A QSAR STUDY OF SOME PCBs' LIGAND-BINDING AFFINITY TO THE CYTOSOLIC Ah RECEPTOR (AhR)

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Introduction

Polychlorobiphenyls (PCBs) represent a class of organohalogen compounds present in the environment and known to give rise to toxic effects mediated by the aryl hydrocarbon (Ah) receptor and, in some cases, other nuclear receptor proteins such as the estrogen receptor (ER) and constitutive androstane receptor (CAR), for example.

Depending on the substitution pattern in the biphenyl nucleus, it is possible for the PCB in question to adopt preferentially either a coplanar or non-planar conformation. For example, *ortho* substitution tends to give rise to non-planarity due to steric hindrance with nearby hydrogen (or chlorine) atoms, whereas *meta* and *para* substitution generally results in relatively planar conformers being energetically favourable. In a previous study, it has been shown that the extent of PCB planarity as described by the area/depth^2 ratio is linearly related (R = 0.89) to the ligand-binding affinity (pEC_{50}) for the Ah receptor[1-3] based on experimental data published by Stephen Safe's group[4]. More recently, it was demonstrated that the inclusion of molecular length improves this correlation (R = 0.95) significantly[5], and it is likely that this parameter represents a measure of overall molecular size. A high degree of molecular planarity is an important characteristic of Ah receptor ligands[5,6] whereas the extent of ligand-binding site desolvation may be related to the molecular size/length parameter. Although this two-variable expression is able to explain 90% of the variation in potency for the relatively small number of congeners studied, it is thought that inclusion of electronic structural descriptors could improve the correlation further and enable additional PCBs to be augmented into the dataset.

Methods

Molecular orbital (MO) calculations by the AM1 method[7] were executed on 14 PCB congeners shown in Table 1. Electronic structural parameters were collated from the MO output and were utilized as part of a larger dataset which included molecular shape parameters, area/depth^2, length/width and the overall molecular dimensions themselves. All structural calculations were carried out on a Silicon Graphics Indigo^2 10000 High Impact graphics workstation running the Sybyl molecular modelling software package (Tripos Associates, St. Louis, MO). Quantitative structure-activity relationships (QSARs) were generated via stepwise multiple linear regression analysis of the structural parameters against biological data in the form of Ah receptor binding affinity[4].

Results and Discussion

Table 1 presents a summary of the results for the structural calculations on 14 PCBs and for the QSARs generated against biological activity in the form of Ah receptor binding (pEC_{50}) expressed as the negative logarithm of affinity[4]. A high degree of molecular planarity is exhibited for

compounds 1 and 2 (3,3',4,4'- and 3,3',4,4',5-PCBs, respectively) whereas those congeners possessing chlorine substituents in the 2 and/or 2' positions were relatively non-planar, with 2,2'- and 2,6-containing PCBs representing the isomers showing least planarity as exemplified by their low area/depth^2 ratios (Table 1). The molecular planarity descriptor, area/depth^2, gave a fairly good correlation with Ah receptor binding affinity (R = 0.80) for the 14 PCBs presented, whereas inclusion of molecular length improved this correlation (R = 0.88) for the same dataset, thus indicating that both molecular size and shape are of relevance to the ability of this class of molecule to fit the Ah receptor ligand binding site.

Table 1 Data for Polychlorobiphenyls Binding to the Ah Receptor

	Compound	Length	Area/depth^2	E(HOMO)	pEC_{50}
1.	3, 3', 4, 4', 5-PCB	14.1	9.8	-8.82	6.89
2.	3, 3', 4, 4'-PCB	14.1	8.5	-.8.69	6.15
3.	2, 3, 4, 4', 5-PCB	14.0	5.0	-8.75	5.39
4.	2, 3, 3', 4, 4'-PCB	14.0	4.0	-8.77	5.37
5.	2, 3, 3', 4, 4', 5'-PCB	14.0	3.1	-8.91	5.33
6.	2, 3', 4, 4', 5-PCB	14.0	4.8	-8.86	5.15
7.	2, 3', 4, 4', 5-PCB	13.9	4.6	-8.73	5.04
8.	2', 3, 4, 4', 5-PCB	13.9	3.6	-8.89	4.85
9.	2, 3', 4, 3', 5, 5'-PCB	13.9	3.2	-8.86	4.80
10.	2, 3, 4, 4'-PCB	13.9	4.4	-8.65	4.55
11.	2, 2', 4, 4', 5, 5'-PCB	13.9	2.2	-8.77	4.10
12.	2, 3', 4, 4', 5, 6-PCB	13.9	2.8	-8.82	4.00
13.	2, 2', 4, 4'-PCB	13.9	4.1	-8.56	3.89
14.	2, 3, 4, 5-PCB	12.7	4.4	-8.74	3.85

Length = Molecular length (Å)
Area/depth^2 = Ratio of molecular area to square of molecular depth
E(HOMO) = Energy of the highest occupied MO (eV.)
pEC_{50} = Negative logarithm of the Ah receptor binding affinity

	Regression Equations	n	s	R	F
1.	pEC_{50} = 0.33 Area/depth^2 + 3.42 (±0.07)	14	0.547	0.80	21.2
2.	pEC_{50} = 0.30 Area/depth^2 + 0.98 length - 10.05 (±0.06) (±0.36)	14	0.443	0.88	19.8
3.	pEC_{50} = 0.36 Area/depth^2 + 3.69 E(HOMO) - 29.13 (±0.06) (±1.22)	14	0.422	0.90	22.3
4.	pEC_{50} = 0.33 Area/depth^2 - 3.22 E(HOMO) + 0.84 Length - 36.44 (±0.06) (±0.90) (±0.26)	14	0.308	0.95	31.5

However, the combination of area/depth^2 and E(HOMO), the energy of the highest occupied MO, gave a somewhat better correlation (R = 0.90) as evidenced by the statistical analysis presented in Table 1. In fact, a significantly improved correlation (R = 0.95) resulted from the combination of all three structural descriptors, namely: area/depth^2, length and E(HOMO), which produced an excellent agreement with the experimental data for 14 PCBs, as shown in Figure 1. Consequently,

it would appear that the HOMO energy is describing an important facet of the ligand binding process which could involve a π-π stacking interaction[8] between benzene rings on the PCB molecule and one or more aromatic amino acid residues (eg. phenylalanine, tyrosine or tryptophan) in the Ah receptor ligand binding site itself. This inference is supported by similar QSAR studies on the Ah receptor binding affinity of TCDDs[5] where the HOMO energy has also been shown to be an important descriptor alongside molecular planarity, together with the overall rectangularity as measured by the length/width ratio. In fact, it is possible to derive a molecular template of Ah receptor ligands, as shown in Figure 2, which indicates that there is an optimal size and shape characteristic (including rectangular envelope dimensions) which best describes structural features of the ligand binding site[5,6].

Conclusion

The variation in Ah receptor binding affinity for planar and non-planar PCBs, an important measure of their biological potency, is related to several factors of their molecular and electronic structure, including: planarity, length and energy of the highest occupied MO. These three descriptors explain over 90% of the variance for 14 PCBs, which includes tetrachloro-, pentachloro- and hexachlorobiphenyls, and indicates that there may be π-π stacking interactions with aromatic amino acid residues within the Ah receptor binding site which preferentially accepts relatively planar aromatic molecules within a specific rectangular envelope. Homology modelling of the Ah receptor ligand-binding domain should assist further in identifying specific binding site interactions and we are currently exploring this area of research in more detail for future studies.

References

1. Lewis, D.F.V; Computer-assisted methods in the evaluation of chemical toxicity, *Reviews in Computational Chemistry*, **1992**, 3, 173-222.

2. Parke, D.V., Ioannides C and Lewis DFV; Structure activity models for toxicity testing, in: *Toxicology in Europe in the Year 2000*, FEST Supplement (C.M. Hodel, ed.) Elsevier, Amsterdam, **1986**, 14-19.

3. Lewis, D.F.V., Gray TJB and Lake BG; The use of structure-activity relationships, in: *Drug Metabolism from Molecules to Man* (D.J. Benford, J.W. Bridges and G.G. Gibson, eds.) Taylor & Francis, London, **1987**, 369-378.

4. Safe, S., Bandiera S, Sawyer T, Zmudzka B, Mason G, Romkes M, Denomme MA, Sparling J, Okey AB and Fujita, T; Effects of structure on binding to the 2,3,7,8-TCDD receptor protein and AHH induction: halogenated biphenyls, *Environmental Health Perspectives*, **1985**, 61, 21-33.

5. Lewis, D.F.V; Quantitative structure-activity relationships in substrates, inducers and inhibitors of cytochrome P4501 (CYP1), *Drug Metabolism Reviews*, **1997**, 29, 589-650.

6. Jacobs, M.N. and Lewis DFV; An examination of recent findings from Seveso on the reproductive system and mechanisms of dioxin toxicity, *Human and Experimental Toxicology*, **1998**, 17, 469-534.

7. Dewar, M.J.S., Zoebisch EG, Healy EF and Stewart JJP; AM1: a new general purpose quantum mechanical molecular model, *Journal of the American Chemical Society*, **1985**, 107, 3902-3909.

8. Lewis, D.F.V; MO-QSARs: a review of molecular orbital-generated quantitative structure-activity relationships, *Progress in Drug Metabolism*, **1990,** 12, 205-255.