Species-specific partial agonism on AhR by 5-fluorinated-3-(4-aminophenyl)benzothiazole (5F 203)

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Introduction

The Aryl Hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that induces expression of a number of genes encoding drug metabolizing enzymes, such as CYP1A1, CYP1A2 and CYP1B1 (Whitlock 1999). It has been suggested that the AhR may mediate the action of anticancer phenylbenzothiazoles analogues (Trapani et al 2003). 5-fluorinated-(4-amino-3-methylphenyl)benzothiazole (5F 203) was the most potent anticancer agent in several cancer cells, and its prodrug, lysylamide Phortress, is in Phase I clinical trial. This study investigates whether the benzothiazole 5F 203 binds to the rat hepatic AhR, quantifies the binding in rat cytosol and the EC_{50} for *CYP1A1* mRNA induction in rat hepatoma H4-II-E cells and human MCF-7 breast cancer cells. A comparison of the binding affinity and potency for inducing CYP1A1 allowed the classification of this drug as a species-specific partial agonist.

Methods

[^{3}H]-TCDD competition assays: the competition assays for AhR using [^{3}H]-TCDD were conducted essentially as described by (Poland et al. 1976), except with buffers as (Bradfield et al. 1988) and using tetrachloroazoxybenzene (TCAOB) as a competitor. Briefly, 1mg of cytosolic protein prepared from male Wistar rat were incubated with a range of concentrations of [^{3}H]-TCDD ± TCAOB (200-fold molar excess) ± a range of concentrations of 5F 203 for 16 h at 4 °C. The assay was terminated by the addition of charcoal, followed by centrifugation and measurement of radioactivity by liquid scintillation.

CYP1A1 induction assay: the general conditions consist of incubating rat hepatoma H4-II-E and human breast carcinoma MCF-7 cells for 24 h to achieve high density. The following day, cultures were treated with vehicle control 0.1% DMSO (negative control), 10 nM TCDD (positive control) and 5F 203 for 4 h. Chemicals were diluted in DMSO then further diluted into 200µl of day-old medium. *CYP1A1* mRNA induction was determined using the TaqMan real-time RT-PCR, with *ß-actin* and *AhR* as reference genes. Maximal levels of *CYP1A1/ß-actin* mRNA ratios were determined as % of control, relative to 10 nM TCDD, which is arbitrarily set at 100%.

Results and discussion

Characterization of the binding of 5F 203 to rat AhR

Under the conditions of the binding assay, the apparent k_d and B_{max} for [^{3}H]-TCDD were 0.37 ±0.05 nM and 40 fmol/mg, respectively (data not shown). Using standard assay conditions, the drug concentration that displace 50% of TCDD binding (IC_{50}) were calculated assuming one site-competition by nonlinear regression (GraphPad Prism 5.0), and a K_i derived therefrom. (Fig. 1) shows the displacement curve of [^{3}H]-TCDD from AhR specific binding sites by increasing concentrations of 5F 203. The K_i derived for 5F 203 was 2.8 nM (95% CI, 2-5). These results revealed that 5F 203 is a potent high-affinity ligand for rat AhR.

Characterization of CYP1A1 induction in H4-II-E cells

5F 203 induced *CYP1A1* mRNA to similar maximal induced levels as seen with TCDD in H4-II-E cells. However, 5F 203 was 50,000-fold less potent than TCDD in rat cells (Fig. 2), with an EC_{50} of 3µM. Two possibilities were proposed to explain the much lower potency of the high-affinity ligand 5F 203 in rat cells:
1) Low intracellular concentration of 5F 203, as a result of metabolism, uptake, excretion, etc.
2) Partial agonism of 5F 203.
The CYP1A1/ß-actin mRNA ratios were still increasing linearly in response to 5F 203 at 4 h treatment, suggesting that 5F 203 is not subjected to significant metabolism at an early time point (4 h) (data not shown).

Interpretation for the low potency of 5F 203 in H4-II-E cells: partial agonsim

To address the second possibility, the antagonistic activity of 5F 203 on TCDD-induced *CYP1A1* mRNA was examined. H4-II-E cells were treated with increasing concentrations of TCDD ± 1µM 5F 203: (Fig. 3) demonstrated that the EC_{50} of TCDD in the absence of 5F 203 was 46 pM, but co-incubation with 5F 203 increases the EC_{50} to 5 nM. These results prove that 5F 203 competitively inhibited TCDD in H4-II-E cells. The strength of the effect of 5F 203 on TCDD-induced AhR signaling in H4-II-E cells excludes the possibility that the lower potency of 5F 203 in rat cells is a function of low intracellular concentration of 5F 203. The partial agonism of 5F 203 quantitatively explains the low potency of this drug as an inducer of *CYP1A1* mRNA in H4-II-E cells (Schild analysis, unpublished data).

Characterization of CYP1A1 induction by 5F 203 in human MCF-7 cells

5F 203 induced *CYP1A1* mRNA to similar maximal induced levels as seen with TCDD with an EC_{50} in MCF-7 (human) cells was 2 nM, which is ~10-fold less potent than TCDD (Fig.4). MCF-7 cells were treated with increasing concentrations of TCDD ± 500 pM 5F 203: (Fig. 5) demonstrated that the EC_{50} of TCDD (300pM) was not affected by 5F 203, thus no antagonism was detected.

To our knowledge, no AhR ligand has previously been shown to be a potent agonist in one species, and a partial (ant)agonist in another species. This study proves that 5F 203 can competitively antagonize TCDD from AhR specific binding sites in rat cytosol, which excludes the possibility of a ligand-independent mechanism of AhR activation. From the other hand, 5F 203 competitively antagonized the induction of CYP1A1 caused by TCDD in rat cells and the antagonizing effect was quantitated. These results prove that 5F 203 competitively inhibited TCDD in H4-II-E cells, but not in MCF-7 cells and reveals species-specific differences in AhR agonism.

Acknowledgments
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References
Bradfield C. A. and Poland, A., Mol. Pharmacol 1988; 34: 682-88.
Poland A., Glover E. and Bradfield C.A., J. Biol. Chem ???. ; 251: 4936-46.
Trapani V., Patel V., Leong C.O., Ciolino H.P., Yeh G.C., Hose C., Trepel J.B., Stevens M.F, Sausville E.A. and Loaiza-Perez A. I. Br. J. Cancer 2003; 88: 599-605.
Whitlock JP Jr. Annu. Rev. Pharmacol. Toxicol 1999; 39: 103-112.

Figure 1: Displacement of [^{3}H]-TCDD from AhR specific binding sites by 5F 203

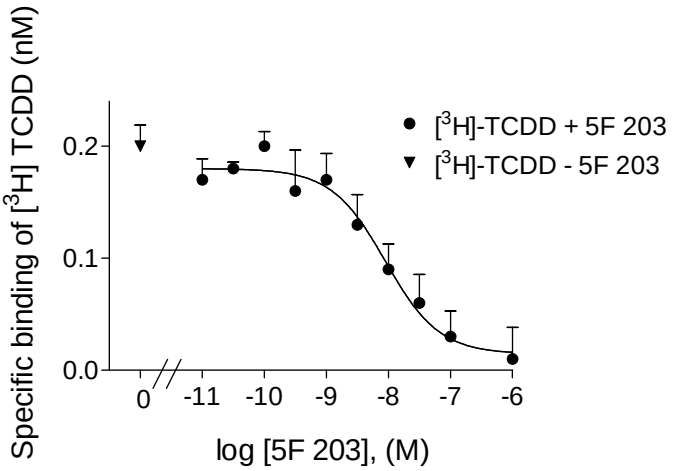

Figure 2: Induction of *CYP1A1* mRNA by TCDD and 5F 203 in H4-II-E cells

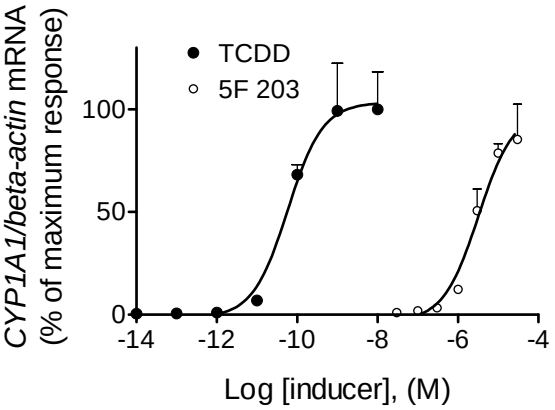

Figure 3: Inhibition of TCDD-induced *CYP1A1* mRNA by 5F 203 in H4-II-E cells

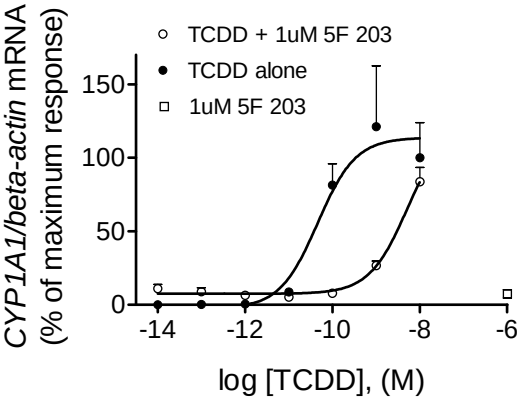

Figure 4: Induction of *CYP1A1* mRNA by TCDD and 5F 203 in MCF-7 cells

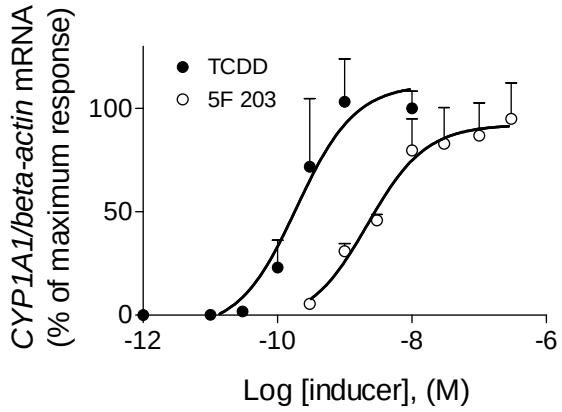

Figure 5: Effect of 5F 203 on TCDD-induced *CYP1A1* mRNA in MCF-7 cells