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Computational Prediction of Drug–Albumin Binding Affinity by Modeling Liquid Chromatographic Interactions[#]

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Abstract

A fast analytical method is required to measure human serum albumin–drug binding affinity in drug discovery research. A liquid chromatographic system was established without albumin based on the conformational flexibility of the albumin molecule and the basic molecular forces, ion–ion and hydrophobic interactions, involved in the molecular recognition of albumin. Using a computational chemical calculation to analyze liquid chromatographic data, the direct interaction between a model–phase and a drug was calculated as energy values with the MM2 calculation. Computational chemistry using a model adsorbent is a new method for quantitative analysis of the retention of acidic drugs on a guanidino phase which was used for ion–exchange liquid chromatography of acidic drugs. Furthermore, the computational chemical method demonstrated the possibility of estimating albumin–acidic drug binding affinity without chemical experiments. The r^2 value was 0.922 (n = 13) between binding affinity values (log nK) and interaction energy values of the final structure (ΔFS).

Keywords. Albumin–drug binding affinity; liquid chromatography; computational chemistry; acidic drugs.

Abbreviations and notations

ES, electrostatic energy	MM, molecular mechanics
FS, final structure energy	nK, binding affinity value
HB, hydrogen bonding energy	ODS, octadecylsilane
HSA, human serum albumin	VW, van der Waals energy

1 INTRODUCTION

The development of new drugs has been accelerated by combinatorial chemistry, and therefore requires faster screening methods. A simple experimental method is required to measure drug–albumin binding affinity, because human serum albumin (HSA) is the most abundant plasma protein and often accounts for all drug binding in plasma. The degree of protein–binding is an important parameter in the evaluation of the pharmacological and pharmacokinetic properties of potential drugs. The pharmacological effect is directly related to the free rather than total

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concentration of drug in plasma. The binding to albumin has physiological significance in the transport, modulation and inactivation of metabolites and drug activities, for example, by providing a protective device for the binding and inactivation of potential toxic compounds to which the body is exposed. The majority of drugs bind to serum albumin quantitatively. Since drug binding to albumin is readily reversible, the albumin–drug complex serves as a circulating reservoir that releases more drug as the free drug is biotransformed or excreted. Albumin binding thus decreases the maximum intensity but increases the duration of activation of many drugs [1–5].

As summarized in a review, Hümmel–Dryer and frontal analyses have been used to measure protein–drug binding affinity by liquid chromatography [6, 7]. The protein binding affinity of drugs was determined using a physically protein–coated ODS column [8] and a chemically bonded bovine serum albumin column [9–12]. The immobilized protein column method is simple but the columns are not stable. The active sites are probably buried by the binding reaction in a process to synthesize packing materials for liquid chromatography. Capillary electrophoresis and ultra–centrifugation are also not sufficient due to poor reproducibility and the requirement of a large quantity of proteins, respectively. These are fundamental problems in measuring protein–drug binding. The reference log nK values vary significantly, probably due to the different qualities of human serum albumin and different analytical systems used.

Drug–albumin binding sites have been studied, but albumin also functions as a scavenger. This indicates that the albumin structure has the flexibility to carry a variety of compounds and the affinity may not be specific. The main binding forces are hydrophobic interactions and ion–ion interactions, and specific steric effects may not be important. Previously, acidic drug–HSA and basic drug–HSA binding affinities were successfully determined by a combination of reversed–phase and ion–exchange liquid chromatographies [13,14]. Guanidino groups of arginine should work as anion–exchange groups and carboxyl groups of aspartic and glutamic acids should work as cation–exchange groups. The chromatographic behavior of acidic and basic drugs was studied using these columns, and their retention factors were correlated with their log nK values measured by the modified Hümmer–Dreyer method. A pentyl–bonded silica gel was more stable than a butyl–bonded silica gel [15], and was simplified to estimate the binding affinity compared to a previous study with a butyl–bonded silica gel column [14]. Such a simple liquid chromatography may be useful to measure the albumin–drug binding affinity without albumin. However, a faster analytical method is required. The retention time of acidic drugs was measured using a guanidino–phase with pH–controlled eluent to determine molecular forms in pH 7.4 eluent. The log nK values of the drugs were investigated with a computational chemical analysis using a molecular mechanics calculation program (MM2).

2 EXPERIMENTAL

2.1 Liquid Chromatography

Drugs were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Wako Pure Chemical Industries (Osaka, Japan). Their properties are summarized in Table 1. Sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate $12H_2O$ were purchased from Wako Pure Chemical Industries. HPLC–grade methanol was obtained from Kanto–Kagaku (Tokyo, Japan). The water used was of Milli–Q grade.

Table 1. Molecular properties and retention factors of acidic drugs

Drugs	log P [a]	nK [b]	pKa [c]	pKa [d]	Retention factor	
					pH 2.0 [e]	pH 3.0 [e]
p–aminohippuric acid	0.232	3.12	3.83	5.90	1.017	4.819
amoxicillin	–2.502	3.12	9.60	(8.53)	–0.288	0.285
benzoic acid	1.485	4.09	4.20	5.73	3.788	5.697
furosemide	1.901	5.70	3.90	5.83	33.477	86.353
p–hydroxybenzoic acid	1.002	4.06	9.46	(8.67)	4.770	5.007
ibuprofen	3.550	6.61	5.20	6.15	5.235	9.605
indomethacin	3.426	7.32	4.50	6.03	3.824	6.477
mefenamic acid	4.971	7.84	4.20	6.43	55.758	56.393
nalidixic acid	0.966	4.18	6.00	(6.99)	2.206	2.263
naproxen	3.047	5.75	4.20	6.13	13.592	21.622
nicotinic acid	0.477	3.20	4.95	6.27	0.502	2.418
phenylbutazone	3.251	6.05	4.40	6.44	3.682	6.681
salicylic acid	1.060	4.81	3.00	5.93	2.842	4.620
sulfamethoxazole	0.791	4.15	5.81	7.53	1.112	3.876
tolazamide	1.448	5.16	5.70	7.27	1.181	1.609
tolbutamide	2.266	5.29	5.30	6.62	2.542	2.624
warfarin	2.866	5.38	5.10	6.76	3.542	6.294

Table 1. (Continued)

Drugs	Retention factor					
	pH 4.0 [e]	pH 5.0 [e]	pH 6.0 [e]	pH 7.0 [e]	pH 8.0 [e]	pH 9.0 [e]
p–aminohippuric acid	9.119	5.368	2.839	1.818	1.161	0.676
amoxicillin	0.515	0.738	1.214	1.149	0.886	0.538
benzoic acid	11.787	8.426	4.401	2.275	1.576	0.869
furosemide	170.947	95.132	50.287	25.100	16.985	9.400
p–hydroxybenzoic acid	4.958	5.046	5.064	5.098	3.701	2.317
ibuprofen	17.273	13.730	7.793	3.274	2.238	1.196
indomethacin	13.585	19.369	9.073	4.188	2.971	1.726
mefenamic acid	212.660	213.408	127.122	50.094	24.648	12.826
nalidixic acid	2.556	2.887	3.119	2.686	2.902	2.671
naproxen	39.181	32.407	17.173	6.959	5.160	2.786
nicotinic acid	4.678	2.751	1.483	1.297	0.658	0.385
phenylbutazone	14.869	15.801	9.928	4.390	3.749	2.532
salicylic acid	9.947	34.956	14.797	6.498	5.311	2.947
sulfamethoxazole	4.179	5.821	6.482	3.757	2.913	1.661
tolazamide	2.347	3.829	3.751	1.989	1.626	0.939
tolbutamide	3.783	5.998	5.069	2.577	1.984	1.167
warfarin	14.295	19.713	13.807	9.938	7.267	4.549

[a] Vlog *P* from Ref. [14]; [b] measured binding affinity from Ref. [14]; [c] dissociation constant from Ref. [14]; [d] measured p*K*a by liquid chromatography; [e] column, 50 x 4.6 mm ID packed guanidino–bonded polyvinylalcohol gel, eluent: pH controlled 50 mM sodium–phosphate and methanol (1:1); flow rate: 1.0 mL/min at 37 ºC.

The liquid chromatograph was constructed with a model LC–10AD pump and a model SIL–10AXL auto–injector, a model SPD–10AV UV detector from Shimadzu (Kyoto, Japan) equipped with a model UZ–SH–MIC μflow cell from LC Packing (The Netherlands), and a model ERC–3522 degasser from ERC (Tokyo). The aluminum block column heater was made to specifications and controlled with a model 965 Temperature & Process Controller from Sakaguchi E.H. Voc Co. (Tokyo). The operation and chromatographic data analysis were performed with a CLASS–LC10 workstation from Shimadzu.

The retention factors of acidic drugs were measured by ion–exchange liquid chromatography. A guanidino–bonded polyvinylalcohol gel column, 50 × 4.6 mm I.D., was used for the ion–exchange liquid chromatography of acidic drugs with various pH eluents. The preparation of guanidino–bonded polyvinylalcohol gel columns was detailed previously [16]. The column temperature was 37 °C. The void volume marker was fructose. The eluent was a mixture of 50 mM sodium phosphate solution and methanol (1 : 1). The flow rate was 1.0 mL/min.

Table 2. Energy values of drugs and their complexes with ionized guanidino–phase

Drug	FS1	ES1	HB1	VW1
Benzoic acid (I)	–2.5511	0.000	0.000	4.746
Benzoic acid (M)	–17.8396	–7.304	–4.183	4.983
Furosemide (I)	13.8365	–2.594	–2.736	9.998
Furosemide (M)	10.1725	–0.837	–5.606	5.981
p–Hydroxybenzoic acid (I)	–4.9589	–0.050	–1.462	4.463
p–Hydroxybenzoic acid (M)	–16.0982	–6.603	0.000	4.471
Ibuprofen (I)	–1.4639	3.151	0.000	5.220
Ibuprofen (M)	–15.6010	–5.040	–3.732	5.318
Indomethacin (I)	–7.2472	–4.273	0.000	6.009
Indomethacin (M)	–24.0717	–12.458	–5.284	5.883
Mefenamic acid (I)	20.2949	–8.420	–0.654	19.891
Mefenamic acid (M)	–6.1680	–11.690	–5.732	13.353
Nalidixic acid (I)	–44.4161	–55.760	0.000	11.793
Nalidixic acid (M)	–33.4226	–39.918	–3.335	11.741
Naproxen (I)	–13.5376	3.156	0.000	6.681
Naproxen (M)	–27.7018	–5.025	–3.755	6.778
Nicotinic acid (I)	–7.2772	–7.301	0.000	3.586
Nicotinic acid (M)	–14.5865	–3.271	–9.865	3.626
Phenylbutazone (I)	31.6122	–1.218	0.000	20.031
Phenylbutazone (M)	18.4233	0.000	–11.276	19.712
Salicylic acid (I)	–4.1495	–0.150	–1.487	5.234
Salicylic acid (M)	–15.3507	–6.437	–5.355	5.438
Sulfamethoxazole (I)	1.4759	1.067	–2.230	3.175
Sulfamethoxazole (M)	7.0614	2.679	–2.202	3.090
Tolazamide (I)	–3.9984	–4.823	–10.955	7.312
Tolazamide (M)	–3.1534	–12.721	–2.847	8.547
Tolbutamide (I)	–24.5760	–14.334	–11.046	6.193
Tolbutamide (M)	–29.9856	–25.539	–2.920	4.886
Warfarin (I)	–10.5399	–7.668	0.000	8.840
Warfarin (M)	–17.5045	–2.808	–5.999	7.411
Guanidino–phase	1686.6850	–154.119	–23.239	247.238

Table 2. (Continued)

	FS2	ES2	HB2	VW2
Benzoic acid (I)	1651.8096	–174.793	–30.182	246.880
Benzoic acid (M)	1658.8115	–165.312	–35.974	248.219
Furosemide (I)	1653.5079	–176.388	–39.635	241.312
Furosemide (M)	1677.1578	–149.734	–44.426	240.977
p–Hydroxybenzoic acid (I)	1650.9736	–164.737	–40.575	246.352
p–Hydroxybenzoic acid (M)	1647.2808	–162.100	–43.938	246.383
Ibuprofen (I)	1640.4757	–179.419	–31.437	240.163
Ibuprofen (M)	1643.1719	–165.235	–37.668	240.183
Indomethacin (I)	1614.6758	–199.328	–38.065	244.893
Indomethacin (M)	1632.9172	–168.346	–48.523	242.247
Mefenamic acid (I)	1649.7503	–187.764	–34.556	252.413
Mefenamic acid (M)	1641.5016	–199.918	–25.581	255.629
Nalidixic acid (I)	1603.3127	–235.158	–31.424	250.155
Nalidixic acid (M)	1628.9742	–200.434	–35.421	250.728
Naproxen (I)	1627.7769	–175.634	–34.958	244.604
Naproxen (M)	1640.3849	–160.512	–46.710	248.110
Nicotinic acid (I)	1651.2436	–182.070	–27.660	245.600
Nicotinic acid (M)	1658.0056	–166.875	–34.833	245.689
Phenylbutazone (I)	1661.6602	–168.243	–32.747	250.004
Phenylbutazone (M)	1676.6717	–167.444	–39.202	252.836
Salicylic acid (I)	1649.9164	–176.561	–31.659	247.122
Salicylic acid (M)	1651.6304	–167.869	–44.303	252.315
Sulfamethoxazole (I)	1663.9776	–152.577	–40.181	239.863
Sulfamethoxazole (M)	1670.6025	–153.870	–38.727	240.173
Tolazamide (I)	1644.5191	–186.274	–38.040	246.016
Tolazamide (M)	1647.8821	–176.914	–36.819	240.227
Tolbutamide (I)	1610.6043	–192.189	–46.607	237.236
Tolbutamide (M)	1624.6632	–188.516	–37.063	239.034
Warfarin (I)	1629.9823	–182.248	–35.841	243.857
Warfarin (M)	1640.4280	–172.954	–35.774	244.339

unit: kcal/mol, 1: energy values of drugs; 2: energy values of complexes; FS: final energy; ES: electrostatic energy: HB: hydrogen bonding energy; VW: van der Waals energy; (I): value of ionic form; (M): value of molecular form.

2.2 Computational Chemical Analysis

The computer used was a Dell model Latitude C840 equipped with a 2 GHz processor and 1024 MB memory. The molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) from version 5 of the CAChe program from Fujitsu, Tokyo, Japan. The standard parameters used were bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, hydrogen bond and electrostatic (MM2/MM3 bond dipoles). The van der Waals cut–off distance was 9Å. The energy unit was kcal/mol (1 kj/mol = 4.18 kcal/mol). The Cricket–Graph™ program from Computer Associates (San Diego, CA, USA) and Project Reader of CAChe™ program were used for data handling.

A model guanidino phase was constructed to investigate drug–guanidino phase interactions. The guanidino phase consisted of 316 carbon, 36 nitrogen and 71 hydrogen atoms due to the capacity of the computer and the size of the guanidino phase. The molecular weight was 4,367. The 12 guanidino groups were in 260 $Å^2$, and three guanidino groups might cover one analyte within the

dense guanidino surface. The optimized energy value was less than 0.00001 kcal/mol. The 1:1 adsorption form of phenylbutazone on the guanidino phase is shown in Figure 1 as an example of the adsorption of a drug on the guanidino phase. After subtraction of the individual energies of the drugs and the guanidino phase from the energy values of the complex, the retention factors obtained by liquid chromatography and albumin–drug binding affinity, log n*K*, were related to their final (FS), hydrogen bonding (HB), electrostatic (ES) and van der Waals (VW) energy values calculated by MM2 and are listed in Table 2. The subtracted energy values, Δvalues, were considered to be interaction energy values. The energy values were calculated for both ionic and molecular forms of analytes if the effect of pH was important depending on the p*K*a values.

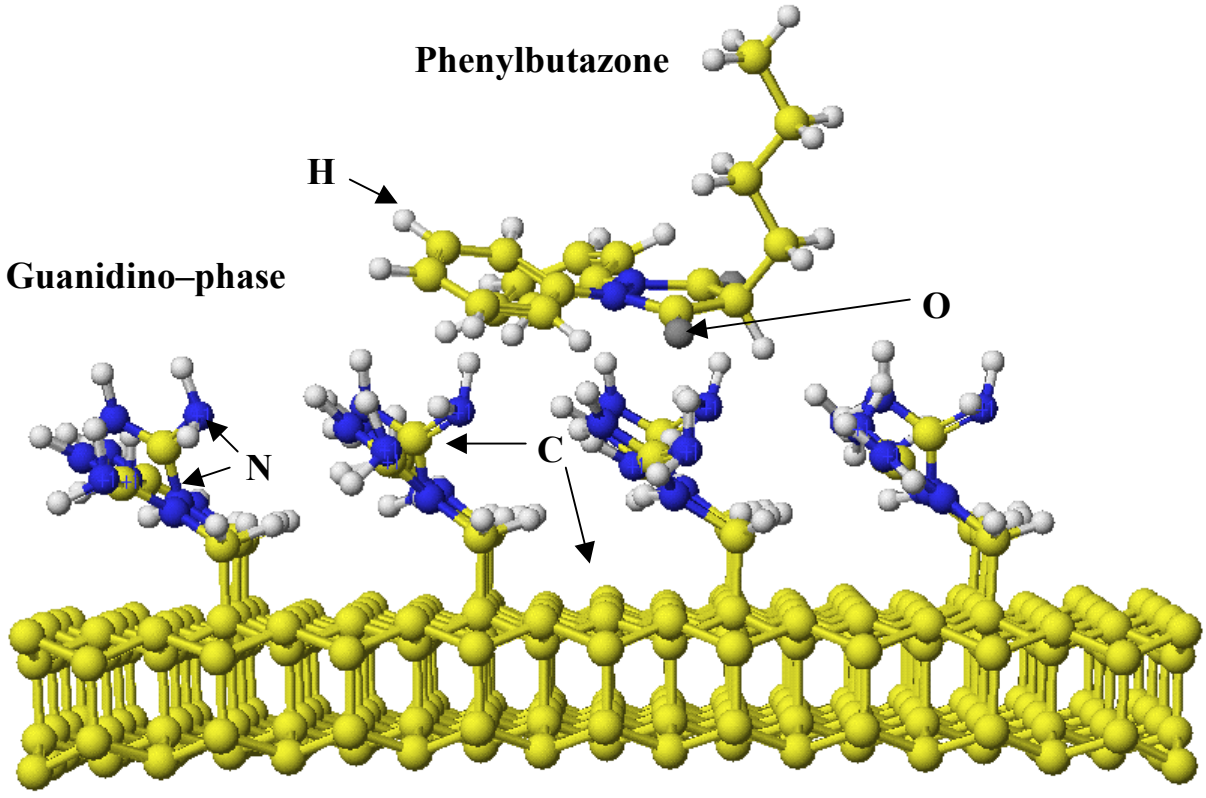

Figure 1. Adsortion of phenylbutazone on guanidino–phase. white small ball, hydrogen; white large ball, carbon; grey ball, oxygen; black ball, nitrogen.

3 RESULTS AND DISCUSSION

A computational chemical analysis using a molecular mechanics calculation program (MM2) was applied to analyze the retention mechanism of acidic drugs on the guanidino phase, and to estimate the albumin–drug binding affinity log n*K* values. Their Δfinal (ΔFS), Δhydrogen bonding (ΔHB), Δelectrostatic (ΔES) and Δvan der Waals (ΔVW) values were used for the analyses. The

maximum retention factors (log k_{max}) measured on the guanidino–bonded polyvinylalcohol gel using a 0.050M sodium phosphate solution containing 50% methanol did not show any meaningful correlation with these Δvalues for the retention of molecular form analytes.

The maximum retention was related to a combination of hydrophobic and ion–ion interaction forces, and could not be related to one type of interaction such as between a molecular form compound and the guanidino phase or an ionic form compound and the guanidino–phase, because construction of a partially ionized form compound is difficult for computational chemical calculations.

The poor correlation is due to that the maximum retention factors, log k_{max}, did not show high correlation coefficients with their Vlog *P* values obtained from references, log k_{max} = 0.247 (Vlog *P*) + 0.671, r^2 = 0.512, n = 17, because the measurement of maximum retention time was difficult due to an ion–exclusion effect at low pH, and log *P* is related to hydrophobicity but not to ion–ion interaction.

The dissociation constant was calculated from retention factors measured in a higher pH eluent than the pH eluent used to obtain the highest retention factors. This phenomenon in ion–exchange liquid chromatography is different from that measured in reversed–phase liquid chromatography where retention factors in low pH eluent are usually constant for acidic compounds. However, the retention factors were smaller in lower pH eluent in ion–exchange liquid chromatography due to an ion exclusion effect. The p*K*a values measured in ion–exchange liquid chromatography are relative p*K*a values of the analytes used. The relative p*K*a values were related to the ionic strength of an ion–exchanger.

The difference between the p*K*a values measured by reversed–phase liquid chromatography and ion–exchange liquid chromatography is a property of the ion–exchanger used. The relative p*K*a values measured on the guanidino–bonded polyvinylalcohol gel column were about 1.4 p*K*a unit higher than their reference values. One reason for this would be the effect of the organic modifier concentration. The p*K*a values were about 1.1 p*K*a units higher in 50% acetonitrile [17] and methanol [18]. However, pH was measured before mixing with an organic modifier in this experiment. Therefore, the relatively high pH values should be a property of the ion–exchanger used [19]. The relation is given by the following equation:

$$\text{p}K\text{a measured} = 0.544\ (\text{p}K\text{a reference}) + 3.862 \qquad r^2 = 0.641,\ n = 14$$

where p*K*a values of amoxicillin, *p*–hydroxybenzoic acid and nalidixic acid were not included due to a lack of experimental data.

The construction of a molecular form of zwitter ion at pH 7.40 was difficult for the computational chemical calculation, therefore *p*–aminohippuric acid and amoxicillin were excluded from further analysis. However, the above results indicated that the calculated energy values required an effect of pH because log *k* values were dependent on pH.

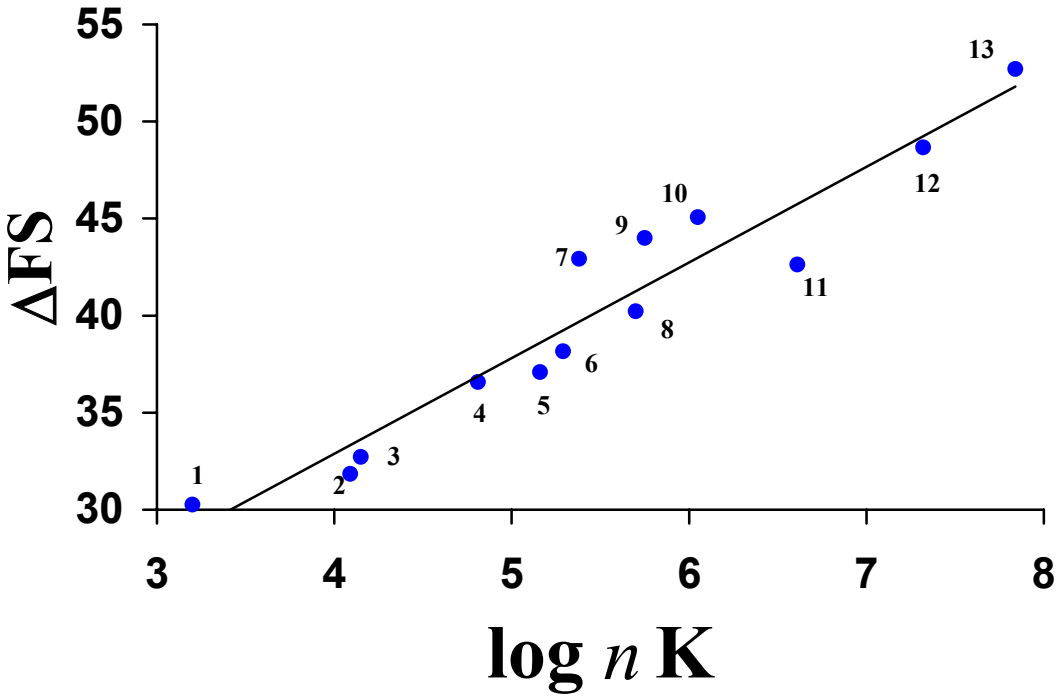

Figure 2. Relationship between log *n*K and ΔFS. No. 1, nicotinic acid; 2, benzoic acid; 3, sulfamethoxazole; 4, salicylic acid; 5, tolazamide; 6, tolbutamide; 7, walfarin; 8, furosemide; 9, naproxen; 10, phenylbutazone; 11, ibuprofen; 12, indomethacin; 13, mefenamic acid; ΔFS = 4.926 (log n*K*) + 13.188, r^2 = 0.922

Further analysis was performed to estimate the log n*K* values for albumin–acidic drug binding affinity. The binding affinity was measured at pH 7.40. The log *k* values measured at pH 7.40 correlated with the predicted log n*K* values from the reference. The log n*K* values may be derived from log *k* values measured on a guanidino phase at pH 7.40. Therefore, the addition of retention factors measured by reversed–phase liquid chromatography improved the correlation as previously described [14]. Finally, the log n*K* values correlated with the energy values without these four compounds. The correlations are shown in the following equations:

$$\Delta VW = 0.609 (\log nK) + 6.037 \qquad r^2 = 0.040,\ n = 13$$
$$\Delta HB = -4.654 (\log nK) + 40.431 \qquad r^2 = 0.160,\ n = 13$$
$$\Delta ES = 8.483 (\log nK) - 28.372 \qquad r^2 = 0.385,\ n = 13$$
$$\Delta FS = 4.926 (\log nK) + 13.188 \qquad r^2 = 0.922,\ n = 13$$

where these energy values were calculated from the following equation. Δenergy = (Δenergy$_i$ +

$\Delta energy_m$ x $[H^+]/[Ka])/(1 + [H^+]/[Ka])$, where $\Delta energy_i$ is the Δenergy value of ionic form analyte, $\Delta energy_m$ is the Δenergy value of molecular form analyte and H^+ is the hydrogen ion concentration at pH 7.4. The dissociation constant p*K*a is measured by ion–exchange liquid chromatography and the values are given in Table 1. The relation between ΔFS and log n*K* values is shown in Figure 2. This result suggested that the albumin–acidic binding affinity, log n*K*, can be estimated by a computational chemical calculation. Further study including an examination of the effect of solvent may improve precision, but this is not simple with the present computational system.

4 CONCLUSIONS

Computational chemical calculations demonstrated the possibility of quantitative analysis of the retention of acidic drugs on a guanidine phase, and estimation of albumin–acidic drug binding affinity without experiments. Further study of the effect of solvent and dissociation for the calculation may improve precision.

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