Differences in Functional Clustering of Endogenous and Exogenous Substrates Between Members of the CYP1A Subfamily

Supplementary Material

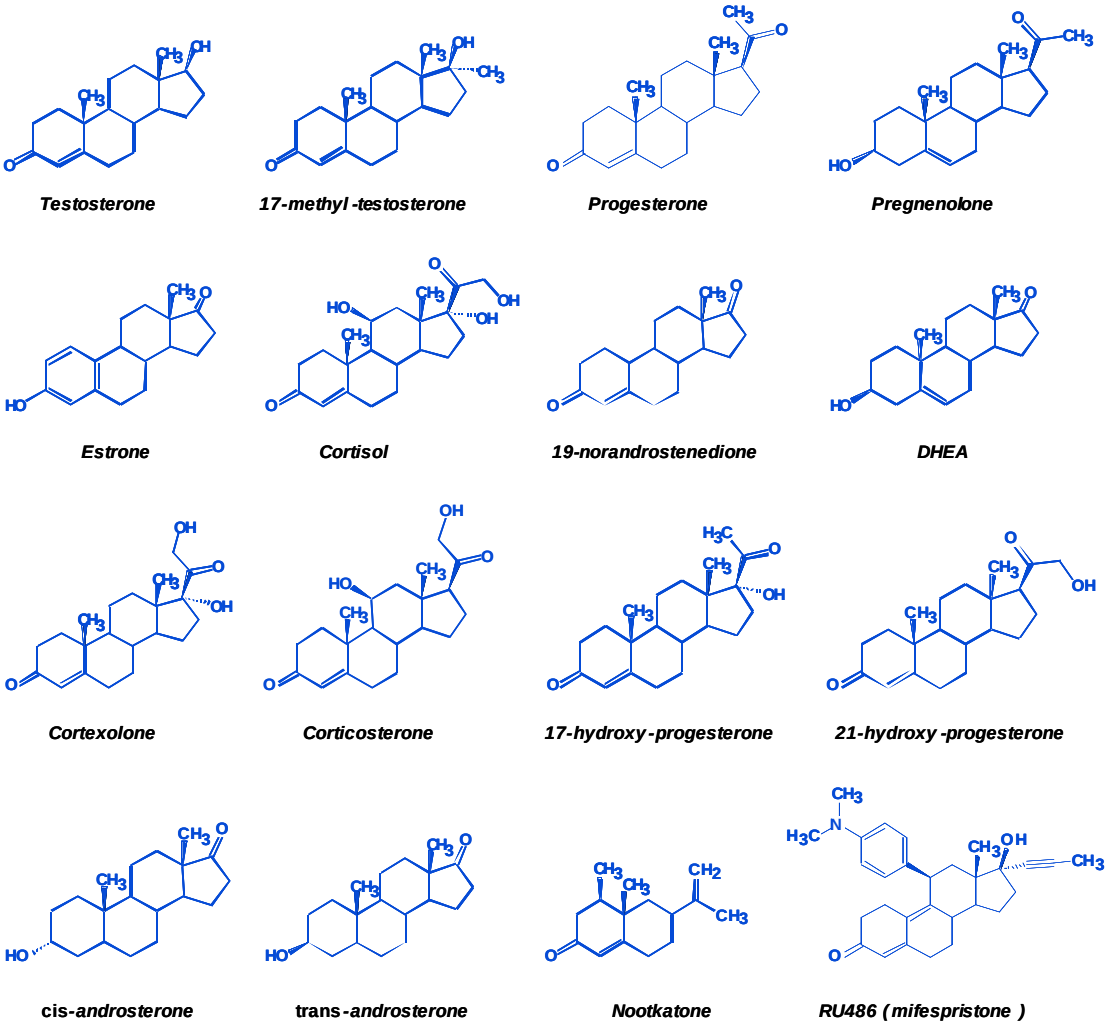

Fig. (S1). Structure of the steroid substrates used in this work.

Table S1. Observed rates of metabolite formation measured at a saturating concentration of steroid substrates. Metabolites were quantified by mass spectrometry after HPLC separation. Activities (units are µV.s/min/mg of microsomal protein) were calculated from initial velocities determined from metabolite concentration observed at different incubation times. The mammalian CYP1A enzymes were assayed in duplicate. An artificial mouse CYP1A1 - rabbit CYP1A2 chimera, the chimera 1ACh4, was also included. Enzymes were produced in yeast and incubations were performed with yeast microsomal fractions. This table is a non-processed data set

	Testosterone			RU486					17-methyl-testosterone			Cortexolone		
	T1	T2	T3	RU1	RU2	RU3	RU4	RU5	MT1	MT2	MT3	MT4	CTX1	CTX2
Hum 1A1-1	15968.6	142577.1	1693.1	207786.2	4229.5	7499.4	2682.8	5838.6	9044.8	113258.7	9658.6	8013.3	13116.4	50786.9
Hum 1A1-2	13320.3	118495.8	1407.1	336744.8	8750.9	5433.2	5061.3	10314.0	15129.7	118540.1	5619.6	12766.3	14679.8	68563.3
Hum 1A2-1	8.9	2082.5	8.9	11259.9	5.7	5.7	5.7	5.7	8.2	8.2	8.2	8.2	11.6	11895.9
Hum 1A2-2	8.9	3658.9	8.9	19089.3	5.7	5.7	5.7	5.7	8.2	8.2	8.2	8.2	11.6	9115.4
Mou 1A1-1	1588.8	67225.1	8.9	359506.6	5.7	5.7	5.7	1422.3	8.2	81412.3	8564.7	2429.0	28459.8	46343.1
Mou 1A1-2	980.2	45659.4	8.9	173040.0	5.7	5.7	5.7	967.0	8.2	53643.5	5378.3	4163.0	14154.4	14347.3
Rab 1A2-1	3596.5	82741.9	2103.9	1447504.5	5.7	5.7	5.7	5.7	8.2	63275.1	6716.7	1418.8	23720.8	61822.6
Rab 1A2-2	897.3	27970.9	2369.5	363536.2	5.7	5.7	5.7	5.7	8.2	36413.1	5792.7	1771.7	7307.9	19680.3
1ACh4	8.9	67955.8	4901.1	262670.3	5.7	5.7	5.7	665.5	8.2	79862.1	8382.6	5004.9	11.6	37463.7

	Progesterone				Corticosterone			Pregnenolone				17-hydroxy-progesterone		DHEA
	PROG1	PROG2	PROG3	PROG4	PROG5	CTC1	CTC2	PREG1	PREG2	PREG3	PREG4	17HP1	17HP2	DH1
Hum 1A1-1	370480.7	281494.4	39430.5	22100.5	16171.6	20736.9	114565.3	259450.6	53248.9	58124.5	72860.5	35829.1	10599.6	17936.6
Hum 1A1-2	285911.5	161149.2	22178.4	17003.9	13193.8	18948.7	71962.2	114860.5	22341.2	32804.7	88967.8	12512.7	11890.2	7690.4
Hum 1A2-1	9.2	1315.8	2142.7	4292.6	19844.3	8.7	8.7	21.5	21.5	21.5	21.5	12.7	12.7	13.6
Hum 1A2-2	9.2	1493.5	2431.1	2845.9	13156.3	8.7	8.7	21.5	21.5	21.5	21.5	12.7	12.7	13.6
Mou 1A1-1	42550.1	158594.5	27784.1	12352.3	11663.8	17719.1	36029.6	16233.9	21.5	8397.0	21.5	11071.2	12.7	9972.8
Mou 1A1-2	15884.1	84049.1	5656.1	1127.2	12436.6	8590.2	23908.0	14379.8	21.5	10115.9	21.5	14179.8	12.7	4011.2
Rab 1A2-1	62340.4	226850.1	57741.0	49273.0	15679.9	24031.0	24289.1	9420.6	21.5	21.5	65718.9	17786.5	12.7	9394.7
Rab 1A2-2	14950.1	105378.6	19904.8	18032.2	2000.7	4930.7	11366.5	8927.0	21.5	21.5	15982.8	1649.3	12.7	1550.6
1ACh4	44029.9	171292.0	23072.1	5381.4	21657.4	36242.6	35247.7	27686.7	4594.4	21.5	21.5	16958.7	12.7	13335.1

	DHEA	21-hydroxy-progesterone					Estrone					Nootkatone		
	DH2	DH3	21HP1	21HP2	21HP3	21HP4	EST1	EST2	NK1	NK2	NK3	NK4	NK5	NK6
Hum 1A1-1	14918.4	10735.9	41598.3	7968.3	167177.7	6.8	925421.8	56.2	18217.7	7378.2	33690.7	6110.2	37762.6	225429.4
Hum 1A1-2	14262.8	11507.1	16951.1	3140.8	168230.9	6.8	691001.1	56.2	19410.3	55321.3	21881.9	5247.4	33906.2	119705.2
Hum 1A2-1	13.6	29732.0	6.8	6.8	6491.1	6.8	56.2	56.2	7.8	7.8	7.8	7.8	7.8	7.8
Hum 1A2-2	13.6	8827.5	6.8	6.8	12780.8	6.8	56.2	56.2	7.8	7.8	7.8	7.8	7.8	7.8
Mou 1A1-1	7770.7	13.6	24070.9	6.8	24463.5	3025.1	422958.4	56153.0	7001.7	7.8	9526.2	7.8	10223.3	133203.7
Mou 1A1-2	3125.7	13.6	7816.6	6.8	16267.3	6413.6	202036.0	57058.5	4328.6	7.8	5890.0	7.8	10783.4	66713.9
Rab 1A2-1	6137.1	23499.7	60198.0	6.8	49081.4	10265.4	34488.2	56.2	7.8	7.8	26930.9	7.8	7.8	73688.3
Rab 1A2-2	1640.4	7808.8	26470.0	6.8	19427.8	1787.4	14083.2	56.2	7.8	7.8	8648.7	7.8	6309.3	23465.8
1ACh4	12540.8	13.6	22622.3	6.8	24688.0	6.8	478144.0	47845.9	7.8	7.8	7535.4	7.8	10630.2	122072.5

	Nootkatone	Cortisol	*cis* -Androsterone					Norandrostenedione				*trans* -Androsterone		
	NK7	NK8	HC1	cAD1	cAD2	cAD3	cAD4	NAD1	NAD2	NAD3	NAD4	NAD5	tAD1	tAD2
Hum 1A1-1	33744.4	22725.2	50649.0	11271.7	4723.5	86624.0	15.8	63350.3	64238.3	5.2	7661.0	6831.3	32122.7	31906.1
Hum 1A1-2	15926.6	22725.2	34940.8	4627.2	8445.5	44510.3	15.8	38837.8	21644.7	5.2	4570.3	4075.4	11328.5	24377.3
Hum 1A2-1	7.8	34303.7	13.2	15.8	14560.8	38146.9	15.8	9322.9	19108.4	27342.9	5.2	3925.5	18.1	20223.8
Hum 1A2-2	7.8	7036.7	13.2	15.8	6587.7	6061.6	15.8	10944.7	22433.2	32100.7	5.2	4611.5	18.1	9882.7
Mou 1A1-1	19040.8	9186.2	17901.5	8377.6	4998.4	39532.4	15.8	24894.1	14085.8	5.2	776.6	1449.0	2146.2	7065.0
Mou 1A1-2	5044.3	2739.2	7909.4	3050.6	15.8	49447.1	15.8	13882.3	8942.9	5.2	1141.9	2130.8	3115.5	10386.3
Rab 1A2-1	8950.5	56109.4	37051.1	14475.5	7181.7	87842.0	6748.8	64875.9	30281.6	5.2	9783.0	3243.1	6729.2	60889.9
Rab 1A2-2	7.8	11541.2	7214.3	4970.0	1582.9	45154.8	3376.0	27400.2	14894.1	5.2	4629.2	2866.8	19025.3	32424.2
1ACh4	17363.5	778.0	28039.8	8884.7	5128.0	76453.4	15.8	29745.5	59857.4	5.2	5.2	5.2	3794.2	12485.6

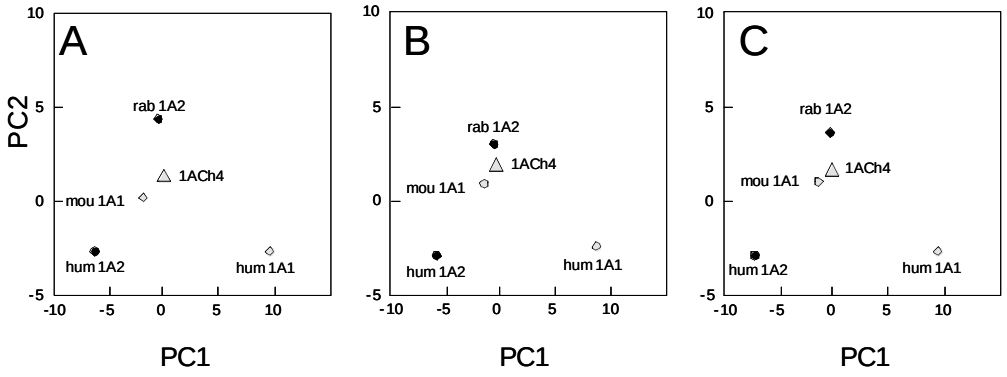

Fig. (S2). Comparison of the principal component analyses on steroid dataset obtained with three different normalization procedures. (A). No normalization. (B). Normalization by the variance. (C). Normalization by the average substrate peak area. The first principal component accounts for 53.5 % of the variance in the initial dataset for panel A and for 60.2 % for both panels B and C. Grey circles, 1A1s; solid circles, 1A2s; grey triangle, chimera 1ACh4 (mostly mouse 1A1).

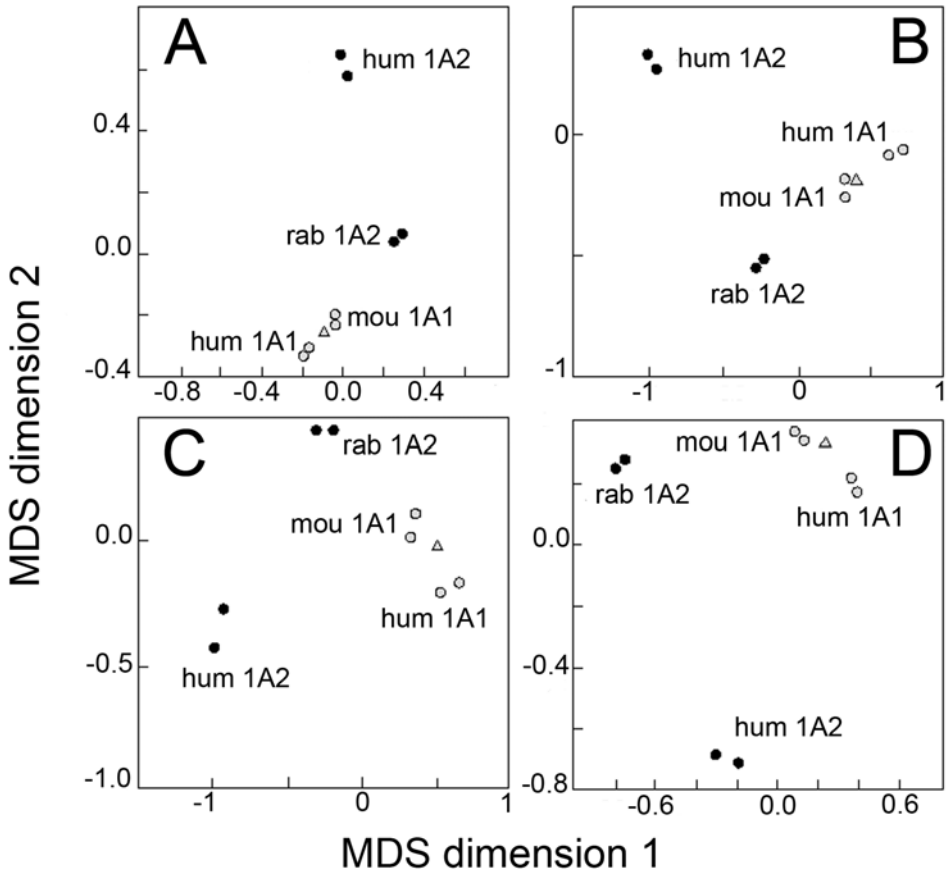

Fig. (S3). Comparison of MDS configuration plots obtained with four different models applied on steroid activity data. (A). Ratio MDS model, *stress* = 0.064. (B). Absolute MDS model, *stress* = 0.063. (C). Interval MDS model, *stress* = 0.064. (D). Polynomial MDS model, *stress* = 0.123. CYP1A1, grey; CYP1A2, black; chimera 1ACh4, triangle.

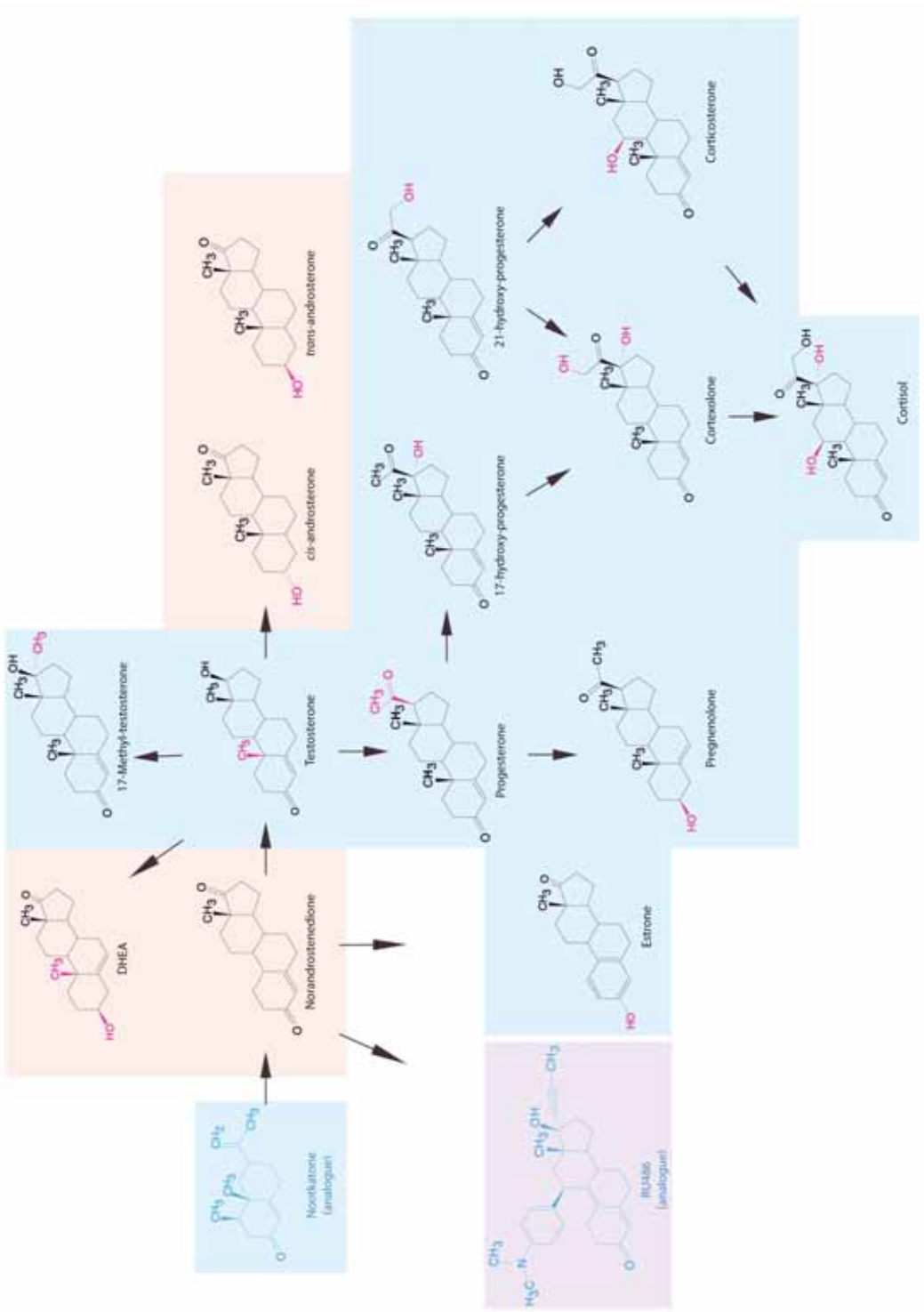

Fig. (S4). Step by step comparison of steroid and analogues from the simplest to the most complex. The background colour refers to CYP1A functional clusters and the substituent that differs from the previous molecule at each step is coloured in magenta.

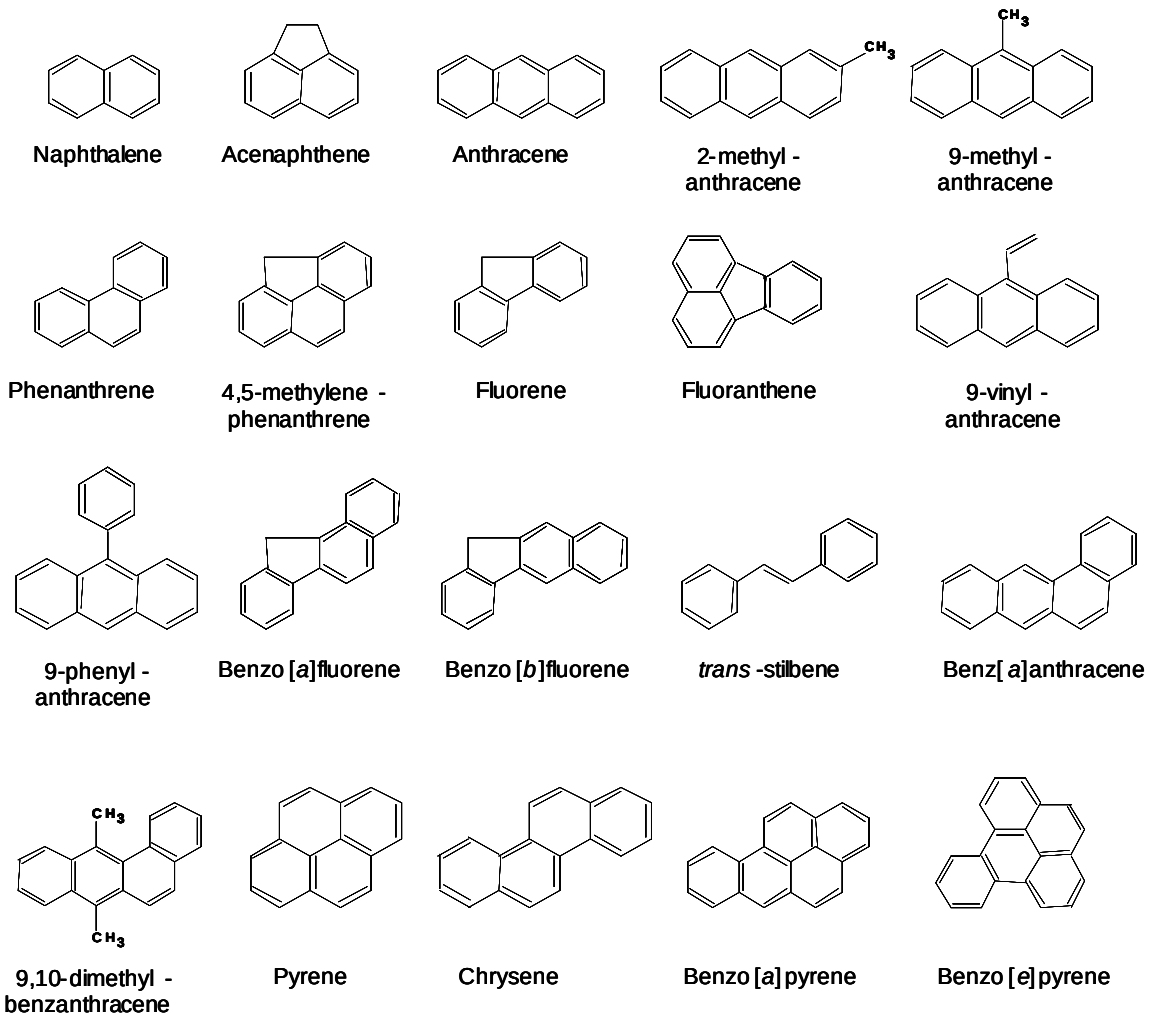

Fig. (S5). Structure of the PAH substrates used in this work.

Table S2. Observed rates of metabolite formation measured at a saturating concentration of polycyclic aromatic hydrocarbon substrates. Metabolites were quantified by fluorescence after HPLC separation. Activities were calculated from initial velocities determined from metabolite concentration observed at different incubation times. Enzymes were produced in yeast and incubations were performed with yeast microsomal fractions at which recombinant human microsomal epoxide hydrolase was added. Metabolites were quantified by fluorescence after HPLC separation. Activities units are fluorescence arbitrary units/min/mg of microsomal protein. This table is a non-processed data set

	EOR	MOR	EFEE	naphthalene		acenaphthene					*trans* -stilbene		
				naphthol-1	naphthol-2	Acen1	Acen2	Acen3	Acen4	Acen5	Stilb1	Stilb2	Stilb3
				Rt= 13.4 min	Rt= 13.8 min	Rt= 10.6 min	Rt= 13.3 min	Rt= 13.8 min	Rt= 16.4 min	Rt= 33.4 min	Rt= 3.8 min	Rt= 6.0 min	Rt= 6.2 min
Hum 1A1	414	123	453	837 111	946 290	10 352 881	5 543 982	5 571 793	1 005 072	1 603 802	102 225	103 336	43 282
Hum 1A2	68	108	36	242 715	278 470	2 397 986	3 147 100	7 587 007	264 116	607 818	3 410	25 336	30 789
Mou 1A1	821	262	59	1 024 309	1 294 024	2 434 277	7 754 512	6 462 494	289 345	331 111	40 413	87 611	59 619
Rab 1A2	17	207	323	471 789	576 444	2 759 935	5 340 558	9 330 774	291 482	350 360	8 880	15 867	23 523

	anthracene	2-methyl-anthracene					9-methyl-anthracene		phenanthrene				
	1,2-Diol	2man1	2man2	2man3	2man4	2man5	9man1	9man2	Phe1	Phe3	Phe5	Phe6	Phe7
	Rt= 12.8 min	Rt= 10.5 min	Rt= 11.2 min	Rt= 11.5 min	Rt= 14.0 min	Rt= 14.4 min	Rt= 2.8 min	Rt= 4.2 min	Rt= 10.2 min	Rt= 12.6 min	Rt= 16.3 min	Rt= 16.7 min	Rt= 17.2 min
Hum 1A1	10 851 304	5 034 649	857 804	5 810 073	19 752 853	24 148 599	37 542 671	20 917 129	1 160 198	575 780	1 725 960	771 272	910 460
Hum 1A2	994 461	57 979	52 781	1 050 105	3 642 542	1 027 601	3 099 816	4 738 593	307 717	42 389	321 945	111 941	175 464
Mou 1A1	15 130 582	1 074 805	294 962	4 684 619	19 811 595	22 934 834	12 527 464	22 687 667	1 475 968	489 406	3 311 146	1 000 236	1 882 976
Rab 1A2	5 324 524	64 842	97 608	858 056	9 186 853	2 825 621	5 125 815	8 706 386	428 531	58 779	588 051	138 288	399 105

	4,5-methylene-phenanthrene			fluorene			fluoranthene				9-phenyl-anthracene		
	Mphe1	Mphe2	Mphe3	Mphe4	Flu1	Flu2	Flu3	Flua1	Flua2	Flua3	Flua4	Pant1	Pant2
	Rt= 3.2 min	Rt= 3.6 min	Rt= 5.0 min	Rt= 5.9 min	Rt= 3.5 min	Rt= 5.3 min	Rt= 5.7 min	Rt= 1.7 min	Rt= 3.2 min	Rt= 3.6 min	Rt= 5.6 min	Rt= 4.7 min	Rt= 5.9 min
Hum 1A1	1 706 036	316 189	1 390 711	5 044 405	247 940	785 375	474 180	282 591	157 925	73 570	1 491 810	425 777	16 519 379
Hum 1A2	453 873	2 378 449	64 051	5 121 140	158 830	4 079 965	247 608	1	4 995	1	40 350	28 209	2 308 200
Mou 1A1	2 334 887	103 153	4 031 200	8 375 819	1 088 120	1 273 435	908 761	315 816	131 240	84 433	1 832 330	196 575	3 972 726
Rab 1A2	611 144	1 286 706	403 704	6 152 602	800 500	1 395 591	1 540 200	112 716	12 020	4 207	582 840	31 370	32 176

	9-vinyl-anthracene						benzo[*a*] fluorene						
	Pant3	Pant4	Vant1	Vant2	Vant3	Vant4	Vant5	BaF1	BaF2	BaF3	BaF4	BaF5	BaF6
	Rt= 8.3 min	Rt= 9.1 min	Rt= 1.5 min	Rt= 2.3 min	Rt= 2.5 min	Rt= 3.2 min	Rt= 4.7 min	Rt= 3.5 min	Rt= 4.0 min	Rt= 4.4 min	Rt= 4.9 min	Rt= 5.7 min	Rt= 6.0 min
Hum 1A1	591 345	257 215	347 721	736 833	3 277 964	11 843 369	4 339 120	1 951 060	616 752	519 212	360 947	9 835 647	2 245 378
Hum 1A2	243 157	267 760	86 461	83 440	254 899	109 559	1 125 415	41 090	37 973	38 991	5 830	515 413	112 843
Mou 1A1	73 386	102 466	122 466	90 178	1 843 203	1 617 903	2 478 950	399 757	793 621	330 031	400 761	7 437 370	2 883 969
Rab 1A2	115 207	210 320	118 366	102 526	124 456	319 660	637 985	24 935	59 881	25 580	7 510	534 032	336 804

	benzo[*b*]fluorene			dimethylbenzanthracene						benzanthracene			
	BaF7	BbF1	BbF2	BbF3	Dmb1	Dmb2	Dmb3	Dmb4	Dmb5	Bza1	Bza3	Bza4	Bza5
	Rt= 6.2 min	Rt= 3.5 min	Rt= 5.7 min	Rt= 5.9 min	Rt= 9.5 min	Rt= 9.8 min	Rt= 12.6 min	Rt= 13.5 min	Rt= 24.2 min	Rt= 9.2 min	Rt= 14.8 min	Rt= 21.9 min	Rt= 22.8 min
Hum 1A1	2 016 339	4 004 031	8 555 598	1 227 491	163 433	94 298	1 554 291	1 269 153	751 156	179 461	5 551 689	311 627	208 555
Hum 1A2	176 254	32 865	927 579	175 060	1 299	503	6 485	29 187	20 081	5 285	77 829	18 166	1 069
Mou 1A1	1 964 002	268 476	3 484 120	1 927 936	452 208	68 416	2 756 928	763 964	843 995	75 733	8 173 778	111 165	54 156
Rab 1A2	246 604	18 264	708 184	228 532	460	113	24 543	17 444	18 229	2 197	175 975	21 972	973

	chrysene						pyrene			benzo[*a*]pyrene			
	Bza6	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Pyr1	Pyr2	9,10diol-BaP	4,5diol-BaP	7,8diol-BaP	9OH-BaP
	Rt= 23.3 min	Rt= 8.2 min	Rt= 10.8 min	Rt= 14.3 min	Rt= 15.2 min	Rt= 16.6 min	Rt= 20.9 min	Rt= 13.5 min	Rt= 19.7 min	Rt= 13.2 min	Rt= 16.3 min	Rt= 17.2 min	Rt= 25.0 min
Hum 1A1	132 469	1 113 286	4 468 349	374 674	302 181	524 079	366 212	2 577 028	23 744 886	277 935	66 456	808 386	1 046 988
Hum 1A2	27 317	14 881	75 703	5 432	1 598	3 562	8 029	50 371	1 702 893	1	1	2 227	1
Mou 1A1	121 335	584 841	3 240 499	218 368	214 032	384 513	412 853	1 975 277	7 435 411	182 550	128 041	424 771	291 468
Rab 1A2	40 844	1 768	145 852	5 939	352	271	11 585	43 548	2 141 165	1	1	9 639	1

	benzo[*e*] pyrene						
	3OH-BaP	BeP1	BeP2	BeP3	BeP4	BeP5	BeP6
	Rt= 25.9 min	Rt= 14.9 min	Rt= 16.5 min	Rt= 17.3 min	Rt= 19.5 min	Rt= 23.4 min	Rt= 24.0 min
Hum 1A1	462 301	147 579	69 903	952 923	72 234	6 520 628	431 123
Hum 1A2	1	132	7 206	165	132	116 909	34 622
Mou 1A1	1 487 534	219 911	247 829	682 507	339 481	1 847 802	237 560
Rab 1A2	1	18 367	12 588	10 571	3 254	196 690	49 702

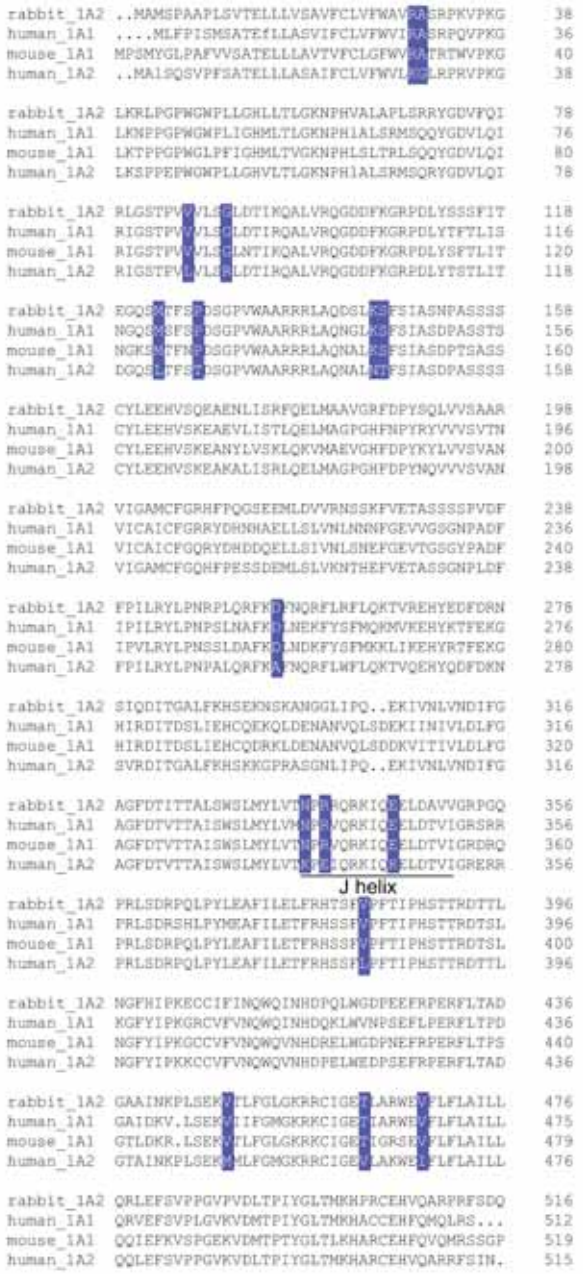

Fig. (S6). Multiple alignment of CYP1A amino acid sequences with positions characterizing differential steroid specificity coloured in blue. The J helix element is underlined.