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Effects of Substituents on the Cytosolic Receptor-Binding Avidities and Aryl Hydrocarbon Hydroxylase Induction Potencies of 7-Substituted 2,3-Dichlorodibenzo-*p*-dioxins

A Quantitative Structure-Activity Relationship Analysis

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SUMMARY

The binding affinities of 16 7-substituted 2,3-dichlorodibenzo-*p*-dioxins for the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) cytosolic receptor protein from male Wistar rats have been determined. The EC_{50} value for each compound was estimated by competitive displacement of [^{3}H]TCDD and the data illustrated that the differences between competitive ligands were dependent on the substituent (X) group. The EC_{50} value for 7-trifluoromethyl-2,3-dichlorodibenzo-*p*-dioxin was 1.95×10^{-8} M and was greater than 1000-fold more active than 7-amino-2,3-dichlorodibenzo-*p*-dioxin ($EC_{50} = 2.88 \times 10^{-5}$ M). Multiple parameter linear regression analysis of the data for 14 different compounds gave the following equation: $\log (1/EC_{50}) = 1.24\pi + 6.11$. This demonstrated that the binding affinity was linearly dependent on the lipophilicity (π) of the 7-X-group. This contrasted with a comparable analysis of the substituent effects on the binding of 13 4′-substituted 2,3,4,5-tetrachlorobiphenyls to the cytosolic receptor which showed that the lipophilicity, electronegativity, and hydrogen-bonding capacity were important physicochemical determinants which facilitated binding to the receptor protein. These data suggest that the halogenated dibenzo-*p*-dioxins and biphenyls may interact with different binding sites on the receptor or they may bind to the same site but exert different conformational effects on the receptor protein. For the 7-X-2,3,-dichlorodibenzo-*p*-dioxins, there was not a rank order correlation between receptor-binding EC_{50} values and the induction of aryl hydrocarbon hydroxylase (AHH) or ethoxyresorufin *O*-deethylase in rat hepatoma H-4-II E cells in culture. However, the data could be correlated with an estimate of substituent width, the STERIMOL factor (B_5), i.e., $\log (\text{AHH}) = 1.29 \log (\text{binding}) + 2.19\Delta B_5 - 1.31 (\Delta B_5)^2 - 1.48$. The importance of a steric factor in the correlation between receptor binding and AHH induction for substituted dibenzo-*p*-dioxins and halogenated biphenyls is consistent with a structure-dependent conformational change(s) in the receptor protein:ligand complex after the initial binding event. Presumably, this latter process is associated with the steps involving interactions between the ligand:receptor complex and nuclear binding sites.

INTRODUCTION

Pharmacogenetic studies have demonstrated that some genetically inbred strains of mice and mammalian cells in culture can be either "responsive" or "nonresponsive" to the biologic and toxic effects of polynuclear aromatic hydrocarbons (1–4). For example, 3-methylcholanthrene, a carcinogenic hydrocarbon, induces hepatic microsomal benzo[*a*]pyrene hydroxylase (AHH)[1] in responsive B6 mice but does not induce this cytochrome P-450-dependent monooxygenase enzyme in nonresponsive D2 mice. It was postulated that the *Ah* locus, which may be comprised of regulatory, structural, and temporal genes (5), controls the induction of numer-

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[1] The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; HB, hydrogen bonding; QSAR, quantitative structure-activity relationship; RB, receptor binding; SAR, structure-activity relationship; TCDD, ?,3,7,8-tetrachlorodibenzo-*p*-dioxin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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ous drug-metabolizing enzymes, including AHH. Like 3-methylcholanthrene, the toxic halogenated aryl hydrocarbon TCDD also induces AHH in responsive strains of mice and mammalian cell cultures; however, in contrast to 3-methylcholanthrene, at higher dose levels, TCDD also induces this enzyme in nonresponsive systems (6–8). The synthesis of [^{3}H]TCDD of high specific activity and the utilization of this ligand in diverse receptor assays has resulted in the identification of a high affinity, low capacity cytosolic receptor protein (9). This receptor has been identified in mammalian cell cultures and in the hepatic and extrahepatic tissues of diverse animals including genetically inbred responsive mice (9–13). Nonresponsive DBA/2 mice contain low levels of the receptor protein in the nuclear fraction and the genetic differences in mice appear to be related to a mutation or defect in the regulatory gene which codes for the receptor protein.

Several studies with TCDD indicate that the *Ah* receptor not only initiates the rapid induction of AHH but also plays a role in mediating many of the toxic effects elicited by this compound. Administration of TCDD to genetically inbred responsive C57BL/6 and nonresponsive DBA/2 mice and their backcrosses confirms that several toxic effects including thymic atrophy, porphyria, body weight loss, teratogenicity, and immunotoxicity all segregate with the *Ah* locus (8, 14–16). Structure-activity studies within the polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls and polybrominated biphenyls all support the role of the receptor in mediating the effects of the halogenated aryl hydrocarbons (16–21).

Although the TCDD receptor protein has not been isolated and purified, the SARs developed for the chlorinated aryl hydrocarbons suggested that the following ligand structural features facilitate interaction with the receptor protein-binding sites (22): 1) co-planarity, 2) substituents (Cl) at lateral positions of the aromatic ring systems, 3) molecular dimensions which are accommodated by a 3 × 10 Å rectangle, and 4) polarizable substituent groups at the lateral positions.

Although these observations describe the SARs for the halogenated aryl hydrocarbons, they do not explain the interaction of PAHs or 7,8-benzoflavone with the cytosolic receptor protein. These compounds also bind to the receptor protein, and extensive binding studies with unlabeled and [^{3}H]TCDD and radiolabeled polynuclear aromatic hydrocarbons (13) confirm that these structurally diverse compounds bind to the same protein. It is possible that their respective binding domains on the receptor protein may not be identical.

Bandiera and co-workers (23) have studied the ligand:receptor interactions of several 4′-substituted-2,3,4,5-tetrachlorobiphenyls (Fig. 1) using QSAR, which relates the effects of variable 4′-substituents in modulating the *in vitro* receptor-binding affinities and AHH induction potencies of a series of homologs (23). The results indicated that hydrophobic (π), electronic (σ), and HB-forming properties are the important substituent properties which facilitate binding of these ligands to the cytosolic receptor protein. The present study utilizes a QSAR approach (23) for determining the substituent physicochemical parameters which influence the *in vitro* receptor-binding affinities and AHH induction potencies of a series of 7-substituted-2,3-dichlorodibenzo-*p*-dioxins (Fig. 1). The parent chloro analog of this series, 2,3,7-trichlorodibenzo-*p*-dioxin, is a highly active chlorinated dibenzo-*p*-dioxin (26); in contrast, the 2,3,-dichlorodibenzo-*p*-dioxin is relatively inactive and this confirms the critical importance of the single lateral (7) substituent in this group of homologs.

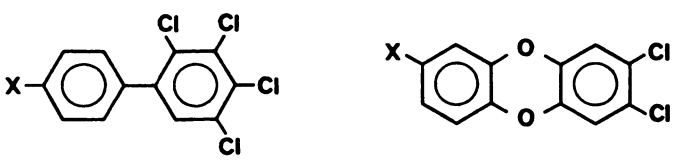

FIG. 1. *Structure of 4′-substituted 2,3,4,5-tetrachlorobiphenyl and 7-substituted 2,3-dichlorodibenzo-p-dioxin.*

MATERIALS AND METHODS

Chemicals and biochemicals. [^{3}H]TCDD (specific activity, 50–52 Ci/mmol) was obtained from ICN Chemical and Radioisotope Division and purified by thin layer chromatography on silica gel using petroleum spirit as the eluant. The radiolabeled compound used in this study was >95% pure; the major contaminants were the 2,3,7-tri- and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (approximately 2 and 3%, respectively). Dextran (average M_r = 82,000), Hepes, NADP$^+$, NADH, D-glucose 6-phosphate, D-glucose 6-phosphate dehydrogenase (baker's yeast), benzo[*a*]pyrene, rhodamine B, bovine serum albumin, and ethylisocyanide were purchased from the Sigma Chemical Co. (St. Louis, MO). Dithiothreitol was obtained from the Eastman Kodak Co., Rochester, NY; 3-methylcholanthrene was from Pfaltz & Bauer, Inc., Stamford, CT; decolorizing charcoal (Norit A) was from BDH Chemicals, Toronto, Ontario; and sucrose (sodium dodecyl sulfate grade) was from Beckman Instruments, Fullerton, CA. Dimethyl sulfoxide, glycerol (ACS grade), and EDTA were obtained from the Fisher Scientific Co., Toronto, Ontario.

Synthesis and purification of 16 7-substituted 2,3-dichlorodibenzo-p-dioxins. 2,4,5-Trichloronitrobenzene, catechol, 4-methylcatechol, 4-chloro-3-nitrobenzofluoride, 2,3-naphthalenediol, aminoveratrole, veratrole, 4-bromoveratrole, and 4-*t*-butylcatechol were purchased from the Aldrich Chemical Co., Milwaukee, WI; 1,2,4-trihydroxybenzene, 4-cyanocatechol, and 4-chloro-3-nitrobenzoic acid methyl ester were purchased from Pfaltz and Bauer, and 4-phenylcatechol was obtained from Eastman Organic Chemicals. 4-Chloro-, 4-iodo-, and 4-fluoroveratrole were all synthesized via diazotization of 4-aminoveratrole in hydrochloric acid with sodium nitrite followed by decomposition of the diazonium salts with cuprous chloride, potassium iodide, or fluoroboric acid, respectively (note that solid fluoroborate was isolated and thermally decomposed to give the fluoro compound). The halocatechols were all prepared by quantitative demethylation of the corresponding 4-haloveratroles in methylene chloride-born tribromide solution; 4,5-dichlorocatechol was synthesized via chlorination of veratrole followed by demethylation as described above.

The condensations of the catechol or substituted catechols with chloronitrobenzenes to give the corresponding dibenzo-*p*-dioxins were carried out using comparable reaction conditions. The catechol (2 mmol) and the chloronitrobenzene (2 mmol) in hexamethylphosphoramide (3 ml) were heated with stirring at 180°. The reaction was monitored by thin layer chromatography and gas-liquid chromatography and terminated when the chloronitrobenzene was completely reacted. The reaction time (30–120 min) varied with the type of substituent on the catechol moiety. The reaction mixtures were adsorbed on silicic acid and purified by column and thin layer chromatography using silica gel as the solid phase as described (23) to give the crude 7-substituted 2,3-dichlorodibenzo-*p*-dioxins which were further purified by crystallization from methanol (yields varied from 10–25%).

The following compounds (and reactants) were synthesized using the coupling procedures noted above: 2,3,7-trichlorodibenzo-*p*-dioxin (4-chlorocatechol and 2,4,5-trichloronitrobenzene); 2,3-dichlorodibenzo-*p*-dioxin (catechol and 2,4,5-trichloronitrobenzene); 7-bromo-2,3-dichlorodibenzo-*p*-dioxin (4-bromocatechol and 2,4,5-trichloronitrobenzene); 7-iodo-2,3-dichlorodibenzo-*p*-dioxin (4-iodocatechol and 2,4,5-trichloronitrobenzene); 7-fluoro-2,3-dichlorodibenzo-*p*-dioxin (7-fluorocatechol and 2,4,5-trichloronitrobenzene); 7-hydroxy-2,3-dichlorodibenzo-*p*-dioxin (1,2,4-trihydroxybenzene and 2,4,5-trichloronitrobenzene); 7-methyl-2,3-dichlorodibenzo-*p*-dioxin (4-methylcatechol and 2,4,5-trichloronitrobenzene); 7-phenyl-2,3-dichlorodibenzo-*p*-dioxin (4-phenylcatechol and 2,4,5-trichloronitrobenzene); 7-trifluoromethyl-2,3-dichlorodibenzo-*p*-dioxin (4,5-dichlorocatechol and 4-chloro-3-nitrobenzotrifluoride); 2,3-dichlorobenznaphtho-*p*-dioxin (2,3-naphthalenediol and 2,4,5-trichloronitrobenzene); 7-cyano-2,3-dichlorodibenzo-*p*-dioxin (4-cyanocatechol and 2,4,5-trichloronitrobenzene); 7-*t*-butyl-2,3-dichlorodibenzo-*p*-dioxin (4-*t*-butylcatechol and 2,4,5-trichloronitrobenzene); 7-carbomethoxy-2,3-dichlorodibenzo-*p*-dioxin (4,5-dichlorocatechol and methyl-4-chloro-3-nitrobenzoate.

7-Nitro-2,3-dichlorodibenzo-*p*-dioxin was prepared by nitration of 2,3-dichlorodibenzo-*p*-dioxin, 2,3-Dichlorodibenzo-*p*-dioxin (200 mg) was dissolved in nitromethane (8 ml) and trifluoroacetic anhydride (2 ml) and treated with solid ammonium nitrate until the reaction was complete (monitored by gas-liquid and thin layer chromatography). The 7-nitro-2,3-dichlorodibenzo-*p*-dioxin was recovered by filtration (195 mg). 7-Nitro-2,3-dichlorodibenzo-*p*-dioxin (50 mg) in tetrahydrofuran (5 ml) and hydrazine (0.25 ml) was added dropwise to a mixture of ethanol (20 ml), tetrahydrofuran (5 ml), hydrazine (0.5 ml), and Raney nickel (0.5 g) with stirring at 90°. The reaction mixture was stirred for an additional hour, filtered, and concentrated, and the amine was purified further by TLC to give the reduction product (40 mg). The purities of all the 7-substituted 2,3-dichlorodibenzo-*p*-dioxins were determined by gas-liquid chromatography using a Tracor model 565 instrument equipped with an 0.6 cm × 1.2 m glass column and packed with 3% OV 101 Ultrabonded Carbowax 20 (80–100 mesh, RFR Corp., Hope, RI) using a flame ionization detector. The purity of all the substituted dibenzo-*p*-dioxins was >97%. Mass spectra were determined by gas chromatography—mass spectrometry using a Finnigan OAW 1000 instrument.

Cytosol receptor assays. The cytosol receptor assay was performed as described (23) using the combined dextran-charcoal treatment to remove unbound organic ligand followed by sucrose density gradient analysis centrifugation of the protein:ligand complex. The binding of the 7-substituted 2,3-dichlorodibenzo-*p*-dioxins were determined by adding different concentrations of the ligands in dimethyl sulfoxide (10 ml) to a 10 nM solution of [^{3}H]TCDD in 1 ml of rat hepatic cytosol (5–6 mg of protein) which was incubated for 1 hr at 0–5°. The ligand binding was determined by the competitive displacement of the radiolabeled [^{3}H]TCDD from the protein receptor (23). After gradient centrifugation, the distribution of radioactivity in each gradient was determined by collecting 40 × 0.12 ml fractions followed by liquid scintillation counting of each fraction (23).

Enzyme induction studies. Rat hepatoma H-4-II E cells were kindly supplied by Dr. J. Bradlaw, Food and Drug Administration, Washington, D. C. The culture conditions, enzyme induction experiments, and the methods used for the AHH and EROD assays were carried out as previously described (23). The EC_{50} values for each compound were determined in a dose-response fashion.

Data analysis. The multiple linear regression analysis of the data was determined using the physical chemical substituent parameters given in Table 1 using the FACOM M200 computer at the Data Processing Center of Kyoto University, Kyoto, Japan. The hydrophobic parameter for each substituent was estimated using the equation:

$$\pi(X/\text{2,3-dichlorodibenzo-}p\text{-dioxin}) = 0.94\ \pi_{X/\text{PhH}} + 0.27\sigma_x^0(m + p) + 0.30\rho_x$$

where the suffix X/2,3-dichlorodibenzo-*p*-dioxin indicates π values for the 7-substituted 2,3-dichlorodibenzo-*p*-dioxins, the suffix X/PhX refers to the π values for monosubstituted benzenes (24), the $0.27\sigma_x^0$ term describes the solubility-modifying effects of the 7-substituents on the ether oxygen functions at positions 5 and 10 (the susceptibility value 0.27 is taken as that of OCH_3), and the 0.30 ρ_x represents the effect of "*p*-phenoxy" and "*m*-phenoxy" groups on the hydrogen-bonding interaction of 7-substituents with octanol and water (25). ρ_x is the susceptibility value, and the electron-withdrawing effects of two phenoxy groups are expressed as the summation of the σ^0 value in a modified Hammett from which the "through-resonance" effect of substituents on functional side chain groups is eliminated. The ΔB_5 value is the STERIMOL maximum width parameter relative to that of H and is derived from published data (26).

RESULTS

The binding affinity EC_{50} values for the individual 7-substituted 2,3-dichlorodibenzo-*p*-dioxins were determined by the dose-response competitive displacement of [^{3}H]TCDD using the sucrose density gradient centrifugation and fractionation assay technique as described (23). The results are summarized in Table 1. The EC_{50} of the most active compound, 7-trifluoromethyl-2,3-dichlorodibenzo-*p*-dioxin, was 1.95×10^{-8} M and was only slightly less active than TCDD as a competitor for the receptor protein. In contrast, the EC_{50} for the least active homolog, 7-amino-2,3-dichlorodibenzo-*p*-dioxin was 2.88×10^{-5} M which was >1000-fold less active than the 7-trifluoromethyl-substituted ligand. Correlation of the activity indices of the 16 substituted homologs with respect to the physicochemical properties (e.g., lipophilicity, electronegativity, and hydrogen bond-forming characteristics) of each substituent was determined by multiple parameter regression analysis as described (23). The substituent effects on receptor binding could be expressed by Eq. 1

$$\log (1/EC_{50}) = 1.24\pi + 6.11 \quad (1)$$
$$(\pm 0.26)\ \ (\pm 0.19)$$
$$n = 14,\ s = 0.29,\ r = 0.950$$

where n is the number of compounds used to develop the equation, s is the standard deviation, r is the correlation coefficient, the figures in parentheses are 95% confidence intervals, and π represents the substituent lipophilicity. Two compounds ($X = C_6H_5$ and -*t*-C_4H_9) were outliers (Fig. 2) and data for these compounds were not used to derive Eq. 1. The van der Waals volumes for the C_6H_5 and -*t*-C_4H_9) substituents were 45.8 and 41.8 cm^3/mol, respectively, and exceeded the ideal volumes (<35 cm^3/mol) for substituents which facilitated ligand:receptor interactions (23). Previous QSAR studies with 4′-substituted 2,3,4,5-tetrachlorobiphenyls showed that, although there was no apparent correlation between steric factors and receptor binding, there was a limiting molecular volume which was exceeded by both $X = C_6H_5$ and *t*-C_4H_9 (23).

The relative AHH and EROD induction activities of the 7-substituted 2,3-dichlorodibenzo-*p*-dioxins are summarized in Table 1; the AHH and EROD induction potencies (EC_{50}) of the most active inducer, 7-bromo-2,3-dichlorodibenzo-*p*-dioxin, were 1.31 and 1.00×10^{-8} M, respectively, and were considerably less active than TCDD. The range of homolog induction potencies was

TABLE 1

Substituent parameters and analysis of binding constant and enzyme induction of 7-substituted 2,3-dichlorodibenzo-p-dioxins

Substituent	π^a	ΔB_5^b	σ_m^{0c}	σ_p^{0c}	Binding EC_{50}	AHH induction (EC_{50})	EROD induction (EC_{50})
					M	*M*	*M*
F	0.26	0.35	0.34	0.15	1.12×10^{-7}	7.4×10^{-8}	6.70×10^{-8}
CF_3	1.10	1.61	0.46	0.53	1.95×10^{-8}	2.01×10^{-7}	1.27×10^{-7}
7,8-$(CH)_4$[d]	1.28	3.31	0.08	0.08	1.85×10^{-8}	7.46×10^{-7}	1.05×10^{-6}
OME	0	2.07	0.10	−0.12	3.09×10^{-7}	2.62×10^{-5}	1.43×10^{-5}
Cl	0.83	0.80	0.37	0.24	4.68×10^{-8}	8.94×10^{-8}	6.54×10^{-8}
Br	0.98	0.95	0.37	0.26	4.79×10^{-8}	1.31×10^{-8}	1.00×10^{-8}
I	1.22	1.15	0.34	0.28	5.37×10^{-8}	7.13×10^{-8}	1.25×10^{-7}
CN	−0.18	0.60	0.62	0.71	1.2×10^{-6}	3.72×10^{-6}	4.41×10^{-6}
C_6H_5[e,f]	1.86	2.11	0.04	0.05	2.4×10^{-7}	3.92×10^{-7}	2.06×10^{-6}
t-C_4H_9[e,f]	1.80	2.17	−0.09	−0.15	3.02×10^{-7}	2.04×10^{-8}	1.43×10^{-8}
CH_3	0.48	1.04	−0.06	−0.14	3.72×10^{-7}	1.13×10^{-6}	9.2×10^{-7}
NO_2	0.11	1.44	0.71	0.81	4.6×10^{-7}	2.38×10^{-6}	1.34×10^{-6}
COOMe[d]	0.16	2.36	0.35	0.44	5.37×10^{-7}	1.29×10^{-6}	1.89×10^{-6}
H	0	0	0	0	7.59×10^{-7}	2.36×10^{-5}	3.65×10^{-5}
OH	−0.43	0.93	0.02	−0.22	4.47×10^{-6}	1.49×10^{-5}	1.54×10^{-5}
NH_2	−1.07	0.97	−0.09	−0.30	2.88×10^{-5}	Inactive	Inactive

[a] Calculated as described (see Materials and Methods).

[b] Obtained from compilations of Verloop (26).

[c] Derived from published data.

[d] Not included in Eq. 2. For the 7,8-$(CH)_4$ and COOMe derivatives, the steric parameter, ΔB_5, may not represent the real situation, since it is a "disubstituted" compound. The COOMe compound or its complex with the receptor may recognize the nuclear receptor site from the CO side. The ΔB_5 value for the CHO group is 1.36, which predicts well the log AHH value of the COOMe compound.

[e] Not included in Eq. 1. The van der Waals volume of these substituents is larger than a threshold value.

[f] Not included in Equation 2. The enzyme induction is much higher than predicted from their receptor binding. Although the receptor binding is lower than predicted from hydrophobicity of substituents, the complex may behave as if it is the "regular" complex. Thus, the value of log (binding) calculated by Equation 1 is used instead of the observed value in Eq. 2; the log AHH value predicted for the C_6H_5 derivative agrees with the observed value.

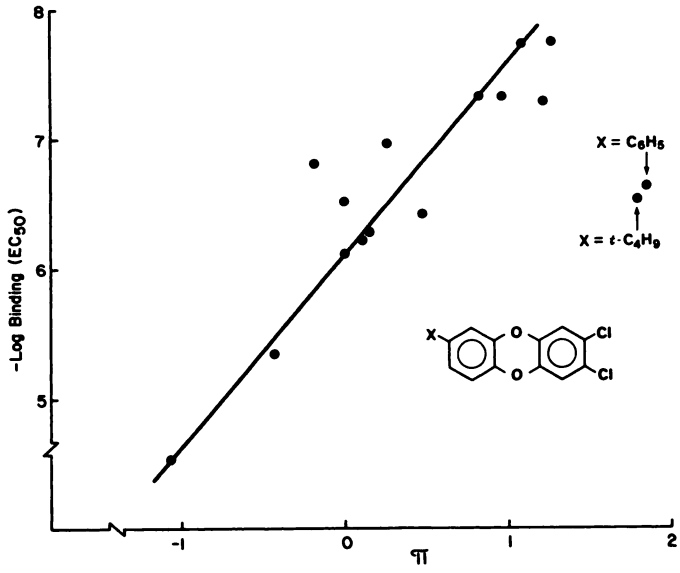

FIG. 2. *A plot of the −log EC_{50} receptor-binding data for the 7-substituted 2,3-dichlorodibenzo-p-*dioxins versus the π values for these substituents.

substituent dependent and varied over 3 orders of magnitude; the 7-amino derivative was inactive as an inducer of AHH and EROD at all doses tested using the rat hepatoma H-4-II E cell culture system. Inspection of the results clearly showed that there was not an obvious correspondence between rank order AHH or EROD induction potencies and receptor-binding avidities for this series of substituted dichlorodibenzo-*p*-dioxins. However, if the *t*-butyl, phenyl, fused naphthalene, and carboxymethyl-substituted compounds were treated as outliers, the following correlation between AHH induction and receptor binding was derived.

$$\log(\text{AHH}) = \underset{(\pm 0.40)}{1.29}\log(\text{RB}) + \underset{(\pm 1.53)}{2.19}\Delta B_5 - \underset{(\pm 0.69)}{1.31}(\Delta B_5)^2 - \underset{(\pm 0.78)}{2.96} \quad (2)$$

$$n = 11 \quad s = 0.27 \quad r = 0.963$$

AHH and RB refer to the $1/EC_{50}$ values obtained for AHH induction and receptor binding, respectively. The equation indicates that the correlation between the receptor-binding and AHH induction activities of the 7-*X*-2,3-dichlorodibenzo-*p*-dioxins is dependent on a parabolic relationship with the STERIMOL parameter ΔB_5 (25, 26). The ΔB_5 values for the substituent groups used in this study are summarized in Table 1 and represent the maximum width of the substituents from the axis connecting the 7-substituent to the rest of the molecule. Previous studies with 4′-*X*-2,3,4,5-tetrachlorobiphenyls also show that the correlation between their receptor binding and AHH induction activities was also related to the STERIMOL values for the substituent groups.

Analysis of the AHH and EROD induction potencies for the 7-*X*-2,3-dichlorodibenzo-*p*-dioxins gave Eq. 3.

$$\log (\text{EROD}) = 0.99 \log (\text{AHH}) - 0.07 \quad (3)$$
$$(\pm 0.10) \qquad (\pm 0.11)$$
$$n = 15 \; s = 0.19 \; r = 0.985$$

This equation clearly demonstrates the excellent correlation between the induction of these two enzymes by the 7-*X*-2,3-dichlorodibenzo-*p*-dioxin and supports the contention that the oxidation of ethoxyresorufin and benzo[*a*]pyrene is catalyzed by the same cytochrome P-450 monooxygenase enzyme system (27).

DISCUSSION

Previous *in vitro* QSAR studies with a series of 4′-*X*-2,3,4,5-tetrachlorobiphenyls demonstrated that this approach can be used as a sensitive probe for understanding the substituent parameters which facilitate interaction of these ligands with the TCDD cytosolic receptor protein (23). For 15 different substituents, a multiparameter linear regression analysis gave Eq. 4, which relates receptor binding to the lipophilicity (π), electronegativity (σ) and HB capacity of the substituents.

$$\log (1/EC_{50}) = 1.39\sigma \pm 1.31\pi + 1.12\text{HB} + 4.20 \quad (4)$$

This suggests that the critical 4′-lateral substituent in the biphenyl moiety interacts with both hydrophobic and polar functionalities which must be located in the region of the receptor site.

Our observation for the substituted biphenyls is in partial agreement with other studies which suggest that substituent electron-accepting or polarizability properties are important factors for facilitating binding with the cytosolic receptor protein (22). However, in contrast to the substituted biphenyls, multiparameter linear regression analysis of the 7-*X*-2,3-dichlorodibenzo-*p*-dioxins indicates that only the lipophilic property of these lateral substituents influences relative binding affinities as illustrated by Eq. 1.

The multiparameter linear regression analysis of the effects of substituents on binding affinities showed that, for both the dibenzo-*p*-dioxin and biphenyl series of ligands, the two bulky phenyl and *t*-butyl groups were classified as outliers in the derivation of Eqs. 1 and 4. This provides indirect confirmation on the steric limits for the lateral substituents and is consistent with a binding site which has both area and volume restrictions (22) (see Eq. 1). Molecular overlap of the substituted biphenyl and dibenzo-*p*-dioxin moieties in their planar conformation does not illustrate major differences in the relative positions of the two variable substituent positions. The observed differences may be related to several factors including the nonco-planarity of the substituted biphenyls or the existence of different binding domains for the biphenyl and dibenzo-*p*-dioxin ligands.

Previous *in vivo* and *in vitro* studies have shown that, after initial binding to the receptor, there is first a rapid induction of *m*-RNA for cytochrome P_1-450 and this is followed by the rapid induction of the monooxygenase enzyme (29, 30). Not surprisingly, the 7-*X*-2,3-dichlorodibenzo-*p*-dioxins induce AHH (and EROD) in the responsive rat hepatoma H-4-II E cells in culture and their induction potencies (EC_{50} values) are dependent on the structure of the substituent. Previous studies with chlorinated biphenyls and dibenzo-*p*-dioxins suggest that for these compounds there is a rank order correlation between AHH induction potencies and receptor-binding affinities (16). This correlation is not evident for the 7-*X*-2,3-dichlorodibenzo-*p*-dioxins nor for the previous studies with the 4′-*X*-2,3,4,5-tetrachlorobiphenyls (23). QSAR analysis of the data gave Eq. 2 which indicates that the relationship between AHH induction and receptor binding is dependent on the STERIMOL parameter which is related to substituent width. Similar results were observed for the 4′-*X*-2,3,4,5-tetrachlorobiphenyls. The STERIMOL parameters have proven useful in several studies which involve the interaction of substituted ligands with macromolecules. The relationship expressed in Eq. 2 is consistent with substituent effect(s) which may influence some aspect of the mechanism which occurs after the initial ligand:receptor-binding process.

Eqs. 3 and 5 illustrate the correlation between the effects of substituents on the AHH and EROD induction potencies of the substituted dibenzo-*p*-dioxins and biphenyls (Fig. 1), respectively.

$$\log (\text{EROD}) = 0.94 \log (\text{AHH}) + 0.43 \quad (5)$$

For a total of 27 compounds in both series, there was a linear correlation between the EC_{50} values for the two enzyme activities in which the slopes for both equations were not significantly different from 1. These results confirm that the oxidation of both ethoxyresorufin and benzo[*a*]pyrene is catalyzed by the same cytochrome P-450 isozyme(s).

The *in vitro* studies reported in this paper demonstrate the applications of QSAR in probing the effects of lateral substituents on the receptor-binding and enzyme induction activities of 7-*X*-2,3-dichlorodibenzo-*p*-dioxins. The data illustrate that there are major differences in the binding forces which facilitate the interaction of substituted dibenzo-*p*-dioxins and biphenyls with the TCDD cytosolic receptor protein. Future QSAR studies are planned with other substituted halogenated aromatics to investigate further the structural effects which are important for ligand-receptor interactions and these data will be used as a guide for the design of affinity labels for this protein.

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