Suppression of cytokine-mediated complement factor gene expression through selective activation of the Ah receptor with 3´, 4´-dimethoxy-α-naphthoflavone

Iain A. Murray, Colin A. Flaveny, Christopher R. Chiaro, Arun K. Sharma,Rachel S. Tanos, Jennifer C. Schroeder, Shantu G. Amin, William H. Bisson, Siva K. Kolluri and Gary H. Perdew.

Molecular Pharmacology

Supplemental Figure S1. Chemical synthesis of 3´-methoxy-α-naphthoflavone. Reaction scheme for the synthesis of 3´-methoxy-α-naphthoflavone. A detailed synthesis protocol is described in materials and methods.

Supplemental Figure S2. Huh7 cytotoxicity assays. The effect of DiMNF on Huh7 cell viability was assessed through MTS and clonogenic colony formation assays. (**A**) Cells were incubated with vehicle, 10 μM αNF, or 4 and 10 μM DiMNF. Following 48 h incubation cell viability was determined. Data represent mean ± SEM survival expressed as percentage of vehicle-treated cells. (**B**) Huh7 cells were seeded at 1 x 10^3 cells/plate, after overnight incubation cells were treated as indicated with vehicle (DMSO), 10 μM αNF, 4 and 10 μM DiMNF or 10 μM SGA360. After 24 h, cells were washed and cultured for an additional 14 days, after which time cells were stained with Coomassie Brilliant Blue for 2 min and colonies counted. Data represent percentage colony number ± SEM compared to vehicle-treated controls. Statistical significance is indicated by an asterisk (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). Inter-treatment statistical comparisons are indicated by lower case letters.

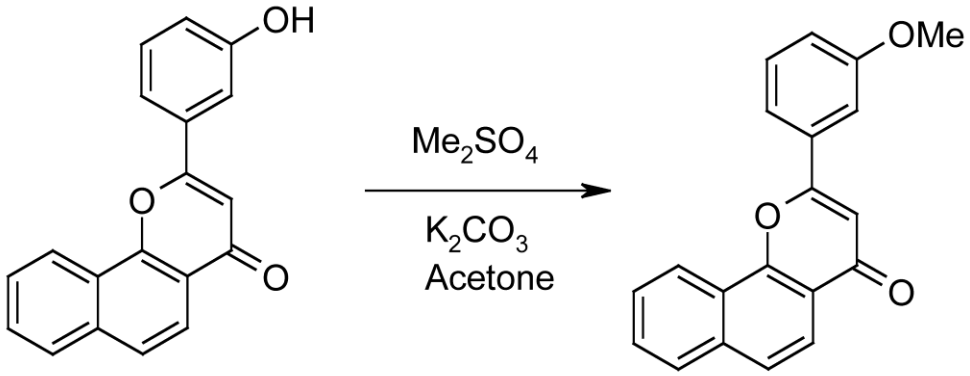
Supplemental Figure S3. Characterization of siRNA-mediated AHR knockdown in Hep3B cells. (**A**) Scrambled or AHR-specific siRNA were introduced into Hep3B cells through electroporation. 48 h post transfection, protein was isolated and resolved by SDS-PAGE. Following transfer to PVDF membrane, blots were probed as indicated for AHR and β-actin. (**B**) Hep3B cells targeted with scrambled or AHR-specific siRNA were incubated with vehicle (DMSO) or 10 nM TCDD for 4 h and

CYP1A1 mRNA expression analyzed by quantitative PCR. Data represent mean *CYP1A1* mRNA ± SEM normalized to the constitutively expressed ribosomal *L13A* mRNA. Statistical significance is indicated by an asterisk (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). Inter-treatment statistical comparisons are indicated by lower case letters.

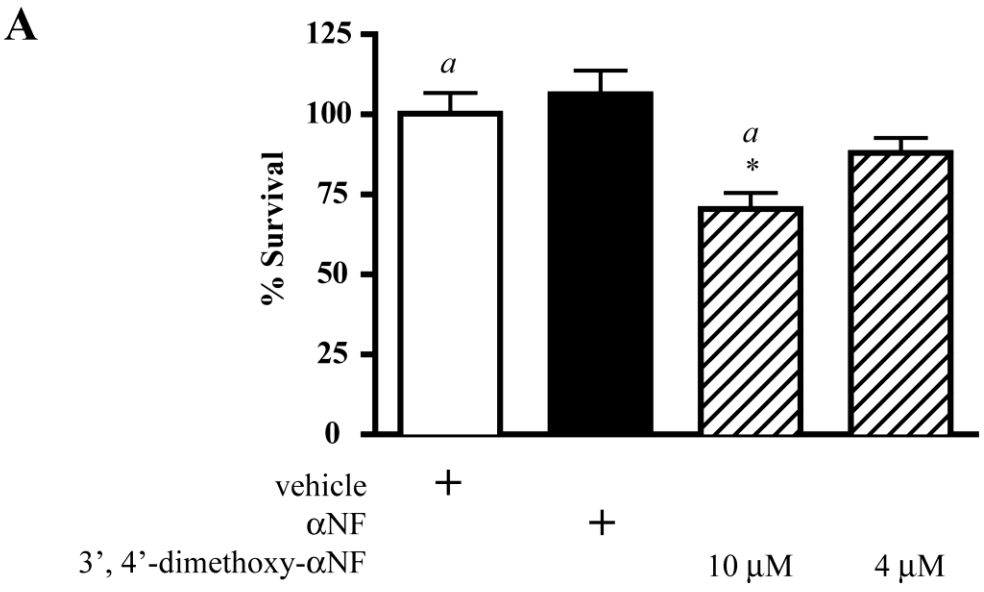
Supplemental Figure S4. In silico ligand docking algorithm predicts differential orientations between αNF and the SAhRM DiMNF in the ligand binding pocket of AHR (Color version of Figure 10.). Predicted docking orientations of (**A**) α-NF and (**B**) DiMNF within the ligand binding pocket of human AHR highlighting common interaction with S365 but divergent interaction with T289 with 3´, 4´-dimethoxy-αNF.

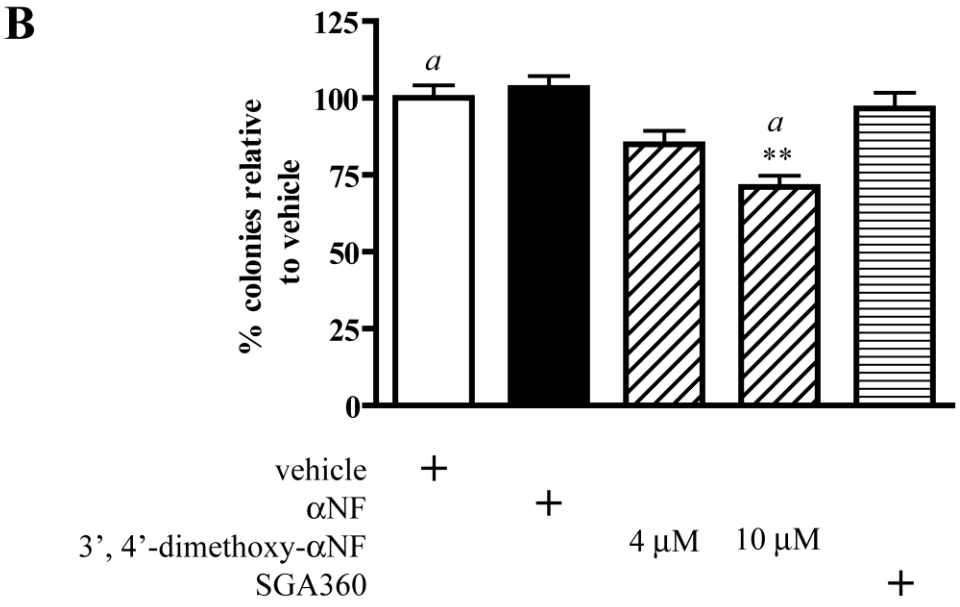
Supplemental Figure S5. Chemical structures of all chemicals used in this manuscript.

Supplemental Figure 1

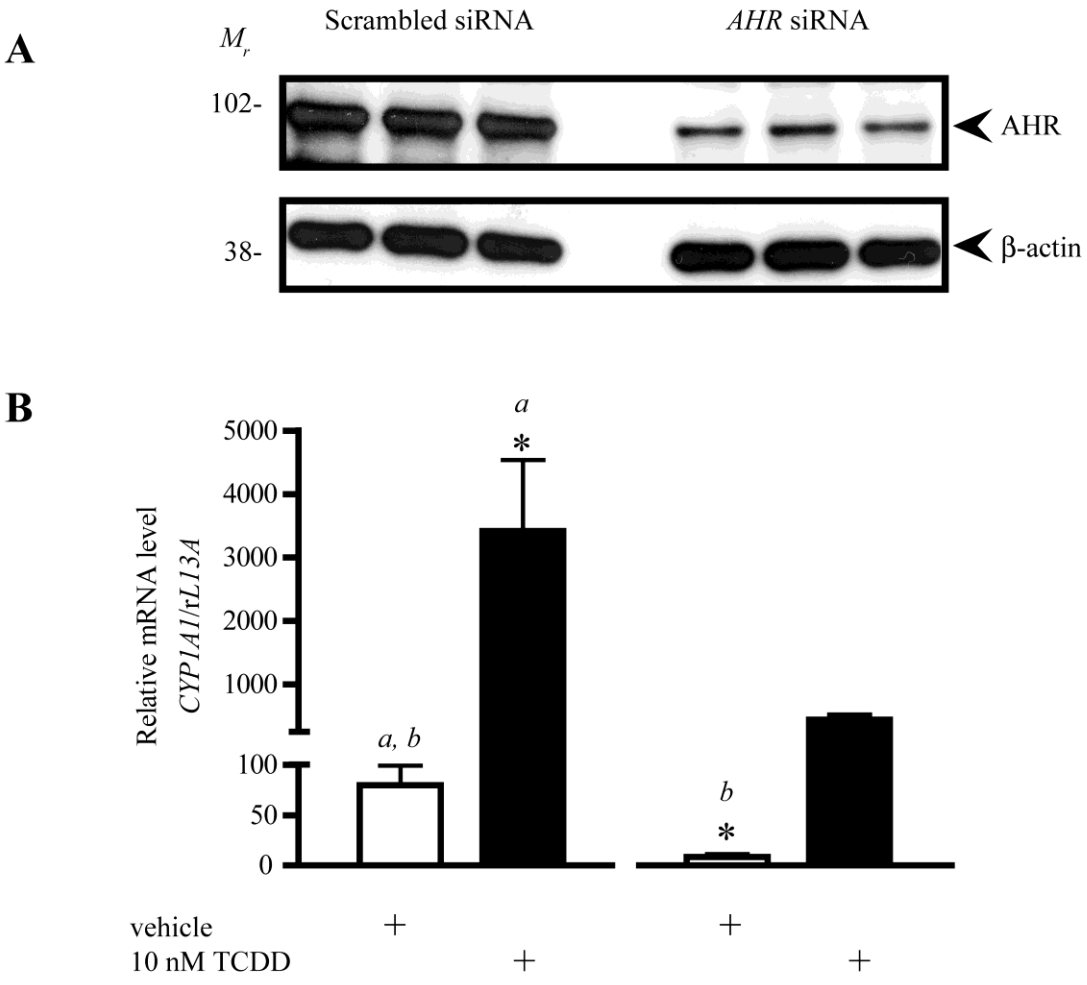


Supplemental Figure 2





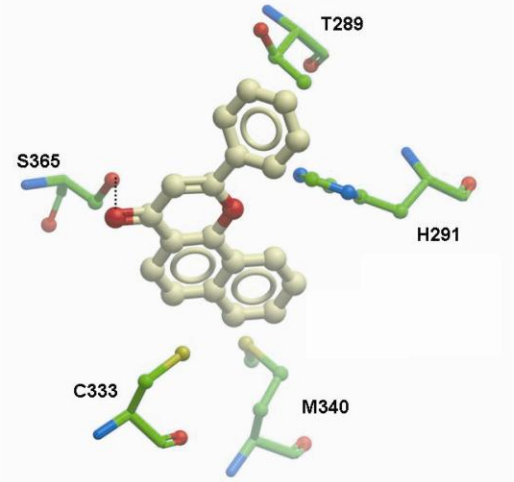
Supplemental Figure 3



Supplemental Figure 4

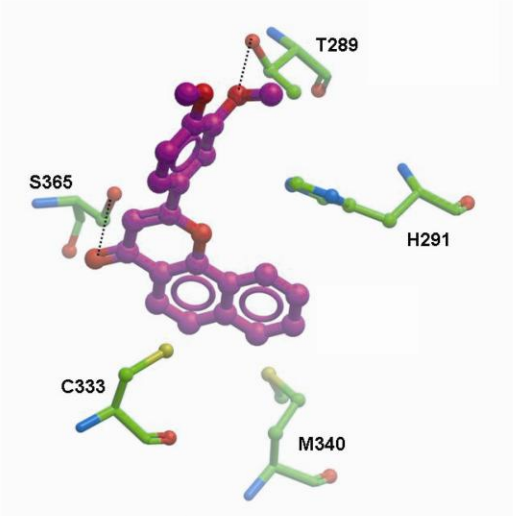
Predicted binding of αNF in the binding pocket of the human AHR

Binding Energy: - 4.06 kcal/mol

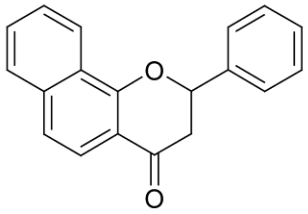


Predicted binding of DiMNF in the binding pocket of the human AHR

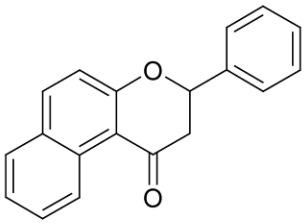
Binding Energy: - 5.44 kcal/mol



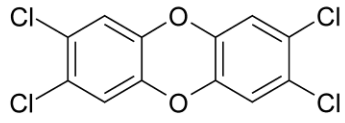
Supplemental Figure 5



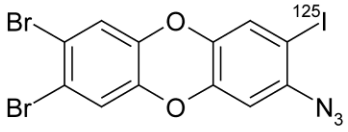
α-naphthoflavone



β-naphthoflavone



TCDD



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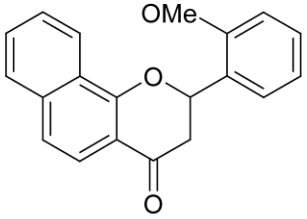
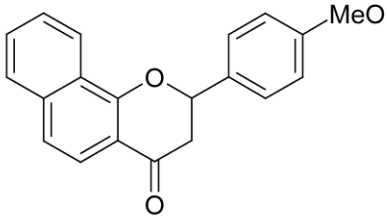


2‘-methoxy-α-NF



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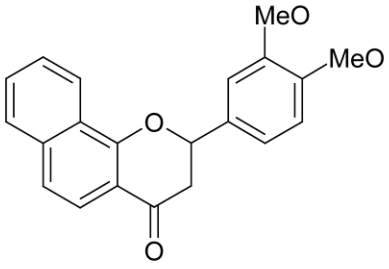
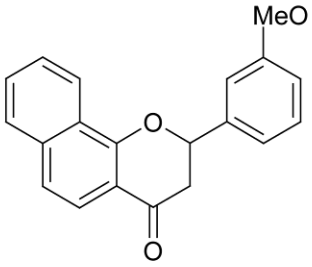


3‘, 4‘-dimethoxy-α-NF



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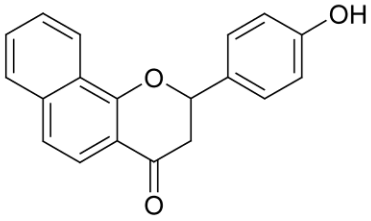

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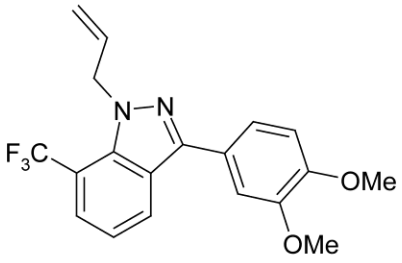

SGA360