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Mechanistic Insights into the Specificity of Human Cytosolic Sulfotransferase 2A1 (hSULT2A1) for Hydroxylated Polychlorinated Biphenyls Through the Use of Fluoro-tagged Probes

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Abstract

Determining the relationships between the structures of substrates and inhibitors and their interactions with drug-metabolizing enzymes is of prime importance in predicting the toxic potential of new and legacy xenobiotics. Traditionally, quantitative structure activity relationship (QSAR) studies are performed with many distinct compounds. Based on the chemical properties of the tested compounds, complex relationships can be established so that models can be developed to predict toxicity of novel compounds. In this study, the use of fluorinated analogues as supplemental QSAR compounds was investigated. Substituting fluorine induces changes in electronic and steric properties of the substrate without substantially changing the chemical backbone of the substrate. *In vitro* assays were performed using purified human cytosolic sulfotransferase hSULT2A1 as a model enzyme. A mono-hydroxylated polychlorinated biphenyl (4-OH PCB 14) and its four possible mono-fluoro analogues were used as test compounds. Remarkable similarities were found between this approach and previously published QSAR studies for hSULT2A1. Both studies implicate the importance of dipole moment and dihedral angle as being important to PCB structure in respect to being substrates for hSULT2A1. We conclude that mono-fluorinated analogues of a target substrate can be a useful tool to study the structure activity relationships for enzyme specificity.

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Conflict of Interest

The authors state that no conflict of interest exists.

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Keywords

F-tagged probes; QSAR; hSULT2A1; polychlorinated biphenyls; hydroxylated polychlorinated biphenyls; sulfotransferase; computational chemistry; 4-hydroxy-3,5-dichlorobiphenyl

Introduction

There is a great need to predict the uptake, metabolism, and subsequent toxicity of xenobiotic agents, such as drugs, dietary supplements, and environmental pollutants (Pery *et al.* 2010, Soffers *et al.* 2001, Winkler *et al.* 2013). Quantitative structure activity relationship (QSAR) studies are used to define critical factors of substrates and inhibitors of enzymes. Most commonly, after testing a large variety of compounds, several different properties important to enzyme specificity are identified as well as the varying degrees to which these parameters contribute to that specificity. Defining these properties is especially important in toxicology where determining the potential of specific xenobiotics and their metabolites to negatively affect endogenous enzymes is crucial. One of these enzymes that is involved in both the metabolism of xenobiotics as well as physiologically important endogenous molecules is the human cytosolic sulfotransferase 2A1 (hSULT2A1).

Human cytosolic sulfotransferase 2A1 is an important enzyme both for hydroxysteroid hormonal homeostasis and for the metabolism of many drugs and other xenobiotics. Among the physiologic functions of hSULT2A1, the enzyme catalyzes the 3'-phosphoadenosine 5'-phosphosulfate (PAPS)-dependent sulfation of dehydroepiandrosterone (DHEA), other endogenous hydroxysteroids, and many xenobiotic alcohols, phenols, and amines (Duffel 2010, Gamage *et al.* 2006, James & Ambadapadi 2013, Pacifici & Coughtrie 2005). Such sulfation reactions facilitate the transport, redistribution, and/or excretion of these molecules. Previous studies have determined that hydroxylated polychlorinated biphenyls (OH-PCBs) can serve either as competitive substrates or as inhibitors for the hSULT2A1-catalyzed sulfation of dehydroepiandrosterone (DHEA) (Ekuase *et al.* 2011, Liu *et al.* 2006). Such interactions might either alter systemic distribution of DHEA or interfere with its cellular role as a precursor to androgens and estrogens within specific tissues. The importance of electrostatic and steric effects on the specificity of hSULT2A1 was investigated using a range of 15 OH-PCBs, and a QSAR for inhibition of the enzyme was developed (Ekuase *et al.* 2014, Ekuase *et al.* 2011). While the findings of these studies likely have broader applicability to many other OH-PCB congeners, such extrapolation would be aided by development of additional methodology to both verify currently available studies and extend them to other OH-PCBs.

An alternative QSAR strategy involves introduction of fluorine atoms to a more limited set of the molecules of interest. Fluoro-tagging is accomplished by substituting a single hydrogen atom with a fluorine atom at defined locations in the molecular structure (Luthe *et al.* 2002a). This is a unique method to alter a molecule, since functional groups between molecules remain similar, the carbon backbone is identical for all compounds studied, fluorine is a generally inert substituent, and these substituents are not commonly found in nature nor can they be introduced by human metabolism. The introduction of a fluorine atom

does however induce steric (*e.g.*, dihedral angle, rotational barrier) as well as electronic (*e.g.*, electron density, dipole moment, Ionization constant and lipophilicity) differences in a molecule. Depending on the hydrogen atom chosen for substitution, the magnitude of these differences can be controlled (Klösener *et al.* 2008, Luthe *et al.* 2007). This technique has been successfully used to generate non-isotopic tracers or internal standards for mass spectroscopy (Kim *et al.* 2014, Klösener *et al.* 2009, Luthe *et al.* 2006, van 't Erve *et al.* 2010), and investigate the properties of environmental pollutants (Klösener *et al.* 2008, Luthe & Brinkman 2000, Luthe *et al.* 2008a, Luthe *et al.* 2008b, Luthe *et al.* 2007). Thus, we hypothesize that fluoro-tagged OH-PCBs would provide unique insight into the QSARs of their interactions with hSULT2A1.

Interest in the toxicology of OH-PCBs derives both from their occurrence as metabolites of polychlorinated biphenyls (PCBs) and from the diversity of toxicities associated with them. PCBs are legacy environmental pollutants that were manufactured and used for many varied applications over the span of decades (Erickson & Kaley 2011). In addition to these legacy sources of PCBs, it is increasingly being recognized that PCBs are currently being produced inadvertently in some manufacturing processes (Hu & Hornbuckle 2010). Adverse health effects of PCBs include carcinogenesis, endocrine disruption, neurodevelopmental and reproductive effects, cardiovascular disease, immunological disorders, and other toxicities (Brouwer *et al.* 1999, Hansen 1998, Lauby-Secretan *et al.* 2013, Ludewig & Robertson 2013, Schantz 1996, Seegal 1996). Although there are 209 possible congeners of PCBs, we are particularly interested in those with lower numbers of chlorine atoms. These PCBs are especially represented in outdoor and indoor air samples (Currado & Harrad 1998, Herrick *et al.* 2004, Persoon *et al.* 2010) and are readily metabolized to OH-PCBs in reactions catalyzed by cytochrome P450s (Bergman *et al.* 1994, Grimm *et al.* 2015, James 2001, Matthews & Kato 1979). In human blood, an increasing number of OH-PCBs have been identified, and the detected levels of some congeners are equal or greater than some unmetabolized PCB congeners (Bergman *et al.* 1994, Grimm *et al.* 2015, Quinete *et al.* 2014, Sandau *et al.* 2000). In addition to their presence as *in vivo* metabolites of PCBs, OH-PCBs have also been found in water and sediment samples as well as in original aroclor mixtures (Ueno *et al.*, 2007, Marek *et al.*, 2013 (Marek *et al.* 2013, Ueno *et al.* 2007)). As recently reviewed (Grimm *et al.* 2015, Quinete *et al.* 2014), OH-PCBs have been associated with most of the adverse health effects that are also observed with PCBs themselves.

Our interest in the biological activities of the OH-PCBs has led to investigations of their interactions with sulfotransferases that may either be involved in the further metabolic conversion of OH-PCBs or for which interaction with OH-PCBs may inhibit sulfation of endogenous molecules that are important in endocrine and other physiologic functions. In the current study, four mono-fluorinated analogues of 4-OH PCB 14 were investigated to identify key properties for the substrate specificity displayed by hSULT2A1. Predicting the potency for legacy as well as novel xenobiotics and their metabolites to disrupt endogenous systems is of increasing importance to the evaluation and design of industrial processes as well as decision-making by government regulatory agencies.

Materials and Methods

Synthesis

Four fluorinated analogues of 4-hydroxy-3,5-dichlorobiphenyl (4-OH PCB 14) were synthesized (*i.e.*, 2'-fluoro-4-hydroxy-3,5-dichlorobiphenyl (4-OH PCB 14 F2'), 3'-fluoro-4-hydroxy-3,5-dichlorobiphenyl (4-OH PCB 14 F3'), 4'-fluoro-4-hydroxy-3,5-dichlorobiphenyl (4-OH PCB 14 F4') and 2-fluoro-4-hydroxy-3,5-dichlorobiphenyl (4-OH PCB 14 F2)) using a modified Suzuki cross-coupling, and subsequent demethylation (Luthe *et al.* 2009). For 4-OH PCB 14 F2, the starting bromobenzene was synthesized from 4-bromo-3-fluorophenol as described below.

Di-ortho-chlorination

27 mmol (5 g) of 4-bromo-3-fluorophenol was dissolved in 100 mL dry acetonitrile. The solution was stirred until all phenol was dissolved. 60 mmol (8 g) N-chlorosuccinimide was added in three portions to the stirring mixture. The mixture was allowed to stir for a minimum of 24 h, at which time the reaction was monitored using either TLC or GC-FID. Additional N-chlorosuccinimide was added when necessary. In general, yields of over 90% were reached between 24 and 48 h. The reaction mixture was washed with 1M HCl (5 × 20 mL) to remove succinimide and unreacted N-chlorosuccinimide. Flash silica gel column chromatography with hexane/ethyl acetate (9.9:0.1) was used to remove remaining contaminants.

Methylation

20 mmol (3.78 g) of 4-bromo-2,6-dichloro-3-fluorophenol was dissolved in 50 mL of dried acetone. The solution was heated to (50 °C) dissolve the phenol. When dissolved, 30 mmol (4.15 g) of dry potassium carbonate and 30 mmol (4.26 g) of methyl iodide was added to the reaction. The mixture was refluxed for a minimum of 6 h. The volume of acetone was reduced to 10 mL, and the residue was extracted with 3 × 10 mL of dichloromethane. The organic layers were combined and washed with 3 × 10 mL of water. The solution was dried with MgSO₄ and NaSO₄ overnight, filtered, and the solvent was evaporated. The crude product was recrystallized from methanol.

GC-MS characterization

Analysis of 4-OH PCB 14 F2', 4-OH PCB 14 F3', 4-OH PCB 14 F4' and 4-OH PCB 14 F2 was carried out on a Thermo Trace 2000 GCMS (Thermo Fisher, San Jose, CA, USA) coupled with a Thermo Voyager inert MS detection and auto sampler (Thermo AS 3000). 1 µL aliquots were injected split less into the device. The injector temperature was set to 225 °C. Separation was performed on an SLB-5ms capillary column (60 m 0.25 mm I.D., 0.25 µm film thickness). Helium was used as the carrier gas at a flow of 1.2 mL/min. The split was opened after 2 min. The column temperature was programmed from 100 °C to 250 °C with 10 °C/min. The final temperature was held for ten minutes. Detection was based on EI-MS-mode in the total ion count (TIC) mode (m/z 50–500) over the entire time range. The ion source temperature was 200 °C.

Chemicals and reagents

Adenosine-3'-phosphate-5'-phosphosulfate (PAPS), adenosine-3',5'-diphosphate sodium salt (PAP), potassium phosphate monobasic, phenylmethylsulfonyl fluoride (PMSF), and dehydroepiandrosterone (DHEA) were purchased from Sigma-Aldrich (St. Louis, MO). PAPS was further purified (>98% as analyzed by HPLC) using a previously described procedure (Sekura 1981). Bacto tryptone and yeast extract were obtained from Becton Dickinson, Co. (Sparks, MD) and Hydroxyapatite (Bio-Gel HT) from Bio-Rad Laboratories (Hercules, CA). Tween 20 was purchased from J.T. Baker Chemicals (Phillipsburg, NJ). Isopropyl-1-thio-D-galactopyranoside (IPTG) was from AMRESCO Inc. (Solon, OH). All other chemicals used were of the highest purity commercially available.

Enzyme expression and purification

Overexpression of hSULT2A1 was achieved using recombinant *Escherichia coli* BL21 (DE3) cells as described previously (Ekuase *et al.* 2011, Sheng & Duffel 2003). After cells were disrupted by sonication to release the enzyme, purification of hSULT2A1 was carried out using a previously described procedure (Gulcan *et al.* 2008). The purity of the resulting enzyme preparation was judged by SDS-PAGE that was stained by Coomassie Blue. A modified Lowry assay using bovine serum albumin (BSA) as standard was used to determine protein concentrations during purification of the enzyme (Bensadoun & Weinstein 1976). Catalytic activity of hSULT2A1 was determined throughout the purification process using a paired ion extraction assay (Sekura & Jakoby 1979, Sheng *et al.* 2001) with DHEA as substrate.

Enzyme kinetics

A previously described HPLC assay (Liu *et al.* 2009) was used to determine the kinetic characteristics of the fluorine-substituted congeners of 4-OH PCB 14 as substrates for hSULT2A1. Assay mixtures contained 200 μ M PAPS, 0.25 M potassium phosphate at pH 7.0, and 7.5 mM 2-mercaptoethanol. Following incubation at 37 °C for 2 min, either 4-OH-PCB 14 or one of the indicated fluorinated congeners of 4-OH PCB 14 dissolved in acetone were added to the reaction mixture (final concentration of 5 μ M to 267 μ M in the assay, with a final concentration of acetone less than 2 % v/v). A total of 1 μ g purified hSULT2A1 was added, and the reaction mixture (total volume of 30 μ L) was incubated for 5 min at 37 °C. Reactions were terminated by the addition of 30 μ L HPLC grade methanol, mixed vigorously, and 20 μ L of this mixture was analyzed by HPLC for enzyme- and substrate-dependent formation of PAP (Sheng *et al.* 2001). An assay utilizing a final concentration of 50 μ M DHEA served as positive control.

In silico determination of chemical properties

The conformations of substituted biphenyls are described by the dihedral angle of the C1-C1' bond. The changes in the conformation by the introduction of fluorine were calculated using *semi empirical* as well as *ab initio* methods. *Semi empirical*, SCF-MO (self-consistent field molecular orbital) calculations using an Austin Model 1 (AM1) Hamiltonian (18 (Dewar *et al.* 1985)) contained in the Spartan '02 package (19 (Shao *et al.* 2006)) were used to find the optimized structures of the OH-PCBs in the gas phase. The Gaussian 03 program

was used for both *semi empirical* and *ab initio* calculations. We used the AM1 optimized structure as starting point for the *ab initio* optimization. *Ab initio* calculations were carried out using HF level of theory with 3-21G basis sets. *ab initio* calculations were also performed using density functional theory (DFT) B3LYP with 6-31G** basis sets. To determine the effects of solvation of OH-PCBs in water, we applied the surface tension model (SM5) (21 (Hawkins *et al.* 1998)) at AM1 and HF level of theory implemented in Spartan software package. We also used the polarized continuum model (PCM) at the DFT level ((*et al.* 1997, Cossi *et al.* 1996)). The gas phase structures were optimized with either SM5 or PCM in water as an implicit model, to optimize and to determine the structures of the OH-PCBs in aqueous solution. In addition to the dihedral angles, we computed rotation energies, dipole moments, polarized solute-solvent interaction energies, cavitation energies, repulsion energies, dispersion energies and solvation free energies for all compounds. The solvation free energy was defined as the difference in SCF energy of the model compounds between the gas phase and aqueous solutions.

Results

Preparation of the four fluorinated analogues of 4-OH PCB 14

Syntheses and characterizations of 4-OH PCB 14 2'-F, 4-OH PCB 14 3'-F, 4-OH PCB 14 4'-F, and 4-OH PCB 14 2-F were carried out as described in the Materials and Methods. All compounds were >99% pure and stored as their methoxy-derivatives. De-methylation occurred no more than 1 week prior to utilization.

Enzyme kinetics

The four fluorine-substituted analogues of 4-OH PCB 14 were analyzed for their ability to serve as substrates for the purified hSULT2A1. Due to the electronic effects of fluorine in the ring system, the kinetic characteristics of the interactions of these congeners with hSULT2A1 were altered as seen in Figure 1 and Table 1. The maximum reaction velocity, V_{max} , ranged between 30 – 69 nmol/min/mg with 4-OH PCB 14 4'-F and 4-OH PCB 14 2'-F exhibiting the lowest and highest values, respectively. The concentration of substrate at half maximal velocity, K_m , ranged from 3.7 to 25.6 μ M. It is notable that substitution of a fluorine atom at the meta position with respect to the ring junction (4-OH PCB 14 3'-F) did not have any effect on the kinetics of the enzyme-catalyzed reaction. That is, similar V_{max} , K_m and K_i values to those of the non-fluorinated compound, 4-OH PCB 14, were obtained. Fluorine substitution on the non-phenolic ring at the ortho position relative to the ring junction (4-OH PCB 14 2'-F) resulted in increased substrate inhibition (*i.e.* a decreased K_i value), and this may be due to an increased affinity of the substrate for binding to the enzyme-PAP complex. The resulting formation of a dead-end complex is commonly seen in the substrate inhibition of hSULT2A1 by DHEA (Gulcan & Duffel 2011). Along with an increase in substrate inhibition, there was also an increase in the catalytic efficiency (K_{cat}/K_m) of the enzyme with 4-OH PCB 14 2'-F as substrate. Fluorine substitution at the para position relative to the ring junction (*i.e.*, 4-OH PCB 14 4'-F) resulted in an opposite effect on the affinity of the substrate for the enzyme. As seen in Table 1, 4-OH PCB 14 4'-F had a decreased affinity for the enzyme as seen by its exhibition of the highest K_i and K_m

values coupled with a low V_{max} . Thus, the catalytic efficiency of the enzyme with this substrate was the lowest of the congeners examined.

Calculated molecular properties and correlation analysis

Computer aided calculations AM1, HF/3-21G and PCM (DFT/6-31G**) were used to calculate the conformations, dihedral angles, dipole moments, non-electrostatic and polarized solute-solvent energies (polarization, cavitation, dispersion, repulsion, electrostatic), and acidity of the parent 4-OH PCB 14 and the four fluorinated analogues. The properties listed above are shown in Table 2. To determine which calculated properties of the fluorinated OH-PCBs influence substrate specificity for reactions catalyzed by hSULT2A1, a correlation matrix was generated. Linear regression was performed for all kinetic properties measured and compound properties calculated through the various *in silico* models. As seen in Table 3, from all properties included in this study, statistically significant correlations were observed for dipole moment with K_i and V_{max} in all three models and dihedral angle with K_{cat}/K_m from the AM1 model, Figure 3. The correlation observed between V_{max} and dipole moment is positive indicating a higher dipole moment has a positive effect on catalysis at lower concentrations of the OH-PCB. However, compounds with a high dipole moment also exhibit lower K_i values (*i.e.*, increased substrate inhibition characteristics). This would mean that at higher substrate concentrations, the reaction velocity would be more dramatically decreased due to substrate inhibition. The correlation between dihedral angle and K_{cat}/K_m was only significant in one model; therefore the significance of this correlation needs to be investigated further before any conclusion can be drawn.

Discussion

Establishing the fundamental molecular characteristics for substrates of enzymes using some forms of QSAR can take dozens of compounds and multiple tests. In our current studies we have shown that a limited number of fluorinated analogues of a hydroxylated PCB can provide useful information on the molecular parameters involved in the substrate specificity of hSULT2A1. The inclusion of fluorine in the structure of a known substrate prompts substantial changes to the electronic properties of the molecule. Varying the position of the fluorine substituent within the molecule altered the magnitude of this effect. It is noteworthy that with the example presented here (*i.e.*, 4-OH PCB 14 and its four fluorinated analogues acting as a substrates for hSULT2A1), we found results that were remarkably similar to those obtained utilizing a more traditional QSAR study that employed 15 OHPCBs with hSULT2A1 (Ekuase *et al.* 2011).

Fluorine is the most electronegative element and is thus thought of as a negative induction factor. However, due to its hardness, fluorine can push its partial negative charge to overlapping π -orbitals, resulting in hyperconjugation. This is antagonistic to the permanent dipole moment and reduces the London forces, resulting in a lower overall polarity than expected from electronegativity alone. In fact fluoro-substituted aromatic compounds exhibit lower boiling points and higher lipophilicity due to this effect. This hyperconjugation

effect is important in creating a wide range of dipole moments within the same chemical backbone, thus facilitating the elucidation of structure-activity relationships.

An additional advantage of this approach for enzyme-related studies is the comparable van der Waals radii of fluorine and hydrogen, especially if C-F secondary binding (hyperconjugation) is taken into account. Therefore, the overall dimensions and the steric fit in the active site of an enzyme will not be greatly affected by mono-fluorine substitution (Luthe *et al.* 2002b, Luthe *et al.* 2007). The substitution of fluorine only makes a subtle change in the steric properties of a compound, and this enables more precise definition of other factors in a structure-activity relationship. This should, in turn result in the need for fewer numbers of compounds to make accurate predictions of these parameters on catalysis.

Due to the fact that there are 837 potential congeners of OH-PCBs (Grimm *et al.* 2015), the ability to predict interactions of these PCB metabolites with human cytosolic sulfotransferases using a more limited number of compounds becomes an important aspect of risk identification and risk assessment for the lower chlorinated PCBs. This is particularly evident with respect to inhibition of sulfotransferases regulating steroid hormone concentrations (Ekuase *et al.* 2011, Kester *et al.* 2000) as well as with respect to recent findings of high-affinity interactions of the sulfuric acid esters formed from OH-PCBs with the thyroid hormone transport protein, transthyretin (Grimm *et al.* 2013). Our current results indicate that the inclusion of mono-fluorinated analogues of a single OH-PCB congener augments traditional QSAR experiments and can complement, and independently validate, these approaches for the prediction of specificity of a sulfotransferase that is important in metabolism of OH-PCBs as well as endogenous molecules.

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Fig 1. Sulfation of 4-OHPCB 14 and its fluorinated analogues by purified recombinant hSULT2A1

Assays were conducted at pH 7.0 with 200 μM PAPS as described in methods section. Data points are the means $\pm$ standard error of duplicate reactions, and curves represent the fit of the data to a substrate inhibition equation ($v = V_{\max}/(1 + (K_m/[S]) + [S]/K_i)$), where S is substrate concentration, v is the initial velocity, K_m is the Michaelis constant, K_i is the substrate inhibition constant and $V_{\max}$ is the maximal velocity

Fig 2. A strong correlation between K_i and V_{max} with dipole moment is observed in 4-OH PCB 14 and its 4 fluorinated analogues

A: Visual demonstration of the correlation between the dipole moment of the four fluorinated 4-OH PCB 14 analogues and the parent 4-OH PCB 14 with their respective K_i (substrate inhibition constant) and V_{max} (maximal velocity) parameters. B: Visual demonstration of the correlation between the dihedral angle of the four fluorinated 4-OH PCB 14 analogues and the parent 4-OH PCB 14 with their respective K_{cat}/K_m parameters.

Fig 3. Summary of correlation findings

A positive correlation was observed between $V_{\max}$ and dipole moment, while a negative correlation was observed between K_i and dipole moment. Other chemical parameters calculated for these substrates did not have significant correlations with kinetic constants for the enzyme-catalyzed reaction.

Table 1

Summary of kinetic constants for 4-OH PCB 14 and its fluorinated derivatives as substrates for hSULT2A1

	$V_{\max}$ (nmol/min/mg)	K_m (μM)	K_i (μM)	k_{cat}/K_m^a ($\text{min}^{-1} \text{mM}^{-1}$)	DHEA rate ^b (nmol/min/mg)
4-OH PCB 14	44.3 $\pm$ 3.9	8.5 $\pm$ 1.8	221 $\pm$ 56.4	354	70 $\pm$ 1.1
4-OH PCB 14 2-F	33.2 $\pm$ 1.7	3.7 $\pm$ 0.7	259 $\pm$ 43.4	611	62 $\pm$ 1.6
4-OH PCB 14 2'-F	69.0 $\pm$ 7.9	6.3 $\pm$ 1.7	77.5 $\pm$ 17.9	744	83 $\pm$ 5.5
4-OH PCB 14 3'-F	47.7 $\pm$ 5.5	12.1 $\pm$ 3.0	219 $\pm$ 70.1	268	88 $\pm$ 2.2
4-OH PCB 14 4'-F	29.7 $\pm$ 7.0	25.6 $\pm$ 11.0	323 $\pm$ 231.8	79	78 $\pm$ 0.4

^a k_{cat} determined using M_I for hSULT2A1 = 68,000 (*i.e.*, the total M_I of the dimer)

^b Rate determined during each set of assays at a concentration of 50 μM DHEA

Table 2

Chemical properties

Parameters		Model compounds			
Structural formula
		4-OH PCB 14	4-OH PCB 14-2F	4-OH PCB 14-2F	4-OH PCB 14-4F
		Dihedral angle (grad)			
AM1		41.0127	45.6420	45.4072	41.1862
HF/3-21G		45.5648	48.8031	48.7243	46.3255
PCM (DFT/6-31G**)		43.4924	49.3873	51.0508	44.8495
		Dipole moment (Debye)			
AM1		2.97	1.23	4.71	1.68
HF/3-21G		2.75	1.93	3.80	1.53
PCM (DFT/6-31G**)		3.33	1.71	4.87	2.02
		Non electrostatic and polarized solute-solvent energy in DMSO (kcal/mol)			
Polarization		-11.02	-11.02	-11.57	-11.62
Cavitation		27.21	27.97	28.05	28.22
Dispersion		-23.64	-24.05	-24.04	-24.24
Repulsion		1.99	2.02	2.02	2.03
Electrostatic		5.56	5.94	6.04	6.00
		Solvation free energies in DMSO (kcal/mol)			
PCM (DFT/6-31G**)		-0.9117 × 10 ⁶	-0.9706 × 10 ⁶	-0.9737 × 10 ⁶	-0.9737 × 10 ⁶
		Acidity (pKa)			
AM1		6.36	5.82	6.47	6.34

Table 3

Complete correlation overview

CORRELATION	Dihedral angle α			Dipole momentum			Polarization		Cavitation		Solv Energy		Acidity	
	AMI	HF	PCM	AMI	HF	PCM	PCM	PCM	PCM	PCM	PCM	PCM	PCM	PCM
$V_{\max}$	0.343	0.237	0.469	0.963(**)	0.980(**)	0.958(*)	-0.303	-0.031	-0.031	-0.37	0.58			
K_m	-0.653	-0.485	-0.534	-0.284	-0.538	-0.307	-0.577	0.383	0.383	0.815	0.367			
K_i	-0.494	-0.382	-0.586	-0.895(*)	-0.962(**)	-0.891(*)	0.146	0.089	0.089	0.532	-0.424			
K_{cat}/K_m	0.896(*)	0.805	0.868	0.401	0.601	0.398	0.304	-0.103	-0.103	-0.839	-0.217			

Correlation is significant (p<0.01)
 Correlation is significant (p<0.05)