Study of 202 Natural, Synthetic, and Environmental Chemicals for Binding to the Androgen Receptor

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A number of environmental and industrial chemicals are reported to possess androgenic or antiandrogenic activities. These androgenic endocrine disrupting chemicals may disrupt the endocrine system of humans and wildlife by mimicking or antagonizing the functions of natural hormones. The present study developed a low cost recombinant androgen receptor (AR) competitive binding assay that uses no animals. We validated the assay by comparing the protocols and results from other similar assays, such as the binding assay using prostate cytosol. We tested 202 natural, synthetic, and environmental chemicals that encompass a broad range of structural classes, including steroids, diethylstilbestrol and related chemicals, antiestrogens, flutamide derivatives, bisphenol A derivatives, alkylphenols, parabens, alkyloxyphenols, phthalates, siloxanes, phytoestrogens, DDTs, PCBs, pesticides, organophosphate insecticides, and other chemicals. Some of these chemicals are environmentally persistent and/or commercially important, but their AR binding affinities have not been previously reported. To the best of our knowledge, these results represent the largest and most diverse data set publicly available for chemical binding to the AR. Through a careful structure−activity relationship (SAR) examination of the data set in conjunction with knowledge of the recently reported ligand−AR crystal structures, we are able to define the general structural requirements for chemical binding to AR. Hydrophobic interactions are important for AR binding. The interaction between ligand and AR at the 3- and 17-positions of testosterone and R1881 found in other chemical classes are discussed in depth. The SAR studies of ligand binding characteristics for AR are compared to our previously reported results for estrogen receptor binding.

Introduction

Androgens, such as testosterone (T) and dihydrotestosterone (DHT), play a crucial role at several stages of male development and in the maintenance of the male phenotype. They control specific responses in the reproductive tract, muscle, liver, skin, nervous system, and immune system. The functions of T include muscle mass increase, penis, scrotum, and vocal cord enlargement, support of spermatogenesis, and male sex drive and performance. DHT promotes the appearance of facial and body hair, acne, scalp hair recession, and prostate enlargement (*1*). Diminished or excessive production of androgens is a major problem related to prostate cancer, spinal bulbar muscular atrophy, and male pattern baldness.

The androgen receptor (AR) is a member of the nuclear receptor superfamily, a class of receptors that function through ligand-dependent transcription of specific genes (*2*). Receptor binding of androgens is the primary and critical intracellular step for androgen-dependent gene expression in vitro and in vivo. Estrogenic effects are likewise mediated through the estrogen receptor (ER). For estrogens, ER binding affinity correlates strongly with results from assays measuring estrogenic responses through downstream events, such as a yeast-based reporter gene assay and MCF-7 cell proliferation assay (*3*). These and other findings demonstrate that ligand binding is the major determinant for receptor-mediated effects for the nuclear receptor superfamily (*4*).

A number of environmental and industrial chemicals are reported to possess androgenic or antiandrogenic activities. These include the organophosphate insecticide fenitrothion (*5*, *6*), the phenylurea herbicide linuron (*7*), chemicals in pulp mill effluent (*8*), the industrial chemical phthalates (*9*), and some of the long-lived environmental contaminants, such as PCBs (*10*), DDTs (*11*, *12*), and cyclic hydrocarbons (*13*). These have been designated as endocrine disrupting chemicals (EDCs) because they disrupt the endocrine systems of humans and wildlife by mimicking or antagonizing the functions of natural hormones (*14*−*16*). Most androgenic chemicals activate AR-mediated transcription in mammalian cells through receptor-mediated mechanisms. Only a few chemicals, such as tributyltin and triphenyltin, may target a novel site other than the ligand binding site of AR (*17*). Thus, the AR competitive binding assay could be used to identify many potential EDCs through binding to AR.

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In principle, chemicals sharing essential structural characteristics may have similar biological actions. Structurally diverse androgens possess a certain degree of structural commonalities essential to exhibit AR binding. Efforts have been initiated to evaluate the chemical structural resemblance to T and DHT or the strong synthetic antiandrogens (e.g., flutamide, anandron, and casodex) for a number of chemical classes (*1*). However, structure−activity relationships (SAR) of androgens have not been as thoroughly evaluated as have estrogens. Particularly, few examinations of the structural commonalties between different chemical classes of xenoandrogens have been reported. This appears mainly due to the fact that there is no large and reliable data set obtained with a well-designed and controlled assay covering a broad range of chemical classes for evaluating the chemical structural requirements for AR binding.

In this paper, we report results from an AR binding assay using a recombinant rat AR. AR binding affinities for 202 natural, synthetic, and environmental chemicals from a broad range of structural classes are presented. To the best of our knowledge, this is the largest and most diverse data set that has been reported for chemical binding to the AR. This AR data set (termed NCTR [National Center for Toxicological Research] AR data set hereafter to distinguish it from other reported AR data sets in the literature) covers most known androgenic chemical classes as well as some classes not previously known to include AR ligands. A careful SAR examination of this data set in conjunction with knowledge of the recently reported ligand−AR crystal structures has increased our understanding of the general structural requirements for a chemical's binding to AR. In turn, this knowledge can be used to develop in silico predictive toxicology models to rapidly identify potential androgenic EDCs.

Materials and Methods

Chemicals. The radiolabeled ligand, 17α-methyl-^{3}H]methyltrienolone (R1881; 85 Ci/mmol) was purchased from New England Nuclear (Boston, MA). The sources and purity (when available) for the 202 test chemicals are found in Table 1 along with the results from the assay.

Trizma base, Trizma hydrochloride, glycerol, EDTA, dithiothreitol, and bovine serum albumin were obtained from Sigma (St. Louis, MO). Hydroxylapatite (HAP) was obtained from Bio-Rad Laboratories (Hercules, CA). UltimaGold scintillation cocktail was obtained from the Packard Instrument Company (Meriden, CT).

AR Protein. The AR protein used in the assay was purchased from PanVera LLC (Madison, WI). The PanVera AR is a recombinant rat protein expressed in *Eshcerichia coli*. The amino acid sequence of the PanVera AR ligand binding domain (LBD) is identical to that of the human AR LBD. It has a molecular mass of 48.4 kDa and contains both the hinge region and the LBD, which is fused to thioredoxin.

The AR protein (e.g., 750 pmol in a volume of 286 μL) was aliquoted to 21 test tubes stored at −78 °C; each tube contained 13.32 μL of protein with 34.93 pmol of AR. Cold buffer (19 mL at 4 °C) was added to one of the 21 tubes immediately before use, which provided 1.84 nM AR (e.g., 34.93 pmol/19 mL) in the assay. Scatchard analysis of binding data provided a K_d = 2.03 ± 0.15 nM (SD; n = 4) for R1881, the standard AR ligand in the assay.

AR Competitive Binding Assay. The competitive receptor binding assay protocol in this study is similar to that for the ER binding assay used in our lab (*18*). Briefly, radiolabeled [^{3}H]-R1881, a competitor (test chemical), and AR protein were combined in a 12 mm × 75 mm test tube on ice. The [^{3}H]R1881 concentration was at 1 nM (5 μL of 7×10^{-8} M), the competitor concentration ranged from 4.28×10^{-9} to 4.28×10^{-4} M (5 μL of 3×10^{-7} to 3×10^{-2} M) by one log unit concentration intervals, and the AR concentration was 1.84 nM (340 μL). A total of 350 μL of reaction mixture was incubated at 4 °C for 18−20 h in duplicate tubes. In addition to the radioinert competitor, each assay included a zero tube (no competitor added; represents total binding of [^{3}H]R1881; average approximately 18 000 dpm/350 μL) and a R1881 standard curve (1×10^{-7}, 1×10^{-8}, 1×10^{-9}, 1×10^{-10}, and 1×10^{-11} M concentrations) for quality control purposes. The 1×10^{-6} M R1881 tube contained radioinert R1881 that was at a 1000-fold molar excess over the [^{3}H]R1881 (1 nM); thus, it represented nonspecific binding (NSB; average approximately 2000 dpm).

Following the 18−20 h of incubation at 4 °C, 0.75 mL of a HAP slurry (60% HAP in 50 mM TRIS) was added to each assay tube. The tubes were vortexed on ice at 5 min intervals for 20 min and centrifuged at 600*g* for 4 min, and the supernatant was discarded. After addition of 2 mL of 50 mM TRIS buffer to each tube, the HAP pellet was resuspended by vortexing and then centrifuged as above; this wash was repeated two times. After the removal of the final TRIS supernatant, 2 mL of 100% cold ethanol was added and the HAP pellet was again resuspended. The HAP was vortexed three times at 5 min intervals and centrifuged at 600*g* for 4 min to release the bound AR. The ethanolic supernatant containing the AR plus bound radioactivity was decanted to a liquid scintillation vial, and 10 mL of scintillation cocktail was added. Radioactivity counts (dpm) of the NSB tubes were subtracted from all tubes prior to calculation of percent [^{3}H]R1881 bound. Data for each competitor and the R1881 standard curve were plotted as percent [^{3}H]R1881 bound (relative to the standard) vs molar concentration, and the IC_{50} (50% inhibition of [^{3}H]R1881 binding) for each competitor was determined.

AR Binding Affinity. The AR binding affinity was expressed as either relative binding affinity (RBA) or log unit of RBA (logRBA) in this study. The RBA for each competitor was calculated by dividing the IC_{50} of R1881 by the IC_{50} of the competitor and was expressed as a percent. The mean IC_{50} was $3.07 \times 10^{-9} \pm 9.12 \times 10^{-10}$ M (N = 82) for R1881; its RBA was set to 100 or its logRBA = 2. All assays were run in duplicate tubes with at least two replications. The RBAs of active chemicals are the means of the replicate values. Chemicals that failed to compete [^{3}H]R1881 in binding were designated as "nonbinders" (NBs). Chemicals that showed binding but did not reach 50% inhibition at maximum concentration were designated as "slight binders" (SBs). A less than IC_{50} value was assigned for both NB and SB to indicate the maximum concentration used.

For the sake of discussion, the NCTR AR data set that contained 202 chemicals with the assay data was classified into four activity groups with respect to their RBA ranges, designated strong (RBA > 1), moderate (1 > RBA > 0.01), or weak binders (0.01 > RBA > 0.0001), and inactive chemicals. The inactive group includes both NB and SB chemicals.

The binding affinities reported by Waller et al. (*19*) in an AR binding assay using a ventral prostate cytosol were compared with our assay results for the chemicals common to both assays. The inhibition constant (pK_i) reported by Waller et al. (*19*) was converted to RBAs using the Cheng−Prusoff equation (*20*).

Molecular Modeling. The crystal structures of R1881 and DHT bound to AR were obtained from the Protein Data Bank (PDB) as entries 1E3G and 1I37. The atom−atom distance was measured using Sybyl 6.7 (Tripos, St. Louis, MO). All molecules were energy-minimized using the standard Tripos force field and parameter setting. The logP value (measuring hydrophobicity) for each chemical was calculated using the atom/fragment contribution method (*21*).

Table 1. NCTR AR Data Set

name	CAS	LogP	IC_{50}	RBA[a]	logRBA	source	purity (%)
steroids: steroidal androgens							
5α-androstan	438-22-2	6.65	6.35E-04	0.0005	−3.32	Sigma	
androsterone	53-41-8	3.07	4.05E-05	0.0076	−2.12	Sigma	99
5,6-didehydroisoandrosterone	53-43-0	2.98	2.95E-05	0.0104	−1.98	Sigma	
5α-androstane-3,11,17-trione	1482-70-8	1.21	1.35E-05	0.0227	−1.64	Sigma	
epitestosterone	481-30-1	3.27	3.10E-06	0.0990	−1.00	Sigma	
3α-androstanediol	1852-53-5	3.98	1.98E-06	0.1554	−0.81	Sigma	
T propionate	57-85-2	4.77	1.90E-06	0.1615	−0.79	Sigma	
5α-androstan-3*β*-ol	1224-92-6	5.12	1.70E-06	0.1805	−0.74	Sigma	
androstenediol	521-17-5	3.90	1.40E-06	0.2192	−0.66	Steraloids	
4-androstenedione	63-05-8	2.76	1.28E-06	0.2407	−0.62	Sigma	98
4-androstenediol	1156-92-9	3.90	6.20E-07	0.4949	−0.31	Steraloids	
etiocholan-17*β*-ol-3-one	571-22-2	3.07	3.85E-07	0.7970	−0.10	Sigma	
DHT benzoate	1057-07-4	5.53	2.60E-07	1.1801	0.07	Sigma	
3*β*-androstanediol	571-20-0	3.98	1.35E-07	2.2728	0.36	Sigma	
11-keto-testosterone	564-35-2	1.92	8.90E-08	3.4475	0.54	Steraloids	
methyltestosterone	58-18-4	3.72	1.60E-08	19.1768	1.28	Steraloids	
T	58-22-0	3.27	1.59E-08	19.2571	1.28	Sigma	
5α-androstan-17*β*-ol	1225-43-0	5.12	1.10E-08	27.8936	1.45	Sigma	
methyltrienolone (R 1881)	965-93-5	3.10	3.07E-09	100.0000	2.00	NEN[b]	
trenbolone	10161-33-8	2.65	2.75E-09	111.5743	2.05	Sigma	98
DHT	521-18-6	3.07	2.23E-09	137.9008	2.14	Sigma	
mibolerone	3704-09-4	3.69	1.65E-09	185.9571	2.27	Steraloids	
steroids: steroidal estrogens							
estriol (E_3)	50-27-1	2.81	4.30E-04	0.0007	−3.15	Sigma	98
17α-estradiol	57-91-0	3.94	7.70E-05	0.0040	−2.40	Sigma	
3-methylestriol	3434-79-5	3.37	5.43E-05	0.0057	−2.25	NCI[c]	
17-deoxyestradiol	53-63-4	5.48	4.15E-05	0.0074	−2.13	Steraloids	
16*β*-OH-16α-Me-3-Me-estradiol	5108-94-1	3.83	3.70E-05	0.0083	−2.08	NCI	
2-OH-estradiol	362-05-0	3.46	8.50E-06	0.0361	−1.44	Steraloids	
ethynylestradiol (EE)	57-63-6	4.12	8.00E-06	0.0384	−1.42	Sigma	98
4-OH-estradiol	5976-61-4	3.46	2.50E-06	0.1227	−0.91	NCI	
estradiol (E_2)	50-28-2	3.94	4.05E-07	0.7576	−0.12	U.S. Biochemical Corp.	
3-deoxyestradiol	2529-64-8	4.42	8.90E-08	3.4475	0.54	NCI	
ICI 182,780	129453-61-8	9.09	>4.28E-05	NB	NB	Zeneca	
moxestrol	34816-55-2	3.28	>4.28E-05	SB	SB	R.H. Purdy	
estrone (E_1)	53-16-7	3.43	>8.30E-05	SB	SB	Aldrich	99
ICI 164,384		8.94	>4.28E-05	SB	SB	Zeneca	
steroids: others							
cortisol	50-23-7	1.62	1.80E-04	0.0017	−2.77	Sigma	
dexamethasone	50-02-2	1.72	8.00E-05	0.0038	−2.42	Sigma	
corticosterone	50-22-6	1.99	2.30E-05	0.0133	−1.87	Sigma	95
norethynodrel	68-23-5	3.51	1.55E-06	0.1980	−0.70	Sigma	
progesterone	57-83-0	3.67	1.55E-06	0.1980	−0.70	Sigma	99
promegestone	34184-77-5	4.20	1.35E-06	0.2273	−0.64	NEN	
6α-Me-17α-OH-progesterone	520-85-4	3.50	7.95E-07	0.3859	−0.41	Sigma	
spironolactone	52-01-7	3.56	6.85E-07	0.4479	−0.35	Aldrich	99
cyproterone acetate	427-51-0	4.18	6.40E-07	0.4794	−0.32	Sigma	98
norethindrone	68-22-4	2.99	1.20E-07	2.5569	0.41	Sigma	
6α-Me-17α-OH-progesterone acetate	71-58-9	4.01	3.55E-08	8.6431	0.94	Sigma	95
norgestrel	797-63-7	3.48	1.85E-08	16.5854	1.22	Sigma	
aldosterone	52-39-1	0.50	>4.28E-05	NB	NB	Sigma	
prednisolone	50-24-8	1.40	>4.28E-05	NB	NB	Sigma	
pregnenolone	145-13-1	3.89	>4.28E-05	NB	NB	Sigma	98
cholesterol	57-88-5	8.74	>4.28E-04	NB	NB	Sigma	95
sitosterol	83-46-5	9.65	>4.28E-05	NB	NB	Indofine	80
triamcinolone acetonide	76-25-5	2.69	>4.28E-05	SB	SB	Sigma	
DESs: agonist							
4,4′-dihydroxystilbene	659-22-3	3.56	8.40E-05	0.0037	−2.44	NCI	
trans-4-hydroxystilbene	3839-46-1	4.04	4.15E-05	0.0074	−2.13	NCI	
3,3′-dihydroxyhexestrol	79199-51-2	4.64	3.65E-05	0.0084	−2.08	NCI	
3,4-diphenyltetrahydrofuran		4.21	2.90E-05	0.0106	−1.98	Sigma	
dimethylstilbestrol	552-80-7	4.66	1.40E-05	0.0219	−1.66	NCI	
DES	56-53-1	5.64	1.40E-05	0.0219	−1.66	Research Plus	
hexestrol monomethyl ether	13026-26-1	6.16	1.30E-05	0.0236	−1.63	NCI	

Table 1 (Continued)

name	CAS	LogP	IC_{50}	RBAa	logRBA	source	purity (%)
			DESs: synthetic antiestrogens				
clomiphene	911-45-5	6.74	1.35E-05	0.0227	−1.64	Sigma	
nafoxidine	1845-11-0	7.20	1.30E-05	0.0236	−1.63	Sigma	
tamoxifen	10540-29-1	6.30	1.20E-05	0.0256	−1.59	Zeneca	
4-hydroxy-tamoxifen	68047-06-3	5.82	9.45E-06	0.0325	−1.49	Zeneca	
			phytoestrogens				
6-hydroxyflavone	6665-83-4	3.03	1.80E-04	0.0017	−2.77	Indofine	
4′-hydroxyflavanone	6515-37-3	2.80	9.25E-05	0.0033	−2.48	Indofine	
genistein	446-72-0	2.84	8.45E-05	0.0036	−2.44	NCI	
flavone	525-82-6	3.51	7.65E-05	0.0040	−2.40	Sigma	
equol	531-95-3	3.67	7.50E-05	0.0041	−2.39	Spectrum	
chalcone	94-41-7	3.66	6.35E-05	0.0048	−2.32	Indofine	
4′-hydroxychalcone	2657-25-2	3.18	5.75E-05	0.0053	−2.27	Indofine	
flavanone	487-26-3	3.28	5.45E-05	0.0056	−2.25	Indofine	
4-hydroxychalcone	20426-12-4	3.18	4.80E-05	0.0064	−2.19	Indofine	
zearalanone	5975-78-0	4.86	4.22E-05	0.0073	−2.14	Sigma	
β-zearalenol		4.09	3.80E-05	0.0081	−2.09	Sigma	
6-hydroxyflavanone	4250-77-5	2.80	1.85E-05	0.0166	−1.78	Indofine	
β-zearalanol	42422-68-4	5.37	1.60E-05	0.0192	−1.72	Sigma	98
zearalenol	71030-11-0	4.09	1.35E-05	0.0227	−1.64	Sigma	
coumestrol	479-13-0	1.57	>4.28E-05	NB	NB	Spectrum	
7-hydroxyflavone	6665-86-7	3.03	>4.28E-05	NB	NB	Indofine	
naringin	10236-47-2	−0.52	>4.28E-05	SB	SB	Indofine	
			phenols				
3-chlorophenol	108-43-0	2.16	4.55E-04	0.0007	−3.17	Aldrich	98
propyl parabene	94-13-3	2.98	3.10E-04	0.0010	−3.00	Aldrich	99
4-benzyloxylphenol	103-16-2	3.30	2.40E-04	0.0013	−2.89	Aldrich	99
isoeugenol	97-54-1	2.65	2.00E-04	0.0015	−2.81	Aldrich	98
4-*tert*-butylphenol	98-54-4	3.42	1.45E-04	0.0021	−2.67	Aldrich	99
4-chloro-2-methyl phenol	1570-64-5	2.70	1.20E-04	0.0026	−2.59	Aldrich	97
2-*sec*-butylphenol	89-72-5	3.46	1.02E-04	0.0030	−2.52	Aldrich	98
4-*sec*-butylphenol	99-71-8	3.46	8.50E-05	0.0036	−2.44	Aldrich	93
4-*tert*-amylphenol	80-46-6	3.91	7.60E-05	0.0040	−2.39	Aldrich	99
4-dodecylphenol	104-43-8	7.46	2.00E-05	0.0153	−1.81	Aldrich	
4-*n*-octylphenol	1806-26-4	5.50	1.95E-05	0.0157	−1.80	Aldrich	99
igepal CO-210		5.30	1.85E-05	0.0166	−1.78	Aldrich	
4-heptyloxyphenol	13037-86-0	4.54	1.50E-05	0.0205	−1.69	Aldrich	97
nonylphenol	25154-52-3	5.99	1.15E-05	0.0267	−1.57	Aldrich	
vanillin	121-33-5	1.05	>4.28E-04	NB	NB	Aldrich	99
phenol	108-95-2	1.51	>4.28E-04	NB	NB	Aldrich	99
methyl paraben	99-76-3	2.00	>4.28E-04	NB	NB	Aldrich	99
2-chlorophenol	95-57-8	2.16	>4.28E-04	NB	NB	Aldrich	99
4-ethylphenol	123-07-9	2.55	>4.28E-04	SB	SB	NCI	
			flutamides				
4′-chloroacetoacetanilide	101-92-8	1.65	8.95E-04	0.0003	−3.46	Aldrich	98
procymidone	32809-16-8	2.59	1.25E-04	0.0025	−2.61	Riedel-DeHaen	
metolachlor	51218-45-2	3.24	1.25E-04	0.0025	−2.61	Supelco	
vinclozolin	50471-44-8	3.03	9.65E-05	0.0032	−2.50	Supelco	
flutamide	13311-84-7	3.51	8.00E-05	0.0038	−2.42	Sigma	
linuron	330-55-2	2.91	5.50E-05	0.0056	−2.25	Supelco	
propanil (DCPA)	709-98-8	2.88	5.10E-05	0.0060	−2.22	Sigma	
fenpicionil	74738-17-3	3.48	1.25E-05	0.0246	−1.61	Riedel-DeHaen	
p-lactophenetide	539-08-2	0.62	>4.28E-05	NB	NB	Sigma	
			diphenylmethanes: benzophenones				
4-hydroxybenzophenone	1137-42-4	2.67	1.85E-04	0.0017	−2.78	Aldrich	98
4,4′-dihydoxybenzophenone	611-99-4	2.19	1.45E-04	0.0021	−2.67	Aldrich	99
benzophenone	119-61-9	3.15	1.30E-04	0.0024	−2.63	Aldrich	99
2,4-dihydroxybenzophenone	131-56-6	2.96	1.05E-04	0.0029	−2.53	Aldrich	99
			diphenylmethanes: bisphenol A derivatives				
bisphenol A	80-05-7	3.64	7.50E-05	0.0041	−2.39	Aldrich	99
p-cumyl phenol	599-64-4	4.12	3.95E-05	0.0078	−2.11	Aldrich	99
bisphenol B	77-40-7	4.13	3.75E-05	0.0082	−2.09	Aldrich	
			diphenylmethanes: DDTs				
o,p′-DDE	3424-82-6	6.00	2.00E-05	0.0153	−1.81	Supelco	
p,p′-DDT	50-29-3	6.79	1.78E-05	0.0173	−1.76	Supelco	
p,p′-DDD	72-54-8	5.87	1.55E-05	0.0198	−1.70	Supelco	
p,p′-DDE	72-55-9	6.00	1.53E-05	0.0201	−1.70	Supelco	

Table 1 (Continued)

name	CAS	LogP	IC_{50}	RBAa	logRBA	source	purity (%)
			diphenylmethanes: DDTs				
o,p′-DDT	789-02-6	6.79	1.50E-05	0.0205	−1.69	Supelco	
o,p′-DDD	53-19-0	5.87	1.03E-05	0.0299	−1.52	Supelco	
			diphenylmethanes: methoxychlors				
p,p′-methoxychlor olefin	2132-70-9	4.87	4.90E-05	0.0063	−2.20	Supelco	
p,p′-methoxychlor	72-43-5	5.67	2.70E-05	0.0114	−1.94	Sigma	95
monohydroxymethoxychlor olefin	75938-34-0	4.31	2.10E-05	0.0146	−1.84	Tom Burka (NIEHS)	
HPTE	2971-36-0	4.55	9.10E-06	0.0337	−1.47	Tom Burka (NIEHS)	
dihydroxymethoxychlor olefin	14868-03-2	3.75	6.30E-06	0.0487	−1.31	Tom Burka (NIEHS)	
			PCBs				
3,3′,5,5′-tetrachloro-4,4′-biphenyldiol	13049-13-3	5.37	3.85E-05	0.0080	−2.10	Ultra Scientific	
2,2′,4,4′-tetrachlorobiphenyl	2437-79-8	6.34	1.70E-05	0.0180	−1.74	Ultra Scientific	
2,3,4,5-tetrachloro-4′-biphenylol		5.85	1.65E-05	0.0186	−1.73	Ultra Scientific	
2,4′-dichlorobiphenyl	34883-43-7	5.05	1.60E-05	0.0192	−1.72	Ultra Scientific	
4-hydroxybiphenyl	92-69-3	3.28	8.30E-06	0.0370	−1.43	Ultra Scientific	
4,4′-dichlorobiphenyl	2050-68-2	5.05	>4.28E-05	NB	NB	Ultra Scientific	
			organochlorines				
2,4,5-T	93-76-5	3.26	4.65E-04	0.0007	−3.18	Supelco	
lindane (γ-HCH)	58-89-9	4.26	4.05E-05	0.0076	−2.12	Supelco	
aldrin	309-00-2	6.75	3.20E-05	0.0096	−2.02	Supelco	
endosulfan (technical grade)	115-29-7	3.50	2.30E-05	0.0133	−1.87	Supelco	
heptachlor	76-44-8	5.86	1.35E-05	0.0227	−1.64	Supelco	
kepone	143-50-0	4.91	1.18E-05	0.0260	−1.58	Supelco	
chlordane	57-74-9	6.26	1.00E-05	0.0307	−1.51	Supelco	
2,4-D (2,4-dichlorophenoxyacetic acid)	94-75-7	2.62	>4.28E-05	NB	NB	Supelco	
hexachlorobenzene	118-74-1	5.86	>4.28E-05	NB	NB	Supelco	
mirex	2385-85-5	7.01	>4.28E-05	NB	NB	Supelco	
			phthalates				
diisononylphthalate	28553-12-0	9.37	1.12E-03	0.0003	−3.56	Fluka	
diethylphthalate	84-66-2	2.65	8.40E-04	0.0004	−3.44	Aldrich	99
bis(*n*-octyl)phthalate	117-84-0	8.54	5.80E-04	0.0005	−3.28	Fluka	98
di-*i*-butyl phthalate (DIBP)	84-69-5	4.46	5.15E-05	0.0060	−2.22	Fluka	98
butylbenzylphthalate	85-68-7	4.84	3.60E-05	0.0085	−2.07	Aldrich	98
di-*n*-butyl phthalate (DBuP)	84-74-2	4.61	2.75E-05	0.0112	−1.95	Aldrich	99
bis(2-ethylhexyl)phthalate	117-81-7	8.39	>4.28E-04	SB	SB	Aldrich	99
			aromatic hydrocarbons				
triphenylethylene	58-72-0	5.49	2.90E-05	0.0106	−1.98	Aldrich	99
sec-butylbenzene	135-98-8	3.94	>4.28E-04	NB	NB	Aldrich	99
n-butylbenzene	104-51-8	4.01	>4.28E-04	NB	NB	Aldrich	99
1,6-dimethylnaphthalene	575-43-9	4.26	>4.28E-05	NB	NB	Aldrich	99
1,3-butadiene, *trans*,*trans*-1,4-diphenyl	886-65-7	5.29	>4.28E-05	NB	NB	Aldrich	98
chrysene	218-01-9	5.52	>4.28E-05	NB	NB	Aldrich	98
1,1,2-triphenylpropane	94871-36-0	6.28	>4.28E-05	NB	NB	Sigma	
			noncyclic compounds				
diisobutyl adipate	141-04-8	4.19	2.10E-04	0.0015	−2.84	Aldrich	99
dibutyl adipate	105-99-7	4.33	1.65E-04	0.0019	−2.73	Aldrich	96
spermidine	124-20-9	−0.66	>4.28E-04	NB	NB	Sigma	
suberic acid	505-48-6	1.21	>4.28E-05	NB	NB	Sigma	
2-ethyl-1,3-hexanediol	94-96-2	1.60	>4.28E-04	NB	NB	Aldrich	97
1,2-octanediol	1117-86-8	1.67	>4.28E-04	NB	NB	Aldrich	98
1,8-octanediol	629-41-4	1.75	>4.28E-04	NB	NB	Aldrich	98
1-octen-3-ol	3391-86-4	2.60	>4.28E-04	NB	NB	Aldrich	98
palmitic acid	57-10-3	6.96	>4.28E-04	NB	NB	Aldrich	99
di-2-ethylhexyl adipate	103-23-1	8.12	>4.28E-04	NB	NB	Fluka	99
			aromatic acids				
4-amino butylbenzoate	94-25-7	2.78	2.15E-04	0.0014	−2.85	Aldrich	99
4-heptyloxybenzoic acid	15872-42-1	4.90	1.70E-04	0.0018	−2.74	Aldrich	98
4-aminosalicylic acid, sodium salt	6018-19-5	0.98	>4.28E-04	NB	NB	Sigma	
salicylamide	65-45-2	1.03	>4.28E-04	NB	NB	Sigma	99
cinnamic acid	140-10-3	2.07	>4.28E-04	NB	NB	Aldrich	99

Table 1 (Continued)

name	CAS	LogP	IC_{50}	RBAa	logRBA	source	purity (%)
			aromatic acids				
methyl salicylate	119-36-8	2.60	>4.28E-05	NB	NB	Sigma	
			phenollike chemicals				
1-methoxy-4-[1-propenyl]benzene	4180-23-8	3.39	4.75E-04	0.0006	−3.19	Sigma	
carbaryl	63-25-2	2.35	4.00E-04	0.0008	−3.12	Supelco	
nordihydroguaiaretic acid	500-38-9	4.64	5.90E-05	0.0052	−2.28	Aldrich	97
4-(3,5-diphenylcyclohexyl)phenol	33330-65-3	7.60	5.77E-05	0.0053	−2.27	Sigma	
2,6-dihdroxyanthraquinone	84-60-6	2.38	>4.28E-05	NB	NB	Sigma	
2-naphthol	135-19-3	2.69	>4.28E-05	NB	NB	Aldrich	99
			others: isoindole				
2-benzyl-isoindole-1,3-dione	2142-01-0	3.22	4.05E-04	0.0008	−3.12	Aldrich	99
2-(4-OH-benzyl)isoindole-1,3-dione	24124-24-1	2.74	1.75E-04	0.0018	−2.76	Sigma	
2-(4-nitro-benzyl)isoindole-1,3-dione	62133-07-7	3.03	8.95E-05	0.0034	−2.46	Sigma	
			others: organophosphate				
methylparathion	298-00-0	2.75	5.55E-05	0.0055	−2.26	Supelco	
ethylparathion	56-38-2	3.73	3.45E-05	0.0089	−2.05	Supelco	
triphenyl phosphate	115-86-6	4.70	1.50E-05	0.0205	−1.69	Aldrich	98
			others: siloxane				
1,3-diphenyltetramethyldisiloxane	56-33-7	7.20	4.10E-04	0.0007	−3.13	United Chem. Tech.	
triphenylsilanol	791-31-1	4.79	3.45E-05	0.0089	−2.05	U.S. Biochemical Corp.	
1,3-dibenzyltetramethyldisiloxane	1833-27-8	8.18	>4.28E-04	NB	NB	United Chem. Tech.	
			others: triazine				
simazine	122-34-9	2.40	>1.40E-05	NB	NB	Supelco	
atrazine	1912-24-9	2.82	>4.28E-05	NB	NB	Supelco	
prometon	1610-18-0	3.57	>4.28E-04	SB	SB	Supelco	
			others: triphenylmethane				
aurin	603-45-2	3.03	1.55E-05	0.0198	−1.70	Sigma	
phenol red	143-74-8	3.21	>4.28E-05	NB	NB	Aldrich	
phenolphthalin	81-90-3	3.95	>4.28E-05	NB	NB	Sigma	95
			others				
folic acid	59-30-3	−2.00	>4.28E-05	NB	NB	Sigma	98
caffeine	58-08-2	0.16	>4.28E-05	NB	NB	Sigma	
amaranth	915-67-3	1.63	>4.28E-05	NB	NB	Aldrich	
melatonin	73-31-4	1.65	>4.28E-05	NB	NB	Aldrich	99
4,4′-methylenebis(*N*,*N*-dimethylaniline)	101-61-1	4.37	>4.28E-05	NB	NB	Aldrich	98
doisynoestrol	15372-34-6	5.76	>4.28E-05	NB	NB	NCI	
4,4′-sulfonyldiphenol	80-09-1	1.65	3.75E-04	0.0008	−3.09	Sigma	

[a] $IC_{50} = 3.07 \times 10^{-9}$ M for R1881. [b] NEN, New England Nuclear. [c] NCI, National Cancer Institute.

Results

A number of AR competitive binding assay protocols have been reported in the literature. They vary in AR source, protein concentration, radiolabeled standard androgen ligand, incubation time, and temperature. These assays can be divided into two categories with respect to the source of AR protein: the prostate cytosol AR (*13*, *19*, *22*−*25*) or a recombinant AR protein (*26*−*28*). Table 2 summarizes these assay protocols along with K_d values. In general, our assay based on a rat recombinant AR protein is comparable with others with respect to the K_d value. The differences in amino acid sequence between the PanVera's rat recombinant AR and human AR are found in the hinge region (*29*). Because the AR in the prostate undergoes proteolysis near the hinge region (*27*), this assay should be similarly effective for measuring AR binding affinity as the ones that use the cytosol.

One step for validating an assay is to compare the data with literature values for similar assay protocols based on common assayed chemicals. This was found to be effective for comparisons across assay types or across laboratories (*3*). In this study, the NCTR AR data set was compared with the data set reported by Waller et al. (*19*) that used the rat prostate cytosol AR in a competitive receptor binding assay. A total of 20 chemicals are shared between the two data sets (Figure 1). Fifteen chemicals are active in both assays; the regression equation was logRBA (NCTR) = 0.92 logRBA(Waller) + 0.21 with R^2 = 0.92, when excluding progesterone that is an outlier. Pregnenolone is inactive in both assays. Four chemicals (*p*,*p*′-methoxychlor, vinclozolin, corticosterone, and procymidone) were active in our assay but inactive in Waller's assay. The results indicate that both assays were generally comparable for, specifically, chemicals with RBA > 0.001. For the chemicals with lower RBAs, our assay is more sensitive.

There is a large RBA discrepancy between our assay and Waller's report for progesterone, which might be due to an artifact in the prostate assay. More than a 100-fold difference in RBA for progesterone was also observed in the early reports (*19*, *25*). The low AR activity of

Table 2. Parameters of AR Competitive Binding Assay Protocols

AR sources	rat prostate cytosol				recombinant human expression COS cell or *E. coli*			[c]recombinant rat expression *E. coli*
parameters	Dalton (*22*) and Kirkovsky (*23*)	Kelce (*24*) and Waller (*19*)	Chang (*13*)	Wilson (*25*)	Wong (*26*)	Roehrborn (*27*)	Young (*28*)	NCTR
labeled ligand (nM)	mibolerone 1 nM	R1881 0.5−3 nM	R1881 10 nM	DHT 15−20 nM	R1881 5 nM	mibolerone 1 nM	mibolerone 4 nM	R1881 1 nM
AR concn	500 mg/mL	10−20 mg/mL (∼0.2 nM)	1:7.5 vol tissue:buffer	5−12 mg/mL (∼0.5 nM)		2 mg/mL	0.2 nM	1.2−1.8 nM
incubation	18 h, 4 °C	20 h, 4 °C	1 h, 37 °C	18−24 h, 0 °C	2 h, 37 °C	12−18 h, 4 °C	18 h, 4 °C	18 h, 4 °C
triamcinolone acetonide[a]	1000-fold	500-fold	no	no	no	no	no	no
K_d (nM)	0.19	0.53		0.2−0.5		0.89 or 3.42[b]	1.2	2.0
no. of compds	7 (*22*) and 20 (*23*)	28	80	8	7	1	7	202

[a] Triamcinolone acetonide is used to prevent interaction of radioactive R1881 with PR and glucocorticoid. [b] $K_d = 0.89$ nM for the truncated [GSThAR(472−917)] protein while $K_d = 3.43$ nM for the complete [SThAR(1−917)] fusion proteins. [c] Recombinant rat clone is comprised of amino acids 606−902, N-linked to thioredoixin and expressed in *E. coli*.

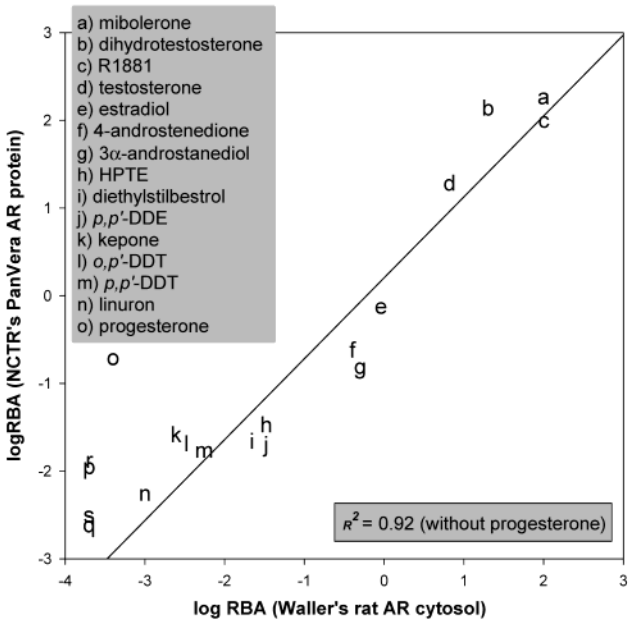


Figure 1. Correlation between the competitive receptor binding assay results using two different AR sources, the pure recombinant AR reported in the paper vs the ventral prostate cytosol reported by Waller et al. (*19*).

progesterone could be due to a 500-fold excess of triamcinolone acetonide in the prostate assay (*24*). In prostate cytosol preparations, other nuclear receptors, such as progesterone receptor (PR) and glucocorticoid receptors, are present. Furthermore, the concentration of AR relative to these other receptors is considerably lower unlike the high ER levels in the uterus. Given the fact that R1881 binds PR, a 500-fold excess of triamcinolone acetonide was used in the prostate assay to suppress PR binding of AR ligands (*24*). In contrast, the assay using the recombinant AR protein does not suffer from this possible artifact.

SAR Studies

A total of 202 chemicals were evaluated in this study. The chemical structures and RBA values of these chemicals are found in Figures 4−17. More detailed information on each chemical, i.e., the chemical name, CAS number, binding affinity (IC_{50}, RBA, and log RBA), and the chemical purity and source can be found in Table 1,

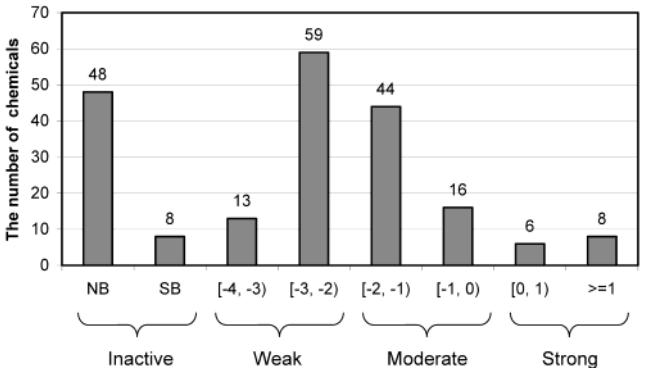


Figure 2. AR binding affinity (RBA) distribution for 202 tested chemicals. The NCTR AR data set covers over 6 orders of magnitude of RBA range, where RBA = 100 for R1881.

Table 3. Comparison of the Active/Inactive Distribution and Mean RBA Across 14 Chemical Classes in the NCTR AR Data Set

chemical classes	no. of compds	active/ inactive	mean RBA[a]
steroids	54	44/10	14.7
DESs	11	11/0	0.018
phytoestrogens	17	14/3	0.0081
phenols	19	14/5	0.0082
flutamides	9	8/1	0.0061
diphenylmethanes	18	18/0	0.015
PCBs	6	5/1	0.02
organochlorines	10	7/3	0.016
phthalates	7	6/1	0.0045
aromatic hydrocarbons	7	1/6	0.011
noncyclic chemicals	10	2/8	0.0017
aromatic acids	6	2/4	0.0016
phenollike chemicals	6	4/2	0.003
others	22	10/12	0.0071

[a] Mean RBA values were calculated for AR binders only.

where the calculated logP is also provided. The RBA distribution across all tested chemicals is shown in Figure 2. One hundred forty-six chemicals are active while 56 are inactive. Of the 146 active chemicals, 14 are strong binders, all of which are steroids. The majority of active chemicals are in the moderate (60 chemicals) and weak (72 chemicals) categories (Figure 2).

The NCTR AR data set was divided into 14 structurally distinct classes. The description of each class is presented in the figure legends. The active/inactive distribution and mean RBA value for each class are summarized in Table 3. Except for aromatic hydrocarbons, noncyclic chemicals, aromatic acids, and others, there are more AR

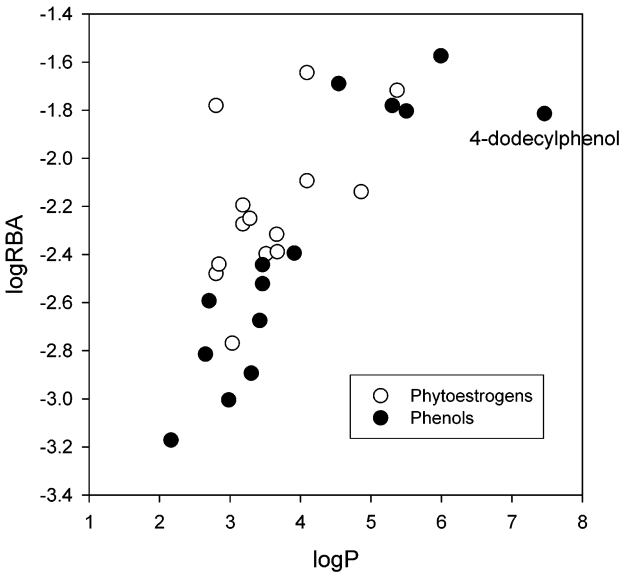


Figure 3. LogP vs logRBA for phenols and phytoestrogens. AR binding affinity increases as logP increases, but hydrophobicity that is too high might not be of further benefit for binding.

binders than NBs for the other 10 chemical classes, indicating that AR, like ER, is promiscuous. Excluding these four classes, because their mean RBA values are not representative, the relative AR binding tendency for the rest of chemical classes based on their mean RBAs follows the order of steroids > diethylstilbestrols (DES), diphenylmethanes, PCBs, organochlorines > phytoestrogens, phenols, flutamides, phthalates, and phenol-like chemicals. It is not surprising that steroids have a much higher mean RBA value as compared to all other classes. Interestingly, the large portion of tested environmental and industrial chemicals, such as methoxychlors, bisphenol A derivatives, DDTs, and PCBs, also possess reasonable binding affinity for AR.

Steroids (Figure 4). All 22 steroidal androgens (Figure 4A) were active. RBA values covered a wide range of activity (>10^5-fold), from the highest affinity chemical, mibolerone (RBA = 186.00), to the lowest affinity chemical, 5α-androstane (RBA = 0.0005). Interestingly, 10 out of 14 steroidal estrogens (Figure 4B) were also active in the assay, indicating that some estrogens may regulate both estrogenic and androgenic responses. As expected, estrogens had a lower affinity for the AR as compared to androgens. The major structural difference between androgens and estrogens is in the A-ring, where a 3-keto group favors AR binding while a 3-phenol group favors ER binding. Progesterone and its derivatives (Figure 4C), such as promegestone, 6α-Me-17α-OH-progesterone, and progesterone mimics (i.e., norethynodrel, norethindrone, and norgestrel) were moderate binders to AR. We earlier demonstrated that progesterone derivatives do not compete with 17β-estradiol (E_2) for ER binding (*18*, *30*). It appears that progesterone derivatives could alter both AR- and PR- but not ER-mediated tissue responses. Besides steroidal androgens and estrogens, we also found that cyproterone acetate and spironolactone (an aldosterone antagonist) were moderate binders in the assay, which is in agreement with early reports on their antiandrogenic effect (*1*, *31*, *32*). Other steroidal chemicals are in the weak or inactive categories. For example, all glucocorticoids (prednisolone, dexamethasone, triamicinolone acetonide, and cortisol) and the mineralocorticoid aldosterone (Figure 4C) were weak binders or inactive. Corticosterone binds to AR with an affinity 1000-fold below T. Cholesterol and sitosterol were inactive.

A total of six chemicals showed binding affinities higher than or close to T. All seven chemicals (including T) are steroidal androgens. Six of them have a distinct structural pattern, with a 3-keto, a 17β-OH, and a 5α-steroidal frame. There are 10 chemicals in the entire data set that have this structure, of which eight are in the strong activity group. The results indicated that a 3-keto in the A-ring and a 17β-OH at the D-ring along with a steroid hydrophobic backbone contribute significantly for steroids to bind to AR with high affinity (*1*). The SAR results of this class are summarized in Table 4; the main results follow.

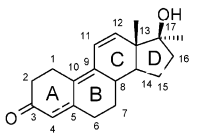
For 17β-OH, any modification or elimination of the 17β-OH reduces the AR binding activity; this appears more effective for T and E_2 derivatives than androstane derivatives. Androstane derivatives are androgenic prohormones, some of which are also found in urine as metabolites. The relatively small degree of activity loss through modifying this class of chemicals would not dramatically change the balance of the hormone level in the metabolic process for the secretion of excess hormone. A reduction in binding affinity was also observed by esterifying the 17β-OH in T with propionate acid (T propionate, RBA = 0.16) and in DHT with benzoate acid (DHT benzoate, RBA = 1.18).

For 17α-substitution, the 17α-OH does not favor AR binding. Moving the OH group from the 17β- to the 17α-position resulted in ~190-fold activity loss for both T and E_2. Blocking the H-bonding potential of 17α-OH in 6α-Me-17α-OH-progesterone through acetation and eliminating the 17α-OH of cortisol showed 22- and 7-fold activity increases for both chemicals, respectively. Small steric substitutions at the 17α-position, such as a methyl group, have no effect on activity; compare the RBAs of two pairs of chemicals, T vs methyltestosterone and trenbolone vs R1881 (Figure 4A). However, a relatively large bulky group, such as an ethynyl group, reduces activity about 20-fold for E_2 and 7-fold for T.

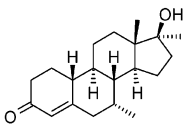
For the 3-keto group, the reduction of the 3-keto to an alcohol (either α or β isomers) is not favorable for binding. For example, both 3α-androstanediol (RBA = 0.16) and 3β-androstanediol (RBA = 2.27) are much less active than DHT. Similarly, going from T to 4-androstenediol (RBA = 0.49) reduces activity about 39-fold. However, elimination of the 3-keto of DHT causes only a 5-fold reduction of binding (5α-androstan-17β-ol, RBA = 27.89). More interestingly, elimination of the 3-OH for both α- and β-androstanediol as well as E_2 even enhanced activity. This indicates that the importance of 3-keto (or 3-OH) for AR binding is less significant than the 17β-OH in the steroids class. This is also evident from the removal of 17β-OH of 5α-androstan-17β-ol; this causes much more activity loss than the removal of 3β-OH of 5α-androstan-3β-ol, and both lead to the same backbone steroidal chemical, 5α-androstane.

For the steroidal framework, the 5α-steroidal framework favors binding, which is illustrated by the fact that DHT (5α-DHT) has about a 173-fold higher binding affinity than 5β-DHT (RBA = 0.80). Reduction of the A-ring distorts the 5α structure and thus reduces the activity. This is evident from DHT (RBA = 138.9) that has ~7-fold higher binding affinity than T (RBA = 19.26)

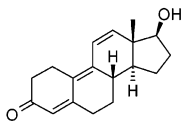
A



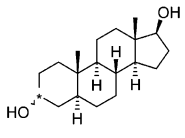
Methyltrienolone (R1881, 100)



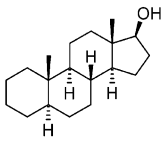
Mibolerone (185.95)



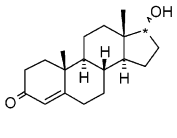
Trenbolone (111.57)



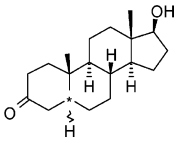
* α - 3α-Androstanediol (0.16)
β - 3β-Androstanediol (2.27)



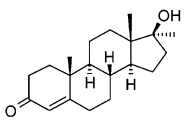
5α-Androstan-17β-ol (27.89)



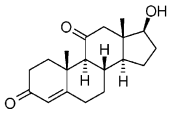
* β - Testosterone (T, 19.26)
α - Epitestosterone (0.099)



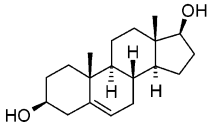
* α – 5α-Dihydrotestosterone (DHT, 138)
β–5β–Dihydrotestosteron (0.80)



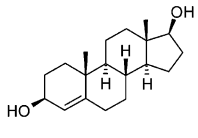
Methyltestosterone (19.18)



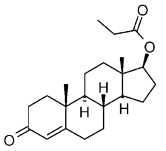
11-Keto-kestosterone (3.44)



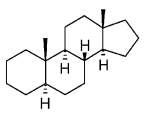
Androstenediol (0.22)



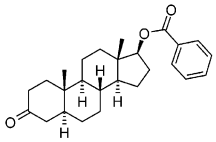
4-Androstenediol (0.49)



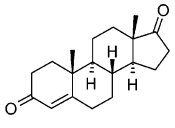
Testosterone propionate (0.16)



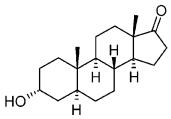
5α–Androstan (0.00048)



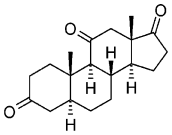
DHT benzoate (1.18)



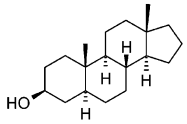
4-Androstenedione (0.24)



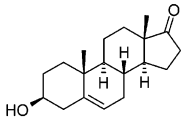
Androsterone (0.0076)



5α-Androstan-3,11,17-trione (0.023)

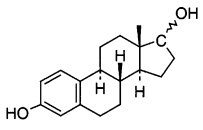


5α-Androstan-3β-ol (0.18)

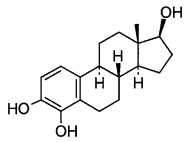


5,6-Didehydroisoandrosterone (0.010)

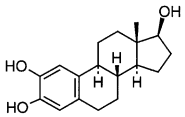
B



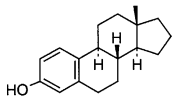
* β - 17β-Estradiol (E_2, 0.76)
α - 17α-Estradiol (0.0040)



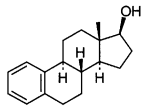
4-OH-Estradiol (0.12)



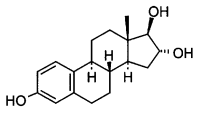
2-OH-Estradiol (0.036)



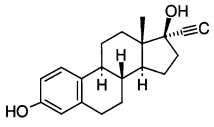
17-Deoxyestradiol (0.0074)



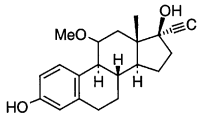
3-Deoxyestradiol (3.44)



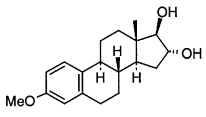
Estriol (E_3, 0.00071)



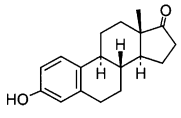
Ethynylestradiol (EE, 0.038)



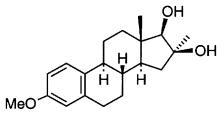
Moxestrol (SB)



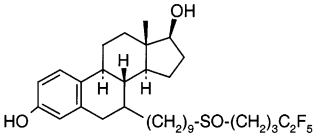
3-Methylestriol (0.0057)



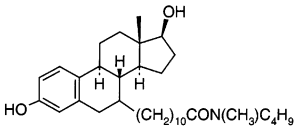
Estrone (E_1, SB)



16β-OH-16α-Me-3-Me-estradiol (0.0083)

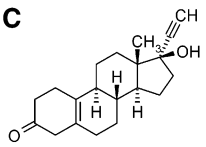


ICI 182,780 (NB)

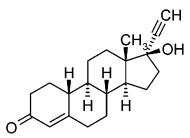


ICI 164,384 (SB)

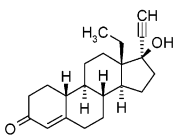
C



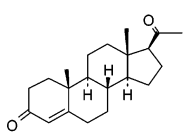
Norethynodrel (0.20)



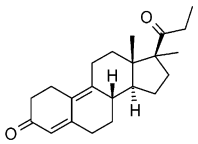
Norethindrone (2.56)



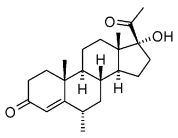
Norgestrel (16.59)



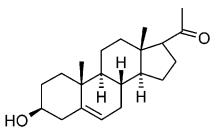
Progesterone (0.20)



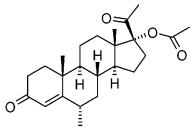
Promegestone(0.23)



6α-Me-17α-OH-progesterone(0.39)



Pregnenolone (NB)



6α-Me-17α-OH- progesterone acetate (8.64)

Cyproterone acetate (0.48)

Corticosterone (0.013)

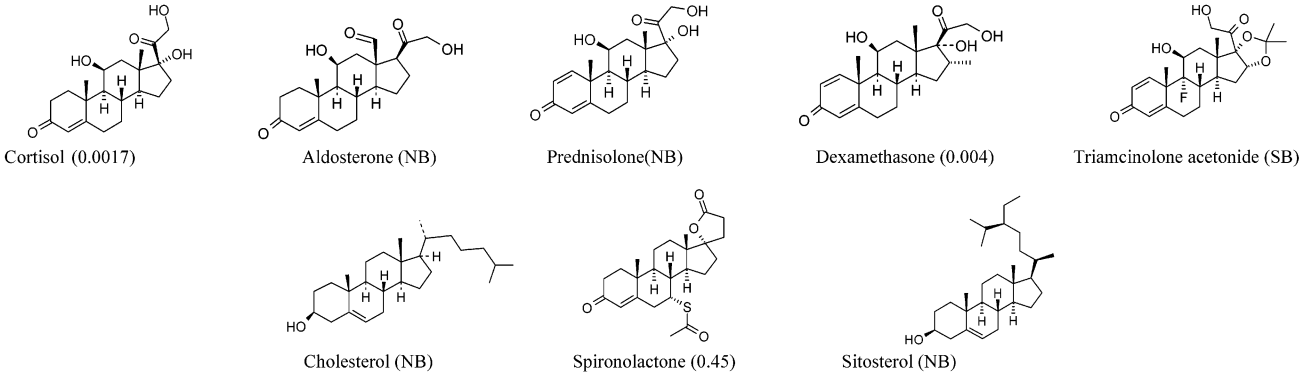


Figure 4. Steroids: chemicals with a steroidal backbone. (A) Steroidal androgens are derivatives of T and DHT; (B) steroidal estrogens have a phenolic A-ring; and (C) the remaining steroids. The chemical abbreviation and RBA value are shown in parentheses.

Table 4. Changes in Binding Affinity (Expressed as Fold Increase [+] or Decrease [−]) by Modifying 17*β*-, 17′α-, and 3-Positions of Steroids

17*β*-OH			
chemical	17*β*-OH → 17-keto	17*β*-OH → 17α-OH	removing 17*β*-OH
T	−80 (4-androstenediol)	−193 (α-epitestosterone)	
3α-androstandiol	−21 (androsterone)		
3*β*-androstandiol			−12 (5α-androstan-3*β*-ol)
5α-androstane-17*β*-ol			<−50 000 (5α-androstan)
androstenediol	−22 (5,6-diehydroiso-androsterone)		
E_2	<−1000 (estrone)	−190 (17α-estradiol)	−103 (17-deoxyestradiol)
17α Substitution			
chemical	steric groups	17α-OH acetation	removing 17α-OH
T	−1.0 (methyltestosterone)		
T	−7.5 (norethindrone)		
trenbolone	−1.1 (R1881)		
6α-Me-17α-OH-PR		+22.1 (6α-Me-17α-OH-PR acetate)	
cortisol			+7.6 (corticosterone)
E_2	−20 (ethynylestradiol)		
3-Keto			
chemical	3-keto → 3*β*-OH	3-keto → 3α-OH	removing 3-keto (or OH)
T	−39.3 (4-androstenediol)		
DHT	−61 (3*β*-androstanediol)	−861 (3α-androstanediol)	−5 (5α-androstan-17*β*-ol)
3α-androstandiol			+174 (5α-androstan-17*β*-ol)
3*β*-androstandiol			+12.3 (5α-androstan-17*β*-ol)
5α-androstane-3*β*-ol			−375 (5α-androstan)
E_2			+4.5 (3-deoxyestradiol)

and from 3*β*-androstanediol (RBA = 2.27) that has ∼5-fold higher binding affinity than 4-androstenediol (RBA = 0.49).

For other positions, the 16α-OH dramatically reduces activity; there is over a 1000-fold decrease from E_2 to estriol. A small steric substitution at the 7α-position enhances activity (10-fold increase from methyltestosterone to mibolerone), but large substituents reduce activity (both ICI 182,780 and ICI 164,384 are NBs). We found that the substitution at the 11*β*-position also reduces activity. For example, 11-keto-testosterone (RBA = 3.44) is 6-fold less active than T, and moxestrol is a SB, but its parent chemical E_2 has a reasonable affinity to AR.

DESs (Figure 5). DESs are strong ER binders (*18*, *30*). Just like steroidal estrogens, they are also capable of AR binding. Two categories of DESs were tested with respect to their agonist and antagonist nature for ER (Figure 5). All four synthetic antiestrogens (tamoxifen, 4-OH tamoxifen, nafoxidine, and clomiphene) had virtually identical RBAs. For seven agonists, binding affinities ranged from weak to moderate. No significant changes in RBA were observed by reducing the alkyl chain length from DES to dimethylstilbestrol (DMS) or 4,4-dihydroxystillbene. This illustrates a significant difference between ER and AR, where substitution of the ethyl group on either side of ethylene is critical for ER binding affinity (*30*).

Phytoestrogens (Figure 6) and Phenols (Figure 7). There are few reports on the androgenic activity of phytoestrogens (*13*) and phenols (*12*, *13*). We examined a wide variety of chemicals in these two classes, including flavones, flavanones, mycoestrogens, chalconoids, isoflavones, coumestans (phytoestrogens), alkylphenols, parabens, and alkyloxyphenols (phenols).

Phytoestrogens (Figure 6) and phenols (Figure 7) share a similar AR binding profile; the RBA of both classes of chemicals correlated positively with logP (Figure 3), and NBs had low logP values. This demonstrates that the contributions of the logP to AR binding are just as important as they are for ER (*30*). However, too high of a hydrophobicity might not be beneficial for binding as observed for an outlier, 4-dodecylphenol (logP = 7.46).

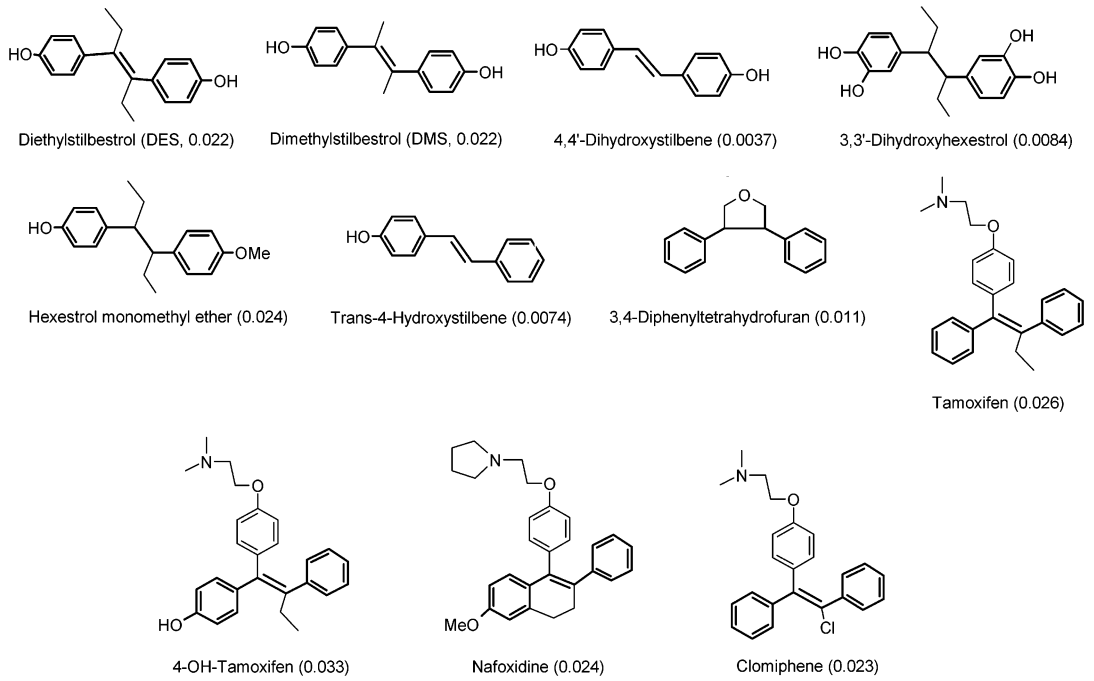


Figure 5. DESs: their distinct structural features are two benzene rings separated by two carbons with either a double or a single bond. The class contains both estrogenic agonists and antagonists.

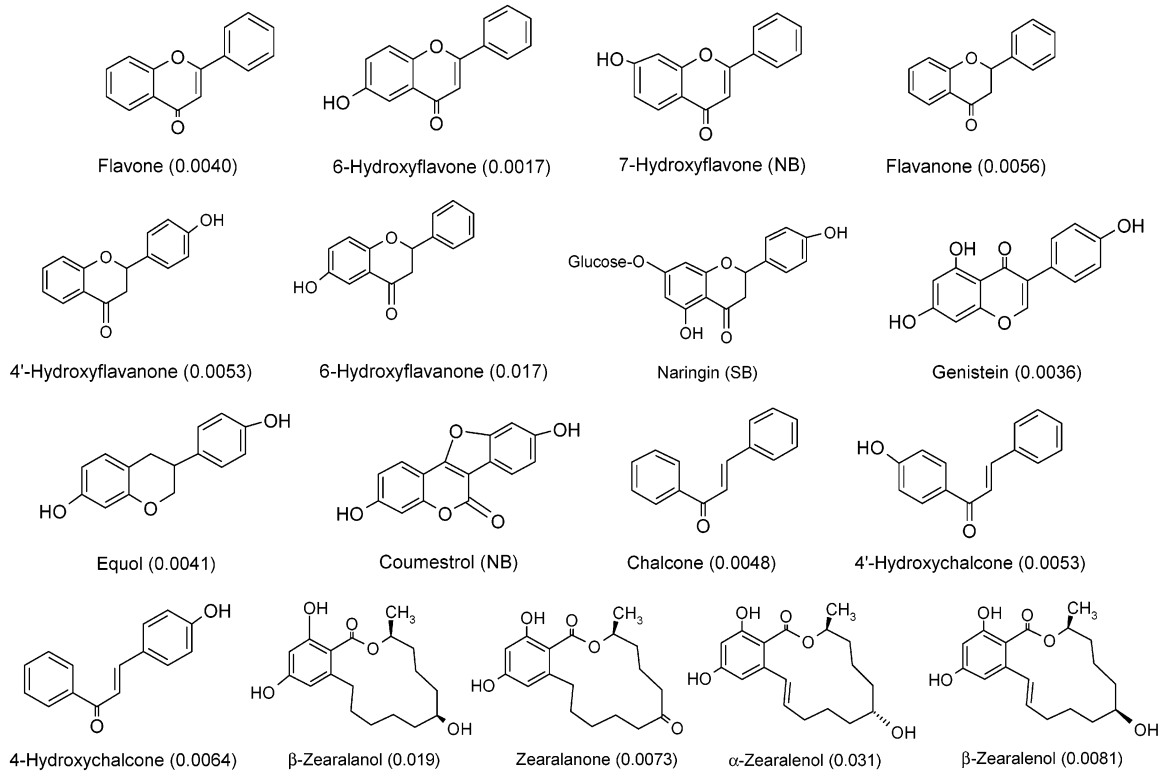


Figure 6. Phytoestrogens: the class contains representative chemicals from six major structurally unique phytoestrogens. These are flavones, flavanones, isoflavones, coumestans, chalconoids, and mycoestrogens.

Phytoestrogens have the potential for two H-bonds at opposite positions of the structure, while most phenols have the ability to form only one H-bond. The similar binding nature of these two classes of chemicals suggests that only one H-bond interaction might be important for binding. That might explain why coumestrol, high in ER binding (100-fold below E_2), is inactive in AR binding; the lowered binding due to its low hydrophobicity (logP = 1.57) cannot be compensated by the enthalpy gained from one H-bonding interaction.

Flutamides (Figure 8). Flutamide is one of the most used and studied antiandrogen (Figure 8A). Its metabolite, hydroxyflutamide (Figure 8B), has proven to have a 50-fold higher AR affinity than flutamide (*24*). Flutamide, along with two other nonsteroidal antiandrogens, anadron and casodex (Figure 8B), have shown clinical benefits in the treatment of prostate cancer (*33*−*36*). The distinct substructure of this class, Ph−N−C═O, might be important for AR binding. Thus, five pesticides (vinclozolin, procymidone, linuron, propanil, and metolachlor) were

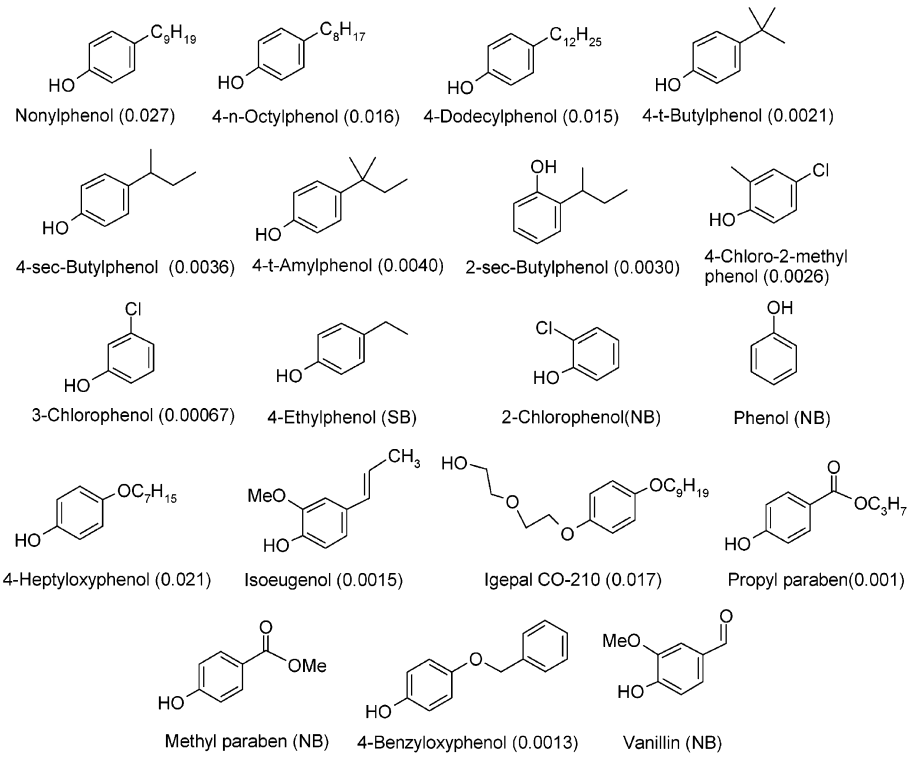


Figure 7. Phenols: alkylphenols, parabens, and alkyloxyphenols are categorized in this category. They all contain a single phenolic ring. Most chemicals in this class have a long alkyl chain substituted at the para position of benzene.

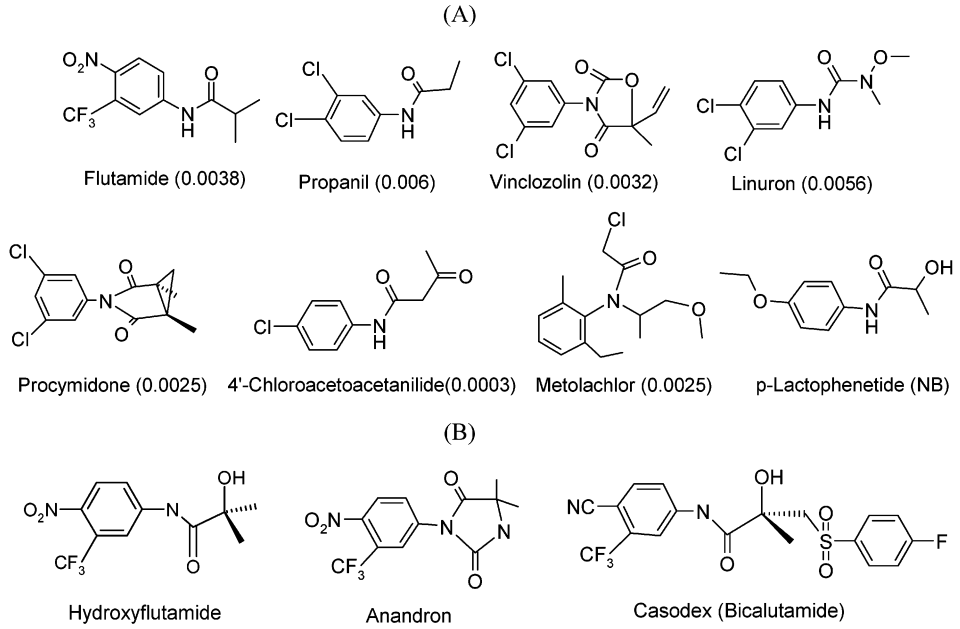


Figure 8. Flutamides: they share a common substructure (Ph−N−C═O), which could be important for androgenic activity. (A) Flutamide-like chemicals and (B) flutamide derivatives.

also included in this class for discussion. It has been demonstrated that vinclozolin, linuron, and procymidone competitively inhibit the binding of androgen to human AR and inhibit androgen-induced gene expression (*37*, *38*). In addition, vinclozolin and linuron also alter androgen-dependent ventral prostate gene expression in vivo (*24*, *39*).

The SAR study indicates that electron-withdrawing groups on the benzene ring, such as F, Cl, NO_2, or CN, favor AR binding (*1*). For example, propanil (RBA = 0.006), which has two electron-withdrawing substituents, was more potent than 4′-chloroacetoacetanilide (RBA = 0.0003), which has only one electron-withdrawing substituent. A chemical with an electron-donating substituent, such as the analgesic drug *p*-lactophenetide, is inactive.

Diphenylmethanes (Figure 9). The chemicals in this class (Figure 9) could be divided into four subclasses: methoxychlors (Figure 9A), DDTs (Figure 9B), bisphenol A derivatives (Figure 9C), and benzophenones (Figure 9D). They all exhibited AR binding affinity. The general binding trend of these four subclasses is methoxychlors = DDTs > bisphenol A derivatives = benzophenones.

DDTs were earlier found to have adverse effects on male reproductive tract development in wildlife (*11*, *37*).

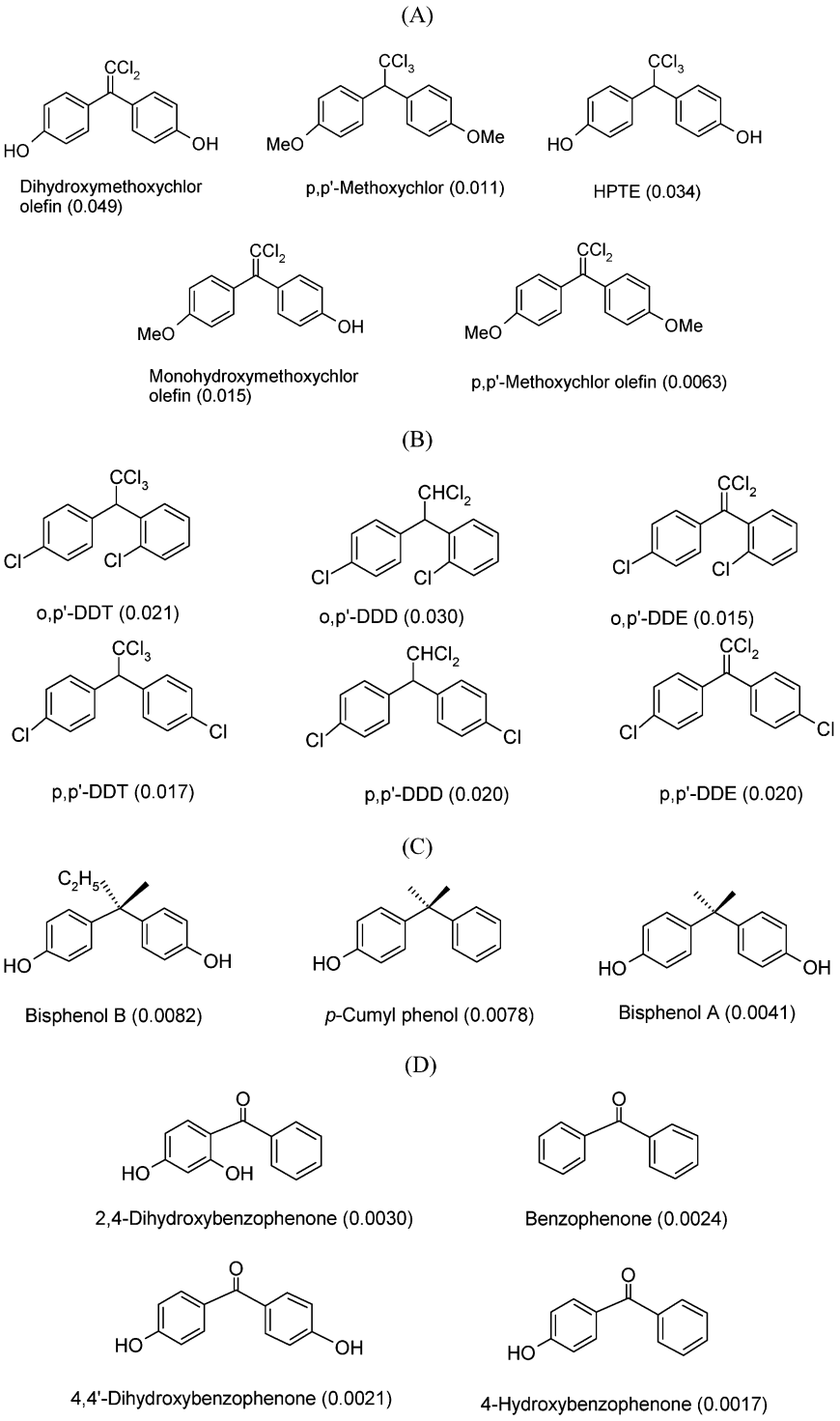


Figure 9. Diphenylmethanes: the class includes four subclasses, including (A) methoxychlors, (B) DDTs, (C) bisphenol A derivatives, and (D) benzophenones.

All six DDTs tested had similar magnitudes of RBA for AR. In contrast, only *o,p′*-DDT binds to ER (*18*, *30*). The different binding profiles between ER and AR across six DDTs indicate that structural features of DDTs important for AR are different from those for ER.

There are two interesting observations of these four subclasses: (i) methoxychlors and DDTs had comparable RBAs, and they share a common structural feature that resides at the methane position; and (ii) methoxychlors and DDTs have a higher binding affinity for AR than bisphenol A derivatives and benzophenones, and the structural difference between these two groups of subclasses was also at the methane position. This implies that the Cl substituent at the methane position of diphenylmethanes is a positive contribution for AR binding. More specifically, the presence of the Cl substituents at both 4-positions of a benzene ring and the methane position for diphenylmethanes is important for AR.

PCBs (Figure 10). Some PCBs are well-known environmental estrogens (*40*) and also exhibit binding to AR (*19*). Treatment of animals with PCBs results in abnormal rodent reproductive tract differentiation (*10*).

Of the six PCBs tested, the chemicals with 2-Cl substituents (2,2′,4,4′-tetrachlorobiphenyl, 2,3,4,5-tetra-

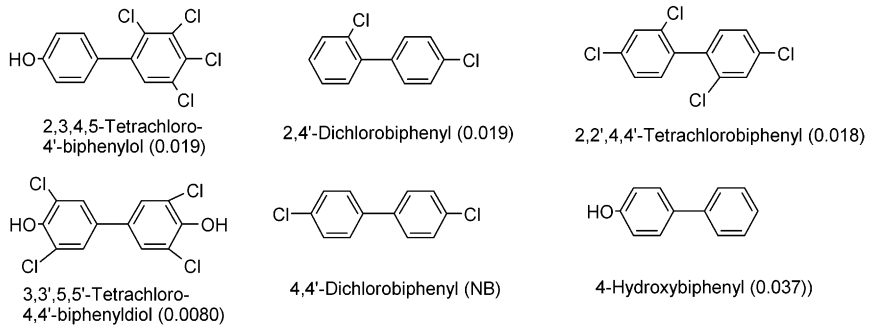


Figure 10. PCBs: chemicals contain two phenyl rings that are often chlorinated directly connected to each other.

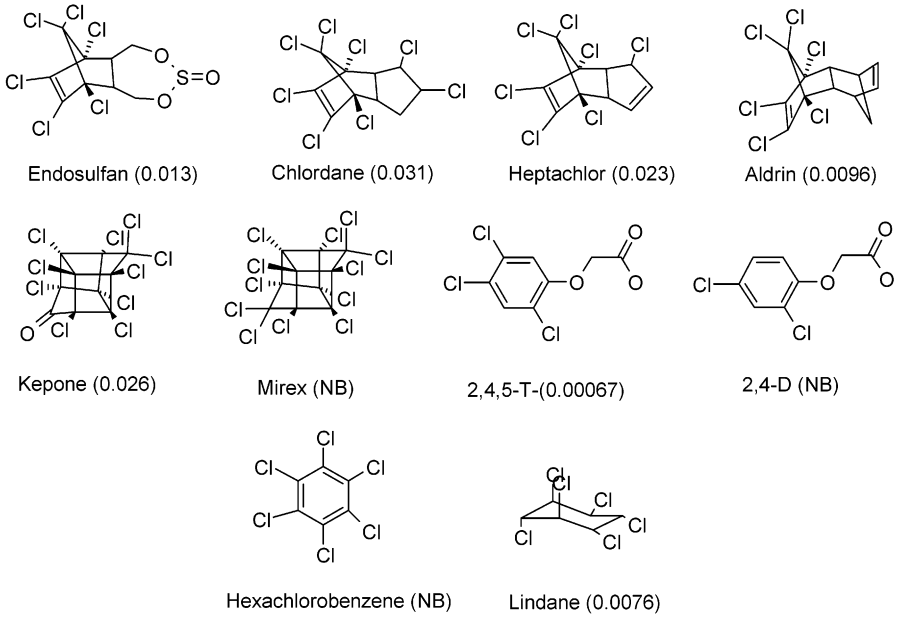


Figure 11. Organochlorines: these are the chlorinated pesticides, not including PCBs, DDTs, and those in the flutemides category.

chloro-4′-biphenylol, and 2,4′-dichlorobiphenyl) are better binders than those without this substituent (3,3′,5,5′-tetrachloro-4,4′-biphenyldiol and 4,4′-dichlorobiphenyl) (Figure 10). This is further emphasized by comparing 2,4′-dichlorobiphenyl with 4,4′-dichlorobiphenyl. The positive contribution of 2-Cl substituent of PCBs to ER binding is suspected to enhance the rigidity of the structures (*30*), which might play a similar role in AR binding. In addition, the 2-Cl substitution could also contribute to AR binding through H-bonding interaction with the receptor, which might play a more significant role. It is evident that the Cl−Cl distance (d_{Cl-Cl} = 6.48 Å) between the 2- and the 4′-position of PCB is close to that of DDTs between the 4-position and the Cl at the methane position (d_{Cl-Cl} = 6.56−6.66 Å); the latter is suspected to be important for AR binding.

4-Hydroxybiphenyl is technically not a PCB, but it is included in this category because it shares a common structural framework with the rest of the chemicals. It also shows similar binding affinity to the AR as typical PCBs, indicating that chlorinating substitutions may not be a unique determinant for AR binding in PCBs. The structural frame of PCB is also important in contributing to binding.

Organochlorines (Figure 11). We tested a variety of organochlorines for AR binding. Figure 11 listed only 10 organochlorinic pesticides; the rest of the organochlorines (DDTs, PCBs, etc.) were included in other classes according to their structural characteristics. To the best of our knowledge, for the organochlorines shown in Figure 11, there are no AR binding data reported except for kepone (*41*). However, these chemicals are potential endocrine disruptors (*42*). Endosulfan was shown to produce testicular atrophy in male rats (*43*) and to increase the rate of T biotransformation in mice (*44*). Lindane shows reproductive toxicity in the male offspring of rats (*45*). The T level in animals treated with 6 mg/kg lindane was significantly reduced to approximately 50% at both puberty and adulthood. The pesticide 2,4,5-T is toxic in the testis (*46*). These observations of androgenic activity in the animal models are consistent with our AR binding data. Chlordane, heptachlor, kepone, and endosulfan are moderate binders, while lindane, aldrin, and 2,4,5-T are weak binders. The pesticides 2,4-D, mirex, and hexachlorabenzene are the few chlorinated pesticides that did not bind to AR.

It is worthwhile to point out that except for kepone, none of these chlorinated pesticides in Figure 11 display binding affinity for the ER (*18*), indicating that the Cl could be more significant for AR than for ER. Kepone exhibits binding for both ER and AR.

Phthalates (Figure 12). Phthalate esters are widely used plasticizers (*47*). These chemicals leach from plastics and also have been found to be ubiquitously distributed in the environment (*48, 49*). Colon (*50*) found phthalate ester in the serum of young Puerto Rican girls with

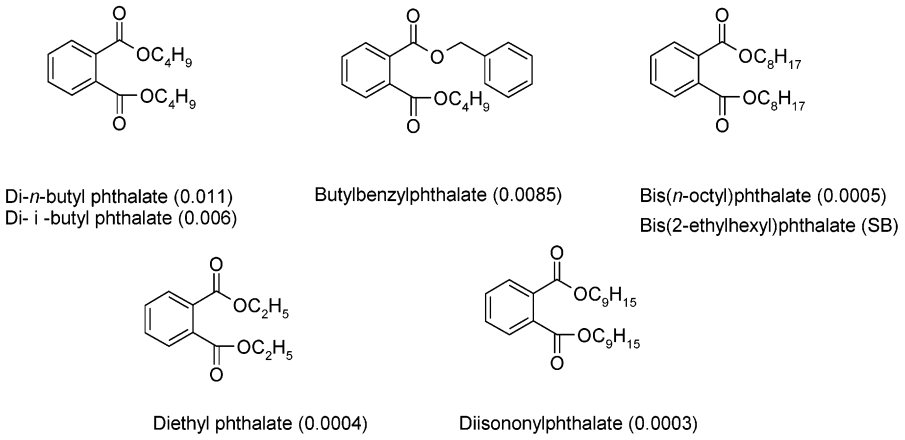


Figure 12. Phthalates: these are phthalate esters.

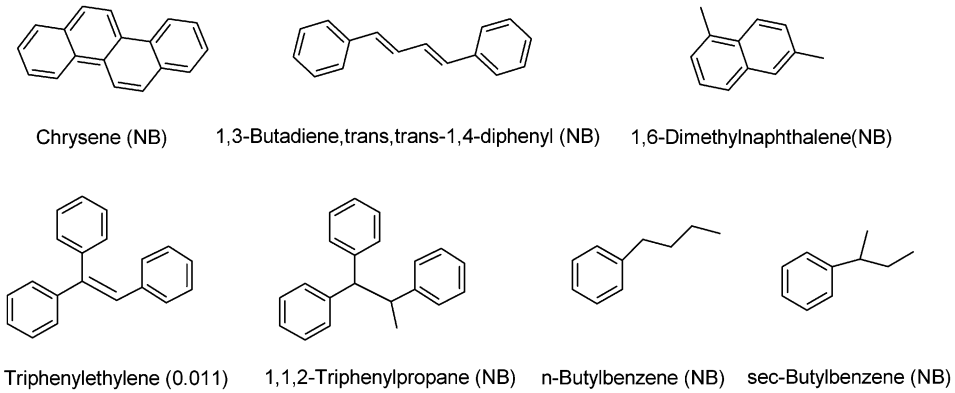


Figure 13. Aromatic hydrocarbons: these chemicals contain only H and C with at least one aromatic ring.

premature breast development. Some phthalate monoesters alter reproductive development in an antiandrogenic fashion (*51*, *52*).

We tested seven phthalates (Figure 12) that can be divided into three groups with respect to their hydrophobicity. Three chemicals (i.e., di-*n*-butyl phthalate, di-*i*-butyl phthalate, and butylbenzylphthalate) with logP values between 4 and 5 have similar RBAs (0.011, 0.006, and 0.0085, respectively). The phthalates that have a logP greater than 8 [bis(*n*-octyl)phthalate, bis(2-ethylhexyl)phthalate, and diisononylphthalate] or less than 3 (diethyl phthalate) were much weaker AR binders. This is consistent with the AR binding characteristics for phytoestrogens and phenols (Figure 3).

It was reported that the mechanism of androgenic effect of some phthalates, such as bis(2-ethylhexyl)-phthalate, might not involve AR binding (*9*, *37*); rather, it inhibits fetal T production and leads to fetal Leydig cell destruction. In our binding assay, most phthalates exhibited some binding to AR. It is reasonable to suspect that phthalates might initiate in vivo androgenic responses through both receptor- and nonreceptor-mediated mechanisms.

Aromatic Hydrocarbons (Figure 13) and Noncyclic Chemicals (Figure 14). Aromatic hydrocarbons lack H-bonding potential while noncyclic chemicals are very flexible, which does not favor binding. It is not surprising that there are not many chemicals in these two classes that exhibit binding affinity for AR.

Of 10 noncyclic chemicals, only dibutyl adipate and diisobutyl adipate (Figure 14) are active. Both chemicals possess an appropriate logP value (around 4−6, Figure 3) that is favorable for AR binding. While it is not clear why triphenylethylene binds to both ER and AR, it might be due to its structural framework that has a proper size and hydrophobicity.

Aromatic Acids (Figure 15) and Phenol-Like Chemicals (Figure 16). One of the common structural characteristics shared by these two chemical classes is the potential to form at least one H-bond with AR, which is considered an essential driving force for AR binding. With that, chemicals with a proper hydrophobicity are likely to exhibit AR activity. This is consistent with our observation that the active chemicals in both these classes are the ones that have relatively high logP values. We tested four chemicals from these two classes for both ER and AR binding (*18*, *30*); nordihydroguaiaretic acid, an antioxidant for fats and oils and a natural product in foods, shows weak binding activities while cinnamic acid shows no binding for either receptor. Both carbaryl and 4-amino-butylbenzoate bind weakly to AR but not to ER.

Others (Figure 17). Bennett reported earlier that some oral organosiloxanes depressed male reproductive functions of mouse, rat, and rabbit (*53*). Three siloxanes were tested in this study. Triphenylsilanol and 1,3-diphenyltetramethyldisiloxane were active while 1,3-dibenzyltetramethyldisiloxane was a nonbinder.

The nonchlorinated triazine pesticides (atrazine, simazine, and prometon) were all NBs, which is consistent with the report that they acted as endocrine disruptors through a direct effect on the central nervous system (*54*).

Two organophosphate insecticides, methylparathion (RBA = 0.0055) and ethylparathion (or called parathion, RBA = 0.0089), were tested. They contain a structural

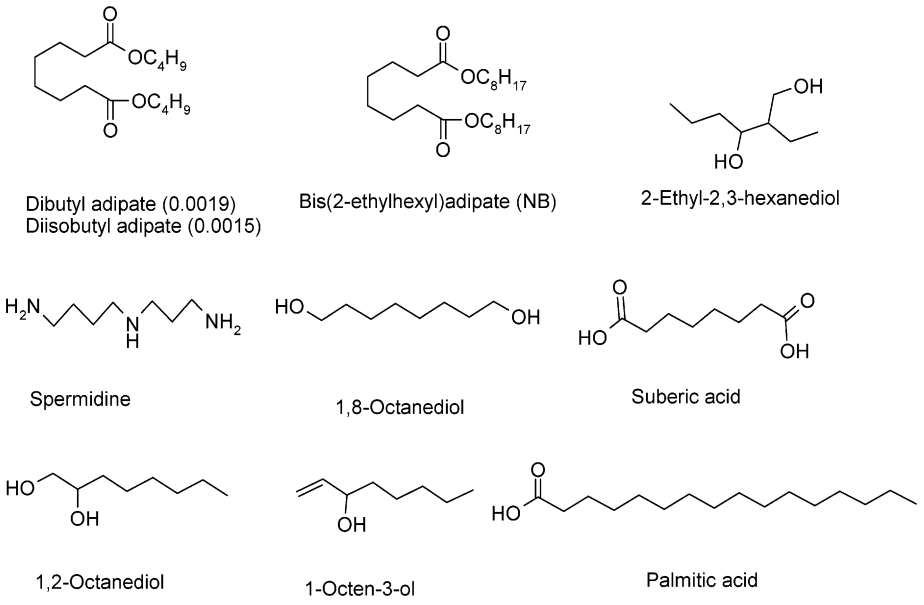


Figure 14. Noncyclic chemicals: these are the chemicals with either linear or branched chains.

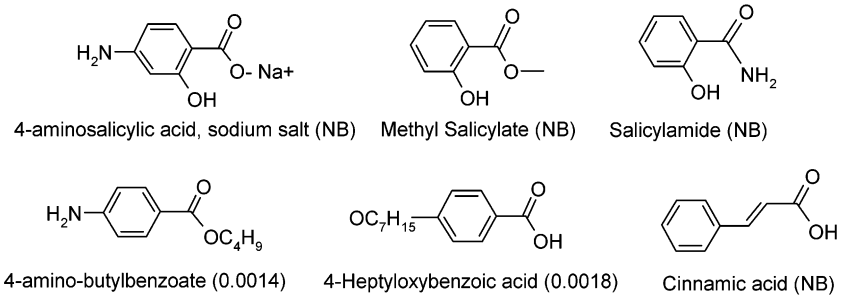


Figure 15. Aromatic acids: it includes aromatic acid, amide, and ester.

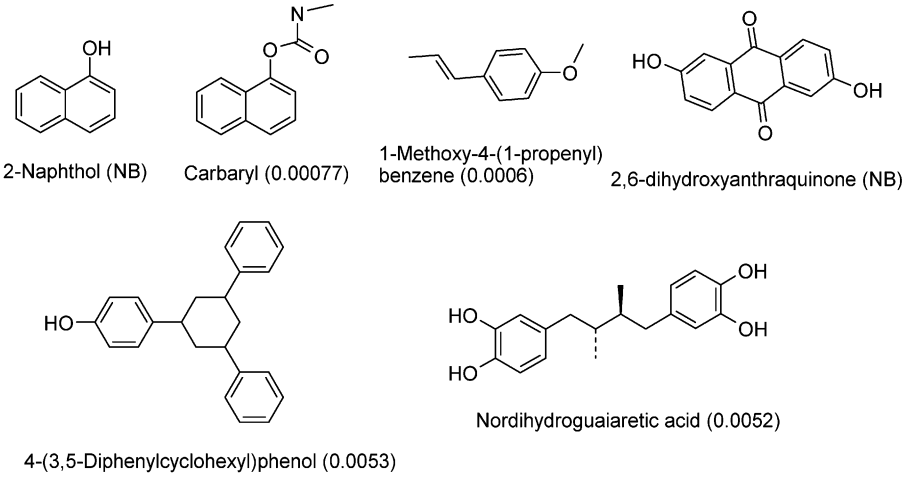


Figure 16. Phenollike chemicals: these chemicals contain a phenollike structure but do not fit into the class of phenols.

framework (Ph−O−P═S) that is similar to the core structure of flutamide (Ph−N−C═O). Both were weak binders, consistent with findings of AR antagonism of another reported organophosphate insecticide, fenitrothion (*5*, *6*). Triphenyl phosphate, which contains the similar substructure, Ph−O−P═O, and has been heavily used in the manufacture of aircraft, roofing material, fireproof plastic, etc., also showed moderate binding (RBA = 0.021). To the best of our knowledge, this is the first report on the AR binding affinity for this chemical. We also demonstrated that this chemical does not bind to ER (*18*, *30*).

Aurin was the only active chemical out of three tested triphenylmethanes (phenolphthalin, aurin, and phenol red). We demonstrated that all three triphenylmethanes are weak binders for ER with RBAs 10 000-fold below E_2 (*18*, *30*), but aurin is 10-fold higher than phenol red and 100-fold higher than phenolphthalein. It seems that a chemical with relatively higher RBA in ER tends to be stronger for AR binding.

The indole derivatives are an important class of nonsteroidal chemicals that could become new hormonal treatments for breast cancer (*55*). This class of chemicals was studied partially with the intention of producing

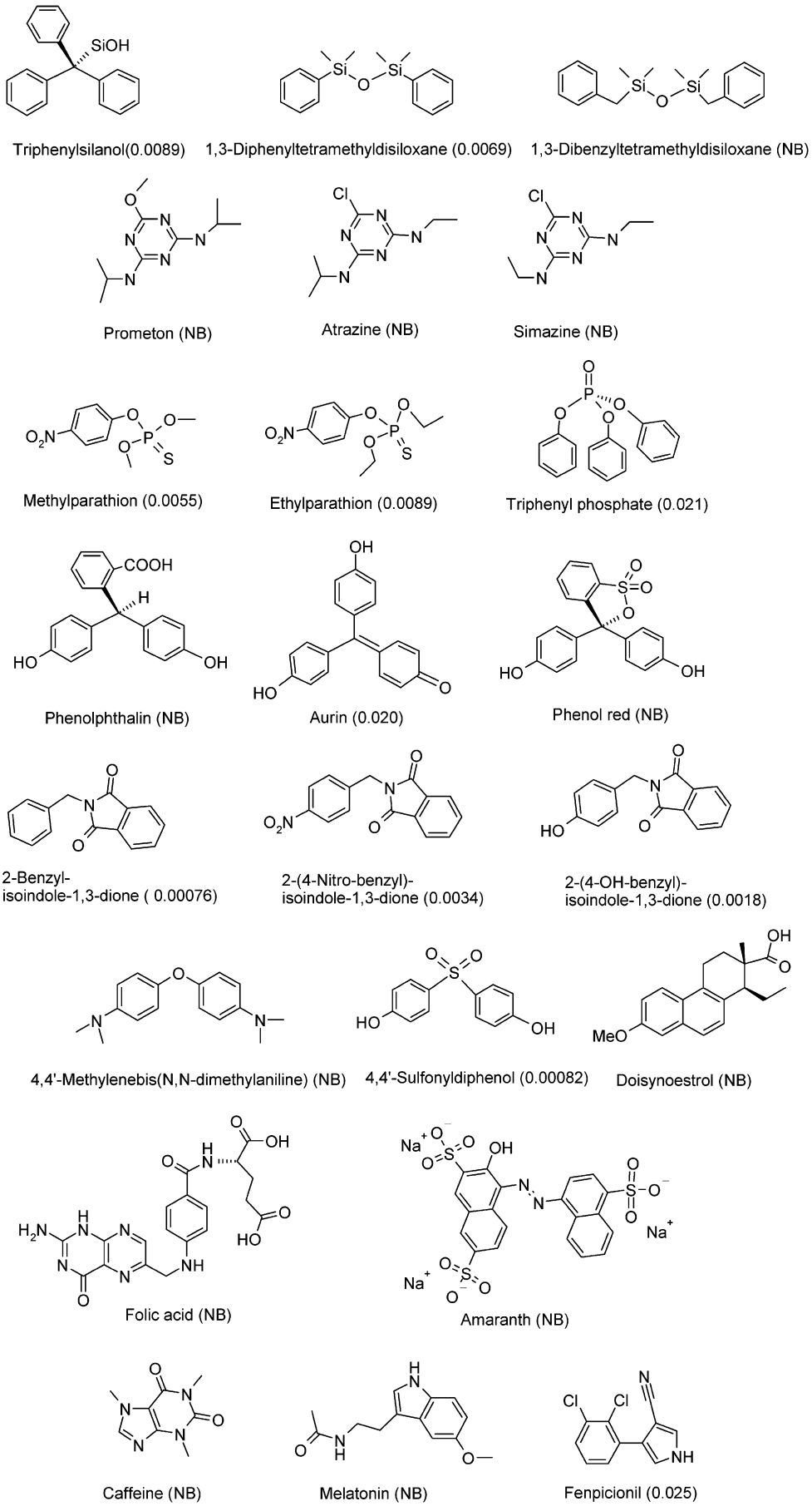


Figure 17. Others: the chemicals that do not belong to any of the classes indicated in Figures 4−16 are found in this category. Some of these chemicals can be further classified based on their distinct structures. For example, the first five trios of chemicals can be separately classified; they are siloxanes, triazines, organophosphates, triphenylmethanes, and isoindoles.

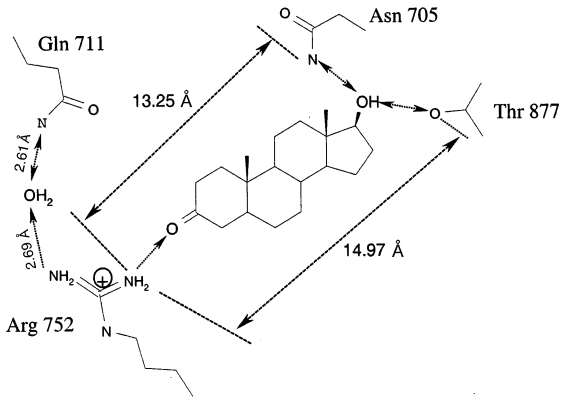


Figure 18. DHT−AR hydrogen-bonding network. The AR−DHT crystal structure is obtained from the PDB with entry 1I37.

Table 5. H-Bond Distances between the 3-Keto and 17β-OH of Ligands and the Key Amino Acids of AR in the Crystal Structure of R1881 and DHT Complexes

	3-keto			17β-OH	
AR ligands	Gln 711 (d_{O-O}) (Å)	Arg 752 (d_{O-N}) (Å)	water (d_{O-O}) (Å)	Thr 877 (d_{O-O}) (Å)	Asn 705 (d_{O-N}) (Å)
DHT	3.36	2.89	3.46	2.70	2.80 (d_{O-N})
R1881	3.87	2.85	3.18	2.87	2.79 (d_{O-O})

molecules that resemble E_2 more closely than such derivatives of triphenylethylene as tamoxifen (*56*). We developed QSAR models to predict their ER binding activities for cross-species comparison (*57*). There are few reports on this class of chemicals for androgenic activity. In this study, three isoindoles were tested, i.e., 2-benzyl-isoindole-1,3-dione, 2-(4-nitro-benzyl)-isoindole-1,3-dione, and 2-(4-hydroxy-benzyl)-isoindole-1,3-dione. They all exhibited weak binding activity for AR, and it showed reduced activity when the 4-position was changed from nitro or OH to no substitution.

Almost all chemicals in this category had activities at least 2000-fold below T. Triphenyl phosphate (an organophosphate pesticide) and fenpicionil (an agricultural fungicide) are only two chemicals that had an activity less than 1000-fold lower than T.

Discussion

Of a total of 146 active AR ligands, only two chemicals, triphenylethylene and 5α-androstane, contain only H and C. The rest of the 144 chemicals contain either an O element (111 chemicals) or a Cl element (13 chemicals) or both (20 chemicals). The O is often associated with H-bonding in receptor binding while the Cl enhances the hydrophobicity that is also critical for binding. Thus, it would be expected that both specific H-bonding and a proper range of hydrophobicity play important roles for AR binding, as we also observed from ER binding (*30*).

H-Bonding. The crystal structures of DHT bound to the rat AR LBD (*58*) and R1881 to the human AR LDB (*59*) are similar with respect to the H-bonding network, as shown in Figure 18. The 3-keto of the ligands interacts with the NH_2 of Arg^{752} as an H-bond acceptor while the 17β-OH forms H-bonds with both Thr^{877} and Asn^{705}. The H-bond distances of the 3-keto and 17β-OH groups to the key amino acids and a water molecule, respectively, are summarized in Table 5. These distance were derived from the AR crystal structures of R1881 and T complexes.

The shorter O−N distance of the 3-keto group to Arg^{752} than the O−O distances to Gln^{711} and the water molecule

Table 6. Distance between the 3-Keto and the 17β-OH for Steroidal Androgenic Agonists and the Distance between the 3-Keto Mimic and the 17-OH Mimic for Three Pure Antagonists

chemical	type	d_{O-O} (Å)
R1881	predominantly agonist	10.7
DHT	predominantly agonist	10.9
T	predominantly agonist	10.9
4-hydroflutamide	pure antagonist	9.77
casodex (d_{N-OH})	pure antagonist	9.80
anandron (d_{O-NH})	pure antagonist	8.45

indicated that the binding contribution at the A-ring of these two ligands is mainly through the 3-keto−Arg^{752} interaction. Arg has a relatively long side chain with two equal resonances of $-NH_2$, which provides a flexible H-bonding site. Its position could be stabilized through an H-bonding network among Arg^{752}, Gln^{711}, and the water molecule, where the N−O distance between the Arg^{752} and the water molecule is 2.69 Å and the O−N distance between the Gln^{711} and the water molecule is 2.61 Å. Although the 3-keto-Arg^{752} interaction acts as an anchor (*60*) for AR binding, it is relatively weak as compared to the similar H-bond anchor at the A-ring for ER binding. This might be due to only one H-bond potential in AR binding at the A-ring as compared to at least two H-bond potentials observed in ER at the same A-ring location. In addition, the unstable water molecule in the binding site might also contribute to the relatively weak 3-keto-Arg^{752} interaction in AR binding. A molecular dynamic simulation showed that the water molecule could migrate into and out of the crevice between helices 3 and 5 (*61*).

The distance of 17β-OH of T and DHT to the Asn^{705} and Thr^{877} is in the range of 2.70−2.90 Å (Table 5), indicating that two strong H-bonds are formed through 17β-OH−Asn^{705} and 17β-OH−Thr^{877} interaction, respectively. Because of the rotation of the Asn side chain, a different H-bonding nature of the 17β-OH−Asn^{705} interaction was observed in two crystal structures. In the R1881−AR crystal complex, 17β-OH binds to the carbonyl O at Asn^{705}, as an H-bond donor, while in the DHT−AR crystal complex, the 17β-OH binds to the NH_2 of Asn^{705}, as either an H-bond donor or acceptor.

The major difference of the steroids from the rest of the classes in the NCTR AR data set is its unique steroidal backbone that provides a perfect positional and spatial orientation to form two anchors (3-keto and 17β-OH) for AR binding. The dramatic decrease in the RBAs by modification and/or elimination of the 3-keto and 17β-OH of DHT and T illustrates the need for a "17β-OH/3-keto" structure for effective AR binding (Table 4). However, the 3-keto may play a less significant role in binding than the 17β-OH, where the modification of the 17β-OH in most cases has a more significant effect than the modification of 3-keto. That might be related to the fact that the 17β-OH can form two strong H-bonds with Thr^{877} and Asn^{705} while 3-keto only forms one strong H-bonding with Arg^{752} (Table 5).

The 3-keto/17β-OH arrangement might also be responsible for the angonist/antagonist nature of a ligand. When the 17-position interacts predominantly with Asn^{705}, antagonism is seen (*61*). As shown in Figure 18, the Arg^{752}−Asn^{705} distance ($O_{Arg}-O_{Asn}$ = 13.25 Å) is about 1.7 Å shorter than the Arg^{752}−Thr^{877} distance ($O_{Arg}-O_{Thr}$ = 14.97 Å). The distances of two anchors for two sets of representative androgen antagonists and agonists are listed in Table 6. These three pure antagonists have

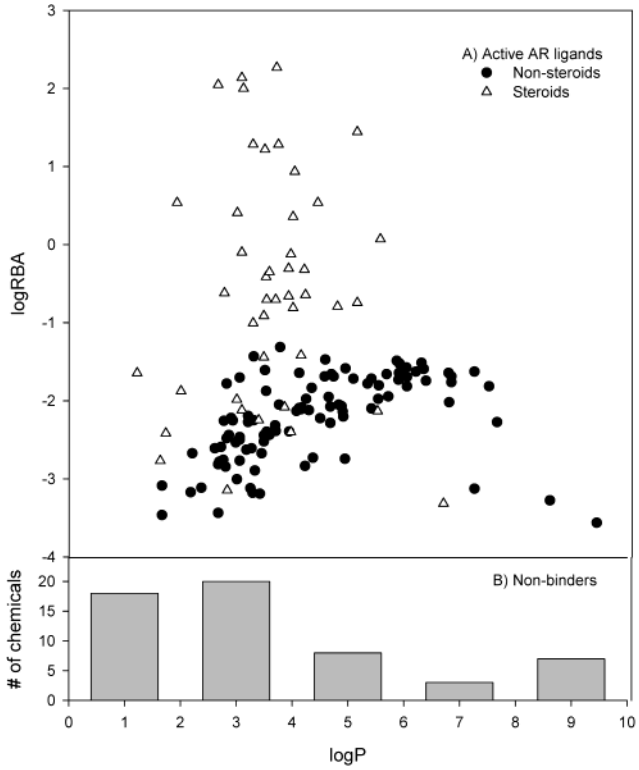


Figure 19. Relationship of AR binding affinity with hydrophobicity: (A) logRBA vs logP for 146 active chemicals and (B) logP distribution for 56 NBs.

smaller distances than the agonists. Although the OH of hydroxyflutamide and casodex and the NH of anandron could interact with both Asn^{705} and Thr^{877}, it appears that they favor binding to Asn^{705} and are less favorable for binding to Thr^{877}. The importance of the Asn^{705} in binding with hydroxyflutamide and casodex has been demonstrated by a site-directed point mutant of Asn^{705} to Ala, resulting in a complete inability of these ligands to act as antagonists (*60*). This suggests that H-bonding of Asn^{705} is a critical structural feature of AR antagonists.

Hydrophobicity. The relationship of AR binding affinity with hydrophobicity for all 202 tested chemicals is shown in Figure 19, where logRBA vs logP for 146 active AR ligands was plotted in Figure 19A while the logP distribution of 56 NBs was displayed in Figure 19B. As shown in Figure 19A, the steroidal chemicals have a much narrower range of logP (between 1 and 6.5) but have a wide range of RBA. All strong binders were found in this class, most of which have the potential to form two anchors. The steroidal framework itself is important with respect to hydrophobicity. A bare 5α-steroidal backbone has an optimal logP value and exhibits binding affinity (5α-androstane, logP = 6.65, RBA = 0.00048).

Excluding steroids, the correlation of logRBA with logP fits the pattern of the inverse U (or V) shape for the active AR ligands (Figure 19A); as logP increases, so does the AR affinity, but the trend is reversed above logP greater than 7. An increase of ∼2.5 RBA (logRBA = 0.4) units per logP unit for nonsteroidal chemicals could be estimated from Figure 19A in the logP range of 1−7. The optimal logP for a good nonsteroidal AR binder is in the range of 4−7, but not all of the chemicals with a logP in the range are active (Figure 19B). There are 48 binders in the logP range of 4−7, of which 36 contain the O element. In contrast, only one nonbinder in this range has the O element out of a total of 11 chemicals. This indicates that a significant anchor interaction with a proper hydrophobicity range is a prerequisite for a chemical to bind to AR.

Chlorinated Chemicals. Chlorinated chemicals active in AR are of concern for environment and public health (*62*). For example, the adverse effects of DDTs have long had public attention. Research has been focused on the mechanism of AR actions in vivo and in vitro (*37*, *63*). However, SAR studies have not fully explored this category of chemicals.

We tested 15 chlorinated chemicals (three PCBs, six pesticides, and six DDTs) that contain no other heteroatoms, of which 12 are active for AR binding while three chemicals are NBs. The tendency of most chlorinated chemicals examined to bind to AR indicates the presence of the Cl-related features important for binding. Because the logP values of all of these chemicals are in the optimal range of 4−7, the hydrophobicity should contribute insignificantly to distinguish active from inactive chlorinated chemicals. It would be reasonable to suspect that there might be a distinct structural feature of the chlorinated chemicals that serves as the two anchors that are observed for most AR binders.

It is known that Cl is a weak H-bond acceptor (*64*, *65*). The common structural features shared by all DDTs are two potential anchors between Cl at the 4-position of the A-ring and Cl at the methane position, which has a wide range of distance 6.5−8.7 Å. A similar distance was also observed for most active chlorinated chemicals. This distance is closer to those of pure antiandrogens than those of agonists, indicating that the chlorinated chemicals might act as AR antagonists (Table 6). Kelce et al. (*11*) report that DDTs act as environmental androgen antagonists.

Because Cl could both act as a weak H-bond acceptor and/or enhance hydrophobicity, it is reasonable to expect that chemicals containing both O and Cl may likely be AR binders. Of 22 chemicals (one steroid, one DES derivative, six flutamids, five methoxychlors, two PCBs, four pesticides, and three phenols) that contain both heteroatoms, 20 chemicals are active for AR; the two NBs have low logP values (2.62 and 2.16, respectively).

AR vs ER. Both strong ER and AR ligands require two anchors residing at the 3-position of the A-ring and the 17-position of the D-ring. The structural differences between androgens and estrogens are primarily at the A-ring. Steroidal estrogens contain an aromatic A-ring, whereas androgens are a 3-keto-cyclohexane (or cyclohexene). The phenolic ring in ER is more critical than the 3-keto in AR. For example, numerous moderate to weak AR ligands are active without a 3-keto group. Eliminating the 3-keto of DHT causes a small degree of activity loss, and eliminating the 3-OH of some steroids even results in increased AR binding. However, very few chemicals are active for ER without a phenolic ring. Unlike the 3-position, the significance of the 17β-OH for ER as compared to AR is reversed; it plays a more important role in AR than it does in ER (*18*, *30*, *66*).

We found that some typical ER ligands (e.g., steroidal estrogens, DES derivatives, phenols, and phytoestrogens) are also active in AR binding. However, several classes of chemicals behaved differently between the two receptors. For example, as expected, steroidal androgens that are normally weak or inactive for ER binding are very active for AR binding. Likewise, some progesterone

derivatives that are weak or inactive for ER binding are moderate binders for AR. The ER partial antagonists tamoxifen and 4-hydroxytamoxifen are weak AR binders, and the complete ER antagonists (ICI 182,780 and ICI 164,384) do not bind to AR. Most chlorinated chemicals that do not contain other heteroatoms are inactive for ER binding but active in AR binding. We demonstrated that all phthalates examined were inactive in ER binding (*18*, *30*, *66*) but active in AR binding. Flutamides are active in AR binding, but little data exists for ER binding.

The AR binding pocket (volume = 341 Å^3) is smaller than ER (369 Å^3) (*60*). However, the volume of the AR ligands (DHT = 299.42 Å^3 with d_{O-O} = 10.92 Å and R1881 = 281.16 Å^3 with d_{O-O} = 10.70 Å) is actually larger than E_2 (270.22 Å^3 with d_{O-O} = 11.08 Å). There is less free space in the AR binding pocket than in the ER binding pocket. Thus, a slightly larger O−O distance of some chemicals, such as DES (d_{O-O} = 12.17 Å), genistein (d_{O-O} = 12.16 Å), or coumestrol (d_{O-O} = 11.44 Å) favor ER binding but weaken AR binding. In contrast, relatively small chemicals such as lindane, flutamide, and fenpicionil favor AR binding.

AR and ER are also different in key amino acids that are located in the vicinity of the A-ring of the ligands for hydrophobic interaction (*60*); AR has methionine 745 and 749 that correspond to Leucine 387 and 391 in ER. The methionine has a more flexible side chain than leucine, which may contribute to the fact that a great variety of A-ring structures have been observed to bind to AR. For instance, an electron deficient A-ring in flutamide; a 4-chlorinated aromatic A-ring in DDT and PCB; a 3-OH aromatic A-ring in E_2, DES, bisphenol A, and phenol; a simple aromatic A-ring in 3-deoxy-estradiol and triphenylethylene; chlorinated cyclohexane in lindane; and cyclohexene chlordane are all possible A-rings that could interact with AR as an active ligand.

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