2-SUBSTITUTED PHENANTHRIDINONES AS ARYL HYDROCARBON (AH) RECEPTOR ANTAGONISTS

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Abstract:

A new synthetic route was utilized to prepare 2-t-butyl-, phenyl-, isopropyl-, iodo-, bromophenanthridinone and phenanthridinone. The relative affinites of these congeners for the aryl hydrocarbon (Ah) receptor were determined using rat hepatic cytosol and [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the radioligand. The effects of these ligands on the TCDD-induced transformation of the rat hepatic cytosolic receptor were also determined by gel mobility shift assays using a consensus ^{32}P-labeled dioxin resposive element (DRE). All of the 2-substituted phenanthridinones inhibited TCDD-induced transformation. The most potent inhibitor of TCDD-induced transformation was 2-phenylphenanthridinone which at 5 μM inhibited 83% of TCDD-induced transformation to a DRE-binding form. The results suggested that these congeners may exhibit partial Ah receptor antagonist activities and this was investigated by determining the inhibitory effects of these congeners on TCDD-induced ethoxyresorufin *O*-deethylase (EROD) activity in rat hepatoma H4II E cells in culture. All of the congeners inhibited the TCDD-induced response. The most potent inhibitor of TCDD-induced EROD activity was 2-phenylphenanthridinone which at 1 μM inhibited 91% of TCDD-induced EROD activity.

Introduction:

One of the hallmarks of Ah receptor-mediated responses is the correlation between structure-receptor binding versus structure-activity relationships for both polynuclear aromatic and chlorinated aromatic hydrocarbons[1-2]. Recent studies have reported that several structurally diverse compounds can partially antagonize some of the responses elicited by TCDD[3-7]. For example, the commercial PCB mixture Aroclor 1254 partially antagonized TCDD-induced aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities in H4II E cells and mouse hepatic microsomes, and immunotoxicity and teratogenicity in C57BL/6 mice. To date all of the antagonists possess several common properties which include moderate in vitro binding affinity for the Ah receptor and low Ah receptor agonist activity.

It has previously been reported that commercially-available phenanthroline derivatives inhibited TCDD-induced transformation of the Ah receptor to a DRE binding

form[8]. The phenathrolines are structurally similar to ethidium bromide, psoralens, and other DNA-intercalating agents, which might inhibit protein-DNA interactions. It has been hypothesized that the binding of phenanthroline to the AhR may impede transformation to the DRE-binding form by inducing a conformation that has reduced affinity for DNA[8]. Recently phenanthrolines and commercially available phenanthridinones were shown to inhibit poly (ADP-ribose)-synthetase[9]. It has been suggested that the role for this enzyme includes implications in DNA repair, cell differentiation, control of cell cycle, transformation, transcription and alteration of chromatin architecture. The objective of this study was to determine the Ah receptor agonist and partial antagonist activities of a series 2-substituted phenanthridinones.

Materials and Methods:

Chemicals: The 2-substituted phenanthridinones were synthesized via initial reaction of *o*-nitrobenzoyl chloride with 4-substituted anilines, followed by reduction with iron/acetic acid and internal diazocyclization with amyl nitrite to give the 2-substituted phenanthridinones (details of the synthesis will be reported separately).

Treatment of cells: Rat hepatoma H4II E cells were grown as a continuous cell line in α-MEM medium supplemented with 2.2 mg/ml tissue culture grade sodium bicarbonate, $NaHCO_3$, 5% fetal calf serum, 10 ml/l antibiotic/antimycotic solution. Stock cultures were grown in 150 cm^2 tissue culture flasks and incubated in a humidified mixture of 5% CO_2 and 95 % air under atmospheric pressure.

Treatment of Animals: Male Long-Evans rats (21 days old, ≈100g) were obtained from Harlan Laboratories (Houston, TX).

Preparation of Hepatic Cytosol: Rat hepatic cytosol was prepared according to the described procedure[10].

Hydroxyapatite (HAP) Assay: The IC_{50} values for competitive receptor binding affinities were determined using freshly prepared rat hepatic cytosol (2 mg protein/ml) and the HAP procedure essentially as described[10]. Different concentrations of the ligands were used to determine displacement curves; the IC_{50} values were determined from a probit plot of the percentage of [^{3}H]TCDD bound versus log concentrations of the ligands.

Gel Shift Assay: DNA binding was measured using a gel shift assay with a complementary pair of oligonucleotides containing the sequence 5'-GATCTGGCTCTTCTCACGCAACTCCG-3'[11].

Ethoxyresorufin O-deethylase (EROD) Induction Assay: EROD activity was determined fluorometrically as described[12].

Statistics and Controls: The statistical differences between different treatment groups was determined by ANOVA and are noted ($p < 0.05$). The data were expressed as means ± standard errors for at least 4 determinations for each experiment.

Results:

The IC_{50} values for the binding of the 2-substituted phenanthridinones were determined using 1 nM [^{3}H]TCDD as the radioligand and rat cytosolic Ah receptor. The IC_{50} values were derived from the concentration-dependent competitive displacement of [^{3}H]TCDD from the rat cytosolic Ah receptor by the various unlabeled ligands using the HAP assay procedure. Logit analysis of the competitive binding data gave IC_{50} values of

1.0±0.2, 1.6±0.4, 0.8±0.2, 0.6±0.2, 0.4±0.1, 0.7±0.1 μM for the 2-t-butyl-, phenyl-, isopropyl-, iodo-, and bromophenanthridinones and phenanthridinone. All of the congeners exhibited modest affinty for the Ah receptor.

The effect of these congeners on the TCDD-induced transformation of the rat hepatic cytosolic receptor, as determined by gel mobility shift assays, are summarized in Table I. The intensity of the retarded DRE-complex after incubation of cytosol with 5 nM TCDD alone was arbitrarily set at 100% and the relative band intensities in the different tretment groups were compared to this value. Cotreatment of the cytosol with 5 nM TCDD and 5 μM concentrations of the 2-substituted phenanthridinones resulted in a decrease in the intensity of the TCDD-induced retarded band The most active inhibitor of TCDD-induced transformation was the 2-phenyl analog which inhibited 83% of the TCDD-induced transformation to a DRE-binding form.

The activities of 2-substituted-phenanthridinones as partial antagonists of TCDD-induced EROD activity were also investigated in rat hepatoma H4II E cells treated with 1 nM TCDD and 1 μM concentrations of the phenanthridinone analogs. The results in Table I summarize the interactive effects of these congeners on the induction of EROD activity by 1 nM TCDD. All of the congeners caused a decrease in TCDD-induced EROD activity. 2-Phenylphenanthridinone was the most active compound and 91% of the TCDD (1 nM)-induced response was inhibited by a 1 μM concentration.

This data shows that the 2-substituted phenanthridinones represent a new structural class of Ah receptor ligands and some of these componds may also be active as partial Ah receptor antagonists (Supported by National Institutes of Health ES03843).

Table I Effect of 2-Substituted Phenanthridinones on TCDD-Induced Transformation of the Rat Hepatic Cytosolic Receptor and TCDD-Induced EROD Activity.

Treatment	DRE Binding[A]	EROD Activity[B]
TCDD	100.0±0.0	100.0±0.0
TCDD + 2-t-butylphen.	55.9±7.6*	41.3±0.4**
TCDD + 2-phenylphen.	17.4±2.1*	9.0±0.1**
TCDD + 2-isopropylphen.	24.7±1.3*	66.1±0.3**
TCDD + 2-iodophen.	24.3±3.9*	57.2±2.3**
TCDD + 2-bromophen.	37.3±7.3*	43.6±1.7**
TCDD + phenanthridinone	45.4±4.5*	71.5±11.0

A: % of Response to 5 nM TCDD for DRE Binding Activity
B: % of Response to 1 nM TCDD for EROD Activity

*Statisitically lower ($p<0.05$) than cytosol transformed with 5 nM TCDD alone.
**Statistically lower ($p<0.05$) than H4II E cells treated with 1 nM TCDD alone.

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