Modeling of the Aryl Hydrocarbon Receptor (AhR) Ligand Binding Domain and Its Utility in Virtual Ligand Screening to Predict New AhR Ligands

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor; the AhR Per-AhR/Arnt-Sim (PAS) domain binds ligands. We developed homology models of the AhR PAS domain to characterize previously observed intra- and interspecies differences in ligand binding using molecular docking. In silico structure-based virtual ligand screening using our model resulted in the identification of pinocembrin and 5-hydroxy-7-methoxyflavone, which promoted nuclear translocation and transcriptional activation of AhR and AhR-dependent induction of endogenous target genes.

Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated member of the basic helix-loop-helix (bHLH) family of transcription factors.1-3 The AhR is activated by a variety of compounds, both synthetic and natural, including halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and mediates their biological activity.1-3 The AhR is a cytosolic transcription factor bound to several co-chaperones. Upon ligand binding, the AhR translocates from the cytoplasm to the nucleus and regulates genes, including several drug metabolizing enzymes that can influence the therapeutic activity of a number of compounds.1-4 The AhR regulates proliferation and differentiation of cells.4-6 The AhR also induces immunosuppressive regulatory T cells with therapeutic implications in hyperimmune disorders.7-9 The PAS (Per-AhR/Arnt-Sim) domain of the AhR is the ligand binding domain (LBD).1-3 In the present study, homology models of the AhR-LBD were built to study the inter and intraspecies differences in ligand binding and to identify AhR ligands by virtual ligand screening.

Results and Discussion

To construct the homology models, a multiple sequence alignment of the AhR-LBD domain from mouse, human, rat, guinea pig, rabbit, and zebrafish (Figure 1A) was performed. The sequence alignment produced sequence identities of greater than 85% between the different species, with the exception of zebrafish isoform 2, which shared a consensus sequence identity of approximately 70% (Table 1). The AhR-LBD of mouse and rat had the highest sequence identity at nearly 98% (Table 1), suggesting a common evolutionary path and a recent species splitting as depicted by the evolutionary tree in Figure 1B. The mouse AhR-LBD residues His285, Cys327, Ile332, Met334, Ala375, and Gln377, which have been characterized by mutagenesis studies and are known to influence TCDD binding,10 are highlighted in red in Figure 1A. These residues are well conserved with the exception of Met337 in guinea pig and Val381 in human AhR. These amino acid residues are replaced with Ile and Ala in all other species (Figure 1A).

Several PAS domain structures are currently available in the Protein Data Bank (PDB). The sequence similarity between the structures of the PAS domain containing protein family is low; however, within the family there is a high structural conservation of the α and β folds. We selected the PAS domain structure of hypoxia-inducible factor 2α (Hif-2α) (PDB 1p97)11 for generating a homology model of the mouse AhR-LBD based on its closest similarity in this region. The model was built using the primary amino acid sequence [241-384] of the AhR-PASB domain, producing a sequence identity of 30% from the alignment with Hif-2α. The backbone geometry was preserved while loops were rebuilt using a database search for matching loop geometries.12 The side chain conformations of amino acids different in the alignment were globally optimized using the biased probability Monte Carlo procedure.13 The human, rat, and zebrafish 1 and 2 AhR-LBD homology models were built starting from the mouse model and using the respective sequences of the other species homologous to that of the mouse primary sequence [241-384].

Each of the AhR ligand binding domains were built using the homology modeling protocol described in detail by Car- dozo et al.14 The initial model was built by using the template backbone and side chains in their most likely conformation.
Next, the side chains were globally optimized using the modified ECEPP/3 energy function described by Abagyan et al.\textsuperscript{15} This energy function includes the following energy terms: van der Waals, hydrogen bonding, torsion energy, distance dependent electrostatic term, disulfide bond constraints, side chain entropy, and the implicit solvent accessible surface dependent solvation term.\textsuperscript{14} After the side chain placement, the whole model was energy optimized using all the above energy terms but with strong positional restraints. The refined homology models were then geometrically corrected such that all of the residues were in sterically allowed regions of the Ramachandran plot. Following development and refinement of the model, we first analyzed the mouse AhR-LBD homology model to characterize the ligand binding pocket. Using ICM Pocket Finder (Molsoft ICM v3.5-1p),\textsuperscript{16} a binding pocket was identified (volume 201.5 Å\textsuperscript{3}, area 178.3 Å\textsuperscript{2}, and radius 3.637 Å) in the region of the protein surrounded by β-sheets Hβ and Iβ (Ala375 and Gln377) at the bottom, by the helix-connector Fα (Cys327and Met334) on the left, by Aβ on the right (His285), and at the top by the loop connecting Aβ and Bβ (Figure 2A). In the helix connector Fα, the backbone NH of Ile332 established an intra H-bond with the backbone carbonyl oxygen of Ala328, keeping the helical secondary structure stable. Replacing Ile332 with a proline in our mouse AhR-LBD homology model resulted in a loss of intra H-bond interactions. These results suggested that the decrease of TCDD binding in the I332P mutant\textsuperscript{10} is due to an indirect effect caused by the disruption of the Fα helix connector in the vicinity of the binding pocket region. Thus, our AhR-LBD homology model provides a structural explanation for the decreased TCDD binding observed with the I332P substitution in the AhR.\textsuperscript{10}

We should note that while other groups have attempted modeling of the AhR-LBD,\textsuperscript{10,17} there are noteworthy differences between these models and our characterized binding pocket. The ligand binding pocket in our model is more compact, surrounded mainly by the residues Cys327, Gln377, His285, Met334, and Ala375. The side chains of these amino acids are all pointing toward the binding pocket, indicating structurally their direct involvement in TCDD binding (Figure 2A). The model by Pandini et al.\textsuperscript{10} is more spread, involving the region between residues Gln377 and His285 surrounded by Dα and Eα helices in addition to the area characterized in our model. Similar to Pandini et al., we observed that the side chain of residues Thr311 and Lys350 point outside the modeled LBD and do not affect AhR ligand binding.\textsuperscript{10} The side chain of residues Ile319 and His320 also pointed toward the pocket cavity, confirming their key role in ligand binding specificity.\textsuperscript{17} To study TCDD binding capacity across species and to provide a structural explanation for the biological data reported

| Table 1. Interspecies Percentage of Sequence Identity in the AhR-LBD Domain\textsuperscript{a} |
|-----------------|-----|-----|-----|-----|-----|
|                  | M   | R   | H   | GP  | Z2  | RB  |
| M                | 100 | 97.91 | 88.19 | 84.72 | 72.22 | 88.19 |
| R                | 97.91 | 100 | 87.5 | 86.11 | 72.22 | 88.19 |
| H                | 88.19 | 87.5 | 100 | 86.11 | 72.22 | 90.97 |
| GP               | 84.72 | 86.11 | 86.11 | 100 | 71.52 | 86.11 |
| Z2               | 72.22 | 72.22 | 72.22 | 71.52 | 100 | 75.69 |
| RB               | 88.19 | 88.19 | 90.97 | 86.11 | 75.69 | 100 |

\textsuperscript{a} M, mouse; R, rat; H, human; GP, guinea pig; Z2, zebrafish isoform 2; RB, rabbit.

Figure 1. (A) Interspecies multiple sequence alignment of the AhR-LBD performed with the program ClustalW.\textsuperscript{28} (B) Evolutionary tree of different species.
in the literature,\textsuperscript{1–3} TCDD was docked into mouse, human, and zebrafish isoform 2 AhR-LBD models. TCDD docked similarly in all three species binding pockets (Figure 2B–D) and the binding energy calculations (see methodology) were in agreement with previous interspecies biological characterizations.\textsuperscript{1–3} Lower binding energy values indicate a higher binding affinity of a particular ligand in our model. The values obtained for TCDD binding in mouse and zebrafish were $-3.796$ and $-3.972$ kcal/mol, respectively, confirming that TCDD has similarly high binding affinity in mice and zebrafish.\textsuperscript{18} In contrast, the value obtained with the human model was $-2.33$ kcal/mol, which was significantly higher than those for the mouse and zebrafish models, indicating a decreased binding affinity compared with the other two species investigated. TCDD docking orientation was similar in all three species. Further, the residues that were important for ligand binding and facing the ligand pocket cavity were conserved in mouse and zebrafish isoform 2. In the human AhR, one of the residues, corresponding to mouse Ala375, was replaced by a valine; the presence of this bulkier residue would likely sterically impair TCDD binding ability.

The mouse AhR-LBD homology model was used to structurally characterize some of the intraspecies experimental biological data available for the AhR. The C57BL/6j mouse strain exhibits a 10-fold higher TCDD susceptibility compared to the DBA/2 mouse strain.\textsuperscript{1–3} Both the mouse DBA/2 AhR-LBD and human AhR-LBD share the A375 V substitution (Figure 1A). Hence, the effect of this amino acid substitution on TCDD docking was analyzed in our mouse homology model by mutating A375 into a valine or a leucine. In the WT and A375 V model, TCDD was able to dock into the binding pocket, whereas in the A375L model, TCDD failed to dock completely. Thus, the residue at position 375 located in the middle of the ligand binding area appears to directly impact TCDD binding. The replacement of the amino acid alanine with bulkier residues like valine (as present in mouse DBA/2 and human sequences) or leucine decreased the volume of the binding pocket ($195$ Å$^3$ (WT), $186.1$ Å$^3$ (A375 V), and $148.6$ Å$^3$ (A375L)) (Figure 2E) with the consequence of partially (valine) or totally (leucine) sterically blocking TCDD binding. By changing the tautomerization of one of the key residue for binding His285 in both WT (194.4 Å$^3$) and the A375 V mutant (185.9 Å$^3$) models and by redocking TCDD, the binding energy calculations generated values of $-3.617$ and $-3.413$ kcal/mol, respectively. Decreased volume of the binding pocket might result in weaker binding energy that contributes to decreased affinity of TCDD to AhR from human or mouse DBA/2 strain. Indeed, substitution of alanine at 375 with leucine has been reported to affect TCDD binding.\textsuperscript{10}

Figure 2. (A) Homology model of the mouse AhR-LBD with the protein backbone displayed as ribbon and colored by secondary structure (ICM v3.5-1n, Molsoft). The residues are displayed as sticks and colored by atom type, with the carbon atoms in white. The binding pocket volume is colored in green and was calculated using the program ICM Pocket Finder.\textsuperscript{16} The protein backbone is displayed as ribbon and colored by secondary structure. The residues are displayed as sticks and colored by atom type, with the carbon atoms in white. TCDD is displayed as sticks and colored by atom type (carbon atoms in yellow). (B–D) TCDD docking orientation into mouse (B), human (C), zebrafish isoform 2 (D) AhR-LBD binding pocket. The protein backbone is displayed as ribbon and colored by secondary structure. The residues are displayed as sticks and colored by atom type, with the carbon atoms in white. TCDD is displayed as sticks and colored by atom type (carbon atoms in yellow).

Figure 3. ITE (A,C) and FICZ (B,D) docking orientation respectively into mouse (A,B), human (C,D) AhR-LBD homology model. The protein backbone is displayed as ribbon and colored by secondary structure. The residues are displayed as sticks and colored by atom type, with carbon atoms in white. H-bonds are represented by white dashed lines.
We next used homology modeling to study AhR in zebrafish. Interestingly, zebrafish have three AhR isoforms, namely, AhR1a, AhR1b, and AhR2, with AhR2 being the dominant isoform that binds TCDD, whereas AhR1a (zfAhR1a) exhibits impaired TCDD binding. The sequence identity between zfAhR1a- and zfAhR2-LBD is 65%. The amino acid residues His285, Ala375, and Gln377 (Figure 2F), which are highly conserved in most species (Figure 1A) and contribute to ligand binding, are present in zfAhR2; however, in zfAhR1a, these amino acids are substituted with threonine, histidine, and tyrosine, respectively (Figure 2G). Thus, the difference between zfAhR1a and zfAhR2 in TCDD binding may be attributed to these dissimilarities in the ligand binding pocket region. To understand the differences in the ligand binding pockets of zfAhR1a and zfAhR2, homology models for the zfAhR1a- and zfAhR2-LBD were built and energetically minimized using the ICM method. TCDD docking was attempted with both zebrafish AhR isoforms models; however, TCDD was able to dock only into zfAhR2-LBD. By analyzing the binding pockets of zfAhR1a and zfAhR2 identified with ICM Pocket Finder (Figure 2F,G), zfAhR1a was found to have a decreased binding area that may contribute to its lower affinity for TCDD. Substitution of zfAhR2 residues Gln388 and His296 with a histidine and tyrosine, respectively (as present in zfAhR1a), modified the type of electrostatic interactions established between the protein and TCDD, resulting in decreased binding affinity. Thus, these specific amino acid replacements (Ala386Thr, Gln388His, and His296Tyr) in the zfAhR1a-LBD appear to play a direct role in the considerable decrease of TCDD binding by modifying the electrostatic environment of the binding pocket region and the volume of

Figure 4. (A) Mouse Hepa1 cells transfected with the AhR response element (AhRE)/xenobiotic response element (XRE)-luciferase reporter were treated with compounds at the indicated concentrations for 6 h and assayed for reporter gene activity. (B) Docking orientation of 5-hydroxy-7-methoxyflavone and pinocembrin into mouse AhR-LBD binding pocket (ICM v3.5-1n, Molsoft). The protein backbone is displayed as ribbon and colored by secondary structure. The residues are displayed as sticks and colored by atom type, with the carbon atoms in green. The ligands are displayed as sticks, colored by atom type, with carbon atoms in yellow. H-bonds are represented as black dashed lines. (C) Western blot showing the expression of AhR in the mouse Hepa1 derivative cell line C12, which has a significantly decreased abundance of AhR, and the C12 + AhR, wild-type AhR re-expressing cells. The blot shows 30 μg of whole cell lysate from each lane (upper panel). Semiquantitative RT-PCR for AhR target genes following exposure to vehicle (0.1% DMSO), TCDD (1 nM), 5-hydroxy-7-methoxyflavone (20 μM), and pinocembrin (20 μM) for 18 h. GAPDH expression control was used to indicate similar amount of RNA usage (lower panel). CYP1A1 (cytochrome p450 1A1) and NAPDH-QO (NADPH quinone-oxidoreductase). PCR cycle numbers are indicated.
the binding pocket itself. Indeed, electrostatic and van der Waals forces are the driving force in the total binding energy of many halogenated aromatic hydrocarbons toward the AhR.20 In addition to characterization of the prototypical AhR ligand TCDD, we also used our homology models to study the binding of structurally diverse molecules to the AhR. Recently, formylindolo [3,2-b]carbazole (FICZ) and 2-(1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) have been described as potent AhR agonists both in vitro and in vivo.8,9 These two ligands were docked into our human and mouse AhR-LBD models to investigate any interspecies differences in their respective binding modes. The two molecules docked into both the human and mouse AhR-LBD models to test any differences in their respective binding modes. The two molecules docked into both the human and mouse AhR-LBD models in a similar orientation (Figure 3A–D), establishing H-bond interactions with protein residues facing the binding cavity. FICZ made two H-bonds between the formyl carbonyl oxygen and the side chain of Ser359 (mouse) and Ser365 (human) and between the nitrogen of the carbazole group and the side chain of Gln377 (mouse) and Gln383 (human) (Figure 3B,D). On the other hand, ITE made a single H-bond between its 3-carbonyl group and the side chain of Ser359 (mouse) and Ser365 (human) (Figure 3A,C). The binding affinity of FICZ and ITE is higher for mAhR than hAhR, as estimated by the binding energy calculations of the ligand and protein complexes. The binding energies for the ligand-mAhR-LBD complex formation for FICZ and ITE were $-4.714$ and $-4.214$ kcal/mol, respectively. The estimated values for hAhR-LBD–ligand binding were $-4.12$ kcal/mol for FICZ and $-3.46$ kcal/mol for ITE. The H-bond pattern established between FICZ and ITE with mouse Ser359 and human Ser365, which are not present for TCDD, may play an important role in increasing AhR binding of these two ligands.

To further validate and apply our AhR-LBD homology model, a database of 498 natural compounds was docked into mouse AhR-LBD model binding pocket for the purpose of identifying new ligands of the AhR by virtual ligand screening (VLS).21 After the docking results were obtained, we arbitrarily set a binding energy cutoff of $-2.5$ kcal/mol, which resulted in the selection of 25 compounds that docked into the binding pocket in an allowable orientation; these 25 compounds were then tested in cell-based experiments. The list of the 25 docked structures and their respective AhR transcriptional activation results are provided in Supporting Information (SI) Table 1. Six of the 25 compounds were able to activate AhR transcription, and at least three of the nonactivating compounds strongly antagonized TCDD-induced AhR transcription (Table 2 and data not shown). The top two AhR activators that we identified by VLS were 5-hydroxy-7-methoxyflavone and pinocembrin, both of which are flavonoids. Both compounds strongly activated the AhR transcription in a dose-dependent manner (Figure 4A). The docking orientation of the two potent AhR activators identified was similar, with the 2-phenyl ring allocated in the small hydrophobic cleft surrounded by residues Phe281, Leu309, and Leu347 (Figure 4B). The 1,4-benzopyrone structure of these two flavonoids bound instead in the middle of the binding pocket surrounded by residues Cys327, His285, Met334, and Ala375 (Figure 4B). Both ligands shared

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Structure</th>
<th>Binding Energy</th>
<th>Induction of AhR Transcriptional Activity</th>
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<tr>
<td>Pinocembrin (5,7-Dihydroxyflavanone, R-form)</td>
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<td>7,8,2'-Trihydroxyflavone</td>
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</table>

+++ Very Strong activation (≥8 fold); ++ Strong activation (>4 fold); + Activation (<3 fold).
on AhR activity. 23 methoxyflavone strongly inhibited both TCDD- and 5-OH-7-transcriptional antagonist. Indeed, cotreatment with 6-OH-7-form. We tested whether this compound can act as an AhR to AhR but may not bring AhR in to a transcriptionally active activate AhR, suggesting that 6-OH-7-methoxyflavone binds to AhR transcription, whereas 3-OH-7-methoxyflavone modestly activated AhR transcription. 6-OH-7-methoxyflavone did not transcription, whereas 5-OH-7-methoxyflavone strongly induced AhR-activated AhR-LBD homology model was also utilized successfully to identify new AhR agonists by in silico structure based VLS. 15

**Conclusions**

In summary, homology models of AhR-LBD in the agonist bound conformation were built using the ICM method. 14,15 Using these models, differences in ligand binding across various species was structurally characterized. The mouse AhR-LBD homology model was also utilized successfully to identify new AhR agonists by in silico structure based VLS. 21

**Experimental Section**

**Multiple Sequence Alignment.** The AhR-LBD sequences in FASTA format for mouse, human, rat, guinea pig, rabbit, and zebrafish were retrieved from NCBI database. Multiple sequence alignment was performed online with the ClustalW program. 27

**Homology Modeling.** We used the nuclear magnetic resonance (NMR) structure of the human PAS domain of the hypoxia-inducible factor 2α (HIF-2α) available in the Protein Data Bank (PDB) 1P97 as the 3D coordinate template for the homology modeling of mouse, human, and zebrafish isoforms 1 and 2 AhR-LBD. All models were energetically refined using the internal coordinate space with Molsoft ICM v3.5.1p. 14,15

**Molecular Docking.** The receptor model is represented by five types of interaction potentials, namely, (i) the van der Waals potential for a hydrogen atom probe, (ii) the van der Waals potential for a heavy-atom probe (generic carbon of 1.7 Å radius, (iii) an optimized electrostatic term, (iv) hydrophobic terms, and (v) loan-pair-based potential, which reflects directional preferences in hydrogen bonding. The energy terms were based on the all-atom vacuum force field ECEPP/3 with appended terms from the Merck Molecular Force Field to account for solvation free energy and entropic contribution. 15 Modified intermolecular terms such as soft van der Waals and hydrogen-bonding as well as a hydrophobic term are added. Conformational sampling was based on the biased probability Monte Carlo (BPMC) procedure, which randomly selects a conformation in the internal coordinate space and then makes a step to a new random position independent of the previous one according to a predefined continuous probability distribution. It has also been shown previously that after each random step, full local minimization greatly improves the efficiency of the procedure. In the ICM-VLS (Molsoft ICM v3.5–1p) screening procedure the ligand scoring was optimized to obtain maximal separation between binders and nonbinders. 26–29 Each compound was assigned a score according to its fit within the receptor, which accounts for continuum and discrete electrostatics, hydrophobicity, and entropy parameters. 15,28,29

**Binding Energy Calculations.** The free binding energy of a ligand was estimated according to the protocol described. 30 In this method, the estimate consists of three essential terms and an additional constant term: 

$$
\Delta G_{\text{binding}} = \Delta G_{\text{H}2} + \Delta G_{\text{EL}} + \Delta G_{\text{S}} + C
$$

Every term (except for the constant) is a difference between the corresponding energy in bound and unbound states. The hydrophobic, or cavity term $\Delta G_{\text{C}}$, which accounts for the change in solvent accessible area upon complexation, is calculated as the solvent accessible area, calculated with a 1.4 Å water probe, and multiplied by 30 cal/A°. The electrostatic term $\Delta G_{\text{EL}}$, was composed of the coulombic interactions and electrostatic energy of solvation estimated with the continuum dielectric model using the boundary element method with MMFF94 partial charges, a protein dielectric constant of eight, water dielectric constant of 80, and probe radius of 1.4 Å. The entropic term was a sum of the side chain and ligand entropic terms. The contribution of each rotatable bond frozen upon binding was estimated as 0.5 kcal/mol. The constant value of 3 kcal/mol was used.

**Library of Compounds.** A database of 498 natural compounds from TimTec (Newark, DE) was used for in silico VLS. Twenty-five compounds identified by VLS from TimTec were dissolved in DMSO and tested at a final concentration of 20 μM. The structures and ≥95% purity of the tested natural compounds were confirmed by TimTec using NMR.

**Cell Culture.** All cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with L-glutamine (Mediatech Inc., VA) supplemented with 10% FBS (Tissue Culture Biologicals, CA), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Mediatech Inc., VA) in a humidified 5% CO2 atmosphere. Cells were typically passaged every three days at a dilution of 1:5.

**Figure 5.** Nuclear localization of AhR in Hepa1 cells. Cells were treated with the indicated compounds for 60 min, fixed, and then immunostained with AhR followed by fluoresceinisothiocyanate-(FITC)-conjugated secondary antibody. Cells were stained by 4,6-diamino-2-phenylindole (DAPI) to visualize nucleus and were imaged on a Zeiss Axiovert S100TV microscope.

H-bond interaction between the carbonyl group of the benzopyrone template and the side chain of residue Ser359 (Figure 4B). Furthermore, 5-hydroxy-7-methoxyflavone established a second H-bond between its hydroxyl group adjacent to the carbonyl group and the side chain of Ser359 (Figure 4B). We further tested the ability of 5-hydroxy-7-methoxyflavone and pinocembrin to activate endogenous target genes of AhR. 22 Both the compounds activated CYP1A1 and NADPH quinone reductase in an AhR dependent manner (Figure 4C). Furthermore, both the compounds promoted nuclear translocation of endogenous AhR from cytosol after one hour of exposure, confirming their status as AhR agonists (Figure 5). Out of the different flavones predicted to bind to AhR ligand binding pocket by the virtual ligand screen, 5-OH-7-methoxyflavone strongly induced AhR transcription, whereas 3-OH-7-methoxyflavone modestly activated AhR transcription. 6-OH-7-methoxyflavone did not activate AhR, suggesting that 6-OH-7-methoxyflavone binds to AhR but may not bring AhR in to a transcriptionally active form. We tested whether this compound can act as an AhR transcriptional antagonist. Indeed, cotreatment with 6-OH-7-methoxyflavone strongly inhibited both TCDD- and 5-OH-7-methoxyflavone-induced AhR transcription (SI Figure 1). There is precedence for differential effects of different flavones on AhR activity. 23–25 β-Naphthoflavone is a strong AhR agonist, whereas α-naphthoflavone is a partial agonist/antagonist of AhR transcription. 26 By comparing the binding pattern of 7-methoxyflavone derivatives to AhR ligand binding pocket, we were unable to distinguish agonists from potential antagonists.
Semiquantitative Polymerase Chain Reaction (PCR). For analysis of AhR target gene induction, a pair of Hepa1c1c7 derived cell lines were used, one with significantly reduced AhR expression (C12) and the same cell line stably expressing a WT AhR construct (Hepa1 C12 + AhR), as described in the SI.

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Supporting Information Available: Binding energy calculations from molecular docking of natural compounds database into mouse AhR ligand binding domain and activation of AhR transcription by compounds identified by virtual ligand screening to bind the AhR ligand binding pocket (Supplemental Table 1). 6-Hydroxy-7-methoxyflavone is an AhR antagonist (Supplemental Figure 1). Semi-quantitative polymerase chain reaction (Experimental Methods). This material is available free of charge via the Internet at http://pubs.acs.org.

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