Supplemental Data

Title: *In Silico* Identification of an Aryl Hydrocarbon Receptor (AHR) Antagonist with Biological Activity *In Vitro* and *In Vivo*

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Journal Title: Molecular Pharmacology

Legends for Supplemental Table and Figures

Table S1. Compounds screened for AHR antagonism

Chemical identifiers as defined by the suppliers are presented with the screening code used in the AHR antagonist screening assay (Figure 1).

Figure S1. Chemical synthesis of (E)-1-Methyl-N-(2-methyl-4-(o-tolyldiazenyl)phenyl)-1H-pyrazole-5-carboxamide (CH223191)

Figure S2. Structure of prototypic AHR-binding flavonoid pharmacophore

Figure S3. CB7993113 has no effect on PPARγ-dependent or CMV-driven reporter activity.

A) Cos-7 cells were transiently transfected with human PPARγ1 and RXRα vectors and with PPRE x3-TK-luc and CMV-eGFP reporter constructs. Transfected cultures received no treatment (Naïve) or were treated with Vh (DMSO, 0.1%) or rosiglitazone (1 μM) and Vh, CB7993113 (10 μM) or CH223191 (10 μM) and incubated for 24 hrs. Luminescence and fluorescence were determined, and the data was normalized as described in the methods. Data are presented as means + SE from 3 independent experiments. **B)** Hs578T breast cancer cells were transfected with a CMV-driven, GFP reporter construct and received no treatment (Naïve) or were treated with Vh (DMSO, 0.1%) or 10^{-8} M TCDD and Vh, CB7993113 (10 μM) or CH223191 (10 μM) and incubated for 24 hrs. GFP fluorescence was measured and the data were normalized to naïve controls. Data are presented as means + SE from 3 independent experiments.

Figure S4. Titration of AHR-driven reporter activity induced by two AHR agonists, β-NF and TCDD, in the presence of increasing concentrations of CB7993113.

H1G1.1c3 cells were cultured in 24 well plates at 80% confluency. Eight culture wells were treated with DMSO (vehicle) or 10^{-8} – 10^{-5} β-NF or 10^{-13} – 10^{-9} M TCDD and DMSO or 10^{5} – 10^{-7} M CB7993113. EGFP fluorescence was analyzed 24 hours later. For each plate, specific fluorescence

was calculated by subtracting the background fluorescence in untreated wells from the fluorescence in vehicle- or compound-treated wells. AHR reporter activity was calculated by dividing by the specific fluorescence in the vehicle + agonist wells by the specific fluorescence in cultures treated with agonist alone for each given agonist concentration x 100%. Data are presented as mean specific fluorescence $\pm$ SE from 6 experiments. The EC_{50}s of β-NF in the presence of vehicle, 10^{-7} M, 10^{-6} M, or 10^{-5} M CB7993113 were 1.3×10^{-7}, 1.3×10^{-7}, 2.9×10^{-7}, and 8.3×10^{-7} M respectively. The EC_{50}s of TCDD in the presence of vehicle or 10^{-7} M, 10^{-6} M and 10^{-5} M CB7993113 were 4×10^{-11} M, 4.6×10^{-11} M, 8.5×10^{-11} M, and 2.7×10^{-10} M respectively.

Figure S5. Amino acid alignment of human HIF-2α and AHR PAS-B Domain.

Supplementary Table 1

Screening Assay #	Enamine ID	Screening Assay #	Enamine ID
1	T0501-9773	51	T0505-8385
2	T0507-2370	52	T0508-7364
3	T0508-7672	53	T5276832
4	T5302486	54	T5529912
5	T5536847	55	T5665053
6	T5681118	56	T5717822
7	T5726634	57	T5805518
8	T5838025	58	T5862559
9	T5875879	59	T5976989
10	T5986510	60	T6032701
11	T0501-9818	61	T6033155
12	T0507-2873	62	T6061108
13	T0515-7358	63	T6106824
14	T5450127	64	T6118415
15	T5603773	65	T6164360
16	T5693589	66	T6247359
17	T5727286	67	T6077698
18	T5841903	68	T6033206
19	T5908589	69	T6060685
20	T6002589	70	T6107516
21	T0501-9907	71	T6118586
22	T0506-3864	72	T6170751
23	T5230304	73	T6301455
24	T5468580	74	T6159188
25	T5622457	75	T6033534
26	T5707977	76	T6086942
27	T5762336	77	T6112909
28	T5845992	78	T6131098
29	T5939191	79	T6171936
30	T6014132	80	T6364988
31	T0503-8345	81	T0501-1630
32	T0506-3804	82	T6035815
33	T5261692	83	T6088078
34	T5476786	84	T6115956
35	T5624621	85	T6131670
36	T5706093	86	T5448133
37	T5771531	87	T6398500
38	T5852139	88	T6037099
39	T5945123	89	T6101869
40	T6030469	90	T6117358
41	T0504-1409	91	T6132178
42	T0506-3959	92	T6222062
43	T5261702	93	T6107523
44	T5508023	94	T6047668
45	T5634895	95	T6097419
46	T5714320	96	T6118411
47	T5795841	97	T6144982
48	T5856326	98	T6241149
49	T5950758	99	T6265240
50	T6030776		

Screening Assay #	ChemBridge ID	Screening Assay #	ChemBridge ID
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4	5705738	53	5677214
5	5976634	54	5805653
6	6059391	55	6059088
7	6558694	56	6161011
8	6621406	57	6618593
9	6918072	58	6788192
10	7163277	59	7151789
11	5173554	60	7360091
12	5468610	61	7369144
13	5667663	62	7926807
14	5705739	63	7936252
15	6047828	64	7953721
16	6062948	65	7981918
17	6559445	66	9002969
18	6664782	67	9151226
19	6922452	68	7818296
20	7179549	69	7928457
21	5213139	70	7939693
22	5474257	71	7957821
23	5668526	72	7984223
24	5705740	73	9003564
25	6049391	74	9153126
26	6064523	75	7821536
27	6595639	76	7928604
28	6670861	77	7950050
29	6944233	78	7960033
30	7266786	79	7993113
31	5217850	80	9074501
32	5474898	81	7888075
33	5675160	82	7928611
34	5766077	83	7950985
35	6053584	84	7961424
36	6128127	85	7996236
37	6611231	86	9115460
38	6676538	87	7895416
39	7126201	88	7933482
40	7297569	89	7950998
41	5222560	90	7975312
42	5533454	91	7999051
43	5675746	92	9121866
44	5769003	93	7918857
45	6054232	94	7933763
46	6131413	95	7952392
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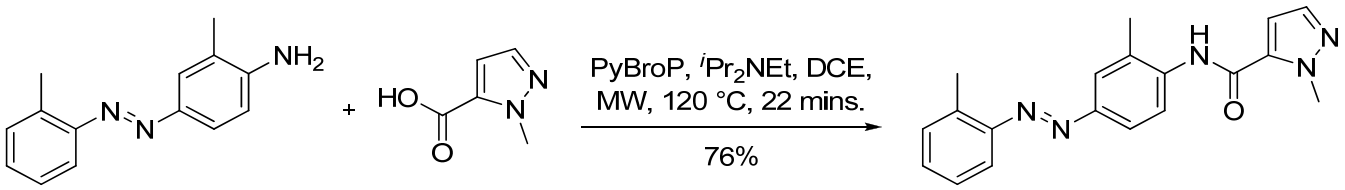
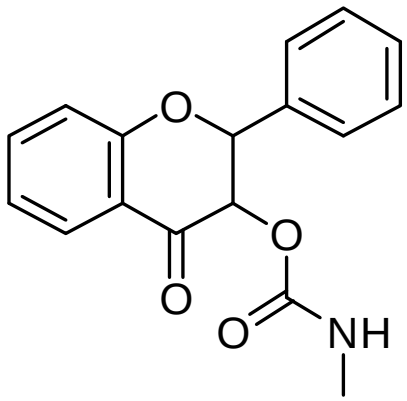
Supplementary Figure 1

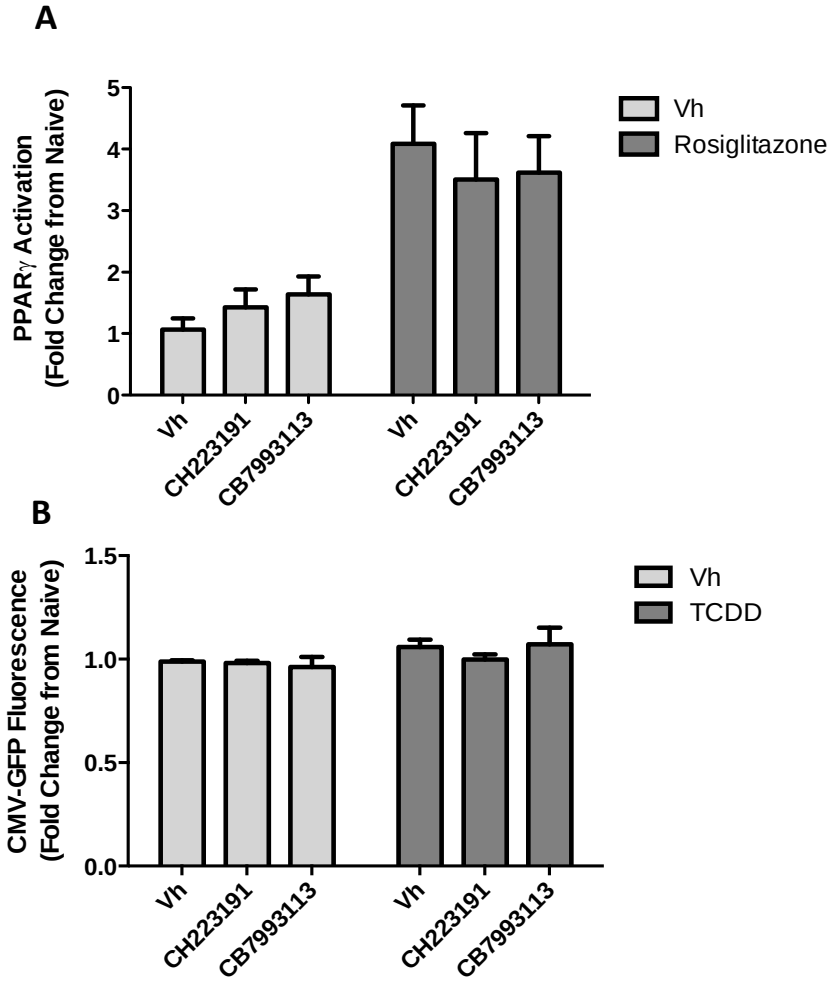


Supplementary Figure 2

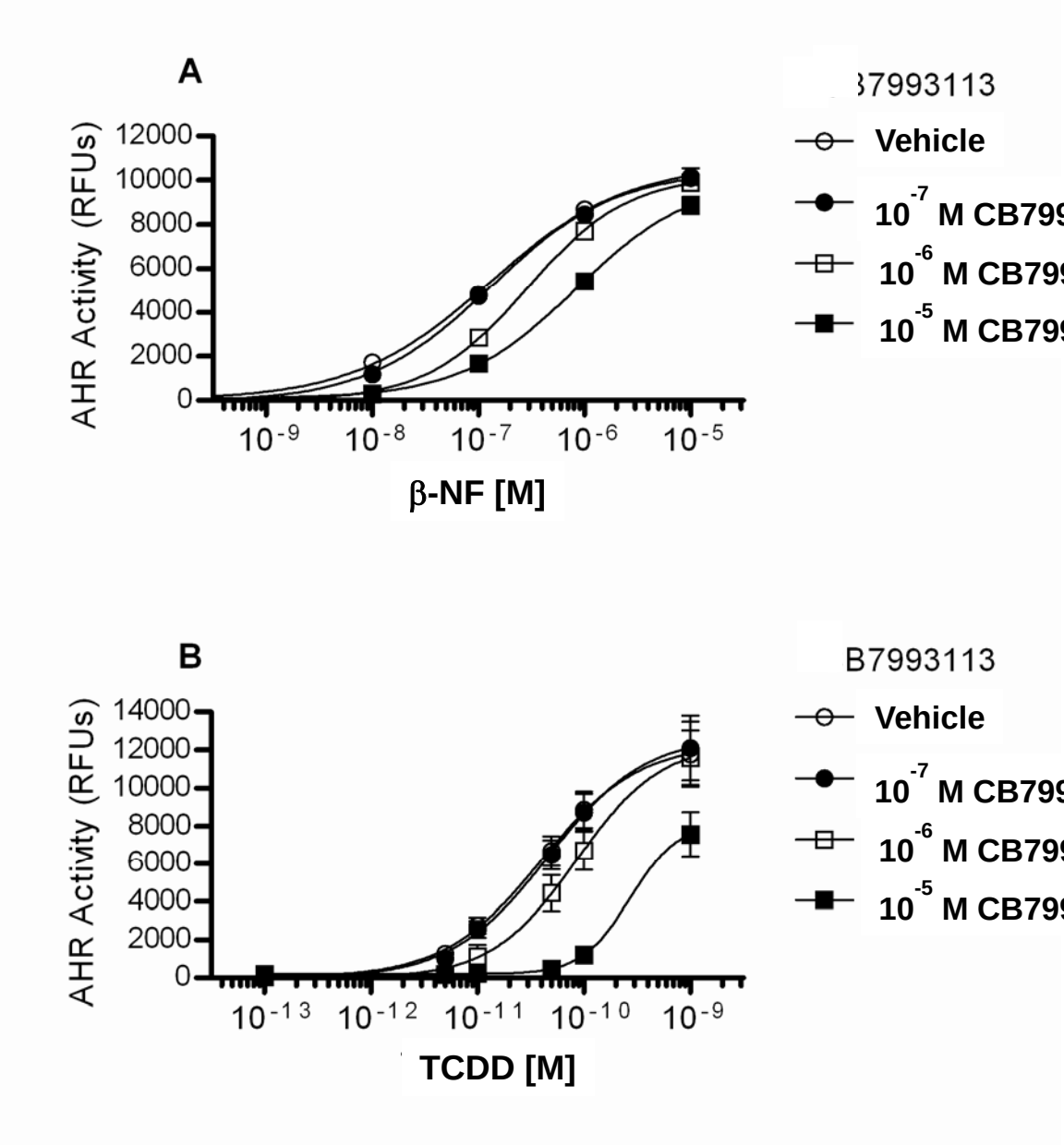


4-oxo-2-phenylchroman-3-yl methylcarbamate

Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Range 1: 244 to 348 Graphics ▼ Next Match ▲ Previous Match

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Sbjct  244  FLSRHSMDMKFTYCDDRITELIGYHPEELLGR-SAYEFYHALDSENMTKSHQNLCTKGQV  302

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