A QSAR study of organochlorine and isoflavenoid compounds ligand binding affinity to the human Oestrogen Receptor α

Miriam N. Jacobs and David F. V. Lewis

Molecular Toxicology Group, School of Biological Sciences, University of Surrey, Guildford, Surrey,GU2 5XH UK

Introduction

PCBs and phytoestrogens are known to bind to oestrogen receptors (ERα and β)[1-4] some having oestrogenic effects across species by stimulating the transcriptional activity of the ER, others having antioestrogenic activity. They are known to mediate effects in other nuclear receptor proteins, phytoestrogens within the pregnane-X-receptor (PXR) and PCBs within the constitutive androstane receptor (CAR). PCBs are also Ah receptor agonists with additional varied toxicological endpoints, which include developmental defects, neurotoxicity and immunotoxicity.

A three-dimensional model and crystal structure of the oestrogen receptor (ER) was used to estimate the binding energies of a 9 oestrogenic agonists and antagonists including 17β-oestradiol (agonist); phytoestrogens; environmental oestrogen mimics, synthetic pharmacological ER agonists and antagonists in the ER binding site. Quantitative Structure Activity Relationship (QSAR) models were established for the 9 compounds. The models demonstrated a significant correlation (R>0.95) between the calculated RBA and observed RBAs, giving a good predictive capability based on linear regression. The models and QSARs obtained provide a rational basis for ligand selectivity.

Materials and Methods: Modelling[5, 6]

The multiple sequence alignments between RAR-γ [7]and several ER sequences including hER were generated by using the GCG package. The hER crystal structure[8], was also used as a template for homology modelling of the hERα ligand binding domain via molecular mutagenesis using the Sybyl Software Suite and specifically employing the Biopolymer module to mutate amino acid residues required by the alignment, together with deletions and insertions, shown in the alignment.

The raw structure of the hER was refined using molecular mechanics to perform energy minimisation of the entire three dimensional structure, to achieve a low energy, stable optimised geometry. Each minimisation took 100 iterations to produce a minimum energy geometry for each docked molecule. Ligand binding interaction energies were estimated from the differences between calculated minimum energies of the ligand-receptor complex and its individual components. The ligands chosen for these binding studies were 17β-oestradiol (agonist), genistein, daidzein and coumestrol, hydroxy PCB153 and hydroxy PCB118 and 4-hydroxytamoxifen (partial agonist) and ICI 182780 (pure antagonist) and latterly 2,2,',4,4,' tetra brominated diphenyl ether (2,2'4,4' TBDE) because of recent reports on rapidly increasing breast milk levels. The data set included a spread of both structural diversity and a wide range of RBAs to give a robust model that can correlate the variation in RBA with chemical structure. The three dimensional structures of each compound were produced using the build and edit/sketch option in Sybyl (Tripos Associates, St Louis, MO). The putative binding site of the hER was located and 17β-oestradiol was fitted into the site. This ligand binding pocket is well defined within a loop of peptide containing complementary amino acids for binding interactions with agonists and antagonists.

Combined with a predominance of leucine, the site is in an optimum position for dimerization leading to DNA interaction[5]. Hydrogen bonds were displayed to ensure the binding orientation was consistent with their formation. The fitted 17β-oestradiol became the template to position the other compounds. The following variables were calculated:

- Surface area, the area of the solvent accessible (hydrocarbon) surface ($Å^2$) of the Connolly surface, around the molecule , based on a 1.4 Å radius solvent sphere (SA).
- Relative molecular mass of the ligand (Mr)
- Log P Calculated (in Pallas, Compudrug Ltd, Budapest). Both Ah receptor binding affinity and EROD activity are related to log P and molecular planarity of PCBs. Log P values of PCBs range from about 5 to 7, broadly increasing with the number of substituting chlorines and planarity of position. 2,2',4,4,',5,5' PCB log P (Obs) = 6.72
- Distance between H bonds of donor acceptors (DHB)
- Molecular weight (MW)
- Docking interaction energy (DI) i.e. the distribution of charge and the shape of the molecule.
- Total energy in HER after minimisation (Kcals/mol)$E_{complex}$ (TEM)
- Minimisation final energy of compound (Kcals/mol) E_{ligand} (MFE)
- $E_{binding} = {=}E_{complex-} E_{receptor-} E_{ligand}$ (EEE)
- Ionisation potential eV (IP)
- Dipole moment Debye (DM)
- E_{HOMO} eV (EH)
- E_{LUMO} eV (EL)
- ΔE eV(DEL E) = E_{LUMO} -E_{HOMO}
- Biological activities: RBA and Log RBA Observed
- RBA and log RBA calculated
- $\Delta G_{part.}$ using calc log P
- Distances for binding with amino acid residues in the binding site

From the data generated, a multiple linear regression analysis for the QSAR of the test compounds in both the model and the crystal structure was conducted using the mainframe program ALLREG. 16 variables were entered for 9 test compounds, then 8 that correlated well (checked for cross-correlation) were selected for further analysis. The experimental values came from various sources[1-3], and where a range of values were found, the most recent one was used [2]. For some compounds experimental values for ligand binding in the ER are not yet available. This was the case for PCB 153 and 2,2',4,4' TBDE. The closest structurally similar value available for PCB 153 was that for another hexachlorobiphenyl, PCB141. For the same reasons the value used for 2,2',4,4' TBDE was that for *op* DDT[3]. These two values may therefore be less reliable.

Results and Discussion

E2, and all the test compounds, formed three hydrogen bonds with Glu 353, His 524 and Arg 394 (in agreement with other studies[4]. In the crystal structure complex of E2 and human ER-α, the A–ring phenolic hydroxyl makes direct bonds to the carboxylate of Glu 353, the guanidinium group of Arg 394 and a water molecule. The D-ring's 17-β hydroxyl makes a single hydrogen bond with His 524. The A-ring is sandwiched between the side-chains of hydrophobic residues on its α- and β- faces, and the D-ring has non-polar contacts with hydrophobic residues. Where bonding occurs, OH charges on the phenyl ring may be significant. The other main interactions of the ligand with the protein are believed to be non-polar. Analogs of oestradiol that have a 3-position moiety that is only capable of acting as a hydrogen bond acceptor and not a donor, have poor binding affinities. This may be due to electrostatic repulsion between the 3-position heteroatom of the ligand and the carboxylate of the Glu 353 . The hydrogen

Table 1: Selected variables calculated from the hER model and the computer programme Pallas, for the QSAR.

Compounds	Obs. LogRBA[3]	Obs. RBA[3]	DI	MFE	EEE	IP	DM	EL	DELE
1. Hydroxy PCB153	-1.523	0.03	-8.270	-4.360	-11.736	9.514518	1.3720	0.5949	8.9196
2. Hydroxy PCB118	0.0	1.0	-3.776	-3.330	-3.163	9.189840	2.3010	-0.7207	8.4691
3. 17 β– oestradiol	2.0	100	-6.455	11.627	-22.375	8.804693	1.5250	0.4215	9.2261
4. Coumestrol	1.301	20	-10.004	17.933	-18.18	8.667859	1.586	-1.2381	7.4297
5. Daidzein	-1.0	0.1	-8.479	-10.417	-1.163	8.741259	2.550	-0.5968	8.1444
6. Genistein	0.602	4.0	-7.639	-10.418	-1.162	8.686854	3.169	-0.4864	8.2004
7. 4-hydroxytamoxifen	2.410	257.0	-7.8392	11.386	-25.332	8.446314	1.5180	0.0506	8.4969
8. Hydroxy 2,2,',4,4,'TBDE	-1.0	0.1	-8.392	-2.886	-15.023	9.286730	2.90	-0.4742	8.8125
9. ICI 182780	0.79	6.15	-2.368	9.508	-41.801	8.930579	3.662	0.2004	9.1309

Table 2: QSAR correlations
Where: n = number of variables; R = multiple correlation coefficient; s = standard error of Y estimate

Equation	n	s	R	F
Log RBA =0.104(+/-0.037)**MFE**-2.926 (+/- 0.739) **IP**+0.044(+/-0.03)**EEE**+1.157(+/-0.0569)**DEL E** +17.078	9	0.579	**0.956**	10.48
Log RBA =1.157 (+/-0.05693)**EL**-0.1.042(+/-0.3.738) **MFE**+-1.769(+/-0.7069)**IP**+0.0439(+/-0.0325)**EEE**+17.078	9	0.579	**0.956**	10.48
Log RBA =2.926(+/-0.7393) **EL**-0.104(+/-0.0.374) **MFE**+0.0438(+/-0.0.033)**EEE** +-1.768(+/-0.707)**DEL E** +17.078	9	0.579	**0.956**	10.48
Log RBA = 0.06375(+/-0.02918)**MFE**+-2.7731(+/-0.7823) **IP**+0.6360(+/-0.4392)**DEL E**+19.565	9	0.62	**0.935**	11.67
Log RBA =-0.3744(+/-0.3717)**DM**+0.1880(+/-0.11909) **DI**+0.051183(+/0.1191)**MFE**-2.6653(+/-0.7753)**IP**+26.237	9	0.648	**0.944**	8.159

bond-accepting feature of His 524 seems to be quite flexible when comparing agonist and antagonist structures, whereas Glu 353 forms a salt bridge with Arg 394 and defining its location [4]. Genistein and daidzein were outliers for all the combinations of parameters except Glu353, suggesting that the distance of binding to this residue is a key variable for the two phytoestrogens, which otherwise appear to have a low biological potency for hERα binding. It may also be indicative of indirect mechanisms acting upon the binding site for hERα.The phytoestrogens may be more potent in hERβ, RXR and as yet uncharacterized steroid receptors, perhaps as a consequence of evolutionary development. The 6 Å biophore (a distance descriptor) present in endogenous oestrogens and xenoestrogens (related to the A ring region in E2), is not present in phytoestrogens, and they appear to be less conformationally flexible due to a planar polyaromatic structure [9] unlike the other compounds in the data set, thus less likely to have a conformational effect. The MFE appears to be more significant for ligand binding affinity than previously thought. The distances for binding with amino acid residues in the binding site were within the range expected for the formation of two hydrogen bonds (2 to 3Å) for Glu 353, Arg 394 and His 524, at 3.0Å.. Receptor flexibility or the presence of a bridging water molecule might account for the ability of the ER to accommodate

ligands with variation in the D-ring and, differing oxygen-oxygen distances. Species, tissue specificity and dose are also among the multiplicity of mechanisms that are believed to be involved. Three QSARs showed particularly strong correlations; Minimised Free Energy, Ionisation Potential, $E_{binding}$, Δ E (R=0.956); E_{Lumo}, Minimised Free Energy, Ionisation Potential, $E_{binding}$ (R=0.956) ; and E_{Lumo}, Minimised Free Energy, Δ E (R=0.956).

Ligand binding modulates the hydrogen bonding network which provides interpeptide communication in the hER between ligand binding and DNA activation. When an agonist molecule completes this electron transport circuit, the resulting effect may disrupt the existing framework of hydrogen bonds to free the DNA binding zinc finger domain. Molecular dynamics simulation is a useful tool to investigate the mechanism behind the conformational energy changes following ligand binding which lead to hER activation, particularly when compared to *in vitro* estimations of the ligand binding affinity.

This study has generated theoretical and molecular structural data for the production of relationships (QSARs) which examine and estimate the potency differences within these compounds in human oestrogen receptor ligand interactions based on sequence homology, site directed mutagenesis and crystallographic data. Further development, in relation to experimental and observed data is needed, to provide a useful tool in a complex situation of considerable public concern. It could be invaluable for providing a scientifically sound perspective on the potential risk to human health from environmental and dietary exposure to oestrogenic materials and other endocrine disrupters, and be of potential relevance in the design of novel anti-oestrogens.

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