# ACTIVATION OF THE Ah RECEPTOR AND Ah RECEPTOR SIGNAL TRANSDUCTION PATHWAY BY TRYPTOPHAN-DERIVED AGONISTS

Michael S. Denison[1], Dal-Ho Han[1], Sharon Heath-Pagliuso[1], John Scovill[2], Laurent Meijer[3], Leandros Skaltounis[4], Elizabeth M.J. Gillam[5] and Scott R. Nagy[1]

[1]Department of Environmental Toxicology, Meyer Hall, University of California, Davis, CA, USA
[2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA
[3]C.R.N.S, Cell Biology Group & UPS-2682, Station Bioloique, Roscoff cedex, Bretagne, France
[4]Department of Pharmacognosy, University of Athens, 15771 Athens, Greece
[5]Department of Physiology and Pharmacology, University of Queensland, Brisbane, Australia.

## Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related halogenated aromatic hydrocarbons (HAHs), such as polychlorinated dibenzo-p-dioxins (PCDDs), biphenyls (PCBs) and dibenzofurans (PCDFs), exert the majority of their effects via their bind to and activation of the Ah receptor (AhR) and AhR-dependent gene expression.[1] Recently, it has become abundantly clear that the AhR can be bound and activated by structurally diverse range of chemicals and numerous studies have described and characterized a variety of naturally-occurring dietary chemicals that can directly bind to and activate and/or inhibit the AhR signaling pathway.[2,3] Given that the greatest source of exposure of animals and humans to AhR ligands (both synthetic and natural) comes from the diet, an understanding of the structural diversity of AhR ligands and their biological and toxicological activity and potency is needed. Numerous naturally-occurring indoles and indole-containing chemicals have been identified that can activate the AhR signaling pathway (Figure 1). The majority of these AhR ligands are formed from tryptophan (TRP) as a result of various biological and physiochemical processes. Potent AhR activators have been observed to be formed from the photooxidation of TRP and the acidic condensation of dietary indole-3-carbinol, while other naturally-occurring TRP metabolic and biosynthetic products (i.e. tryptamine, indole acetic acid, indirubin and indigo) were shown to bind to and activate the AhR and AhR-dependent gene expression.[2,3] Given the lack of information regarding the structural diversity of AhR ligands as well as the lack of information with respect to endogenous physiological ligands for the AhR, further analysis of this rather large group of naturally occurring indole ligands is warranted. Accordingly, we have used several AhR-based bioassay systems to examine the ability of a variety of natural and synthetic indoles and indole-containing chemicals to activate the AhR and AhR-dependent gene expression.

## Materials and Methods

The ability of various chemicals to stimulate AhR-dependent transformation and DNA binding was determined by gel retardation analysis using guinea pigs cytosol.[4] Determination of the ability of various chemicals to stimulate AhR-dependent gene expression was carried out using a recombinant mouse hepatoma cell line that contains an AhR-responsive green fluorescent protein (GFP) reporter plasmid pGreen1.1 (H1G1.1c3 cells). These cells respond to AhR agonists with the induction of GFP in a time-, dose-, ligand-dependent manner and GFP fluorescence activity is measured in intact cells as described.[5]

**Results and Discussion**

Numerous indoles and indole-containing chemicals have been shown to be AhR agonists (figure 1).[2] We have examined a series of derivatives of several of these indole-containing ligands in order to better understand the structural diversity of AhR ligands and to determine whether key structural characteristics exist in this class naturally-occurring ligands. Tryptanthrins are a class of naturally occurring compounds found in plants as well as being a microbial metabolite that has some therapeutic uses. Given their high degree of structural similarity to one of the most potent indole AhR agonists, indolo[3,2-b]carbazole, and previous studies demonstrating their activity at the AhR,[6] we first examined a series of trytpanthrins.[7] These results revealed several compounds (2, 4, 5, 11) that were relatively potent activators of AhR-dependent gene expression (with EC50s of between 14-30 nM compared to TCDD at 0.01 nM). Subsequent studies demonstrated the ability of these specific compounds to stimulate AhR DNA binding in gel retardation analysis and to induce CYP1A1 expression as determined by rtPCR (data not shown). Our results also demonstrate that these compounds were significantly more potent than the classical PAH inducers beta-naphthoflavone and 3-methylcholanthrene. Additional analysis was focused on the ability of a series of 10 substituted indoles to activate AhR-dependent gene expression.[8] The majority of these chemicals were relatively weak activators, with only 2-methylindole and oxazolodiindole inducing reporter gene expression in the low to mid µM concentration range. Previous studies have also reported that indigo and indirubin were potent activators of the AhR in an AhR-Arnt containing yeast cell bioassay system and that they were equipotent or 50-fold more potent than TCDD, respectively.[9] Our results, however, demonstrate that while these compounds are relatively active (figure 3), they are 50,000 to 100,000-fold less potent than TCDD. This significant difference from previously published results likely derives from the metabolic lability of these chemicals in mammalian cells combined with the limitated solubility of TCDD in aqueous yeast growth media. Subsequent analysis of 26 substituted synthetic indirubins resulted in the identification of five compounds that were extremely potent activators of the AhR-dependent gene expression (with EC50s between 1-8 nM, compared to TCDD at 0.015 nM).[10] These results demonstrate that the substituted indirubins represent some of the most potent nonHAH AhR activators that have been identified to date. Current studies are examining the affinity for AhR ligand binding domain and the relative ability to stimulate AhR transformation and DNA binding. While the biological/toxicological significance of these results remain to be elucidated, these results do suggest that indole-containing compounds likely represent one major group of potent naturally-occurring activators of the AhR to which we are exposed.

**References**

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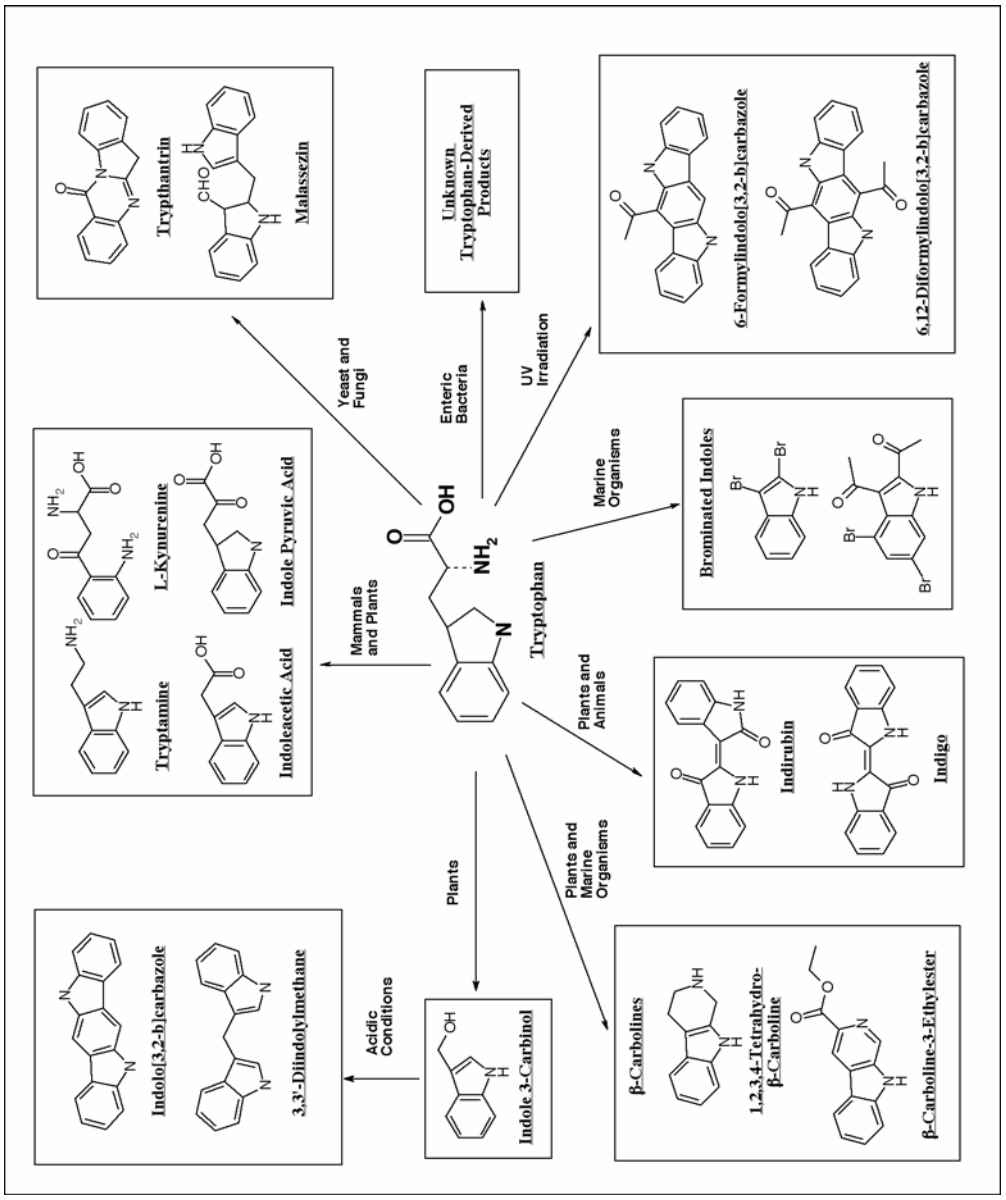
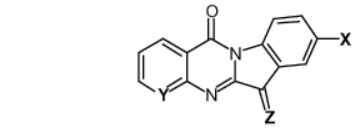



Figure 1. Tryptophan-derived chemicals which have been shown to bind to and/or activate the AhR and AhR-dependent gene expression. Reprinted from reference 2.



| Compound | X | Y | Z | $EC_{50}$(nM) | IEF[a] |
|---|---|---|---|---|---|
| TCDD | - | - | - | 0.01 | 1 |
| 1 | H | C | O | >10,000 | ND[b] |
| 2 | I | C | O | 30 | 3,000 |
| 3 | F | C | O | >10,000 | ND |
| 4 | Cl | C | O | 30 | 3,000 |
| 5 | Br | C | O | 20 | 2,000 |
| 6 | $NO_2$ | C | O | >10,000 | ND |
| 7 | $OCF_3$ | C | O | 1,000 | 100,000 |
| 8 | H | N | O | 10,000 | 1,000,000 |
| 9 | F | N | O | >10,000 | ND |
| 10 | Cl | N | O | 60 | 6,000 |
| 11 | Br | N | O | 14 | 1,400 |
| 12 | H | C | $C(CN)_2$ | 1,000 | 100,000 |
| 13 | H | C | $CHC_6H_5$ | >10,000 | ND |

a. Induction equivalency Factor (IEF) was calculated by dividing the $EC_{50}$ of TCDD by the $EC_{50}$ of the test compound.

b. Not determined.

Figure 2. Determination of the relative potency of series of tryptanthrins to induce AhR-dependent GFP reporter gene expression in H1G1.1c3 cells.

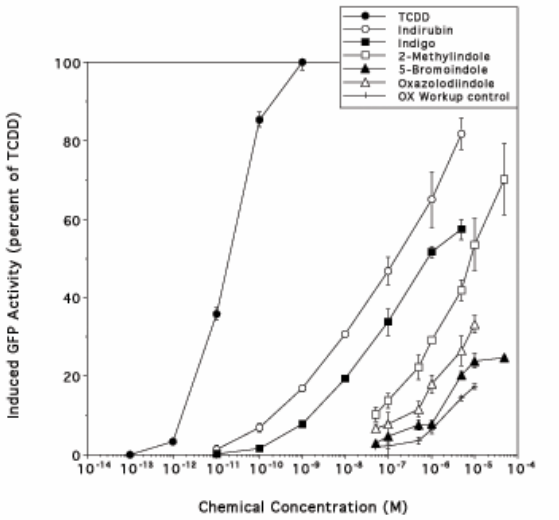



Figure 3. Dose-dependent induction of AhR-dependent GFP reporter gene expression in H1G1.1c3 cells by TCDD, indirubin, indigo and several substituted indoles.