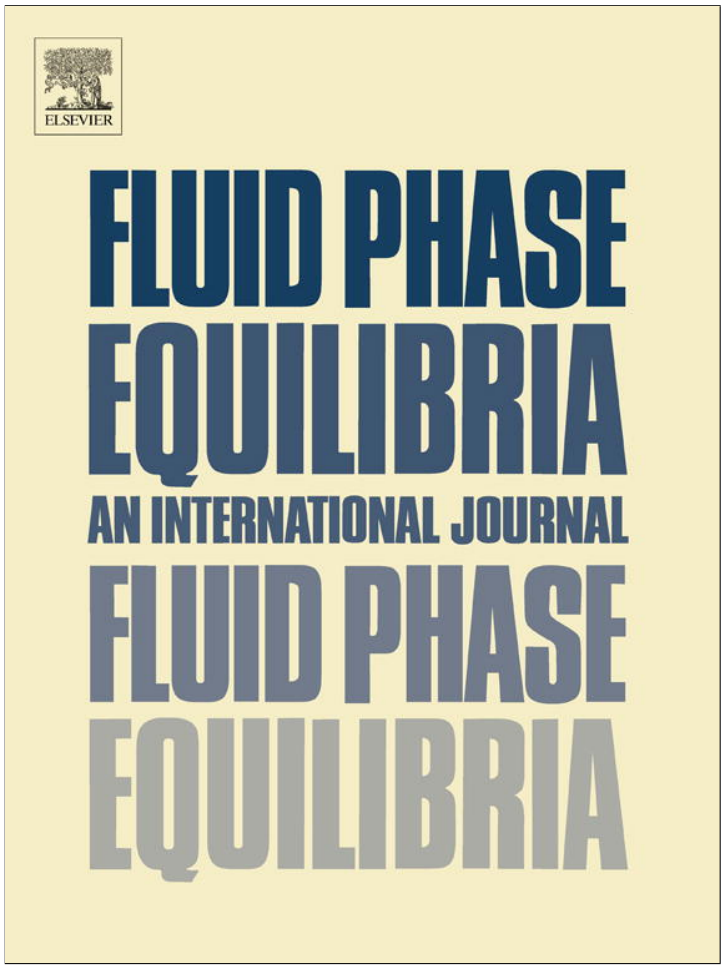

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Enthalpy–entropy compensation for some drugs dissociation in aqueous solutions

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ABSTRACT

The observed enthalpy–entropy compensation seems to be strongly indicative of a common mechanism of drugs dissociation. The regression triplet (data, model, method) is used for the estimation of dependencies between thermodynamic parameters ΔH^0, ΔS^0, ΔG^0 derived from dissociation constant measurements at two different temperatures and Van't Hoff equation. The orthogonal regression analysis involves 87 experimental pK_a values performed on 44 drugs to give unbiased parameter estimates for a linear dependence $\Delta H^0 = 40.43 + 0.966\, T\Delta S^0$ with the Pearson's correlation coefficient $r = 0.8953$. The standard free energy change ΔG^0 for the dissociation reaction may be decomposed on ΔH^0 *vs.* pK_a dependence and $T\Delta S^0$ *vs.* pK_a dependence. The ΔH^0 is sensitive to changes in pK_a because of electrostatic effects. Increasing the Brönsted basicity of the drug causes an increase of the ΔH^0 term and decreases the $T\Delta S^0$ term. When ΔH^0 is negative, then the value $T\Delta S^0$ is the dominant factor, which determines that ΔG^0 is positive. The ionization of a neutral acid involves formation of two ions so that the entropy decreases. On the second ionization of the same acid, there are now three ions and the anion has a charge, so the entropy again only decreases.

1. Introduction

Acid dissociation constants pK_a are essential for understanding many fundamental reactions in chemistry. As they reveal the deprotonation state of a molecule in a particular solvent. They are very important both in the analysis of drugs and in the interpretation of their mechanisms of action. The present study is a continuation of our investigation of dissociation equilibria of various drugs in aqueous solutions at temperatures 298.15 K and 310.15 K (Refs. [1–21]) for which chemical structures are in Fig. 1.

It was shown that the spectrophotometric method can be used for the determination of dissociation constants pK_a, enthalpy, entropy, and Gibbs free energy even for sparingly soluble drugs. Investigation of the entropic and enthalpic properties of dissociation process of drugs in water is very important for correctly understanding their acidic behaviour [22–26]. The concept of QSAR/QSPR is to transform searches for compounds with desired properties using chemical intuition and experience into a mathematically quantified and computerized form. The energy of protonation, defined as the difference between the total energies of the protonated and neutral forms of a molecule, can be considered to be a good measure of the strength of hydrogen bonds (the higher the energy, the stronger the bond) and can be used to determine the correct localization of the most favourable hydrogen bond acceptor site. Such descriptors enable reflection upon simple molecular properties and thus can provide insight into physicochemical nature of the activity/property under consideration [27–30]. Changes in the free energy, enthalpy and entropy, and certain other quantities (such as the heat capacity), are *thermodynamic parameters.* Relationships between these thermodynamic parameters for a series of reactions are termed '*extrathermodynamics*'. They are not derived from first principles and, hence, lie outside the usual domain of traditional thermodynamics. Yet they can yield insight, when properly interpreted, into mechanistic questions. Included in 'extrathermodynamics' are linear free-energy relationships and enthalpy–entropy compensation. These are all approximate and statistical relationships, rather than strict or by mathematical law. Consequently, their validity for a set of experimental data must be established prior to interpretation of their possible meaning [31].

A linear relationship between the entropy change (ΔS) and enthalpy (ΔH) has been observed in a variety of processes of small solutes in aqueous solutions. Enthalpy–entropy compensation has been a widely observed effect in physical, biological, chemical and biochemical processes [32–36] and is excellently reviewed by Liu and Guo [37]. The above empirical relationships were discovered very early [38–41] and have been rediscovered time and time again in many different fields, often independently. It has been observed that as long as some conditions such as pH, solvent composition, a reactant molecule, water activity, ionic hydration [42],

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hydrogen bonding [43], etc. is changed, enthalpy and entropy of activation change accordingly. A stronger intermolecular interaction or bonding (related to the enthalpy) will lead to a greater reduction of the configurational freedom, and hence greater order of the system (related to the entropy). This might be the cause of the enthalpy–entropy compensation [37]. The existence of a linear relationship between enthalpy (ΔH^0) and entropy (ΔS^0) of activation indicates compensation, and the equations are expressed

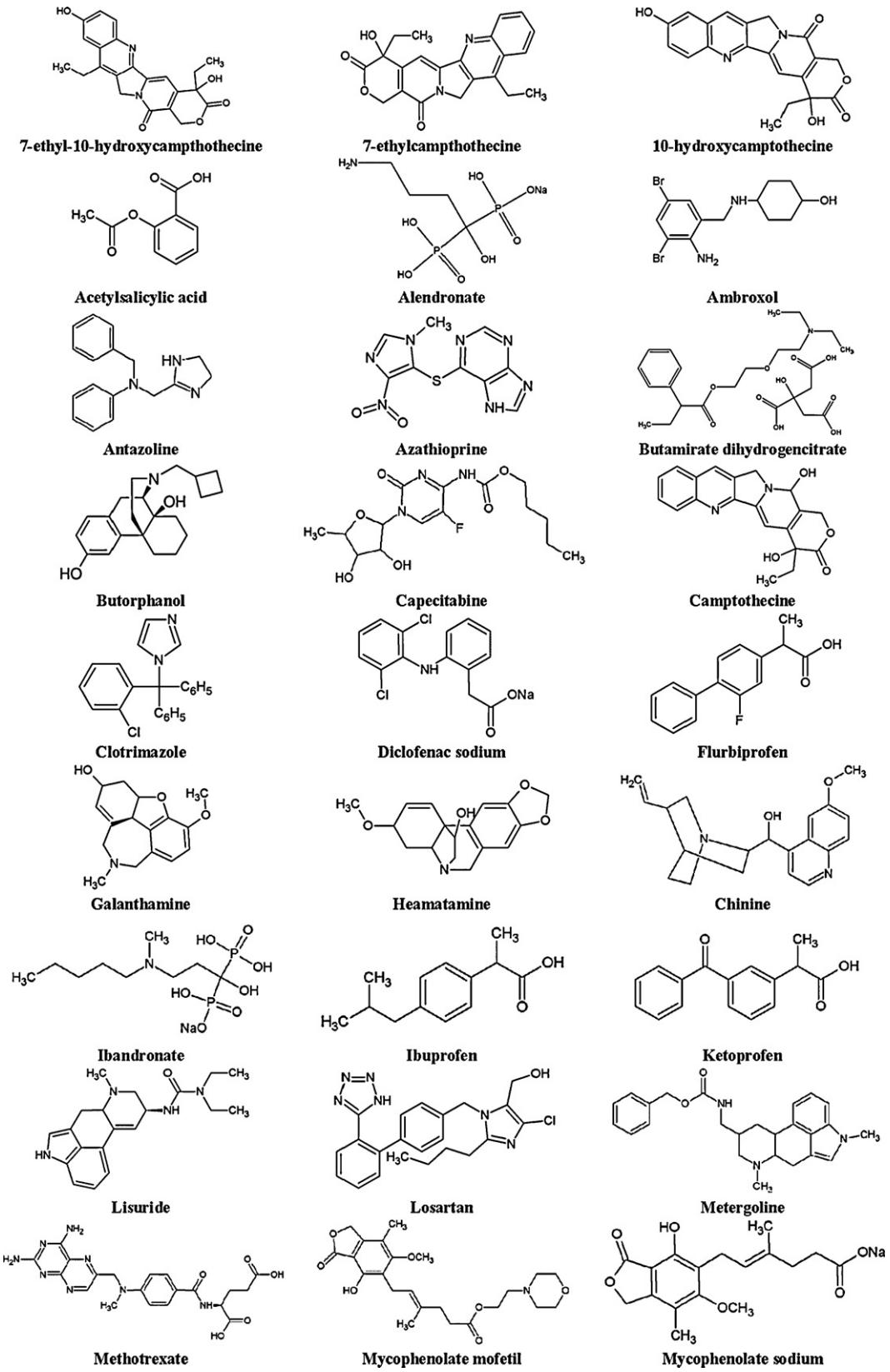

Fig. 1. Structural formula of studied drugs.

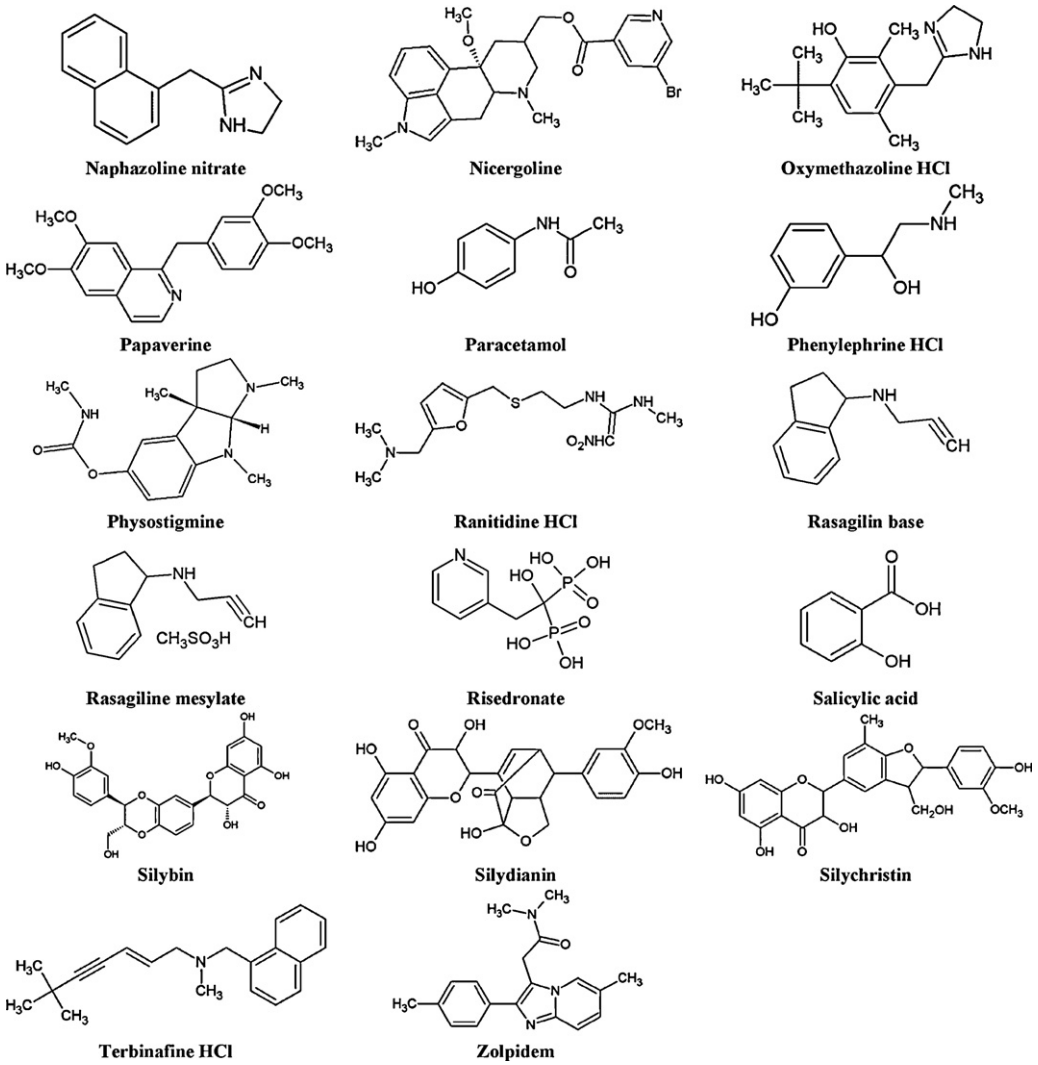

Fig. 1. (*continued*)

as follows: ΔH^0 = slope ΔS^0 + intercept. Enthalpy–entropy compensation has been discussed under several names (for example, isokinetic or isoequilibrium relationship) and from many points of view in countless papers over years. The term refers essentially to the change in enthalpy and the change in entropy in many chemical processes in water involving changes in hydrogen bonding [44–49]. Historically, the isokinetic (or isoequilibrium) relationship and compensation effect were considered to be synonymous or different names for the same phenomenon. However, it will be shown that this truism is misleading. Plotting the enthalpy changes *vs.* the entropy changes gives a straight line. Obviously, there is an excellent linear correlation between the enthalpy and entropy changes and, therefore, an excellent compensation effect. Although enthalpy–entropy compensation has in the past been regarded as a "ubiquitous property of water" [44], it appears to be a property of all weak intermolecular interactions, of which hydrogen bonding in aqueous solution is merely the one most frequently encountered in chemical, biochemical and supramolecular reactions [50].

In this paper, thermodynamic results collected on drug–proton binding equilibria of Fig. 1 are reviewed and discussed from the point of view of their most striking feature: their remarkable enthalpy–entropy compensation behaviour. We review the statistics of the estimation situation for data plotted in the enthalpy–entropy plane. We derive the correlation coefficient and the slope of the regression line for the case in which the chemical variation is small compared to the experimental error.

2. Theoretical

2.1. Extrathermodynamics of drugs dissociation

Acid dissociation constants pK_a are essential for understanding many fundamental reactions in chemistry and biochemistry. The dissociation constants for each of the dissociation reactions may be refined in terms of activity coefficients and concentrations. It is common and useful to calculate enthalpies, entropies, and Gibbs free energies ΔG from dissociation constants pK_a at different temperatures [51]. The standard enthalpy change for each reaction can be obtained from the results of calorimetric measurements extrapolated to infinite dilution when the drug is supposed to be at a steady state at which $\Delta G = 0$ (the process is not capable of producing work). These ΔH values can also be obtained from K_a values at different temperatures by way of the following expressions derived from the Gibbs–Helmholtz equation, also known as the Van't Hoff's equation

$$\Delta H^0 = RT^2 \left(\frac{d \ \ln \ K_a}{dT} \right) = -T^2 \left[\frac{d(G/T)}{dT} \right] \quad (1)$$

where $R = 8.314\,\mathrm{J\,K^{-1}\,mol^{-1}}$ is the universal gas constant and T is the absolute temperature in degrees Kelvin (=°C + 273.15).

Change in enthalpy ΔH sometimes referred to as the 'heat content', this parameter is related to the internal energy by $H = U + pV$ and represents the quantity of energy that is added (*endothermic reaction*) or liberated (*exothermic reaction)* $\Delta H > 0$ and $\Delta H < 0$, respectively, by a process for a system that is at constant pressure and mass. In pharmacologic studies ΔH is commonly interpreted as reflective of changes in intermolecular forces between drug and proton or hydrogen bonds and Van der Waals interactions. A large number of values of the standard free energy of the dissociation equilibrium are available as

$$\Delta G^0 = RT \quad \ln \quad K_a \tag{2}$$

Relatively few measurements of its thermodynamic components

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{3}$$

are known in spite of their remarkable physical importance.

Change in entropy ΔS is defined as $dS = dQ/T$, and has been viewed as a measure of molecular positional unpredictability, uncertainty, 'disorder', 'randomness' or, in statistical terms, as the number of possible microscopic states (molecular configurations) of a reversible reaction. It is not conserved, as is energy. In pharmacologic studies, entropy is often interpreted as reflective of rearrangements in the orderliness of solvent (water) molecules. In fact, the standard enthalpy ΔH^0 can be considered a quantitative indicator of the changes in intermolecular bond energies (hydrogen bonding and Van der Waals interactions) occurring during the binding, while the standard entropy ΔS^0 is most likely a good indicator of the rearrangements undergone by the water molecules during the same process. Methods based on pK_a measurements over a range of temperatures combined with Van't Hoff plots have been successfully applied, as discussed in recent critical appraisals [52–54]. The negative value of ΔS^0 is due to the increased order.

Change in Gibbs free energy ΔG is the amount of work other than the work of expansion that a process of expansion can perform under conditions of constant temperature and pressure which is approximated in chemical reactions in aqueous solution. Chemical reactions are termed *exergonic* when $\Delta G < 0$ and are termed *endergonic* when $\Delta G > 0$. The reaction will occur spontaneously in the direction written if $\Delta G < 0$. Further, ΔG values are positive, thereby indicating that the dissociation process is not spontaneous.

2.2. QSAR to determine the enthalpy–entropy compensation

The application of *a quantitative structure–activity relationship* (QSAR) to the data clarifies the importance of the different structural interactions between drug and proton. The binding of guest to host is determined by the free energy changes of the equilibrium, and the free energy can be separated into two terms: *the enthalpy term* (ΔH^0) and *the entropy term* (ΔS^0). The physical meaning of the two terms is quite different: the enthalpy term originates from the potential energy of the system while the entropy term from the motional freedom of the system. The two terms, however, often counteract each other to ensure stability of the thermodynamic equilibrium. In a number of processes, the potential energy term tends to restrict the motional freedom of the system to lead to smaller entropy. The entropy term tends to disturb the system into a disordered state, and this would result in larger enthalpy. The balance of these two terms finds a thermodynamic equilibrium point, where the free energy becomes minimum.

When a linear relationship between the enthalpy change term (ΔH^0) and entropy change term (ΔS^0) for a reaction series occurs, enthalpy–entropy compensation is said to exist, *i.e.*, that which changes in enthalpy are compensated for by changes in entropy (or *vice versa*) so that free energy change (ΔG^0) remains constant. In practice, the linear relationship obtained may take the form $\alpha = \Delta H^0 - \beta\Delta S^0$ where β does not necessarily equal T. For the compensation effect, the correlation coefficient of the linear plot is usually used as a criterion to judge its existence. It is common sense that the higher the correlation coefficient, the better the compensation. Unfortunately, a complication arises in the interpretation of enthalpy–entropy compensation in situations where ΔH^0 and ΔS^0 are not measured independently, but rather are derived from linear Van't Hoff plots as is typical for most pharmacologic experiments [31]. The uncertainty in the estimates of ΔH^0 and ΔS^0 can be highly correlated and the associated correlation coefficient will tend to near unity so that large experimental errors will tend to increase the correlation coefficient [55,56]. Krug et al. [57] have shown that uncorrelated errors are achieved in a plot of enthalpy *vs.* Gibb's free energy calculated at the harmonic mean of the temperature used in the study. The authors state: "... the significance of an estimated correlation coefficient in the enthalpy–entropy plane is not justification for the detection of a chemically caused compensation, but the significance of an estimated correlation coefficient in the enthalpy–Gibb's free energy plane with estimates evaluated at the harmonic mean of the experimental temperatures is strong justification for the detection of a chemical effect [58].

If a linear relationship exists due to chemical factors rather than to propagation of measurement errors, then the Van't Hoff plot must show a concurrence at some temperature (the lines must intersect) [59].

In 1969, Thorn [60] tried to show the monotonicity of the relation between ΔS^0 and ΔH^0 by considering the entropic and energetic aspects of chemical bonding. It was proven that for a sequence of reactions order → disorder, in which all the significant configurations of the reactants in the sequence are sufficiently equivalent and in which all the significant configurations of the products in the sequence are sufficiently equivalent, ΔS^0 is a non-decreasing function of ΔH^0. Later, Stolov et al. [61–64] studied the compensation effect in the thermodynamics of conformational equilibria, *i.e.*, the enthalpy (ΔH^0) and entropy (ΔS^0) differences of the conformers changed in the same direction when going from one solvent to another.

Recently, Grunwald provided an interesting model to explain the enthalpy–entropy compensation [65]. This theory, though based on the idea similar to that mentioned above, is much easier to understand. According to the theory, the enthalpy–entropy compensation can be caused by two types of interactions, *i.e.*, *solvent reorganization* and *molar shift*. Interestingly, the molar shift can happen within the solvent molecules themselves. Since all the subspecies of the same species are in equilibrium before the change, the free energy change due to the redistribution of the subspecies is zero. This constitutes the origin of the compensation effect.

3. Experimental

3.1. Thermodynamic dissociation constants

Many values of the dissociation constants are reported in literature under conditions of ionic strength I and solution composition where the values of the activity coefficients are not known. Yet it is useful to make some attempt to adjust these pK_a values to $I = 0\,\mathrm{mol\,dm^{-3}}$ for purposes of comparison with other results. The dependence of the mixed dissociation constant $K_a = a_{H^+}[L^{z-1}]/[HL^z]$ on an ionic strength, when both ions HL^z and L^{z-1} have roughly the same ion-size parameter $\mathring{a}$ in the dissociation equilibrium $HL^z \rightleftarrows L^{z-1} + H^+$ with the thermodynamic dissociation constant $K_a^T = a_{H^+}a_{L^-}/a_{HL}$, and suppose that the overall salting-out

Table 1
Thermodynamic dissociation constants of selected drugs studied in our laboratory at two temperatures.

Index	Drug	Drug species abbreviation	pK_a^T 298.15 K	pK_a^T 310.15 K	ΔH^0 (kJ mol^{-1})	$\Delta S^0_{298.15\ K}$ (J mol^{-1})	$\Delta G^0_{298.15\ K}$ (kJ mol^{-1})	Ref.
1a	7-Ethyl-10-hydroxycampthothecine	7E10C	3.11	2.46	95.9	262.1	17.7	[8]
1b		7E10C$^-$	8.91	8.74	25.1	−86.4	50.8	[8]
1c		7E10C^{2-}	9.70	9.47	33.9	−71.8	55.3	[8]
2a	7-Ethylcamptothecine	7EC	3.10	3.30	−29.5	−158.3	17.6	[8]
2b		7EC$^-$	9.94	10.98	−153.4	−704.8	56.7	[8]
3a	10-Hydroxycampthothecine	10HC	2.93	2.84	13.3	−11.5	16.7	[8]
3b		10HC$^-$	8.93	8.92	1.5	−166.0	50.9	[8]
3c		10HC^{2-}	9.45	9.98	−78.2	−443.1	53.9	[8]
4	Acetylsalicylic acid	ASA	3.49	3.41	11.8	−27.2	19.9	[19]
5a	Alendronate	ALE^0	2.60	2.76	−23.6	−128.9	14.8	[17]
5b		ALE$^-$	6.73	6.77	−5.9	−148.6	38.4	[17]
5c		ALE^{2-}	11.51	11.29	32.4	−111.4	65.6	[17]
5d		ALE^{3-}	12.44	11.82	91.4	68.6	71.0	[17]
6a	Ambroxol	AMB	8.05	8.25	−29.5	−253.1	45.9	[1]
6b		AMB$^-$	11.67	11.83	−23.6	−302.6	66.6	[1]
7a	Antazoline	ANT	7.79	7.83	−5.9	−168.9	44.4	[1]
7b		ANT$^-$	9.74	9.55	28.0	−92.4	55.5	[1]
8	Azathioprine	AZA	8.07	7.83	35.1	−36.7	46.0	[18]
9a	Butamirate dihydrogencitrate	BDC	4.51	4.62	−16.2	−140.7	25.7	
9b		BDC$^-$	6.01	6.19	−26.5	−204.1	34.3	
9c		BDC^{2-}	9.72	9.44	41.3	−47.5	55.4	
10a	Butorphanol	BUP	9.46	8.99	69.9	53.3	54.0	[13]
10b		BUP$^-$	9.64	9.34	43.9	−3.1	55.0	[13]
11a	Capecitabine (sp)	CAPs	8.76	8.62	20.65	−98.4	49.9	[14]
12	Capecitabine (pot)	CAPp	8.97	8.74	33.92	−57.9	51.1	[14]
13a	Camptothecine	CAM	2.90	3.02	−17.7	−114.8	16.5	[8]
13b		CAM$^-$	10.18	10.23	−7.3	−219.6	58.1	[8]
14	Clotrimazole	CLO	4.38	4.16	32.4	25.0	24.9	[19]
15	Diclofenac sodium	DCS	4.24	4.41	−25.0	−165.2	24.2	[6]
16	Flurbiprofen	FBP	4.17	4.38	−30.9	−183.7	23.8	[6]
17	Galanthamine	GAL	8.21	7.99	32.4	−48.3	46.8	[19]
18	Haemanthamine	HAE	7.22	7.05	25.0	−54.1	41.2	[2]
19a	Chinine	CHI	4.25	4.12	19.1	−17.0	24.2	[3]
19b		CHI$^-$	8.72	8.46	38.3	−38.2	49.7	[3]
20a	Ibandronate	IBA	2.33	2.5	−25.0	−128.7	13.2	[17]
20b		IBA$^-$	6.31	6.37	−8.8	−150.4	36.0	[17]
20c		IBA^{2-}	10.74	10.65	13.2	−161.0	61.3	[17]
21	Ibuprofen	IBU	4.38	4.51	−19.2	−148.1	24.9	[6]
22	Ketoprofen	KET	4.07	3.87	29.5	21.0	23.2	[6]
23	Lisuride	LIS	7.87	7.59	41.3	−12.1	44.9	[2]
24a	Losartan	LOS	3.63	3.57	8.8	−39.8	20.7	[3]
24b		LOS$^-$	4.84	4.8	5.9	−72.8	27.6	[3]
25	Metergoline	MET	7.62	7.38	35.4	−27.1	43.4	[2]
26a	Methotrexate	MTT	2.89	3.09	−28.6	−151.4	16.5	[12]
26b		MTT$^-$	4.41	4.39	2.6	−75.5	24.1	[12]
26c		MTT^{2-}	5.73	5.58	20.8	−39.8	32.6	[12]
27	Mycophenolate mofetil	MCM	8.29	8.16	19.1	−94.3	47.3	[21]
28	Mycophenolate sodium	MCS	8.32	8.14	26.5	−70.2	47.4	[21]
29	Naphazoline nitrate (sp)	NANs	10.81	10.63	26.5	−117.8	61.7	[1]
30	Naphazoline nitrate (pot)	NANp	10.41	10.13	41.3	−60.7	59.4	[15]
31	Nicergoline	NIC	7.94	7.69	36.8	28.3	45.3	[2]
32a	Oxymetazoline HCl	OXY	10.62	10.77	−22.1	−277.5	60.6	[1]
32b		OXY	12.03	11.82	30.9	−126.3	68.6	[1]
33	Papaverine	PAP	6.42	6.25	25.1	−38.7	36.6	
34	Paracetamol	PAR	9.78	9.65	19.2	−122.9	55.8	[3]
35a	Phenylephrine HCl	PHE	9.17	8.95	32.4	−66.6	52.3	[3]
35b		PHE	10.45	10.22	33.9	−85.2	59.6	[3]
36a	Physostigmine	PHY	2.93	2.61	47.2	102.2	16.7	[16]
36b		PHY$^-$	3.95	3.79	23.6	3.5	22.5	[16]
36c		PHY^{2-}	8.43	8.05	56.0	26.6	48.1	[16]
36d		PHY^{3-}	10.04	10.54	−7.3	−439.5	57.3	[16]
37	Ranitidine HCl	RAN	1.89	1.77	17.7	23.1	10.7	[1]
38	Rasagiline base	RSB	7.12	7.01	16.2	−81.8	40.6	[11]
39	Rasagiline mesylate	RSM	7.07	7.05	2.9	−125.4	40.3	[11]
40a	Risendronate (sp)	RISs	2.37	2.44	−10.3	−80.0	13.5	
40b		RISs$^-$	6.29	6.26	4.4	−105.5	35.9	
40c		RISs^{2-}	7.48	7.46	2.9	−133.2	42.6	
40d		RISs^{3-}	9.31	8.70	89.9	123.5	53.1	
41a	Risendronate (pot)	RISp	2.48	2.43	7.3	−22.7	14.1	
41b		RISp$^-$	6.12	6.10	2.9	−107.2	34.9	
41c		RISp^{2-}	7.25	7.23	2.9	−128.8	41.3	
41d		RISp^{3-}	12.04	11.81	33.9	−116.6	68.7	
42	Salicylic acid	SAL	3.01	3.00	1.4	−52.6	17.1	[19]
43a	Silybin	SLB	7.00	6.86	20.6	−64.7	39.9	[21]
43b		SLB$^-$	8.77	8.77	0	−167.8	50.0	[21]

Table 1 (*Continued*)

Index	Drug	Drug species abbreviation	pK_a^T 298.15 K	pK_a^T 310.15 K	ΔH^0 (kJ mol^{-1})	$\Delta S^0_{298.15\ K}$ (J mol^{-1})	$\Delta G^0_{298.15\ K}$ (kJ mol^{-1})	Ref.
43c		SLB^{2-}	9.57	9.62	−7.3	−207.9	54.6	[21]
43d		SLB^{3-}	11.66	11.38	41.6	−84.6	66.5	[21]
44a	Silydianin	SLD	6.64	7.10	−67.8	−354.7	37.8	[21]
44b		SLD^-	7.78	8.93	−16.9	−717.9	44.4	[21]
44c		SLD^{2-}	9.66	10.06	−59.0	−382.8	55.1	[21]
44d		SLD^{3-}	10.71	10.77	−8.8	−234.7	61.1	[21]
44e		SLD^{4-}	12.26	12.14	17.7	−175.3	69.9	[21]
45a	Silychristin	SLC	6.52	6.62	−14.7	−174.2	37.2	[21]
45b		SLC^-	7.22	7.41	−28.0	−232.2	41.2	[21]
45c		SLC^{2-}	8.96	8.94	2.9	−161.6	51.1	[21]
45d		SLC^{3-}	10.17	10.03	20.6	−125.4	58.0	[21]
45e		SLC^{4-}	11.89	11.63	38.3	−98.9	67.8	[21]
46	Terbinafine HCl	TER	4.19	4.12	10.3	−45.5	23.9	[19]
47	Zolpidem	ZOL	6.335	6.137	29.2	−23.3	36.1	[13]

coefficients are given by $C = C_{HL} - C_L$, is expressed by the extended Debye–Hückel equation (EDH)

$$pK_a = pK_a^T - \frac{A(1-2z)\sqrt{I}}{1 + B\mathring{a}\sqrt{I}} + CI \quad (4)$$

where $A = 0.5112\ mol^{-1/2}\ L^{1/2}\ K^{3/2}$ and $B = 0.3291\ mol^{-1/2}\ m^{-1}\ L^{1/2}\ K^{1/2}\ 10^{10}$ for aqueous solutions at 25 °C. The mixed dissociation constant pK_a represents a dependent variable while the ionic strength I stands for the independent variable. The nonlinear estimation problem is simply a problem of optimization in the parameter space, in which the pK_a and I are known and given values while the parameters pK_a^T, $\mathring{a}$, and C are unknown variables to be estimated. However, for small values of an ionic strength only the pK_a can be estimated. Computation relating to the determination of dissociation constants was performed by regression analysis of the UV/VIS spectra using the SQUAD(84) [66] and SPECFIT/32 [67] programs. Most graphs were plotted using ORIGIN 8.5 [68] and S-Plus [69]. The thermodynamic dissociation constant pK_a^T was estimated with the MINOPT nonlinear regression program in the ADSTAT statistical system (TriloByte Statistical Software, Ltd., Czech Republic) [70].

3.2. Calculation of thermodynamic properties of species

The relationship between change in Gibb's free energy (ΔG^0), change in enthalpy (ΔH^0), and change in entropy (ΔS^0), is given by the equation $\Delta G^0 = \Delta H^0 - T\Delta S^0$, which applies to standard-state conditions. Substitution of the expression relating changes in Gibb's free energy to ΔH^0 and ΔS^0 into the equation $\Delta G^0 = RT\ln(K_a)$ yields the Van't Hoff equation $\ln(K_a) = (\Delta H^0/R)(1/T) - \Delta S^0/R$. A plot of $\ln(K_a)$ against $(1/T)$, where T is degrees in Kelvin, yields a theoretically straight line of slope $\Delta H^0/R$ and y intercept $-\Delta S^0/R$. Because R is known, both ΔH^0 and ΔS^0 can be obtained from such a plot. For K_a values the thermodynamic dissociation constants K_a^T and resulting thermodynamic quantities are shown in Table 1.

3.3. Regression triplet analysis

The parameter estimates were obtained for data sets assuming a linear extrathermodynamic functionality. Three steps of regression procedure according to "regression triplet" [71] were used: (a) the data criticism indicated the influential points, *i.e.*, the outliers and leverages, (b) the model quality for a given data set proves the best regression model proposed, (c) the method criticism checks a fulfilment of all least-squares assumptions. As both variables ΔH^0 and ΔS^0 are loaded with the random error, the orthogonal regression is necessary to apply [71,72]. The procedure for the construction of a linear regression model consists of the following steps:

Step 1. *Proposal of a model for original data*: the procedure usually starts from the simplest model, with individual explanatory controllable variables not raised to powers other than the first. Exploratory data analysis in regression provides a scatter plot of individual variables. Also, in this step the influential points causing multicollinearity are detected.
Step 2. *Significance test of parameter estimates:* the parameters of the proposed regression model and the corresponding basic statistical characteristics of their model are determined by the ordinary least-squares method (OLS). Individual parameters are tested for significance by using the Student t-test. The correlation coefficient R, the determination coefficient or multiplied by 100% being the regression rabat *100D* are computed.
Step 3. *Detection of influential points:* the statistical analysis of ordinary residuals, different diagnostic graphs and numerical measures are used to examine influential points, namely outliers and leverages. If outliers are found, it has to be decided whether these points should be eliminated from the data. If points are eliminated, the whole data treatment must be repeated.
Step 4. *Construction of a more accurate model:* according to the test for fulfilment of the conditions for the least-squares method, and the results of regression diagnostics, a more accurate regression model is constructed.

3.4. Supporting information available

Complete experimental and computational procedures, input data specimens and corresponding output in numerical and graphical form for the programs SQUAD(84), SPECFIT/32 are available free of charge on line at http://meloun.upce.cz and in the menu DOWNLOAD and block DATA.

4. Results and discussion

Thermodynamic parameters of the dissociation reactions of 44 drug acids and bases from Fig. 1 and Table 1 are needed to predict the extent of these reactions and the position of equilibrium for processes in which these reactions occur.

Table 1 contains the following information on each drug considered in this review: (1) the chemical name(s), (2) the abbreviation of an actual drug molecule or anion, (3) the thermodynamic dissociation constant for the dissociation reaction(s) at $T = 298.15$ K and $T = 310.15$ K, (4) the selected values of extrathermodynamic quantities ΔH^0, ΔS^0 and ΔG^0 at 298.15 K that have been adjusted from the reported conditions to $T = 298.15$ K and to the standard state which has been denoted as at an ionic strength equal to zero, "$I = 0$".

Apparently no theory can anticipate at what temperature a given experiment will be carried out. In fact, a number of authors prefer to describe the compensation relationship with the

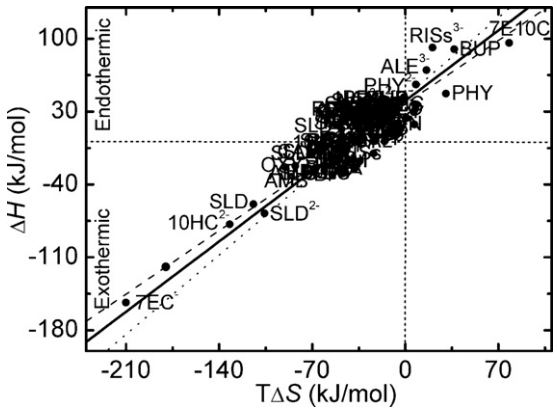

Fig. 2. Graphical presentation of QSARs between ΔH^0 *vs.* $T\Delta S^0$ concerning dissociation of all drugs species of Table 1 which leads to linear regression model: (a) $\Delta H^0 = 37.37(2.26) + 0.868(0.05)\,T\Delta S^0$ with the ordinary least squares, $r = 0.8953$, $p = 0.0000$, (a dashed line). (b) $T\Delta S^0 = -40.73(1.84) + 0.924(0.05)\,\Delta H^0$ with the ordinary least squares (a dotted line). (c) $\Delta H^0 = 40.43 + 0.966\,T\Delta S^0$ with the orthogonal least squares (a full line).

following equation $\Delta H_i = a + b(T_i \Delta S_i)$ instead of $\Delta H_i = \alpha + \beta \Delta S_i$. In this equation, the constant b is usually said to be the "extent" to which the entropy compensates the enthalpy and the "compensation temperature" is usually not mentioned at all [37]. Few enthalpies and entropies in the compensation series are strictly temperature independent. Hence, it is advisable or necessary to contain the temperature information in the compensation model. The equation renders the explanation of the "compensation temperature" unnecessary and the above-raised problem changes to a much simpler one, *i.e.*, why the experimental entropy often offsets the experimental enthalpy to nearly 100%.

Fig. 2 shows the scatter plot of ΔH^0 *vs.* $T\Delta S^0$ for all 44 drugs of Fig. 1 investigated. Using the original set of 89 data points, the ordinary least-squares method OLS finds the two parameters estimates of the linear dependence in which both variables appear to be strongly correlated according to the regression equation $\Delta H^0 = 37.37(2.26) + 0.87(0.05)\,T\Delta S^0$ where standard deviations of the parameters are in brackets. The dependence exhibits a high and significant Pearsonĭs correlation coefficient $r = 0.8953$, $p = 0.0000$ which leads to the regression rabat $100{\cdot}r^2 = 80.16\%$ when 87 values of pK_a were used after elimination of outliers. Also the regression model $T\Delta S^0 = -40.73(1.84) + 0.92(0.05)\,\Delta H^0$ with the same correlation and at last the most rigorous regression equation $\Delta H^0 = 40.43 + 0.966\,T\Delta S^0$ was evaluated with the orthogonal least-squares algorithm. The definition of compensation effect is proposed based on the assumption that the data used in the correlation are error free. In real experiments, errors of the measurements are unavoidable and the data used in the correlation are the estimators of the corresponding parameters. The regression seems remarkable in view of its high correlation coefficient and because of the large number of measurements (87) regarding as many as 44 different drugs. The observed enthalpy–entropy compensation seems to be strongly indicative of a common mechanism of drugs dissociation.

Several authors, however, have remarked that a strong correlation between ΔH^0 and ΔS^0 is not, by itself, a proof of true ΔH^0 *vs.* ΔS^0 compensation arising from *chemical causality*, but that instead it could be an artefact due to the transmission of experimental errors. In fact, ΔS^0 is essentially obtained by calculating it from the thermodynamic dissociation constant, and that of ΔH^0 is determined by Van't Hoff plots. As a consequence, any error on ΔH^0 will affect the error on ΔS^0. Hydrogen bond rearrangement, then, could underlie both ΔH^0 and ΔS^0 in drug–proton interactions and an interrelationship between ΔH^0 and ΔS^0 seems plausible, indeed likely. This suggests that the apparent enthalpy–entropy compensation which is observed experimentally "...arises from an intrinsic property of the hydrogen bond..." namely "...that any tightening of the intermolecular bonds (*the enthalpic factor*) is compensated by a loss of degrees of freedom (*the entropic factor*), or *vice versa*" [73]. Generally speaking, a stronger intermolecular interaction or bonding (related to the enthalpy) will lead to a greater reduction of configurational freedom and hence greater order of the system (related to the entropy). This might be the cause of the enthalpy–entropy compensation. Lumry and Rajender [74]

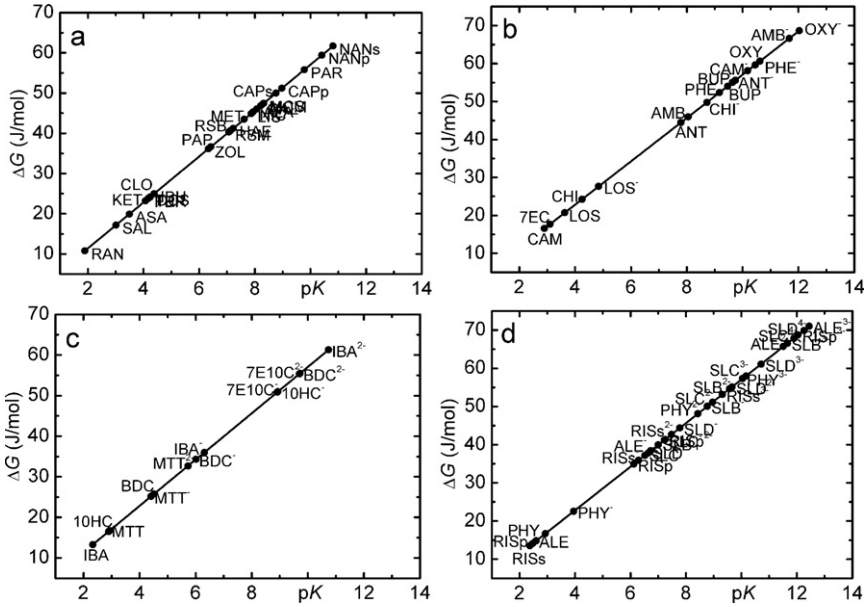

Fig. 3. Graphical presentation of QSARs between ΔG^0 *vs.* pK_a concerning dissociation of HL, H_2L, H_3L, and H_4L species of drugs in Table 1 which leads to linear regression model (in brackets is the standard deviation of the parameter estimate), $RT\ln(10) = 5.705$ for $T = 298.15$ K, $R = 8.314$ J mol^{-1} K^{-1} is the universal gas constant and T is the absolute temperature in degrees Kelvin (=°C + 273.15). (a) Dissociation of HL of various drugs: $\Delta G = 0.00(0.00) + 5.708(0.000)$ pK_a, $r = 1.0000$, $p = 0.0000$; (b) dissociation of H_2L of various drugs: $\Delta G = 0.00(0.00) + 5.708(0.000)$ pK_a, $r = 1.0000$, $p = 0.0000$; (c) dissociation of H_3L of various drugs: $\Delta G = 0.00(0.00) + 5.708(0.000)$ pK_a, $r = 1.0000$, $p = 0.0000$; (d) dissociation of H_4L of various drugs: $\Delta G = 0.01(0.00) + 5.705(0.002)$ pK_a, $r = 0.9999$, $p = 0.0000$.

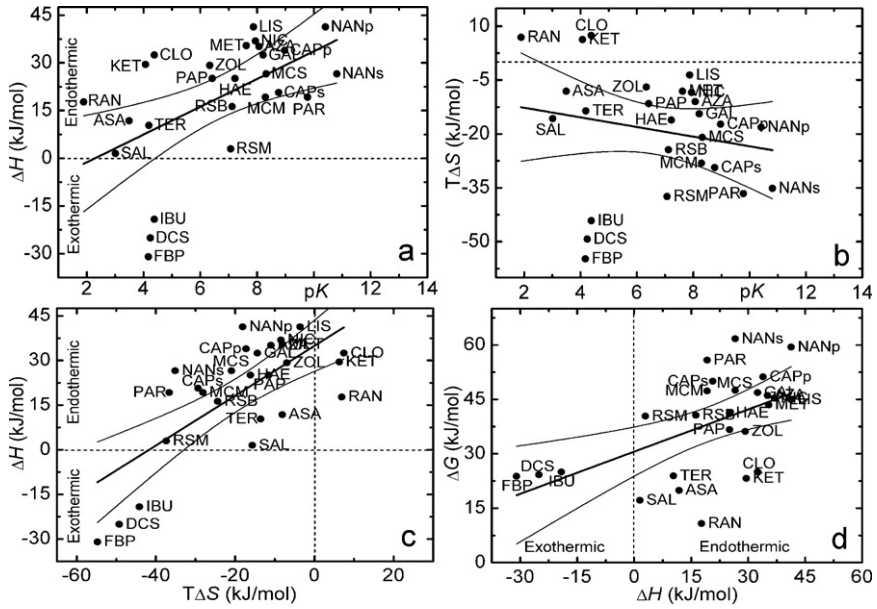

Fig. 4. Graphical presentation of QSARs concerning dissociation of HL of various drugs of Table 1 which lead to linear regression models (in brackets is the standard deviation of the parameter estimate): (a) $\Delta H = -10.01(9.70) + 4.36(1.37)\ \mathrm{p}K_a$, $r = 0.5447$, $p = 0.0040$; (b) $T\Delta S = -10.01(9.70) - 1.34(1.37)\ \mathrm{p}K_a$, $r = -0.1958$, $p = 0.3377$, (*); (c) $\Delta H = 34.90(4.16) + 0.84(0.17)\ T\Delta S$, $r = 0.7158$, $p = 0.0000$; and (d) $\Delta G = 30.60(3.28) + 0.39(0.12)\ \Delta H$, $r = 0.5447$, $p = 0.0040$. The horizontal dashed lines in Fig. 4b and vertical dashed lines in Fig. 4c separate favourable ($T\Delta S^0 < 0$) from unfavourable ($T\Delta S^0 > 0$) entropy binding, (*) means correlation is not significant.

explained enthalpy–entropy compensation often corresponded to aqueous solution reactions, he suggested that the compensation might be a consequence of the properties of water. He employed the idea of two phenomenological species of water, and he supposed that reaction $A \rightarrow B$ was coupled to a second reaction in which n water molecules in state *W1* underwent a transition to state *W2*, *i.e.*, $n\,(H_2O)_{W1} \rightarrow n(H_2O)_{W2}$.

Fig. 3 brings a graphical presentation of QSARs between ΔG^0 *vs.* $\mathrm{p}K_a$ concerning dissociation of HL, H_2L, H_3L, and H_4L species of drugs studied in Table 1 which leads to the linear regression model respecting that $RT\ \ln(10) = 5.705\ \mathrm{J\,mol^{-1}}$ for $T = 298.15\ \mathrm{K}$, $R = 8.314\ \mathrm{J\,K^{-1}\,mol^{-1}}$ being the universal gas constant and T is the absolute temperature in degrees Kelvin (=°C + 273.15). A highly significant correlation led to the resulting slope $5.708\ \mathrm{J\,mol^{-1}}$ in all four cases from (a) to (d) which is very close to theoretical value $5.705\ \mathrm{J\,mol^{-1}}$. This dependence was decomposed into ΔH^0 *vs.* $\mathrm{p}K_a$ dependence (Figs. 4a, 5a, 6a and 7a) and $T\Delta S^0$ *vs.* $\mathrm{p}K_a$ dependence (Figs. 4b, 5b, 6b, and 7b).

Krug [56] have proposed conditions which are sufficient to prove the prevalence of chemical causality over the error transmission effects. The compensation temperature β must be significantly different from the average experimental temperature T. The plots

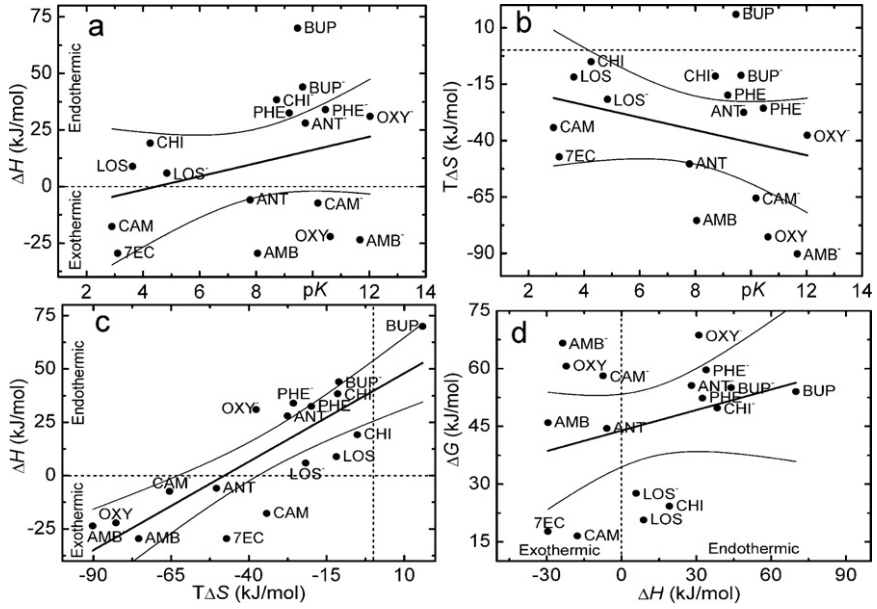

Fig. 5. Graphical presentation of QSARs concerning dissociation of H_2L of various drugs of Table 1 which lead to linear regression models: (a) $\Delta H = -13.07(20.38) + 2.92(2.38)\ \mathrm{p}K_a$, $r = 0.3017$, $p = 0.2393$, (*); (b) $T\Delta S = -13.07(20.38)$ to $2.79(2.38)\ \mathrm{p}K_a$, $r = -0.2889$, $p = 0.2608$, (*); (c) $\Delta H = 39.69(6.66) + 0.83(0.15)\ T\Delta S$, $r = 0.8256$, $p = 0.0000$; and (d) $\Delta G = 43.90(4.44) + 0.18(0.15)\ \Delta H$, $r = 0.3017$, $p = 0.2393$, (*). The horizontal dashed lines in Fig. 5b and vertical dashed lines in Fig. 5c denote separate favourable ($T\Delta S^0 < 0$) from unfavourable ($T\Delta S^0 > 0$) entropy binding, (*) means correlation is not significant.

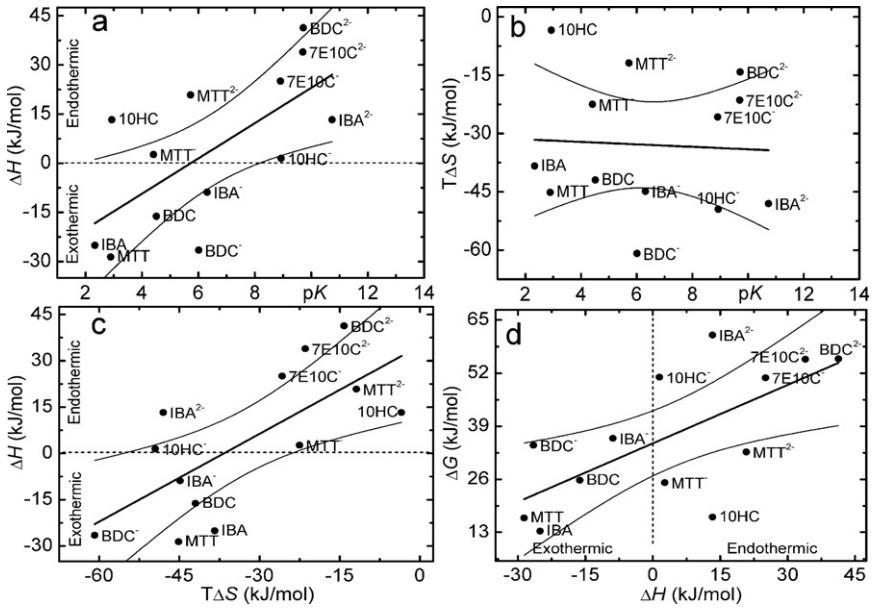

Fig. 6. Graphical presentation of QSARs concerning dissociation of H_3L of various drugs of Table 1 which lead to linear regression models: (a) $\Delta H = -30.89(12.53) + 5.39(1.79)$ pK_a, $r = 0.6713$, $p = 0.0120$; (b) $T\Delta S = -30.89(12.53) - 0.32(1.79)$ pK_a, $r = -0.0531$, $p = 0.8631$, (*); (c) $\Delta H = 34.82(10.65) + 0.95(0.29) T\Delta S$, $r = 0.7045$, $p = 0.0072$; and (d) $\Delta G = 34.79(3.63) + 0.48(0.16)\ \Delta H$, $r = 0.6713$, $p = 0.0120$. The horizontal dashed lines in Fig. 6b and vertical dashed lines in Fig. 6c denote separate favourable ($T\Delta S^0 < 0$) from unfavourable ($T\Delta S^0 > 0$) entropy binding, (*) means correlation is not significant.

of ΔH^0 *vs.* ΔS^0 are shown, which both indicate an excellent compensation. However, plot of ΔH^0 *vs.* ΔG^0 shows no correlation for the complete set of data, $\Delta G^0 = 40.86 + 0.\Delta H^0$, $r = 0.1867$, $p = 0.0781$, 100 $r^2 = 3.48\%$, $n = 87$. Therefore, plot of ΔH^0 *vs.* ΔG^0 (in Figs. 5d, 6d, 7d, and 8d) is not a correct method to examine the compensation effect. The compensation effect can occur when ΔG^0 is approximately constant within the reaction series while ΔH^0 *vs.* ΔS^0 vary significantly [75,76]. In Figs. 5–8 the plots bring some common conclusions which may be commonly described as:

(1) When pK_a is positive, the standard free energy change ΔG^0 for the dissociation reaction is also positive and may be decomposed on while ΔH^0 *vs.* pK_a dependence (Figs. 4a, 5a, 6a, and 7a) and $T\Delta S^0$ *vs.* pK_a dependence (Figs. 4b, 5b, 6b, and 7b). While the while ΔH^0 *vs.* pK_a plot exhibits statistically significant straight line in two cases (for monoprotic acids in Fig. 4a and for triprotic acids in Fig. 6a), the plot $T\Delta S^0$ *vs.* pK_a does not lead to the linear straight line in any of four analysed cases. The while ΔH^0 *vs.* pK_a plot shows that while ΔH^0 is sensitive to changes in pK_a because of electrostatic effects. With increasing the Brœnsted

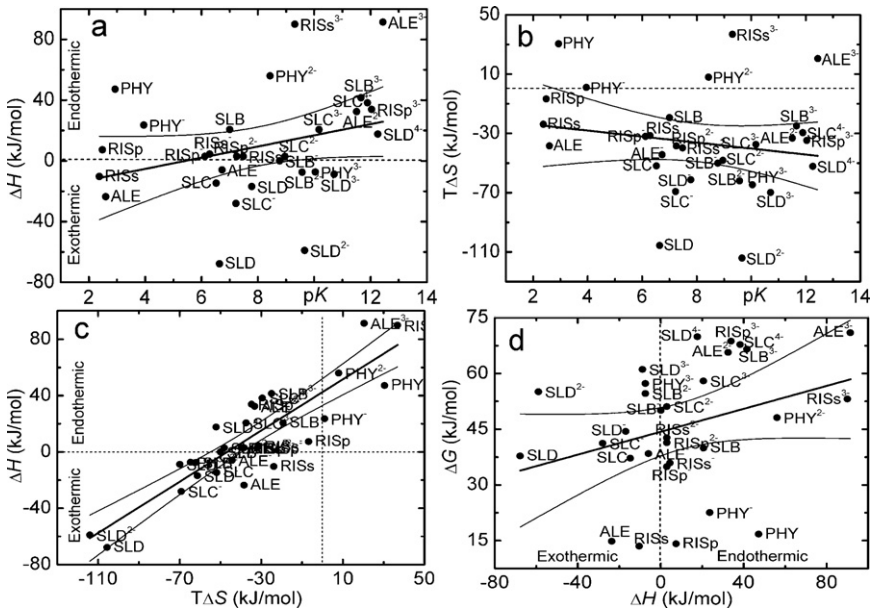

Fig. 7. Graphical presentation of QSARs concerning dissociation of H_4L and H_5L of various drugs of Table 1 which lead to linear regression models: (a) $\Delta H = -19.99(17.94) + 3.68(2.10)$ pK_a, $r = 0.3153$, $p = 0.0896$, (*); (b) $T\Delta S = -19.99(17.94) - 2.02(2.09)$ pK_a, $r = -0.1795$, $p = 0.3427$, (*); (c) $\Delta H = 42.52(4.66) + 0.91(0.09) T\Delta S$, $r = 0.8770$, $p = 0.0000$; and (d) $\Delta G = 44.32(3.17) + 0.15(0.09)\ \Delta H$, $r = 0.3160$, $p = 0.0890$, (*). The horizontal dashed lines in Fig. 7b and vertical dashed lines in Fig. 7c denote separate favourable ($T\Delta S^0 < 0$) from unfavourable ($T\Delta S^0 > 0$) entropy binding, (*) means correlation is not significant.

basicity of drug (*i.e.*, increasing pK_a of an actual drug) while ΔH^0 term increases (Figs. 4a, 5a, 6a, 7a) and $T\Delta S^0$ term decreases (Figs. 4b, 5b, 6b, and 7b).

(2) Second, some reactions are exothermic and some are endothermic as denoted on plots, Figs. 4–7. The positive value of the ΔH^0 indicates that dissociation process is endothermic and is accompanied by absorption of heat. As ΔH^0 becomes more negative (stronger bonding), ΔS^0 tends to decrease due to the tightening up of the system. As ΔH^0 becomes less negative (weaker bonding), ΔS^0 tends to increase as the system becomes increasingly disordered. The hydrogen bond rearrangement, then, could underlie both ΔH^0 and ΔS^0 in drug–proton interactions and an interrelationship between ΔH^0 and ΔS^0 seems plausible, indeed likely. The hydrogen bond as central to a drug–proton interaction also is mechanistically appealing. In water, the hydrogen bonds form a network of continuous chains that are dynamically changing (in a sort of steady state). Because of the dipole created by displacement of the electron from the hydrogen proton, these chains form a sequence of mono- and dipoles that are sensitive to the electrostatic potential of the drug and receptor molecules and provide a mechanism for transmitting information at a distance from drug to receptor [77].

(3) When ΔH^0 is negative, then the value $T\Delta S^0$ is the dominant factor, which determines that ΔG^0 is positive.

(4) Last, the entropy contribution is mostly unfavourable ($\Delta S^0 < 0$) in these reactions. Ions in aqueous solution tend to orient the surrounding water molecules, which orders the solution and decreases the entropy. The contribution of an ion to the entropy is the partial molar entropy which is often negative, especially for small or highly charged ions. The ionization of a neutral acid involves formation of two ions so that the entropy decreases ($\Delta S^0 < 0$). There are now three ions on the second ionization of the same acid and the anion has a charge, so the entropy again decreases only (Figs. 4b, c, 5b, c, 6b, c, 7b, c).

(5) Note that the standard Gibb's free energy change for the reaction is for the changes from the reactants in their standard states to the products in their standard states. The free energy change at equilibrium is zero since the chemical potentials of reactants and products are equal at equilibrium.

5. Conclusions

The *compensation effect* should only mean that there is a linear relationship between the enthalpy and entropy changes of a series of a dissociation of acids in aqueous solutions. For often-observed large compensation effects, especially those involving solution or variously protonated anions, redistribution of the energy-distinguishable subspecies is most likely the physical origin. Usually two forms of redistribution play a major role, *i.e.*, *the solvent recognition* and *the molar shift*. These findings seem to point to the idea that the enthalpy–entropy compensation found arises from an intrinsic property of the hydrogen bond, which is the main force determining the association of the participants (water, drug, binding site) in the drug–receptor binding equilibrium. This idea simply reflects the more basic fact that any tightening of the intermolecular bonds (the enthalpic factor) is compensated by a loss of degrees of freedom (the entropic factor), or *vice versa.* The monotonicity of relation between ΔS^0 and ΔH^0 by considering the entropic and energetic aspects of chemical bonding was proven. When pK_a is positive, the standard free energy change ΔG^0 for the dissociation reaction is also positive and may be decomposed on ΔH^0 *vs.* pK_a dependence and $T\Delta S^0$ *vs.* pK_a dependence. The ΔH^0 *vs.* pK_a plot shows that ΔH^0 is sensitive to changes in pK_a because of electrostatic effects. Increasing the Brönsted basicity of drug causes an increase of the ΔH^0 term and decreases $T\Delta S^0$ term. When ΔH^0 is negative, then the value $T\Delta S^0$ is the dominant factor, which determines that ΔG^0 is positive. The ionization of a neutral acid involves formation of two ions so that the entropy decreases. There are now three ions on the second ionization of the same acid and the anion has a charge, so the entropy again decreases only. The compensation analysis could also be a useful tool for investigating pharmaceutical and chemical processes of relevance to QSAR of drugs.

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