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Structural properties governing drug-plasma protein binding determined by high-performance liquid chromatography method

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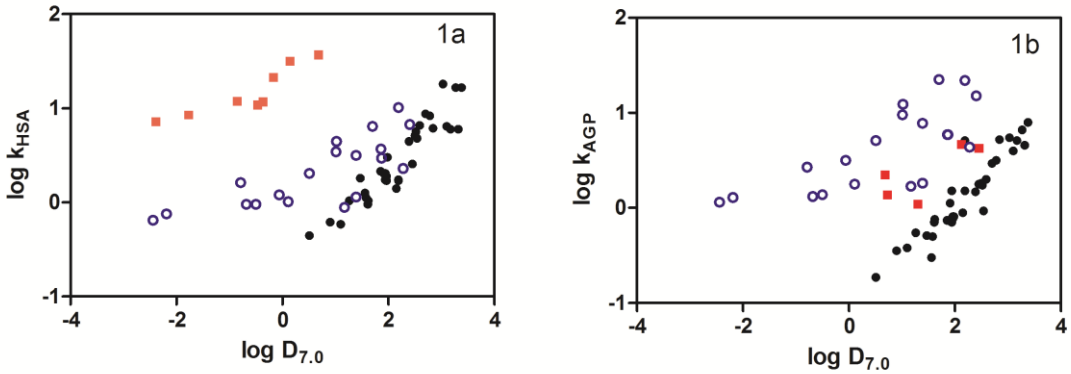
Graphical Abstract



Correlations between log $D_{7.0}$ and log k_{HSA} (1a) as well as log k_{AGP} (1b).
Markers: ●, neutral molecules; ▪, anions; o, cations

Abstract

The high-performance liquid chromatography (HPLC) method employing stationary phases immobilized with plasma proteins was used for this study to investigate the structural properties governing drug-plasma protein binding. A set of 65 compounds with a broad range of structural diversity (in terms of volume, hydrogen-bonding, polarity and electrostatic force) were selected for this purpose. The Abraham linear free energy relationship (LFER) analyses of the retention factors on the immobilized HSA (human serum albumin) and AGP (α_1-acid glycoprotein) stationary phases showed that McGowan's characteristic molecular volume (V), dipolarity/polarizability (S) and hydrogen bond basicity (B) are the three significant molecular descriptors of solutes determining the interaction with immobilized plasma proteins, whereas excess molar refraction (E) is less important and hydrogen bond acidity (A) is not of statistical significance in both systems, for electrically neutral compounds. It was shown that ionised acids, as carboxylate anions, bind very strongly to the immobilised HSA stationary phase and that ionised bases, as cations bind strongly to the AGP stationary phase. This is the first time that the effect of ionised species on plasma protein binding has been determined quantitatively; the increased binding of acids to HSA is due almost entirely to acids in their ionised form.

Keywords

Plasma proteins; Binding; HPLC; Retention factors (k); Linear free energy relationship; Abraham (Absolv) descriptors

1. Introduction

The binding of drugs to plasma proteins is an important process that determines the activity and pharmacokinetics of many drugs in the body. After being distributed around the circulating system, drugs bind to plasma proteins in varying degrees. In general, such binding is reversible, and an equilibrium exists between bound and free molecular forms. In most cases, only the free drug molecules are able to cross membrane barriers, be distributed to tissues and then exert therapeutic effects [1,2]. Drug-plasma protein binding acts as a reservoir for free drug concentration and prolongs the duration of drug action. Therefore drug-plasma protein binding is considered as an important property and needs to be characterized in the early stage of drug discovery [3].

In plasma, two major proteins, human serum albumin (HSA) and α_1-acid glycoprotein (AGP) are present in relatively high quantities. They are able to bind a variety of drugs with considerable affinity [4]. It is found that HSA with the highest concentration ($5\text{-}7.5\times10^{-4}$ mol/L) in plasma has two major binding sites and can bind acidic drugs with high association constants. AGP is an acidic protein with a lower concentration ($0.9\text{-}2.2\times10^{-5}$ mol/L). It exhibits a preference for binding basic and neutral drugs. Lipoproteins which are macromolecular complexes of lipid and proteins also play a role to a lesser degree [5].

There are different techniques to determine drug-plasma protein binding *in vitro*. Among these techniques, equilibrium dialysis, ultra-filtration and ultra-centrifugation have been most widely used. However, these methods show a number of limitations such as a very low throughput and poor reproducibility [6]. In comparison to these conventional methods, the HPLC method that employs stationary phases immobilized with plasma proteins appears an interesting approach because of the speed of analysis and automation capability [7,8]. In this method, drugs with high affinities interact strongly with the immobilized protein and are eluted later than drugs with no or less affinities. The affinity is expressed by the retention factor k, which is calculated through Eq. 1

$$k = (t_r - t_0)/t_0 \qquad \text{Eq.1}$$

where t_r and t_0 are the retention times of the solute and of an unretained compound, respectively.

A number of studies have been carried out to investigate drug-plasma protein interaction by using special classes of drugs [9-13]. In order to understand comprehensively the mechanisms of drug-plasma protein interaction, we selected for this study a set of drugs with a broad range of structural diversity (in terms of volume, hydrogen-bonding, polarity and electrostatic force). The interaction between these drugs and immobilized plasma proteins (HSA and AGP) was measured using the HPLC method. By employing a linear free-energy relationship (LFER) model [14-16], we aim to establish quantitative structure-drug-plasma protein binding relationships and to unravel the structural parameters governing the interaction mechanisms, which are very important for drug design. These quantitative relationships can be used to estimate protein binding of drugs in the early stages of drug discovery, and so will be of considerable use and importance.

2. Materials and methods

2.1. Solutes and reagents

All compounds were obtained from commercial sources (Sigma-Aldrich, Gillingham, UK) and in the highest available purity. Distilled water and HPLC grade isopropanol (Fisher Scientific, Loughborough UK) were used throughout.

2.2. Measurement of retention factors

The retention factors were measured with a Waters 2695 Alliance HPLC system with Waters 996 PDA detector (Waters Millipore, Milford USA). Immobilized CHIRALPAK® HSA (100×4 mm, 5 μm) and AGP (100×4.0 mm, 5 μm) HPLC columns were purchased from DAICEL Chemical Industries, Ltd (Illkirch Cedex, France. The mobile phase was composed of a 0.02 M phosphate buffer pH 7.0, and isopropanol (IPA) in proportions of 15% (v/v). The retention times of the solute t_r and a non-retained compound (IPA) t_0 were measured at room temperature by a 996 PDA detector. The solutions to be injected (10^{-4} to 10^{-5} M) were prepared by dissolving the solutes in the mobile phase; the injection volume was 10 μL. The measurements were carried out at a flow rate of 0.8 ml/min for all compounds. The isocratic retention factor k was calculated by Eq. 1. All log k values were the average of three measurements.

2.3 Linear free-energy relationship (LFER) model

LFER method was used in this study to establish the quantitative structure-drug-plasma protein binding relationships. LFER method was firstly applied to the properties of neutral molecules [17] and subsequently extended to include ions and ionic species by Abraham and Acree [14-16]. The general equation developed by Abraham and Acree is stated as:

$$SP = c + eE + sS + aA + bB + vV + j+J+ + j-J- \quad \text{Eq. 2}$$

The dependent variable SP represents the logarithm of an equilibrium coefficient for a series of solutes in a given system, including partition coefficients, rate coefficients, and in the present work logarithm of retention factors (log k) of the solutes investigated (Table 1). The independent variables are the physicochemical properties or descriptors of the solutes as follows. E is the excess molar refraction in units of $(cm^3/mol)/10$, S is the combined dipolarity/polarizability, A and B are the overall solute hydrogen bond acidity and basicity, and V is McGowan's characteristic molecular volume in units of $(cm^3/mol)/100$; J+ and J- are the additional descriptors that are specific to ionic species. Note that J+ = 0 for anions, J− = 0 for cations, and both J+ and J− = 0 for neutral molecules. In the latter case, Eq. 2 reduces to an equation for neutral molecules. The compound descriptors for neutral molecules are obtained from a variety of experimental processes, as explained in a number of reviews [18,19], and Abraham and Acree have reviewed the methods used to obtain descriptors for ions and ionic species [20]. The coefficients (c, e, s, a, b, v, j+ and j-) in Eq. 2 can be obtained by a multiple linear regression (MLR) of values of SP (log k in this study) in a given system against the known solute descriptors, and used to characterize the system of interest.

It is important to note that for neutral compounds, Eq. 2 with j+ and j- = 0, each term in the equation corresponds to a particular solute-system interaction. Thus the aA term refers to interaction between a neutral solute that is a hydrogen bond acid and the system acting as a hydrogen bond base. However, this simple interpretation does not apply to ionised species. In the equation for neutrals and ions, Eq. 2, the coefficients c, s, a, b and v are fixed as those for the corresponding equation just for neutrals (when the terms in j+J+ + j−J− are both zero). This means that when the descriptors for ions and ionic species are determined they are constrained by the necessity that c, s, a, b and v are fixed. Then the descriptors for ionic species are simply those that reproduce the experimental values, and do not have the theoretical significance that the neutral descriptors have. The j+J+ and j−J− terms in Eq. 2 act as 'correction' terms for the effect of electric charge on the other equation terms, and so only the total effect on SP can be deduced.

3. Results and discussion

In this study, a set of 65 compounds with a broad structural diversity (in terms of E, S, A, B, V, J+ and J-) were selected. The solutes consist of neutrals, acidic and basic compounds which are present as neutral or ionic (cationic or anionic) at the experimental condition of pH 7, which is as close as possible to the physiologic pH and compatible with the stability of the stationary phase (pH no higher than 7.0). Their retention factors log k were determined using immobilized HSA and AGP HPLC columns at the isocratic mobile phase condition. 15% (v/v) of IPA was used as the organic modifier to allow the elution of most of the solutes in a reasonable time. The two columns were stable during the study period. The stability of HSA stationary phase was controlled by injecting 4 compounds (17α-hydroxyprogesterone, salicylic acid, warfarin and penbutolol) throughout the study, whereas the compounds for stability control of AGP column were 17α-hydroxyprogesterone, mefenamic acid, warfarin and penbutolol.

The log k values and other physicochemical parameters including dissociation constant pK_a, n-octanol/water partition coefficient (log P_{oct}), n-octanol/water distribution coefficient at pH 7 (log $D_{7.0}$), as well as the values of the solute descriptors are shown in Table 1.

(Table 1 here)

From Table 1, we can see the large log k values of the acidic compounds on the immobilized HSA HPLC column (log k_{HSA}). At the experimental condition of pH 7, the acidic compounds (35-46 in Table 1) are negatively charged. This indicates the strong interaction of anionic species with HSA stationary phase. Four acidic compounds with high lipophilicity (flurbiprofen, ibuprofen, mefenamic acid and 8-phenyloctanoic acid) could not be eluted from the HSA column (within 4 hours), implying the contribution of lipophilicity of the solute to the binding in addition to their negative charges. In this case, we eluted the injected samples by mobile phase with higher ratio of IPA before next injection. On the contrary, the acidic compounds showed much weaker retention on the AGP HPLC column. All the acidic compounds with log P_{oct} values lower than 3 do not retain in AGP column. The four highly lipophilic acids (flurbiprofen, ibuprofen, mefenamic acid and 8-phenyloctanoic acid) showed a low degree of retention, indicating that the negative charges of the solutes do not favour the interaction with AGP, while lipophilicity plays a role.

Fig 1 shows the correlations between the values of log $D_{7.0}$ and log k on the immobilized HSA (log k_{HSA}) (1a) and AGP (log k_{AGP}) (1b) stationary phases from this study. From Fig 1a, it can be further verified that anions interact much stronger with HSA stationary phase than

cations and neutral compounds. Fig 1b shows that the cations have a higher affinity to AGP stationary phase than anions in general. The neutral compounds bind least to both proteins. Within each set of the solutes, the larger the lipophilicity (expressed by log $D_{7.0}$), the higher the affinity with the proteins, indicating that drug binding to immobilized proteins is controlled by both electrostatic forces and lipophilicity. This is in good agreement with drug binding data from soluble human plasma proteins in previous studies [22].

(Fig 1 here)

To reveal the structural properties that govern the drug interaction with the immobilized HSA and AGP in HPLC system, the multilinear regression (MLR) of log k against the solute descriptors yielded the LFER model shown in Eq 3 and Eq 4.

$$\log k_{HSA} = -0.271(\pm0.298) + 0.470(\pm0.276)E - 0.331(\pm0.190)S - 0.177(\pm0.197)A - 1.233(\pm0.393)B + 1.086(\pm0.0.350)V - 0.231(\pm0.229)J^{+} + 2.024 ((\pm0.460)\ J^{-}$$

N=61, R^2=0.812, SD=0.215, F=33, Press=3.204, Q^2=0.756 Eq. 3

$$\log k_{AGP} = -0.756(\pm0.315) + 0.428(\pm0.275)E - 0.354(\pm0.196)S - 0.156(\pm0.206)A - 1.253(\pm0.397)B + 1.278((\pm0.361)V - 0.051(\pm0.230)J^{+} + 1.562 ((\pm0.426)J^{-}$$

N=58, R^2=0.796, SD=0.228, F = 28, Press=4.143, Q^2=0.674 Eq. 4

In these equations, 95% confidence limits are given in parentheses; N is the number of compounds; R^2 is the squared correlation coefficient; SD is the standard deviation, and F is the Fisher's test. Press and Q^2 are the leave-one-out statistics. The values of Q^2 are good enough to indicate that Eq 3 and Eq 4 are soundly based.

The coefficients of Eq 3 and Eq 4 showed that for the electrically neutral compounds, McGowan's characteristic molecular volume (V), dipolarity/polarizability (S) and hydrogen bond basicity (B) are the three significant molecular descriptors of solutes determining the interaction with immobilized plasma proteins, whereas excess molar refraction (E) is less important and hydrogen bond acidity (A) is not of statistical significance in both systems.

For acids and bases in this study, we compared logarithm of the retention factors of neutral species and the corresponding anions or cations on the two stationary phases as shown in Table 2. For immobilized HSA stationary phase, we use Eq. 3 to calculate log k_{HSA} for the 8 neutral acidic compounds and 19 basic compounds using descriptors for the neutral species. These can then be compared to experimental values of log k_{HSA} of the corresponding anions

and cations, measured in this study. Over the 8 acidic compounds, the average difference log k_{HSA} (anions) – log k_{HSA} (neutral acids) = 0.80 (±0.03) log units, so that on average the retention factor on immobilized HSA column for the anions is 6.3 times that for the neutral acidic compounds, further quantitatively verifying the contribution of the electrostatic interactions between the anions and the immobilized HSA. It also means that for the electrically neutral acids, hydrogen bond acidity (A) is not important for binding with the HSA phase. In the case of 19 basic compounds, the average difference log k_{HSA} (cations) - log k_{HSA} (neutral base) = - 0.14 (±0.01) log units, meaning that retention factor on HSA HPLC column for the protonated base cation is 0.71 times that for the neutral bases. This indicates that positive charge of the solute does not greatly favour the interaction with HSA stationary phase. Thus, for the first time, we are able to show quantitatively the effect of negative or positive charge on binding to HSA.

Valko et al [7] have used a fast gradient HPLC method to study a variety of compounds on an immobilised HSA column. By comparison of log k_{HSA} with calculated values of log P(octanol) at pH 7.4 (log D) they concluded that acid anions were strongly bound. However, they could not quantify any increased binding due to the negative charge.

Table 2 here

In the same way, we use Eq 4 to calculate log k_{AGP} for the neutral species of the acids and bases used in this study, and then compared to experimental values of log k_{AGP} of the corresponding ionic species of neutral acidic compounds and basic compounds obtained from this work on immobilized AGP stationary phase (shown in Table 2). For the 5 acidic compounds, the average difference log k_{AGP} (neutral acids) – log k_{AGP} (anions) = 0.31 (±0.05) log units, so that on average the retention factor on HPLC AGP stationary phase for the neutral acidic compounds is twice that for the anions. This further quantitatively confirms that anions do not favour interaction with the AGP stationary phase. For the 19 basic compounds, the average difference log k_{AGP} (cation) – log k_{AGP} (neutral base) = 0.32 (±0.02), meaning that the retention factor on HPLC AGP stationary phase for the protonated base cations is about 2.1 times that for neutral bases. This indicates the importance of the positive charge in the drug binding with immobilized AGP phase.

4 Conclusion

By using the HPLC method that employs the stationary phases immobilized with plasma proteins (HSA and AGP), the drug-plasma protein binding was investigated for a set of

compounds with a broad range of structural diversity. The obtained LFER model shows that for neutral compounds the structural parameters S, B and V are the prominent factors governing drug binding with HPLC stationary phases immobilized with HSA and AGP, whereas E is less important and A is not of statistical significance in both systems. For ionic compounds, electrostatic interactions play a major role in binding. The ionised acids, as anions, show very strong interaction with immobilized HSA stationary phase compared to immobilized AGP phase, whereas ionised basic compounds, as cations, interact with immobilized AGP phase stronger than the HSA phase.

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References

[1] W.E. Lindup, M.C. L'E Orme, Plasma protein binding of drugs, Brit. Med. J. 282 (1981) 212-214.

[2] K. Vuignier, J. Schappler, J.-L. Veuthey, P.A. Carrupt, Drug-protein binding: a critical review, Anal. Bioanal. Chem. 398 (2010) 53-66.

[3] H.Wan, A.G. Holmen, High throughput screening of physicochemical properties and in vitro ADME profiling in drug discovery, Comb. Chem. High Throughput Screen 12 (2009) 315-329

[4] G.L. Trainor, The importance of plasma protein binding in drug discovery, Expert Opin. Drug Discov. 2 (2007) 51-64.

[5] M.I. Howard, J.J. Hill, G.R. Galluppi, M.A. Mclean, Plasma protein binding in drug discovery and development, Comb Chem High Throughput Screen 13 (2010) 1-18.

[6] J. Oravcova, B. Bohs, W. Lindner, Drug-protein binding studies-New trends in analytical and experimental methodology, J Chromatogr B. 677 (1996) 1-28

[7] K. Valko, S. Nunhuck, C. Bevan, M.H. Abraham, D.P. Reynolds, Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationship with

octanol/water and immobilized artificial membrane lipophilicity. J Pharm Sci, 92 (2003) 2236-2248.

[8] K. Vuignier, D. Guillarme, J.-L. Veuthey, P.A. Carrupy, J. Schappler, High performance affinity chromatography (HPAC) as a high-throughput screening tool in drug discovery to study drug–plasma protein interactions, J. Pharm. Bio. Anal. 74 (2013) 205-212.

[9] F. Barbato, G. di Martino, L. Grumetto, M. Immacolata La Rotonda, Retention of quinolones on human serum albumin and □1-acid glycoprotein HPLC columns: Relationships with different scales of lipophilicity, Eur. J. Pharm.Sci. 30 (2007) 211-219.

[10] D.S. Hage, J. Anguizola, O. Barnaby, A. Jackson, M.J. Yoo, E. Papastavros, E. Pfaunmiller, M. Sobansky, Z. Tong, Characterization of drug interactions with serum proteins by using high-performance affinity chromatography, Curr. Drug Metab. 12 (2011) 313-328.

[11] H.S. Kim, I.W. Wainer, Rapid analysis of the interactions between drugs and human serum albumin (HSA) using high-performance affinity chromatography (HPAC). J. Chromatogr. B 870 (2008) 22-26.

[12] R. Matsuda, J. Anguizola, K.S. Joseph, D.S. Hage, Analysis of drug interactions with modifies proteins by high-performance affinity chromatography: Binding of Glibenclamide to normal and glycated human serum albumin. J. Chromatogr. A 1265 (2012) 114-122.

[13] S.S. Singh, J. Mehta, Measurement of drug-protein binding by immobilized human serum albumin-HPLC and comparison with ultrafiltration. J. Chromatogr. B, 834 (2006) 108-116.

[14] M.H. Abraham, The permeation of neutral molecules, ions, and ionic species through membranes: Brain permeation as an example, J. Pharm. Sci. 100 (2011) 1690-1701.

[15] M.H. Abraham, W.E. Acree Jr., Equations for the transfer of neutral molecules and ionic species from water to organic phases. J. Org. Chem.75 (2010) 1006-1015.

[16] M.H. Abraham, W.E. Acree Jr., The transfer of neutral molecules, ions and ionic species from water to ethylene glycol and to propylene carbonate; Descriptors for pyridinium cations. New J. Chem. 34 (2010) 2298-2305.

[17] M.H. Abraham,. Scales of solute hydrogen-bonding - their construction and application to physicochemical and biochemical processes. Chem. Soc. Rev. 22 (1993) 73-83.

[18] M.H. Abraham, A. Ibrahim, A.M. Zissimos, Determination of sets of solute descriptors from chromatographic measurements, J. Chromatogr. A 1037 (2004) 29-47.

[19] C.F. Poole, T.C. Ariyasena, N. Lenca, Estimation of the environmental properties of compounds from chromatographic measurements and the solvation parameter model, J. Chromatogr. A 1317 (2013) 85-104.

[20] M.H. Abraham, W.E. Acree Jr., Descriptors for ions and ion-pairs for use in linear free energy relationships, J. Chromatogr. A 1430 (2016) 2-14.

[21] K. Zhang, M. Chen, G.K.E.Scriba, M.H. Abraham, A. Fahe, X. Liu, Human skin permeation of neutral species and ionic species: extended linear free-energy relationship analysis, J. Pharm. Sci. 101 (2012) 2034-2044.

[22] S. Urien, J.-P. Tillement, J. Barre, The Significance of plasma-protein binding in drug research, In B. Testa, S.D. Kramer, H. Wunderli-Allenspach, G. Folkers (Eds), Pharmacokinetic Profiling in Drug Research, VCH Publishers: Weinheim, 2006; pp. 189-197.

Figures

Figure 1. Correlations between log $D_{7.0}$ and log k_{HSA} (1a) as well as log k_{AGP} (1b). Markers: ●, neutral molecules; ■, anions; ○, cations

Figure 1

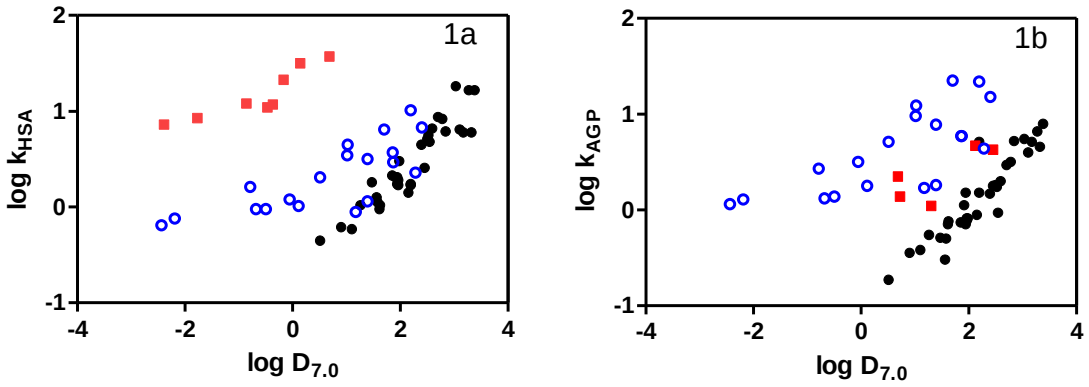

Table 1: Physicochemical parameters of the investigated compounds

No.	Solutes	pKa[a]	Charge state	log k_{HSA}[b]	log k_{AGP}[b]	log P_{oct}[a]	log $D_{7.0}$[c]	E	S	A	B	V	J+	J-
	Neutrals													
1	Acetaminophen	9.50	neutral	-0.35	-0.73	0.51	0.51	1.120	1.63	1.01	0.91	1.1724	0.0000	0.0000
2	Acetophenone	N	neutral	0.05	-0.30	1.58	1.58	0.818	1.01	0.00	0.48	1.0139	0.0000	0.0000
3	Acridine	5.58	neutral	1.22	0.90	3.40	3.38	2.356	1.32	0.00	0.58	1.4133	0.0000	0.0000
4	Aniline	4.60	neutral	-0.21	-0.45	0.90	0.90	0.955	0.96	0.26	0.41	0.8162	0.0000	0.0000
5	2-Chloroaniline	2.64	neutral	0.31	0.05	1.91	1.91	1.033	0.92	0.25	0.31	0.9386	0.0000	0.0000
6	N-Ethylaniline	5.12	neutral	0.15	-0.05	2.16	2.15	0.945	0.85	0.17	0.43	1.0980	0.0000	0.0000
7	Nitrobenzene	N	neutral	0.33	-0.13	1.85	1.85	0.871	1.11	0.00	0.28	0.8906	0.0000	0.0000
8	1-Chloro-2-nitrobenzene	N	neutral	0.75	0.24	2.52	2.52	1.020	1.24	0.00	0.24	1.0130	0.0000	0.0000
9	1-Fluoro-2,4-dinitrobenzene	N	neutral	0.26	-0.29	1.47	1.47	1.006	1.69	0.00	0.45	1.0825	0.0000	0.0000
10	Benzyl alcohol	N	neutral	-0.23	-0.42	1.10	1.10	0.803	0.87	0.39	0.56	0.9160	0.0000	0.0000
11	4-Chlorobenzylalcohol	N	neutral	0.28	-0.09	1.96	1.96	0.911	0.96	0.40	0.50	1.0384	0.0000	0.0000
12	2-Aminobiphenyl	3.84	neutral	0.79	0.72	2.84	2.84	1.600	1.48	0.26	0.41	1.4240	0.0000	0.0000
13	Carbamazepine	N	neutral	0.23	0.71	2.19	2.19	2.154	1.90	0.50	1.15	1.8106	0.0000	0.0000
14	Corticosterone	N	neutral	0.31	0.18	1.94	1.94	1.860	3.43	0.40	1.63	2.7389	0.0000	0.0000
15	m-Cresol	10.10	neutral	0.23	-0.11	1.96	1.96	0.822	0.88	0.57	0.34	0.9160	0.0000	0.0000
16	p-Cresol	10.26	neutral	0.24	-0.15	1.94	1.94	0.820	0.87	0.57	0.31	0.9160	0.0000	0.0000
17	Estradiol	N	neutral	1.26	0.74	3.03	3.03	1.800	1.77	0.86	1.10	2.1988	0.0000	0.0000
18	Estriol	N	neutral	0.68	-0.03	2.54	2.54	1.970	1.74	1.06	1.63	2.2575	0.0000	0.0000
19	Hydrocortisone	N	neutral	-0.02	-0.15	1.61	1.61	2.030	3.49	0.71	1.90	2.7976	0.0000	0.0000
20	Hydrocortisone- 21-acetate	N	neutral	0.24	0.18	2.19	2.19	1.890	2.88	0.46	2.16	3.0951	0.0000	0.0000
21	2- Naphthol	9.57	neutral	0.94	0.47	2.70	2.70	1.520	1.08	0.61	0.40	1.1441	0.0000	0.0000
22	3-Chlorophenol	9.11	neutral	0.71	0.26	2.50	2.50	0.909	1.06	0.69	0.15	0.8975	0.0000	0.0000

23	4-Bromophenol	9.31	neutral	0.82	0.30	2.59	2.59	1.080	1.17	0.67	0.20	0.9501	0.0000	0.0000
24	4-Chlorophenol	9.40	neutral	0.65	0.17	2.39	2.39	0.915	1.08	0.67	0.20	0.8975	0.0000	0.0000
25	4-Chloro-3,5-dimethyl phenol	9.70	neutral	1.22	0.82	3.27	3.27	0.980	0.94	0.61	0.26	1.1793	0.0000	0.0000
26	4-Chloro-2-methylphenol	9.60	neutral	0.92	0.50	2.78	2.78	0.890	0.91	0.63	0.22	1.0384	0.0000	0.0000
27	3-Nitrophenol	8.40	neutral	0.48	-0.09	2.00	1.98	1.050	1.57	0.79	0.23	0.9493	0.0000	0.0000
28	Phenylacetonitrile	N	neutral	0.10	-0.52	1.56	1.56	0.751	1.03	0.00	0.50	1.0120	0.0000	0.0000
29	Phenytoin	8.33	neutral	0.41	0.25	2.47	2.45	1.713	2.23	0.86	1.00	1.8693	0.0000	0.0000
30	Prednisolone	N	neutral	0.02	-0.12	1.62	1.62	2.210	3.10	0.71	1.92	2.7546	0.0000	0.0000
31	17α-Hydroxyprogesterone	N	neutral	0.78	0.71	3.17	3.17	1.640	3.35	0.25	1.31	2.6802	0.0000	0.0000
32	21-Hydroxyprogesterone	N	neutral	0.81	0.60	3.10	3.10	1.740	3.50	0.14	1.31	2.6802	0.0000	0.0000
33	Salicylamide	8.37	neutral	0.02	-0.26	1.28	1.26	1.160	1.58	0.61	0.51	1.0315	0.0000	0.0000
34	Testosterone	N	neutral	0.78	0.66	3.32	3.32	1.540	2.56	0.32	1.17	2.3827	0.0000	0.0000
	Acidic compounds													
35	Aspirin	3.48	Anion	0.86		1.13	-2.39	1.000	4.15	0.00	3.28	1.2664	0.0000	2.2560
36	4-Bromobenzoic Acid	3.97	Anion	1.33		2.86	-0.17	1.150	3.25	0.00	2.60	1.0852	0.0000	2.1480
37	3-Chlorobenzoic Acid	3.82	Anion	1.04		2.71	-0.47	0.990	3.13	0.00	2.57	1.0326	0.0000	2.0340
38	4-Chlorobenzoic acid	3.98	Anion	1.07		2.65	-0.37	0.990	3.37	0.00	2.60	1.0326	0.0000	2.1790
39	Flurbiprofen	3.91	Anion		0.14	3.81	0.72	1.590	4.56	0.07	3.36	1.8174	0.0000	2.5383
40	Ibuprofen	4.43	Anion		0.04	3.87	1.30	0.880	3.50	0.08	3.31	1.7556	0.0000	2.4188
41	Mefenamic acid	4.33	Anion		0.63	5.12	2.45	1.800	4.71	0.09	3.14	1.8996	0.0000	2.6427
42	3-Phenylpropanoic acid	4.25	Anion	1.08		1.89	-0.86	0.900	3.43	0.03	3.02	1.1920	0.0000	2.1879
43	4-Phenylbutanoic acid	4.72	Anion	1.50		2.42	0.14	0.910	3.59	0.04	3.01	1.3829	0.0000	2.2184
44	8-Phenyloctanoic acid	5.03	Anion		0.67	4.09	2.12	0.940	3.87	0.07	3.26	1.8965	0.0000	2.4256
45	Salicylic acid	2.97	Anion	0.93		2.26	-1.77	1.050	3.51	0.14	2.18	0.9689	0.0000	1.6351
46	Warfarin	4.98	Anion	1.57	0.35	2.70	0.68	2.130	5.62	0.00	4.40	2.2862	0.0000	2.7628
	Basic compounds													
47	Acebutolol	9.52	Cation	-0.02	0.14	2.02	-0.50	1.450	6.69	3.62	0.00	2.7771	2.2965	0.0000
48	Alprenolol	9.59	Cation	0.31	0.71	3.10	0.51	1.100	4.46	1.78	0.00	2.1802	2.2574	0.0000
49	Atenolol	9.60	Cation	-0.19	0.06	0.16	-2.44	1.300	6.27	2.91	0.00	2.1978	2.4800	0.0000

50	Bupivacaine	8.10	Cation	0.36	0.64	3.41	2.28	1.170	4.67	2.92	0.00	2.5354	1.5920	0.0000
51	Imipramine	9.40	Cation	0.83	1.18	4.80	2.40	1.000	3.66	2.02	0.00	2.4230	1.6110	0.0000
52	Lidocaine	8.01	Cation	0.06	0.26	2.44	1.39	0.960	4.18	2.12	0.00	2.0804	1.7490	0.0000
53	Mepivacaine	7.70	Cation	-0.05	0.23	1.95	1.17	1.170	4.69	2.98	0.00	2.1127	1.5607	0.0000
54	Metoprolol	9.63	Cation	-0.02	0.12	1.95	-0.68	1.020	5.35	2.16	0.00	2.2819	2.3476	0.0000
55	Oxprenolol	9.57	Cation	0.08	0.50	2.51	-0.06	1.160	5.09	2.35	0.00	2.2389	2.2029	0.0000
56	Penbutolol	9.92	Cation	0.81	1.35	4.62	1.70	0.775	4.66	1.98	0.00	2.6195	1.9630	0.0000
57	Pindolol	9.54	Cation	0.21	0.43	1.75	-0.79	1.550	4.60	2.36	0.00	2.0305	2.2661	0.0000
58	Procaine	9.03	Cation	0.01	0.25	2.14	0.11	0.999	4.58	1.86	0.00	1.9982	2.1487	0.0000
59	Promazine	9.36	Cation	1.01	1.34	4.55	2.19	1.900	3.78	1.76	0.00	2.3047	2.5263	0.0000
60	Propafenone	9.62	Cation	0.65	1.09	3.64	1.02	1.680	5.69	3.08	0.00	2.8467	2.3780	0.0000
61	Propranolol	9.47	Cation	0.54	0.98	3.48	1.01	1.690	4.31	2.07	0.00	2.1695	2.4319	0.0000
62	Quinidine	8.56	Cation	0.47	0.77	3.44	1.87	2.320	5.81	1.23	0.00	2.5727	4.1570	0.0000
63	Quinine	9.05	Cation	0.50	0.89	3.44	1.39	2.319	5.81	1.23	0.00	2.5727	4.1565	0.0000
64	Sotalol	9.43	Cation	-0.12	0.11	0.24	-2.19	1.320	6.18	2.38	0.00	2.1225	2.8119	0.0000
65	Verapamil	8.92	Cation	0.57	0.77	3.79	1.86	1.660	7.70	4.33	0.00	3.8076	2.4040	0.0000

a Taken from [21] and bioloom Software (Biobyte Corporation, Claremont, U.S.A.).
b Calculated from Eq. 1; n=3, s.d. ≤ 0.03
c Calculated according to $\log D = \log P_{oct} - \log\,(1 + 10^{pK_a - pH})$ for bases and $\log D = \log P_{oct} - \log\,(1 + 10^{pH - pK_a})$ for acids.

Table 2. The observed log k values of ionic species and predicted log k values of the neutral species of acids and bases in this study by Eq 3 and Eq 4.

Species	E	S	A	B	V	J^+	J^-	$logk_{HSA}$[a]	$logk_{AGP}$[b]
Acids									
Aspirin, anion								0.86	
Aspirin	0.781	1.69	0.71	0.67	1.2879	0.0000	0.0000	-0.02	
4-Bromobenzoic Acid, anion								1.33	
4-Bromobenzoic Acid	1.000	1.01	0.63	0.26	1.1067	0.0000	0.0000	0.63	
3-Chlorobenzoic Acid, anion								1.04	
3-Chlorobenzoic Acid	0.840	0.95	0.63	0.32	1.0541	0.0000	0.0000	0.45	
4-Chlorobenzoic acid, anion								1.07	
4-Chlorobenzoic acid	0.840	1.02	0.63	0.27	1.0541	0.0000	0.0000	0.49	
Flurbiprofen, anion									0.14
Flurbiprofen	1.440	1.45	0.62	0.76	1.8389	0.0000	0.0000		0.65
Ibuprofen, anion									0.04
Ibuprofen	0.730	0.70	0.57	0.79	1.7771	0.0000	0.0000		0.50
Mefenamic acid, anion									0.63
Mefenamic acid	1.650	1.35	0.65	0.54	1.9211	0.0000	0.0000		1.15
3-Phenylpropanoic acid, anion								1.08	
3-Phenylpropanoic acid	0.750	1.18	0.60	0.60	1.2135	0.0000	0.0000	0.16	
4-Phenylbutanoic acid, anion								1.50	
4-Phenylbutanoic acid	0.760	1.29	0.61	0.57	1.3544	0.0000	0.0000	0.32	
8-Phenyloctanoic acid, anion									0.67
8-Phenyloctanoic acid	0.790	1.28	0.59	0.63	1.9180	0.0000	0.0000		0.70
Salicylic acid, anion								0.93	
Salicylic acid	0.900	0.85	0.73	0.37	0.9904	0.0000	0.0000	0.36	
Warfarin, anion								1.57	0.35
Warfarin	1.980	1.88	0.29	1.57	2.3077	0.0000	0.0000	0.56	0.36
Bases									
Acebutolol, cation								-0.02	0.14
Acebutolol	1.600	2.42	0.90	2.10	2.7556	0.0000	0.0000	-0.08	-0.18
Alprenolol, cation								0.31	0.71
Alprenolol	1.250	1.09	0.15	1.44	2.1587	0.0000	0.0000	0.50	0.32
Atenolol, cation								-0.19	0.06
Atenolol	1.450	1.90	0.62	2.03	2.1763	0.0000	0.0000	-0.47	-0.67
Bupivacaine, cation								0.36	0.64
Bupivacaine	1.320	2.10	0.34	1.33	2.5139	0.0000	0.0000	0.68	0.56
Imipramine, cation								0.89	1.08
Imipramine	1.150	1.45	0.00	1.04	2.4015	0.0000	0.0000	1.12	0.99
Lidocaine, cation								0.06	0.26

Lidocaine	1.110	1.51	0.07	1.24	2.0589	0.0000	0.0000	0.45	0.25
Mepivacaine, cation								-0.05	0.23
Mepivacaine	1.320	2.14	0.34	1.33	2.0912	0.0000	0.0000	0.21	0.00
Metoprolol, cation								-0.02	0.12
Metoprolol	1.170	1.33	0.17	1.76	2.2604	0.0000	0.0000	0.09	-0.07
Oxprenolol, cation								0.08	0.50
Oxprenolol	1.230	1.45	0.15	1.62	2.2174	0.0000	0.0000	0.21	0.04
Penbutolol, cation								0.81	1.35
Penbutolol	0.925	1.15	0.08	1.51	2.5980	0.0000	0.0000	0.73	0.65
Pindolol, cation								0.21	0.43
Pindolol	1.700	1.59	0.30	1.40	2.0090	0.0000	0.0000	0.40	0.18
Procaine, cation								0.01	0.25
Procaine	1.149	1.34	0.26	1.44	1.9767	0.0000	0.0000	0.15	-0.06
Promazine, cation								1.01	1.34
Promazine	2.050	1.49	0.00	1.08	2.2832	0.0000	0.0000	1.35	1.16
Propafenone, cation								0.65	1.09
Propafenone	1.730	2.09	0.29	1.74	2.8252	0.0000	0.0000	0.72	0.63
Propranolol, cation								0.54	0.98
Propranolol	1.840	1.43	0.44	1.31	2.1480	0.0000	0.0000	0.76	0.56
Quinidine, cation								0.47	0.77
Quinidine	2.469	1.23	0.37	1.97	2.5512	0.0000	0.0000	0.76	0.60
Quinine, cation								0.50	0.89
Quinine	2.469	1.23	0.37	1.97	2.5512	0.0000	0.0000	0.76	0.60
Sotalol, cation								-0.12	0.11
Sotalol	1.470	1.55	0.68	2.06	2.1010	0.0000	0.0000	-0.47	-0.68
Verapamil, cation								0.57	0.77
Verapamil	1.807	3.17	0.00	2.37	3.7861	0.0000	0.0000	0.72	0.76

a Observed log k_{HSA} values for ionic species and predicted log k_{HSA} values for neutral species by Eq 3
b Observed log k_{AGP} values for ionic species and predicted log k_{AGP} values for neutral species by Eq 4