CYTOTOXICITY AND ARYL HYDROCARBON RECEPTOR–MEDIATED ACTIVITY OF N-HETEROXYCIC POLYCYCLIC AROMATIC HYDROCARBONS: STRUCTURE–ACTIVITY RELATIONSHIPS

IVA SOVADINOVA,† LUDĚK BLÁHA,‡† JAROSLAV JANOŠEK,† KLÁRA HILSCHEROVÁ,† JOHN P. GIESY,‡§

†RECETOX—Research Centre for Environmental Chemistry and Ecotoxicology, Masaryk University, Kamenice 126/3, CZ62500 Brno, Czech Republic
‡Department of Biology & Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, China
§Department of Zoology, National Food Safety and Toxicology Center, Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824, USA

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Abstract—Toxic effects of many persistent organic pollutants (e.g., polychlorinated biphenyls or polychlorinated dibenzo-p-dioxins and furans) are mediated via the aryl hydrocarbon receptor (AhR). Although polycyclic aromatic hydrocarbons (PAHs) and their derivatives also activate AhR, their toxic effects remain to be fully elucidated. In the present study, we used the in vitro H4IIE-luc transactivation cell assay to investigate cytotoxicity and potencies to activate AhR by 29 individual PAHs and their N-heterocyclic derivatives (aza-PAHs). The aza-PAHs were found to be significantly more cytotoxic and more potent inducers of AhR than their unsubstituted analogues. Several aza-PAHs, such as diben[b]acridine or diben[a]acridine, activated AhR within picomolar concentrations, comparable to the effects of reference 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ellipsoid volume, molar refractivity, and molecular size were the most important descriptors derived from the modeling of quantitative structure–activity relationships for potencies to activate AhR. Comparable relative toxic potencies (induction equivalency factors) for individual aza-PAHs are derived, and their use for evaluation of complex contaminated samples is discussed.

Keywords—Aza-arenes Polycyclic aromatic hydrocarbons Dioxin-like toxicity Aryl hydrocarbon receptor Quantitative structure–toxicity relationships

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a major class of organic contaminants in industrial and urban regions worldwide, and they are ubiquitous in the environment. Sixteen priority PAHs are monitored by the U.S. Environmental Protection Agency, but many compounds remain overlooked in monitoring programs. These include, for example, high-molecular-weight mutagenic PAHs [1,2], nitroderivatives and oxygenated PAHs [3,4], and N-heterocyclic aromatic compounds, such as aza-PAHs or aza-arenes [4,5]. The aza-PAHs may originate from natural sources, such as alkaloids, mycotoxins, or nucleotides. However, they are released predominantly as anthropogenic contaminants by incomplete combustion of fossil fuels, spills, or industrial effluents or as a result of oil drilling and refining, wood preservation, and tobacco smoking [6–8]. The aza-PAHs are concomitantly widespread with their parent analogues, and they have been detected in the air [3], in water and sediments [4,9], and in soil [10]. However, our understanding of their occurrence, environmental fate, biological metabolism, and effects is still limited. Although aza-PAHs outnumber the unsubstituted homocyclic PAHs, their environmental concentrations are lower than those of the parent compounds (1–10% of the total PAH concentrations [11]). However, greater polarity of aza-PAHs, along with higher water solubility and bioavailability may result, in more significant effects, even at lower environmental concentrations [12].

The effects of a limited number of aza-PAHs, particularly low-molecular-weight compounds, have been investigated with algae, invertebrates, and fish [13–15]. Some benzazacridines and dibenzazacridines were found to be mutagens and carcinogens and to cause nongenotoxic effects, such as (anti)estrogenicity [4,16–18]. Modulation of the intracellular aryl hydrocarbon receptor (AhR) is one of the major toxicity mechanisms of many organic environmental contaminants, such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and furans, and the in vivo effects related to the activation of AhR include porphyria, immunotoxicity, developmental, and reproductive failure or carcinogenicity. Polycyclic aromatic hydrocarbons and their derivatives also have been shown to modulate AhR [19], but their in vivo toxicity directly mediated by AhR remains disputable. The risk assessment of polychlorinated dibenzo-p-dioxins and furans and of PCBs uses the concept of toxic equivalency factors—that is, toxic potencies of individual chemicals related to reference 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [20]. A similar approach of relative toxic potencies also has been proposed for other nonhalogenated pollutants, such as PAHs [3,21]. Evidence also suggests that aza-PAHs modulate AhR and induce AhR-dependent hepatic microsomal mixed-function oxidases and cytochromes P450 (CYP450s) [4,5,22–24]. To date, however, only a limited number of aza-PAHs have been studied.

In the present study, we investigated the in vitro effects of 22 individual aza-PAHs and seven parent PAHs. The aim of the present study was to obtain principal information regarding
the cytotoxicity and the potencies to induce AhR of these poorly characterized xenobiotics. Additionally, we studied quantitative structure–activity relationships (QSAR), and we derived induction equivalency factors (IEFs) for evaluation of complex contaminated samples.

**MATERIALS AND METHODS**

**Chemicals**

Quinoline (Chemical Abstracts Service [CAS] no. 91-22-5; purity, 98%), benzo[h]quinoline (CAS no. 230-27-3; purity, 97%), acridine (CAS no. 260-94-6; purity, 97%), quinazoline (CAS no. 91-62-3; purity, 98%), fluorene (CAS no. 91-62-3; purity, 98%), 6-methylquinoline (CAS no. 230-27-3; purity, 97%), 4,7-phenanthroline (CAS no. 230-07-9; purity, 98%), 1,10-phenanthroline (CAS no. 66-71-7; purity, 99%), carbazole (CAS no. 86-74-8; purity, 96%), indole (CAS no. 120-72-9; purity, 98%), 2-methylindole (CAS no. 95-20-5; purity, 98%), 1-methylindole (CAS no. 603-76-9; purity, 97%), 6-methylquinoline (CAS no. 91-62-3; purity, 98%), 1-methylindole (CAS no. 230-27-3; purity, 97%), anthracene (CAS no. 120-12-7; purity, 99%), benzo[a]anthracene (CAS no. 56-55-3; purity, 99%), dibenz[a,h]anthracene (CAS no. 53-70-3; purity, 97%), fluorene (CAS no. 86-73-7; purity, 98%), phthalazine (CAS no. 253-52-1; purity, 98%), naphthalene (CAS no. 91-20-3; purity, 98%), anthracene (CAS no. 120-12-7; purity, 97%), benz[a]anthracene (CAS no. 230-46-6; purity, 99%), phenazine (CAS no. 91-22-5; purity, 99%), phthalazine (CAS no. 253-52-1; purity, 98%), 6-methylquinoline (CAS no. 91-62-3; purity, 98%), 6-methylquinoline (CAS no. 230-27-3; purity, 97%), anthracene (CAS no. 230-27-3; purity, 97%), dibenz[a,h]anthracene (CAS no. 230-27-3; purity, 97%), fluorene (CAS no. 86-73-7; purity, 98%), dibenz[a,h]anthracene (CAS no. 224-41-9), and dibenz[a]pyrene (CAS no. 50-32-8) were purchased from Sigma-Aldrich (Prague, Czech Republic). Benz[a]acridine (CAS no. 225-11-6; purity, 99.5%), benz[c]acridine (CAS no. 225-51-4; purity, 99.8%), dibenz[a,i]acridine (CAS no. 226-92-6; purity, 99.7%), dibenz[a,j]acridine (CAS no. 224-42-0; purity, 99%), dibenz[a,h]acridine (CAS no. 226-36-8; purity, 99.86%), dibenz[c,h]acridine (CAS no. 224-53-3; purity, 99.3%), and 7H-dibenzo[c,g]carbazole (CAS no. 194-59-2; purity, 99.7%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The reference TCDD (CAS no. 1746-01-6) was from Ultra Scientific (North Kingstown, RI, USA). The structures of the studied compounds are shown in Figure 1.

**Toxicity testing**

Cytotoxicity and potency to activate AhR were investigated by the H4IIE-luc rat hepatoma cell line stably transfected with the pGudLuc 1.1 vector containing luciferase reporter gene under the transcriptional control of dioxin-responsive elements [25]. Assessment of cytotoxicity was based on conventional neutral red uptake bioassay, as described elsewhere [26]. Potencies to induce AhR were determined as reported previously [25,27]. Briefly, H4IIE-luc cells were cultured in Dulbecco’s modified Eagle medium supplemented with 10% (v/v) fetal calf serum and antibiotics (all from PAA Laboratories, Pasching, Austria) at 37°C and 5% CO₂. The cells were seeded into 96-well cell culture plates at a density of 2 x 10⁴ cells/well. After 24 h of incubation (~75% cell confluence), tested chemicals diluted in dimethyl sulfoxide were added in three replicates (final concentration of the solvent did not exceed 0.5% v/v). Following 6 or 24 h of exposure, medium was removed, and the cells were washed with phosphate-buffered saline (pH 7.2). Reporter luciferase activity was then determined using a microplate luminometer GENios (Tecan, Mannedorf, Switzerland) with the Steady-Glo Luciferase Assay Kit (Promega, Madison, WI, USA). Blank, solvent control (dimethyl sulfoxide), and a standard curve of the TCDD (0.1-500 pM) were tested on each plate. At least five concentrations of each com-
pound were tested in two independent experiments. The resulting data were pooled and used for further evaluation; coefficient of variance was less than 20% for each individual treatment (concentration) tested.

**Data analyses**

All calculations and statistical analyses were performed with Microsoft Excel and Statistica® for Windows (Ver 6.0; StatSoft, Tulsa, OK, USA). For the assessment of cytotoxicity, data were compared with blank and solvent controls using analysis of variance followed by Dunnet’s test. The lowest data were compared with blank and solvent controls using StatSoft, Tulsa, OK, USA). For the assessment of cytotoxicity, data were compared with blank and solvent controls using analysis of variance followed by Dunnet’s test. The lowest concentration that significantly (p < 0.05) affected cell viability was derived (experimental lowest-observed-effect concentration \(\text{LOEC}_{exp}\)). The H4IE-luc bioassay data (relative luminescence units) were expressed as a percentage of the mean maximum TCDD response (% TCDD-max). Simple log-linear regression models were calculated for linear portions of the dose–response curves of TCDD and tested chemicals. Relative potencies (expressed as IEFs) were calculated using the equieffective approach [21]. Concentrations of the studied compounds inducing the 25 and 50% effect of the TCDD-max \(C_{EQ-25}\) and \(C_{EQ-50}\)) respectively were derived. The \(C_{EQ-50}\) values were compared with the 50% effective concentration of TCDD, and the IEFs of individual PAHs were derived (IEF = \(C_{EQ-50}\) for TCDD/\(C_{EQ-50}\) for PAH).

**Quantitative structure–activity relationships**

Structures of chemicals were built and optimized in the MOE software package (Ver 2003.2; Chemcomp, Montreal, PQ, Canada) and imported into TSAR (Ver 3.3; Accelrys, San Diego, CA, USA). Approximately 180 descriptors were calculated for each individual chemical (electronic and topological descriptors, parameters related to the molecular size and volume, and hydrophobicity descriptors). The bioassay results were expressed as \(\log(1/\text{LOEC}_{exp})\) and \(\log(1/25\%\text{ effective concentration })\) \(\text{EC25}\)) for cytotoxicity and potencies to induce AhR, respectively. The relationships between the chemical descriptors and biological endpoints were analyzed with Statistica for Windows (Ver 6.0; StatSoft). Significant intercorrelations among the descriptors were first determined with principal component analysis, and the subsets of representative and easily interpretable parameters were selected for further analyses. The structure–activity relationships were, at first, described qualitatively, without any particular statistical evaluation (cytotoxic vs noncytotoxic and AhR-active vs nonactive compounds, respectively). Multivariate regression was used for quantitative modeling. Both forward-stepwise and backward-stepwise algorithms were applied to confirm the selection of significant descriptors. The multivariate correlation coefficient \((r)\), the coefficient of multiple determination \((R^2)\), and the Fisher’s test \((F\) value) were used as the quality criteria of calculated QSAR models. The models were validated with training sets there were randomly selected by both leave-one-out and leave-multiple-out techniques.

**RESULTS**

Viability of H4IE-luc cells after the short-term, 6-h exposure was affected by only 2 of the 29 tested chemicals at the highest concentrations (200 \(\mu\)M of 1,10-phenanthroline and 7H-dibenzo[c,g]carbazole) (Table 1). Prolonged, 24-h exposures to most of the four- and five-ring aza-PAHs resulted in significant cytotoxicities (LOEC\(_{exp}\) range, 7–30 \(\mu\)M) (Table 1). Low-molecular-weight compounds generally had weaker effects (LOEC\(_{exp}\) ~ 100–200 \(\mu\)M), with the exception of 1,10-phenanthroline (LOEC\(_{exp}\) 12.5 \(\mu\)M). In general, parent PAHs were less cytotoxic than their N-heterocyclic analogues, the LOEC\(_{exp}\) values differed by more than one order of magnitude (compare, e.g., the 24-h LOEC\(_{exp}\) for benz[a]anthracene [>200 \(\mu\)M] with those of the benzacridines [~8 \(\mu\)M]).

The potencies of 22 N-heterocyclic PAHs and seven homocyclic analogues to induce AhR-dependent luciferase in H4IE-luc bioassay were investigated after 6 and 24 h. The dose–response curves for selected compounds that significantly induced reporter luciferase are shown in Figure 2. Effective concentrations and calculated IEFs are summarized in Table 1. In general, four- and five-ring PAHs were the most potent inducers of AhR in H4IE-luc cells. The effects were more pronounced after shorter, 6-h exposure, followed by a decline after 24 h. Dibenzo[a,h]acridine and dibenz[a,l]acridine had potencies comparable with that of reference TCDD after 6-h exposure (Fig. 2 and Table 1). The aza-PAHs generally were more toxic in comparison with the parent analogues, having IEFs up to three orders of magnitude higher (Table 1).

The evaluation of structure–activity relationships showed relatively poor correlation of cytotoxicity with hydrophobicity as represented by the octanol/water partition coefficient \((\log P)\) (Fig. 3). On the other hand, the combination of the molecule size (number of rings) with the molar refractivity \((MR)\) qualitatively discriminated compounds that were cytotoxic from those with no effects up to 200 \(\mu\)M (when more than three rings and MR > 50 cm$^3$/mol LOEC\(_{exp}\) ≤ 200 \(\mu\)M).

Interestingly, potencies to induce AhR in H4IE-luc cells were significantly correlated with \(\log P\) (Spearman’s \(r = 0.89, p < 0.001, n = 29\) ) (Fig. 3). Detailed selection of the chemical descriptors by stepwise multiple regression revealed that the potencies to induce AhR were best explained by ellipsoidal volume \((EV)\) and/or the combinations of principal axes of inertia (molecular dimensions) with MR (Table 2). The QSAR models were validated with both the leave-one-out and leave-multiple-out algorithms. Each single compound was, first, sequentially removed from the training set, after which the model was recalculated and the log(1/EC25) of the eliminated individual was predicted. The leave-multiple-out validation was based on 10 repeated calculations, with five chemicals randomly removed in each step. Good stability of the calculated QSARs was confirmed, with the maximum relative deviations between the observed and predicted log(1/EC25) values being ±21%.

**DISCUSSION**

Polycyclic aromatic hydrocarbons and their derivatives are major pollutants in many areas worldwide, but our understanding of their toxic effects is still incomplete. For practical reasons, such as relatively high costs of standards and limited commercial availability, (eco)toxicological studies with aza-PAHs have focused mostly on lower-molecular-weight compounds [13–15]. Our study with 22 structurally diverse aza-PAHs and seven parent PAHs allowed comparative toxicological classification of these poorly characterized contaminants. In general agreement with the results of previous studies summarized by Bleeker et al. [7], our investigation confirmed significant toxicities of high-molecular-weight compounds. However, our results did not fully support the previously reported, simple linear correlations between the acute toxicity of aza-PAHs and the hydrophobicity of tested compounds [7,13,28]. Chemicals such as dibenz[a,h]acridine and dibenz[a,l]acridine.
which were not included in previously reported studies, had relatively lower cytotoxicities than those predicted from the log P (Fig. 3). On the other hand, 1,10-phenanthroline was significantly more cytotoxic than that predicted from log P (Fig. 3). Different effects of the outliers might be explained by simultaneous manifestation of multiple cellular mechanisms induced by these chemicals. It generally is accepted that log P, a parameter of hydrophobicity, correlates with basal narcotic toxicity of organic chemicals (i.e., nonspecific accumulation of the compounds into the cell membranes). However, we also observed significant activations of AhR by dibenzacridines. Consequently, cellular events following the activation of AhR, such as inductions of detoxification enzymes and increased cellular proliferation [29], may compensate the direct acute cytotoxic effects (i.e., cell death resulting from the nonspecific membrane damage). Alternatively, the greater toxicity of 1,10-phenanthroline can be explained by known ion-chelating properties of this chemical [30] that might potentiate the acute cytotoxic effects. In general, our results indicate that the acute cytotoxicity of aza-PAHs is better characterized by parameters related to the density of molecules (MR, >50 cm²/mol [31]) and the molecular size (greater than three rings).

We also observed highly significant inductions of AhR-dependent luciferase in H4IIE-luc cells exposed to aza-PAHs. To the best of our knowledge, the present study provides new information regarding the effects of several compounds, such as dibenz[a,i]acridine, dibenz[c,h]acridine, and 7H-dibenzo[c,g]carbazole (Table 1). Significant activations of AhR by these compounds correspond to the effects of structurally related dibenz[a,h]acridine and dibenz[a,j]acridine that also have been observed previously [4,5,23]. The effects of reference TCDD increased with the exposure time (Fig. 2), but PAHs and their derivatives were more active after shorter, 6-h exposures, with decline after 24 h. These differences can be attributed to a greater susceptibility of PAHs to cellular biotransformation, as suggested by Machala et al. [4]. The aza-PAHs were more potent inducers of AhR-dependent luciferase than the parent compounds with IEFs by up to 1,000-fold (compare, e.g., dibenz[a,h]acridine [IEF₈₀ >10] and dibenz[a,h]anthracene [IEF₈₀ ~0.01]) (Table 1). Similarly, whereas anthracene did not modulate AhR, N-heterocyclic analogues, acridine, showed significant inductions after 6 h of exposure. In agreement with the results of previous studies [4,5,23], we observed a high potency of dibenz[a,h]acridine to activate AhR that was comparable or even greater than that of TCDD after short periods. However, the relevance of the in vitro results should be explored further in vivo toxicity studies.

Study of a wider set of individual chemicals allowed detailed investigation of the structure–toxicity relationships. Sig-

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Table 1. Cytotoxicity of the studied compounds and the potencies to induce aryl hydrocarbon receptor (AhR) in H4IIE-luc cell bioassay

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOEC_CYP (μM, 24 h)</th>
<th>C₅₀,50 (M)</th>
<th>IEF²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>24 h</td>
<td>6 h</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>&gt;200</td>
<td>9.4 x 10⁻⁶</td>
<td>3.5 x 10⁻⁶</td>
</tr>
<tr>
<td>Indole</td>
<td>&gt;200</td>
<td>NI ¹</td>
<td>NI</td>
</tr>
<tr>
<td>2-Methylindole</td>
<td>&gt;200</td>
<td>NI ¹</td>
<td>NI</td>
</tr>
<tr>
<td>1-Methylindole</td>
<td>&gt;200</td>
<td>NI ¹</td>
<td>NI</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>≥200</td>
<td>49.8</td>
<td>NI</td>
</tr>
<tr>
<td>Quinoline</td>
<td>&gt;200</td>
<td>4.9 x 10⁻³</td>
<td>2.5</td>
</tr>
<tr>
<td>Quinoxaline</td>
<td>&gt;200</td>
<td>2.0 x 10⁻¹</td>
<td>1.9</td>
</tr>
<tr>
<td>Isonicotole</td>
<td>&gt;200</td>
<td>1.0</td>
<td>NI</td>
</tr>
<tr>
<td>Phthalazine</td>
<td>&gt;200</td>
<td>49.2</td>
<td>WI</td>
</tr>
<tr>
<td>6-Methylquinoline</td>
<td>&gt;200</td>
<td>12.5</td>
<td>WI</td>
</tr>
<tr>
<td>Fluorene</td>
<td>&gt;200</td>
<td>200</td>
<td>WI</td>
</tr>
<tr>
<td>Carbazole</td>
<td>&gt;200</td>
<td>64.2</td>
<td>WI</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>&gt;200</td>
<td>8.0</td>
<td>2.0 x 10⁻¹</td>
</tr>
<tr>
<td>Phenanthroline</td>
<td>&gt;200</td>
<td>30.0</td>
<td>7.4 x 10⁻⁷</td>
</tr>
<tr>
<td>Benz[j]anthracene</td>
<td>&gt;200</td>
<td>7.0</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>Benz[j]acridine</td>
<td>&gt;200</td>
<td>7.0</td>
<td>8.8 x 10⁻⁴</td>
</tr>
<tr>
<td>Benz[j]c[acridine]</td>
<td>&gt;200</td>
<td>7.0</td>
<td>8.8 x 10⁻⁴</td>
</tr>
<tr>
<td>Benz[j]c[acridine]</td>
<td>&gt;200</td>
<td>30.0</td>
<td>7.5 x 10⁻⁶</td>
</tr>
<tr>
<td>Benz[j]c[acridine]</td>
<td>&gt;200</td>
<td>7.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

⁠a Parent unsubstituted polycyclic aromatic hydrocarbons are in italics.

⁠b LOEC_CYP = lowest-observed-experimental concentrations (μmol/L) that significantly inhibited cell viability after 24 h of exposure.

⁠c C₅₀,50 = concentrations (mol/L) inducing AhR-dependent luciferase at levels equivalent to the 50% effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) after 6- and 24-h exposure periods.

⁠d IEF = induction equivalency factors. Calculated as a ratio of C₅₀,50 values of the TCDD and individual tested compounds.

⁠e NI = no significant induction of AhR-dependent luciferase.

⁠f NA = not available.

⁠g WI = weak induction (< 50% of TCDD maximum).
Fig. 2. Concentration–induction curves (6 h, full symbols; 24 h, open symbols) of hydrocarbon receptor (AhR)–dependent luciferase in H4IIE-luc cells. (A) Benzo(a)anthracene and its derivatives. (B) and (C). Effects of five-ring N-heterocyclic derivatives of polycyclic aromatic hydrocarbons (PAHs). Data points represent the mean ± standard deviation of three replicates. The effects of PAHs are compared with the reference 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Fig. 3. Relationships between the hydrophobicity (log \( P \)) and the 24-h cytotoxicity (A) the lowest-observed concentration that significantly affected cell viability [LOEC]) and 6-h potencies to activate aryl hydrocarbon receptor (AhR) in H4IIE-luc cells (B) concentrations that induced 25% effects [EC25] of the reference 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Selected individual compounds are labeled with numbers: 1 = 1,10-phenanthroline; 2 = dibenz[a,h]anthracene; 3 = dibenz[a,i]acridine; and 4 = dibenz[a,h]acridine.

Table 2. Quantitative structure–activity relationships (QSARs) for activation of aryl hydrocarbon receptor (AhR) in H4IIE-luc cell bioassay

<table>
<thead>
<tr>
<th>QSAR model</th>
<th>( n )</th>
<th>( r )</th>
<th>( R^2 )</th>
<th>( F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All compounds that activated AhR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \log(1/EC_{25}) = 0.011 \cdot EV + 1.544 )</td>
<td>14</td>
<td>0.95</td>
<td>0.89</td>
<td>119</td>
</tr>
<tr>
<td>Subset of aza-PAHs that activated AhR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \log(1/EC_{25}) = 0.012 \cdot EV + 1.425 )</td>
<td>11</td>
<td>0.94</td>
<td>0.87</td>
<td>67</td>
</tr>
<tr>
<td>( \log(1/EC_{25}) = 0.34 \cdot \text{length} + 0.091 \cdot \text{MR} - 3.95 )</td>
<td>11</td>
<td>0.94</td>
<td>0.87</td>
<td>49</td>
</tr>
<tr>
<td>( \log(1/EC_{25}) = 1.14 \cdot \text{length} - 2.12 \cdot (l/w) + 2.82 )</td>
<td>11</td>
<td>0.95</td>
<td>0.88</td>
<td>54</td>
</tr>
</tbody>
</table>

\( n = \) Number of chemicals in data set; \( r = \) multivariate correlation coefficient; \( R^2 = \) coefficient of multiple determination; \( F = \) Fisher’s test, (variance ratio); \( EV = \) ellipsoidal volume; \( \text{length} = \) first principal axis of inertia; \( \text{MR} = \) molar refractivity; \( l/w = \) ratio of the length and width (i.e., ratio of the first and the second principal axes of inertia); aza-PAHs = N-heterocyclic derivatives of polycyclic aromatic hydrocarbons; EC25 = concentration that induced 25% of the maximum effect in H4IIE-luc cell bioassay.

Significant correlation between the activation of AhR and log \( P \) was observed (Fig. 3), thus confirming known potencies of hydrophobic toxicants to induce cellular defense mechanisms, including those mediated by AhR [32]. For those compounds that activated AhR, we performed stepwise selection of significant descriptors, and a single parameter (EV) best explained the potency to activate AhR (Table 2). Although EV is not often discussed in toxicological QSARs, a recent study [33] found correlations between EV and the protein-binding ca-
pacity of low-molecular-weight compounds (unsaturated fatty acids). Other models for activation of AhR (Table 2) correspond to those previously published and summarized by Lewis et al. [34]. Significant positive correlations between the inductions of AhR and the length and planarity (area/depth-squared, a/d) as well as hydrophobicity (log P) were observed previously for data sets including PCBs and PAHs [34]. We observed correlations with MR (related to the density and the volume of the molecule) in combinations with molecular length and planarity (the first and second axes of inertia and their ratios; see the second and third equations in Table 2). The importance of molecular size for the activation of AhR by PAHs also was reported previously [4,21]. The derived descriptors explain well the basic steps of AhR activation, such as transport across the membrane (affected by the hydrophobicity) and binding to AhR (described by EV and/or size and density descriptors).

Numerous parent PAHs are routinely analyzed in environmental matrices, but information regarding the occurrence of aza-PAHs is rare, corresponding to the lack of appropriate analytical methods. Some aza-PAHs, such as benz[c]acridine, benz[a]acridine, quinoline, isoquinoline, carbazole, dibenz[a,h]acridine, dibenz[a,j]acridine, and dibenz[c,h]acridine, were identified in the air particulate matter or sediments at concentrations of 1 to 10% those of the parent PAHs [3,9,11]. Relatively lower concentrations can, however, be counterbalanced by properties of aza-PAHs, such as higher water solubility and bioavailability [12], lower biodegradability with a tendency to bioconcentrate [6], as well as higher toxicities in comparison with those of the parent PAHs [13,16,17].

To assess contaminated matrices, the toxic equivalency factor approach is well established for halogenated persistent contaminants [20,27], and methodologies based on relative potencies also have been proposed for dominant PAHs [35,36]. In the following paragraph, we demonstrate the potential use of IEFs derived in the present study (Table 1) for the evaluation of complex contaminated samples. The IEFs were compared with sediment concentrations of aza-PAHs published previously by Kozin et al. [9]: benz[a]acridine, 45 ng/g dry weight; benz[c]acridine, 95 ng/g dry weight; dibenz[a,h]acridine, 17.7 ng/g dry weight; dibenz[a,j]acridine, 3.7 ng/g dry weight; and dibenz[c,h]acridine, 7.6 ng/g dry weight. The final TCDD equivalent (226 ng/g dry wt) reflects the toxic contribution of five individual aza-PAHs, but the value corresponds to the total sediment TCDD equivalents published previously for PAH-contaminated samples. For example, Vondracek et al. [35] reported 24-h bioassay TCDD equivalents for nine sediments ranging from 5.9 to 48 ng/g dry weight. In a study of Hilscherova et al. [27], the bioassay TCDD equivalents for eight sediments ranged from 0.7 to 23 ng/g dry weight. A contribution of dibenz[a,h]acridine to the TCDD equivalents in river sediments also was published by Machala et al. [4]. Polycyclic aromatic hydrocarbons and their derivatives are dominant contaminants in urban areas in concentrations that highly exceed those of persistent chlorinated compounds. Although direct “dioxin-like” in vivo effects of PAHs remain unclear, PAHs and their derivatives certainly modulate AhR and induce CYP450 enzymes. Consequently, chronic exposures to PAHs and their derivatives might lead to increased formation of CYP450-mediated reactive metabolites or enhanced susceptibility to other contaminants that require metabolic activation [7].

In conclusion, the present study revealed significant in vitro toxicities of N-heterocyclic derivatives of PAHs. High potencies to induce AhR in vitro were observed, particularly for dibenzacridine. The principal QSAR descriptors correlated with the potencies to activate AhR were EV, MR, and molecular size. Individual IEFs for aza-PAHs are derived, and their use in evaluation of complex environmental samples is suggested.

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REFERENCES


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