—A series of androstene-3,5-diene derivatives were prepared. Despite lacking the C-3 hydroxyl previously believed necessary for ER activity, some of the analogs retained surprising affinity for ER-β. For example, diene **4** retained excellent selectivity and potency as an ER-β agonist and was more selective for ER-β over the androgen receptor (AR).

The importance of the selective estrogen receptor modulators (SERMs) has prompted extensive research.[1] Much effort has focused on the discovery of ER-α[2] and ER-β[3] subtype-selective SERMs. We have also reported non-selective spiroindene SERMs.[4]



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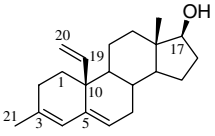

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During the course of our medicinal chemistry program based on the highly ER-β-selective screening hit **1**,[3a] we have discovered and report herein a series of androstene-3,5-diene SERMs (e.g., **4**) with selectivity for ER-β ranging from 6× to 160× in a ligand binding assay.

The 3,5-diene analog **4** was discovered serendipitously during our attempted synthesis of 3-methylated analogs **2** and **3**. Addition of methyl Grignard to ketone **17**[3b] followed by ammonium chloride workup and deprotection afforded the 3,5-diene analog **4**. Similar chemistry has been reported for other steroids.[5] The structure of **4** was confirmed by NMR analysis.[6] Since **4** lacked the A-ring hydroxyl group thought to be essential for ER binding, we expected it to be at best a weak ligand for the ERs. However, to our surprise, **4** retained excellent selectivity and potency as an ER-β agonist (Table 1) with reduced affinity for the androgen receptor AR (Table 2). We prepared the original synthetic targets **2** and **3** by first deconjugating **17** by treatment with base followed by addition of methyl Grignard to the resulting 3-keto-δ-5 intermediate. Subsequent deprotection afforded **2** and **3** which proved to be much weaker ER ligands than **4** (Scheme 1).

Molecular modeling suggests a possible explanation for the surprising ER-β activity of diene **4** (Fig. 1).[7] The terminal carbon of the vinyl group at C-10 of both **1** and **4** appears to have a negative steric interaction with the Leu384 side chain of ER-α which is consistent with the observed decrease in ER-α activity for both series. The vinyl group also forces **1** away from Glu305 (a 0.4 Å shift from what is observed between estradiol and Glu353 in 1ERE[7c]) and toward Phe356 and other hydrophobic residues which line the cavity along the α-face of the steroid. Presumably, these additional hydrophobic interactions compensate for the weakened Hbond interaction. When modeled into the cavity, **4** is similarly shifted toward Phe356 and more deeply toward His475 than is seen with **1** due to the steric bulk of the 3-methyl group along with the C10 vinyl group. It

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* Corresponding author. Tel.: +1 732 594 6212; fax: +1 732 594 9556; e-mail: tim_blizzard@merck.com

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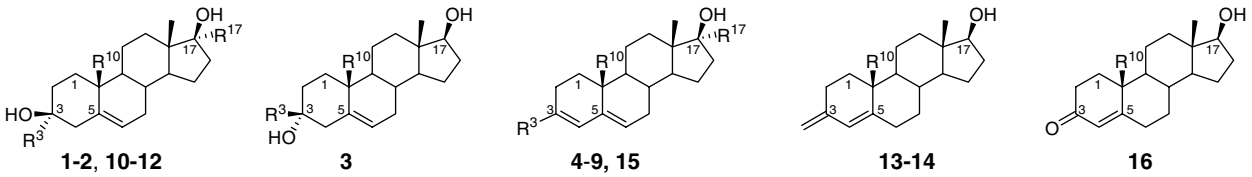
Table 1. ER binding and transactivation data



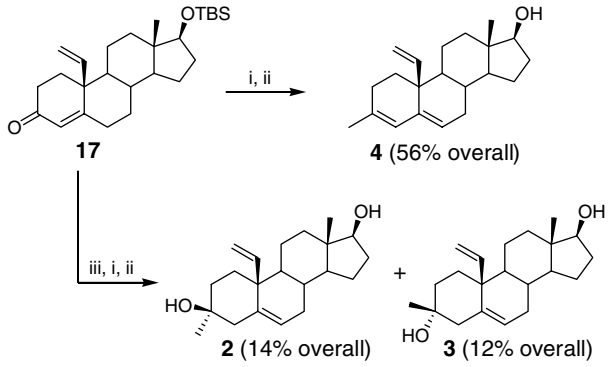
#	R^3	R^{10}	R^{17}	ER binding (IC_{50}, nM)[8]			ER transactivation (EC_{50}, nM)[9]		
				hER-α	hER-β	α/β	ER-α (% Ag)	ER-β (% Ag)	α/β
1	H	CH=CH_2	H	2240	11	204	980 (35)	4.1 (90)	240
2	CH_3	CH=CH_2	H	5300	255	21	>1000 (28)	240 (57)	>4
3	CH_3	CH=CH_2	—	>10000	2490	>4	> 1000 (27)	>1000 (31)	1
4	CH_3	CH=CH_2	H	1440	9	160	>1000 (4)	69 (92)	>14
5	CH_2CH_3	CH=CH_2	H	3110	115	27	> 1000 (3)	565 (54)	>1.8
6	C_6H_5	CH=CH_2	H	>10000	1760	>5.6	>1000 (0)	>1000 (0)	1
7	Cl	CH=CH_2	H	5740	64	90	820 (38)	170 (84)	4.8
8	CH_3	CH=CH_2	CH_3	4380	53	83	>1000 (4)	480 (52)	>2
9	CH_3	CH=CH_2	CH ≡ CH	1850	72	26	490 (15)	31 (80)	15
10	H	CH=CH_2	CH_3	1050	25	42	>1000 (13)	190 (65)	>5.2
11	H	CH=CH_2	CH ≡ CH	150	14	11	130 (82)	24 (78)	5.4
12	H	CH_3	H	180	9.9	18	26 (91)	5.6 (77)	4.6
13	—	CH=CH_2	—	>10000	120	>83	430 (49)	230 (59)	1.9
14	—	CH_3	—	640	34	19	>1000 (29)	210 (67)	> 4.8
15	CH_3	CH_3	H	110	9.4	12	540 (49)	220 (59)	2.4
16	—	CH=CH_2	—	>10000	780	>13	>1000 (12)	380 (78)	>2.6
Estradiol	—	—	—	1.4	1.2	1.2	0.75 (100)	2.1 (100)	0.36

Table 2. Comparison of hER-β and AR binding data

#	hER-β IC_{50} (nM)[8]	AR IC_{50} (nM)[10]	AR/ER-β
1	11	33	3
2	255	842	3.3
3	2490	2610	1
4	9	560	62
5	115	500	4.3
6	1760	3160	1.8
8	53	520	9.8
9	72	1615	22
10	25	170	6.8
11	14	290	21
12	9.9	230	23
13	120	330	2.8
14	34	40	1.2
15	9.4	2250	240
16	780	7.9	0.01
Estradiol	1.2	26	22
Testosterone	>10,000	2.7	<0.0002

may be that the proximity of the planar diene of **4** to Phe356 and increased ability to Hbond with His475 is responsible for maintaining its potency despite the lack of the A-ring hydroxyl group.

The surprising activity of **4** prompted us to prepare additional androstenediene analogs. Reaction of ketone



Scheme 1. Reagents: (i) MeMgBr, NH_4Cl workup; (ii) *n*-Bu_4NBr, THF; (iii) KO^{t}Bu.

17 with ethyl Grignard afforded initially the exo-ethylene analog **18** which was isomerized to the desired 3-ethyl derivative **5** (Scheme 2).

Similarly, treatment of **17** with phenyl Grignard gave the desired 3-phenyl analog **6** (Scheme 3).

The 3-chloro analog **7** was prepared by reaction of ketone **16**[3a] with acetic anhydride (to protect C-17 OH) followed by phosphorus oxychloride (introduce C-3 Cl) and deprotection (Scheme 4).

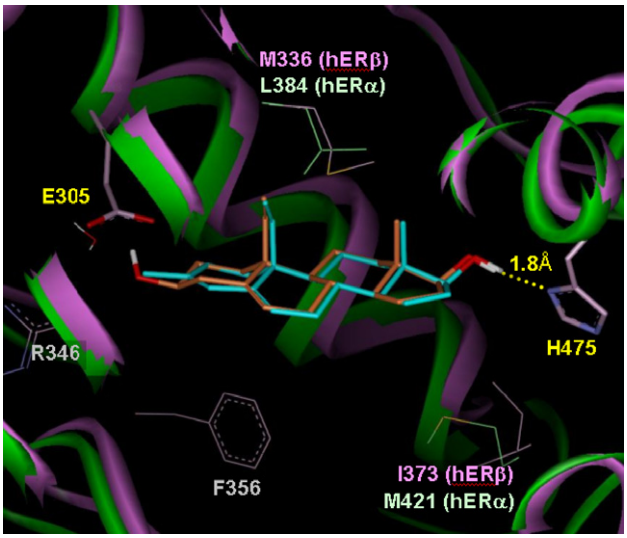
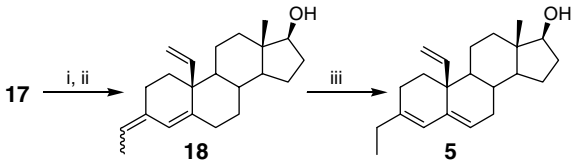
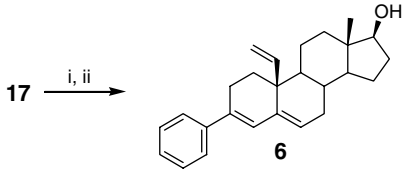


Figure 1. Superposition of crystallographically determined **1** (orange) with **4** (cyan) in the context of hER-α (green) and hER-β (purple) complexed with compound **1**. Unless otherwise indicated, residue numbering is that of hER-β.

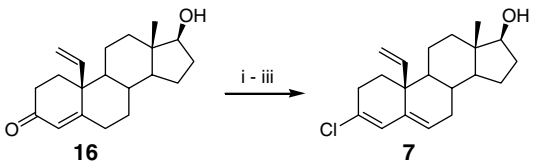


Scheme 2. Reagents: (i) EtMgBr, NH_4Cl workup; (ii) *n*-Bu_4NBr, THF, 94% from **17**; (iii) HCl, EtOH, 61%.

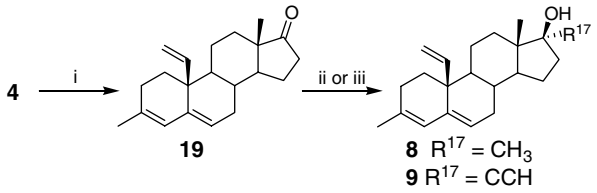


Scheme 3. Reagents: (i) PhMgBr, NH_4Cl workup; (ii) *n*-Bu_4NF, THF, 50% overall.

The 17-methyl (**8**) and 17-ethynyl (**9**) analogs of **4** were readily prepared by oxidation of the 17-hydroxyl to afford ketone **19**, followed by reaction with either methyl Grignard or lithium TMS-acetylide (Scheme 5). We have previously reported the corresponding 3-hydroxy analogs (**10** and **11**).[3a]



Scheme 4. Reagents and condition: (i) Ac_2O, pyridine, DMAP, 100%; (ii) $POCl_3$, AcOH, rt, 50%; (iii) KOH, MeOH, 40%.

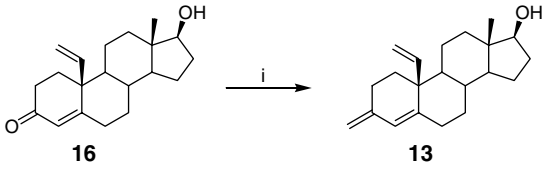


Scheme 5. Reagents: (i) TPAP, NMO, 53%; (ii) MeMgBr, 59%; (iii) LiC ≡ CTMS then MeOH, NaOH, 49%.

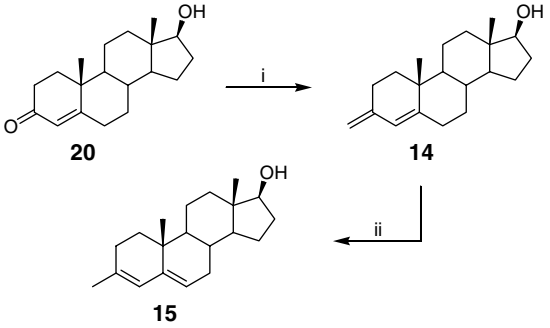
The exo-methylene analog **13** was prepared from ketone **16**[3a] by Wittig olefination[5a] (Scheme 6).

For comparison with the 10-vinyl dienes **4** and **13**, the corresponding 10-methyl analogs[5a] **15** and **14** were prepared from testosterone **20** (Scheme 7). Wittig olefination of **20** afforded the exo-methylene analog **14** which was readily isomerized to the 3,5-diene **15**.

The novel steroids were evaluated in estrogen receptor (ER-α and ER-β)[8] binding assay and in a cell-based ER-β transactivation assay[9] to measure estrogen agonism in HEK293 cells (Table 1). New compounds were also evaluated in an androgen receptor (AR)[10] ligand binding assay (Table 2). Diene **4** was comparable to the lead compound **1** as an ER-β ligand (IC_{50} = 9 nM for **4** vs 11 nM for **1**), despite the lack of a C-3 hydroxyl group, and retained excellent selectivity for ER-β over ER-α although **4** (160×) is a bit less selective than **1** (204×). The excellent binding affinity of **4** was also reflected in the ER-β transactivation assay wherein **4** had a higher EC_{50} (69 nM for **4** vs 4 nM for **1**) but comparable maximum agonism (92% of estradiol for **4** vs 90% for **1**). Interestingly, **4** was much more selective for ER-β over AR (62× for **4** vs 3× for **1**). Similar SAR was observed in the 10-methyl series (compare **12** and **15**); diene **15** is comparable to alcohol **12** as an



Scheme 6. Reagents: (i) Ph_3PCH_3Br, ^{t}BuLi, 56%.



Scheme 7. Reagents: (i) Ph_3PCH_3Br, ^{t}BuLi, 26%; (ii) HCl, EtOH, 99%.

ER ligand (both are less selective for ER-β than the corresponding 10-vinyl analogs **4** and **1**) but is more selective for ER-β over AR. Diene **4** and lead compound **1** were both considerably more active and selective than alcohols **2** and **3**. In the diene series (compounds **4**–**7**), increasing the size of the C-3 substituent is clearly detrimental to ER-β binding and selectivity; with the largest compound, the 3-phenyl analog **6**, being inactive in the ER transactivation assay. In the diene series, addition of a substituent at C-17 (compounds **8** and **9**) results in a slight decrease in ER-β binding and selectivity relative to the unsubstituted analog **4**. A similar result was observed in the 3-hydroxy series (compare compounds **10** and **11**–**1**). The internal diene **4** was clearly a better ER-β ligand than the external diene **13** and was also more selective for ER-β over AR. A similar trend was observed in the 10-methyl series (compare compounds **15** and **14**). The external dienes **13** and **14** are better AR ligands than the corresponding internal dienes **4** and **15** but are weaker ligands than the corresponding ketones **16**[3a] and testosterone. Diene **15** had the best ER-β/AR ratio of all the tested compounds due to its poor AR binding. However, **15** was not very potent in the ER transactivation assay.

In conclusion, androstene-3,5-diene **4** exhibits excellent binding affinity and selectivity for ER-β over ER-α and AR and is a potent ER-β agonist despite lacking the traditional hydroxyl substitution at C-3.

References and notes

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6. All new compounds were characterized by LC–MS and 500 or 600 MHz ^{1}H NMR. HMBC was useful in confirming the carbon skeleton of **4**. Key HMBC correlations observed for **4** were H-21 → C-2, C-3, and C-4; H-4 → C-2 and C-6; and H-6 → C-4, C-10, and C-7. In addition, NOE correlations were observed for H-4 → H-21 and H-6 → H-7 of **4**. Selected ^{1}H NMR data (600 MHz, $CDCl_3$, δ) for **4**: 5.75 (dd, $J = 17$, 10 Hz, 1H, H-19), 5.73 (br s, 1H, H-4), 5.49 (t, $J = 3$ Hz, 1H, H-6), 5.18 (dd, $J = 10$, 2 Hz, 1H, H-20), 4.84 (dd, $J = 17$, 2 Hz, 1H, H-20), 3.63 (t, $J = 9$ Hz, 1H, H-17), 1.67 (s, 3H, H-21), 0.69 (s, 3H, H-18). Selected ^{13}C NMR data for **4**: (125 MHz, $CDCl_3$, δ): 140.1 (C-19), 137.7 (C-5), 133.6 (C-3), 124.5 (C-4), 122.6 (C-6), 117.4 (C-20), 81.8 (C-17), 11.0 (C-18).
7. (a) Models were built using the 1.8 Å resolution crystallographic coordinates of compound **1** as cocrystallized with hER-β (Fitzgerald et al., in preparation). Energy minimization for all of the models within context of the hER-β receptor (**1** 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 force field[7b] with a distance dependent dielectric model of $2r$. Multiple binding orientations of **2** were considered, including those that had the D-ring flipped up to interact with Glu305. However, the most energetically favored binding orientation was found to be the same as estradiol (Fig. 1); (b) Halgren, T. A. *J. Comp. Chem.* **1999**, *20*, 730; (c) 1ERE = pdb code for crystal structure of estradiol in ER-α.
8. The IC_{50} values were generated in a scintillation proximity estrogen receptor ligand binding assay conducted in NEN Basic Flashplates using tritiated estradiol and full length recombinant human ER-α or ER-β proteins. Compounds were evaluated in duplicate in a single assay. This assay provides IC_{50} values that are reproducible to within a factor of 2–3.
9. This assay was run in agonist mode at Merck-CIBE in Spain using the procedure described by Barkhem, T.; Carlsson, B.; Nilsson, Y.; Enmark, E.; Gustafsson, J.; Nilsson, S. *Mol. Pharmacol.* **1998**, *54*, 105, Compounds were tested in triplicate; results were generally reproducible to within a factor of 2–3.
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