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Biorelevant pK_a (37 °C) predicted from the 2D structure of the molecule and its pK_a at 25 °C

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ABSTRACT

Values of the ionization constants at 37 °C, which are scarcely reported, are more meaningful for interpreting mechanisms of cellular transport by ionizable molecules and in mechanistic dissolution studies, which are often performed at the biorelevant temperature. An equation was developed where the pK_a values of drug-like molecules determined at 25 °C can be simply converted to values at 37 °C, without additional measurement. The differences between the values, $\Delta pK_a = pK_a^{37} - pK_a^{25}$, were linearly fitted to a function of pK_a^{25} and the standard entropy of ionization, ΔS°, where the latter term was approximated by the five Abraham linear free energy solvation descriptors using multiple linear regression. The Abraham descriptors (H-bond donor and acceptor strengths, dipolar solute–solvent interactions potential, the pi- and n-electrons dispersion force, and molar volume) were determined from the 2-dimensional structure of the molecules. A total of 143 mostly drug-like molecules (207 pK_a values at 25 °C and at 37 °C) were chosen for the study. The pK_a values of many were determined here for the first time. Included were 34 weak acids, 85 weak bases, and 24 amphoteric compounds (6 ordinary ampholytes, 18 zwitterions).

1. Introduction

The measurement of physicochemical properties of active pharmaceutical ingredients (API) is critical to pharmaceutical development. The ionization constant, pK_a, is one of the most important of such properties for ionizable API. The value of the pK_a can affect the solubility, dissolution rate, absorption across biological membranes, distribution to the site of action, renal elimination, metabolism, protein binding, and receptor interactions [1]. Several methods to determine pK_a values and the control of the experimental details to achieve the maximum precision and accuracy have been described previously in the literature [2–8]. The focus of this paper is to predict the effect of temperature on pK_a from the knowledge 2-dimensional (2D) structure of the molecule and its determined pK_a at 25 °C.

The ionization constant is a thermodynamic parameter [9–11], which depends on temperature. The pharmacokinetics of the API (including absorption, distribution, metabolism, elimination, and toxicity, i.e., ADMET) are evaluated at the physiological relevant temperature of 37 °C. However, pK_a values needed to interpret certain biological mechanisms are most often available only from lower temperature determinations. The majority of the published pK_a values are determined at 'room temperature,' sometimes without ionic strength adjustor [6,12–16]. The most reliable results come from laboratories where the pK_a is determined under standard conditions, i.e., in thermostated 25 °C solutions containing a background electrolyte (e.g., 0.15 M KCl), with special care given to calibrating the pH electrode. Of the published pK_a values of drug-like molecules, scarcely any are reported at 37 °C.

The effect of temperature on pK_a depends on the nature of the functional group. Simple carboxylic acid-containing drugs have nearly the same pK_a at 25 and 37 °C [4,17–19], whereas simple bases usually have a decreased pK_a at the biorelevant temperature ($\Delta pK_a/\Delta T \approx -0.03\ ^\circ C^{-1}$) [2–5,8] (e.g., propranolol has the pK_a values 9.53 and 9.17 at 25 and 37 °C, respectively). Neglecting the temperature effect can lead to inaccurate interpretations of pharmacokinetic mechanisms of ionizable drugs, and potentially contributing to poorer in vitro–in vivo correlations (IVIVC).

In this study we have devised a simple procedure which allows the prediction of the pK_a value at 37 °C, provided the value at 25 °C is known. The differences between the values, $\Delta pK_a = pK_a^{37} - pK_a^{25}$, were linearly fitted to a function of pK_a^{25} and the standard entropy of ionization, ΔS°, where the latter term was approximated by the five Abraham [20] linear free energy relationship (LFER) solvation descriptors using multiple linear regression (MLR). The Abraham descriptors (H-bond donor and acceptor strengths, dipolar solute–solvent interactions potential, the pi- and n-electrons dispersion force, and molar volume) were estimated from the 2D structure of the molecules [38]. A total of 143 mostly drug-like

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Nomenclature

MLR	multiple linear regression
LFER	linear free energy relationships
R_2	Abraham descriptor – excess molar refraction ($dm^3\ mol^{-1}/10$); which models dispersion force interaction arising from pi- and n-electrons of the solute (also called E)
V_x	Abraham descriptor – McGowan molar volume ($dm^3\ mol^{-1}/100$) of the solute
pK_a	negative log, base 10, of the 'concentration' ionization constant (constant ionic medium reference state, 0.15 M KCl)
p_sK_a	mixed-solvent pK_a (constant ionic medium reference state, 0.15 M KCl)
$\bar{n}_H$	Bjerrum function – the average number of bound protons on an ionizable molecule at a particular pH

Greek letters

ΔpK_a	shift in the pK_a on raising the temperature from 25 to 37 °C: $\Delta pK_a = pK_a^{37} - pK_a^{25}$,
$\Sigma\alpha_2^H$	Abraham descriptor – solute H-bond total acidity (also called A)
$\Sigma\beta_2^H$	Abraham descriptor – solute H-bond total basicity (also called B)
π_2	Abraham descriptor – solute polarity/polarizability due to solute–solvent interactions between bond dipoles and induced dipoles (also called S)

molecules (207 pK_a values at 25 °C and at 37 °C) were chosen. The pK_a values of many were determined here for the first time. Included were 34 weak acids, 85 weak bases, and 24 amphoteric compounds (6 ordinary ampholytes, 18 zwitterions).

2. Materials and methods

2.1. Chemicals and materials

Methanol, 1-propanol, and dimethylsulfoxide DMSO (all HPLC purity grade) were purchased from Sigma–Aldrich/RBI (St. Louis, MO, USA). Reverse osmosis de-ionized water ("18 Ω" grade) was used. The drugs whose pK_a values were measured here, including astemizole, carvedilol, chloroquine diphosphate, codeine, diphenhydramine, domperidone, gabapentin, guanabenz acetate, maprotiline hydrochloride, melphalan, omeprazole, oxycodone hydrochloride, pergolide mesylate, perphenazine, pyrilamine mesylate, thioridazine hydrochloride, vinblastine sulfate, and vincristine sulfate were purchased from Sigma–Aldrich/RBI (St. Louis, MO, USA) and Tocris Bioscience (Ellisville, MO, USA). Imatinib mesylate was purchased from Selleck Chemical LLC (Houston, TX, USA). All drugs were used as received without further treatment or purifications. The preparation and standardization of titrants (0.5 M HCl and KOH) follow the procedure described elsewhere [13,21].

2.2. pK_a measurement

The Gemini Profiler™ instrument with software version 3.2 (*p*ION) was used to determine the ionization constants of many of the drugs at pK_a at 37 °C (and in some cases also at 25 °C), as identified in Table 1. The instrument is equipped with three precision dispensers (capable of adding a minimum volume of 0.021 μL) and a high-impedance (10^{15} Ω) pH circuit. For each ionizable drug, at least three replicate titrations were performed at 25 ± 0.5 °C and/or 37 ± 0.5 °C in 0.15 M KCl medium. General details of the procedure have been described elsewhere [6,21,22]. The double-junction combination pH electrode (*p*ION) was standardized *in situ* using the Avdeef–Bucher four–parameter equation [21], in both aqueous and semi-aqueous titrations. As typical procedures, titrations of weak bases and ampholytes begin at low pH and those of weak acids begin at high pH over a range of pH 1.8–12.2. The wide pH range is needed for the *in situ* electrode standardization procedure. This *in situ* procedure eliminates the need for a separate conventional blank titration [6,21]. A Teflon-coated magnetic stir disk was used to mix the solution during titrant addition. The solutions were bathed with argon to minimize the ingress of ambient carbon dioxide. The KOH or HCl (0.5 M) titrant is dispensed accurately and slowly into the solution, to produce about 0.15 pH increments between accepted pH readings. After titrant additions, careful measurements of pH were made until equilibration was established. Sample concentrations were in the range of about 0.1–1.0 mM, with the lower end used for compounds expected to be low in solubility.

Approximate pK_a values were deduced graphically from Bjerrum plots, $\bar{n}_H$, vs. pH [6]. The Bjerrum function, $\bar{n}_H$, is the average number of bound protons at a particular pH, and is defined by $\bar{n}_H = ([HCl] - [KOH] + n_H[drug] - [H^+] + [OH^-])/[drug]$, where n_H is number of dissociable protons introduced by drug, and square brackets designate concentrations. It is a property of Bjerrum plots that the pH at half integral value of $\bar{n}_H$ is approximately equal to the pK_a.

These approximate pK_a values were then refined by a weighted nonlinear least squares procedure in the Gemini Profiler software [6]. A unique feature of the software is that the pK_a can be determined even if there is some precipitation of the drug during titration. Ignoring precipitation can lead to systematic pK_a errors (positive bias for acids and negative bias for bases), as high as a log unit in some cases [6,12–14].

Since many of the drugs studied are practically insoluble in water, the cosolvent procedure [6,23] was also used, where the apparent mixed-solvent pK_a (p_sK_a) values determined at various ratios of cosolvent/water were extrapolated to zero-cosolvent to estimate the aqueous value. Three to six different cosolvent/water mixtures were used, typically in the interval 15–50 wt%. Methanol or DMSO was used for titrations at 25 °C, but 1-propanol or DMSO was used for titrations at 37 °C. The use of methanol (or similarly volatile solvents) for high temperature titrations is not recommended, since the steady rate of its evaporation leads to difficult-to-recognize systematic inaccuracies in the extrapolated values of the ionization constants [24].

For weak bases, the aqueous pK_a was estimated from the linear extrapolation of p_sK_a vs. wt% cosolvent to zero cosolvent [6,23]. However, for weak acids, the *origin-shifted* Yasuda–Shedlovsky procedure was used, which involves the extrapolation of $p_sK_a + \log\{[H_2O]/55.51\}$ vs. $(1/\varepsilon - 1/\varepsilon_0)$ to zero cosolvent, where $[H_2O]$ is the molar concentration of water in the mixed-solvent (55.51 M at zero cosolvent) and ε is the dielectric constant of the mixed-solvent (ε_0 at zero cosolvent). The latter acid–base differentiated procedure appears to be produce smaller bias with practically insoluble acids, compared to bases, as suggested in a comparative mixed-solvent study of 50 compounds by Völgyi et al. [23].

2.3. Literature pK_a data used

In addition to the pK_a values determined here, many values at 25 and 37 °C were also taken from the open literature. For many of the simple molecules used, e.g., amino acids, carboxylic acid and amine buffers, pK_a at various values of temperature and ionic strength were taken from multiple sources compiled in reliable databases

Table 1
Ionization constants and Abraham descriptors[a].

Compounds	Type	ΔpK_a(obs)	ΔpK_a(calc)	pK_a^{25}	Ref[25]	pK_a^{37}	Ref[37]	$\Sigma\alpha_2^H$ (A)	$\Sigma\beta_2^H$ (B)	π_2 (S)	R_2 (E)	McGowan volume (V_x)
1,2-Diaminocyclohexane	B	−0.32	−0.25	6.34	[4]	6.02	[4]	0.44	1.15	0.90	0.61	1.05
1,3-Diamino-2-hydroxypropane	B	−0.32	−0.28	7.81	[4]	7.49	[4]	0.63	1.56	1.00	0.64	0.79
1,3-Diaminopropane	B	−0.37	−0.31	8.49	[4]	8.12	[4]	0.42	1.12	0.82	0.40	0.73
1,4-Diaminobutane	B	−0.36	−0.32	9.20	[4]	8.84	[4]	0.42	1.12	0.82	0.40	0.87
1,6-Diaminohexane	B	−0.41	−0.33	9.83	[4]	9.42	[4]	0.42	1.13	0.83	0.40	1.15
2,4,6-Trimethylpyridine	B	−0.26	−0.30	7.43	[30]	7.17	[30]	0.00	0.40	0.65	0.67	1.10
2,5-Dimethylimidazole	B	−0.30	−0.32	8.36	[30]	8.06	[30]	0.35	0.51	0.93	0.67	0.82
2-Amino-2-ethyl-1,3-propanediol	B	−0.35	−0.31	8.80	[30]	8.45	[30]	0.74	1.30	0.92	0.61	1.03
2-Amino-2-methyl-1,3-propanediol	B	−0.35	−0.31	8.79	[30]	8.44	[30]	0.74	1.30	0.91	0.61	0.89
2-Amino-2-methyl-1-propanol	B	−0.38	−0.34	9.69	[30]	9.31	[30]	0.46	0.98	0.66	0.40	0.83
2-Aminoquinoline	B	−0.30	−0.29	7.29	[4]	6.99	[4]	0.23	0.67	1.47	1.64	1.14
2-Naphthoic acid *	A	+0.15	−0.05	4.18	[41]	4.33	[24,41]	0.57	0.50	1.40	1.47	1.30
2-Nitroaniline	B	−0.05	−0.09	−0.26	[4]	−0.31	[4]	0.18	0.48	1.44	1.16	0.99
3,3-Dimethylglutaric acid (1)	A	+0.09	+0.07	3.70	[30]	3.79	[30]	1.14	0.73	1.01	0.32	1.24
3,3-Dimethylglutaric acid (2)	A	+0.07	+0.01	6.34	[30]	6.41	[30]	1.14	0.73	1.01	0.32	1.24
4-Aminopyridine	B	−0.34	−0.33	9.11	[30]	8.77	[30]	0.23	0.71	1.21	0.90	0.78
4-Hydroxymethlimidazole	B	−0.22	−0.26	6.39	[30]	6.17	[30]	0.66	0.92	1.19	0.87	0.74
4-Methylimidazole	B	−0.26	−0.30	7.52	[30]	7.26	[30]	0.35	0.51	0.99	0.64	0.68
4-Nitroanaline	B	−0.08	−0.11	1.00	[4]	0.92	[4]	0.28	0.53	1.65	1.13	0.99
5,5-Diethylbarbituric acid	A	−0.17	−0.10	7.98	[30]	7.81	[30]	0.52	1.21	1.35	0.98	1.37
6-Aminopurine (1)	X:B	−0.11	−0.02	4.15	[4]	4.04	[4]	0.60	0.98	1.79	1.74	0.92
ACES	B	−0.24	−0.20	6.80	[29]	6.56	[29]	0.93	1.79	2.41	0.85	1.23
Acetic acid	A	+0.00	+0.02	4.52	c	4.53	c	0.57	0.36	0.61	0.17	0.46
ADA	B	−0.13	−0.19	6.55	[28,29]	6.41	[28,29]	1.63	1.73	2.16	0.92	1.32
Alanine (2)	Z:B	−0.32	−0.30	9.87	[30]	9.55	[30]	0.78	0.93	0.92	0.38	0.71
Amitriptyline *	B	−0.32	−0.33	9.49	b	9.17	[47]	0.00	0.77	1.31	1.71	2.40
Ammonia	B	−0.36	−0.36	9.24	c	8.88	c	0.21	0.44	0.42	0.37	0.21
Aniline	B	−0.19	−0.22	4.61	[4]	4.41	[4]	0.23	0.43	1.08	0.86	0.82
Arginine (1)	Z:A	+0.00	−0.03	2.08	[27,28]	2.08	[27,28]	1.26	1.95	1.24	1.06	1.38
Arginine (2)	Z:B	−0.30	−0.30	9.05	[27,28]	8.75	[27,28]	1.26	1.95	1.24	1.06	1.38
Aspartic acid (1)	Z:A	−0.03	+0.01	1.92	[28]	1.90	[28]	1.18	1.26	1.37	0.55	0.92
Aspartic acid (2)	Z:A	−0.01	−0.05	3.67	[28]	3.67	[28]	1.18	1.26	1.37	0.55	0.92
Aspartic acid (3)	Z:B	−0.17	−0.28	9.63	[28]	9.46	[28]	1.18	1.26	1.37	0.55	0.92
Astemizole (1) ● *	B	−0.69	−0.20	5.95	[14]	5.28	b	0.13	1.64	2.70	3.10	3.56
Astemizole (2) *	B	−0.44	−0.27	8.77	[14]	8.34	b	0.13	1.64	2.70	3.10	3.56
Atenolol	B	−0.35	−0.28	9.54	[44]	9.19	[40]	0.78	1.85	1.97	1.48	2.18
Atomoxatine *	B	−0.25	−0.33	9.66	[47]	9.38	[47]	0.13	0.90	1.36	1.37	2.19
Benzoic acid	A	+0.00	−0.02	3.98	[25]	3.98	[25]	0.57	0.44	1.08	0.75	0.93
BES	B	−0.19	−0.21	7.09	[28,29]	6.90	[27,29]	0.79	2.02	1.96	0.92	1.51
β-Alanine (1)	Z:A	+0.09	−0.06	3.55	[27,28]	3.64	[27,28]	0.78	0.9	0.94	0.37	0.71
β-Alanine (2)	Z:B	−0.21	−0.31	10.18	[27,28]	9.97	[27,28]	0.78	0.9	0.94	0.37	0.71
Bicine	B	−0.18	−0.27	8.13	[28,29]	7.96	[28,29]	1.05	1.58	1.25	0.81	1.25
Boric acid	A	−0.09	−0.06	8.98	c	8.89	c	0.94	0.86	0.94	0.51	0.42
Butyric acid	A	+0.03	+0.02	4.67	[27,28]	4.70	[27,28]	0.57	0.36	0.62	0.17	0.75
Carbonic acid (1)	A	−0.06	+0.01	6.12	c	6.05	c	0.97	0.55	0.77	0.30	0.38
Carbonic acid (2)	A	−0.09	−0.08	9.88	c	9.79	c	0.97	0.55	0.77	0.30	0.38
Carvedilol ● *	B	+0.20	−0.24	8.06	[44]	8.25	b	0.62	2.09	3.00	3.08	3.10
Chloroacetic acid	A	+0.03	+0.06	2.88	[30]	2.91	[30]	0.79	0.36	0.76	0.30	0.59
Chloroquine (1)	B	−0.27	−0.28	8.37	[48]	7.99	b	0.13	1.29	1.63	1.85	2.63
Chloroquine (2)	B	−0.47	−0.34	10.76	[48]	10.10	b	0.13	1.29	1.63	1.85	2.63
Cholamine Chloride	B	−0.32	−0.27	6.97	[28,29]	9.64	[28,29]	0.21	0.61	0.42	−0.01	1.03
Cinnarizine *	B	−0.24	−0.26	7.69	[39]	7.45	[39]	0.00	1.37	2.12	2.43	3.11
Citric acid (1)	A	+0.04	+0.09	2.91	c	2.96	c	1.63	1.33	1.50	0.61	1.24
Citric acid (2)	A	+0.05	+0.06	4.34	c	4.39	c	1.63	1.33	1.50	0.61	1.24
Citric acid (3)	A	+0.11	+0.03	5.68	c	5.78	c	1.63	1.33	1.50	0.61	1.24
Codeine	B	−0.25	−0.28	8.24	[43]	7.99	b	0.23	1.58	1.92	2.16	2.21
Creatinine (1)	B	−0.18	−0.22	4.83	[49]	4.66	[50]	0.39	1.31	1.04	1.04	0.84
Creatinine (2)	A	0.03	−0.11	9.20	[49]	9.23	[50]	0.39	1.31	1.04	1.04	0.84
Diethanolamine	B	−0.30	−0.32	8.88	[30]	8.58	[30]	0.64	1.19	0.82	0.58	0.89
Diethylamine	B	−0.41	−0.39	10.93	[30]	10.52	[30]	0.13	0.48	0.35	0.15	0.77
Dimethylamine	B	−0.40	−0.39	10.77	[4,30]	10.37	[4,30]	0.13	0.47	0.34	0.16	0.49
Diphenhydramine	B	−0.23	−0.31	9.10	[51]	8.85	b	0.00	0.95	1.43	1.36	2.19
Dipyridamole ●	B	−1.26	−0.18	6.17	[24]	4.93	[24]	0.95	3.03	2.9	3.74	3.87
Domperidone (1) ● *	X:B	−0.33	−0.22	7.29	[47]	6.91	b	0.72	1.83	3.13	3.11	3.06
Domperidone (2) *	X:A	−0.01	−0.07	9.69	[47]	9.68	b	0.72	1.83	3.13	3.11	3.06
Ephedrine	B	−0.26	−0.33	9.65	[25]	9.39	[30]	0.38	1.12	0.94	0.98	1.44
Ethanolamine	B	−0.34	−0.34	9.53	[27,28]	9.19	[27,28]	0.46	0.94	0.72	0.42	0.55
Ethanolisopropanolamine	B	−0.29	−0.31	8.81	[30]	8.52	[30]	0.64	1.22	0.81	0.59	1.03
Ethylamine	B	−0.38	−0.38	10.63	[4,30]	10.25	[4,30]	0.21	0.57	0.49	0.21	0.49
Ethylenediamine Tetraacetic acid (1)	X:B	−0.00	+0.11	0.58	[27,28]	0.58	[27,28]	2.29	2.35	2.33	1.00	2.01
Ethylenediamine Tetraacetic acid (2)	X:B	−0.06	+0.07	1.62	[27,28]	1.56	[27,28]	2.29	2.35	2.33	1.00	2.01
Ethylenediamine Tetraacetic acid (3)	X:A	+0.12	+0.05	2.06	[27,28]	2.17	[27,28]	2.29	2.35	2.33	1.00	2.01
Ethylenediamine Tetraacetic acid (4)	X:A	+0.09	+0.03	2.63	[27,28]	2.71	[27,28]	2.29	2.35	2.33	1.00	2.01
Ethylenediamine Tetraacetic acid (5)	X:A	−0.10	−0.11	6.12	[27,28]	6.02	[27,28]	2.29	2.35	2.33	1.00	2.01
Ethylenediamine Tetraacetic acid (6)	X:A	−0.22	−0.26	10.05	[27,28]	9.83	[27,28]	2.29	2.35	2.33	1.00	2.01

Table 1 (*Continued*)

Compounds	Type	ΔpK_a(obs)	ΔpK_a(calc)	pK_a^{25}	Ref^{25}	pK_a^{37}	Ref^{37}	$\Sigma\alpha_2^H$ (A)	$\Sigma\beta_2^H$ (B)	π_2 (S)	R_2 (E)	McGowan volume (V_x)
Ethylenediamine (1)	B	−0.31	−0.27	7.15	c	6.85	c	0.44	1.11	0.83	0.38	0.59
Ethylenediamine (2)	B	−0.33	−0.35	9.97	c	9.64	c	0.44	1.11	0.83	0.38	0.59
Fluoxetine *	B	−0.34	−0.33	9.96	[47]	9.62	[45]	0.31	0.78	1.19	1.01	2.24
Formic acid	A	+0.02	+0.03	3.52	[27,28]	3.54	[27,28]	0.57	0.34	0.67	0.20	0.32
Fumaric acid (1)	A	+0.00	+0.06	2.74	c	2.74	c	1.14	0.75	1.16	0.50	0.78
Fumaric acid (2)	A	+0.14	+0.03	4.03	c	4.17	c	1.14	0.75	1.16	0.50	0.78
Furosemide (1)	A	−0.07	−0.05	3.60	[23]	3.53	[40]	1.25	1.50	2.37	2.07	2.10
Furosemide (2)	A	−0.25	−0.19	10.15	[23]	9.90	[40]	1.25	1.50	2.37	2.07	2.10
Gabapentin (1)	Z:A	−0.21	−0.01	3.65	[58,59]	3.44	b	0.78	0.93	0.99	0.56	1.44
Gabapentin (2)	Z:B	−0.49	−0.27	10.73	[58,59]	10.24	b	0.78	0.93	0.99	0.56	1.44
γ-Aminobutyric acid	Z:B	−0.36	−0.32	10.56	[30]	10.20	[30]	0.78	0.91	0.94	0.37	0.85
Glibenclamide *	A	−0.28	−0.27	5.45	[14,39]	5.18	[39,40]	0.85	2.01	3.84	2.64	3.56
Glycerol-2-phosphoric acid	A	+0.00	+0.01	6.65	[30]	6.65	[30]	0.85	1.75	1.12	0.84	1.17
Glycinamide	B	−0.35	−0.29	8.20	[28,29]	7.85	[28,29]	0.70	1.12	1.41	0.62	0.61
Glycine (1)	Z:A	−0.04	−0.02	2.33	c	2.29	c	0.78	0.90	0.93	0.37	0.56
Glycine (2)	Z:B	−0.30	−0.30	9.60	c	9.30	c	0.78	0.90	0.93	0.37	0.56
Glycylglycine (1)	Z:A	−0.06	−0.03	3.23	[27–29]	3.16	[27–29]	1.04	1.46	1.81	0.68	0.96
Glycylglycine (2)	Z:B	−0.29	−0.22	8.14	[27–29]	7.86	[27–29]	1.04	1.46	1.81	0.68	0.96
Glycolic acid	A	+0.00	+0.06	3.60	c	3.60	c	0.74	0.63	0.67	0.30	0.52
Guanabenz ● *	B	+0.10	−0.31	7.98	b	8.08	b	0.48	1.20	1.02	1.73	1.56
Haloperidol *	B	−0.32	−0.27	8.60	[51]	8.29	[40]	0.31	1.45	2.08	2.00	2.80
HEPES (1)	B	−0.17	−0.10	3.01	c	2.84	c	0.54	2.15	2.00	1.07	1.73
HEPES (2)	B	−0.17	−0.22	7.40	c	7.23	c	0.54	2.15	2.00	1.07	1.73
Hexamethylenediamine	B	−0.41	−0.36	10.93	[30]	10.52	[30]	0.42	1.13	0.83	0.40	1.15
Histamine (1)	B	−0.25	−0.24	6.15	[27,28]	5.89	[27,28]	0.56	1.05	1.31	0.84	0.92
Histamine (2)	B	−0.38	−0.34	9.84	[27,28]	9.46	[27,28]	0.56	1.05	1.31	0.84	0.92
Histidine (1)	Z:A	+0.18	+0.04	1.79	[27,28]	1.97	[27,28]	1.13	1.41	1.74	1.02	1.13
Histidine (2)	Z:B	−0.16	−0.12	6.08	[27,28]	5.91	[27,28]	1.13	1.41	1.74	1.02	1.13
Histidine (3)	Z:B	−0.33	−0.24	9.19	[27,28]	8.86	[27,28]	1.13	1.41	1.74	1.02	1.13
Hydrochlorothiazide (1)	A	−0.24	−0.24	8.75	[13]	8.54	[40]	1.01	1.76	2.77	2.15	1.73
Hydrochlorothiazide (2)	A	−0.18	−0.26	9.96	[13]	9.80	[40]	1.01	1.76	2.77	2.15	1.73
Hydroxproline	B	−0.28	−0.33	9.66	[30]	9.38	[30]	0.95	1.20	1.08	0.77	0.94
Imatinib (1) *	B	−0.15	−0.08	3.04	b	2.89	b	0.54	2.63	3.64	3.83	3.85
Imatinib (2) ● *	B	−0.36	−0.12	4.34	b	3.98	b	0.54	2.63	3.64	3.83	3.85
Imatinib (3) *	B	−0.15	−0.21	8.03	b	7.88	b	0.54	2.63	3.64	3.83	3.85
Imidazole	B	−0.25	−0.28	7.10	[27,28]	6.85	[27,28]	0.35	0.51	1.04	0.62	0.54
Imipramine *	B	−0.35	−0.32	9.52	b	9.18	[47]	0.00	0.95	1.59	1.81	2.40
Indomethacin *	A	−0.34	−0.14	4.45	[39,47]	4.13	[24,39]	0.57	1.24	2.49	2.44	2.53
Ketoprofen *	A	+0.01	−0.10	3.99	[50]	4.00	[40]	0.57	0.87	1.97	1.56	1.98
Labetolol (1)	Z:A	−0.03	−0.05	7.28	[13]	7.25	[40]	1.00	1.72	2.30	2.15	2.64
Labetolol (2)	Z:B	−0.24	−0.13	9.27	[13]	9.03	[40]	1.00	1.72	2.30	2.15	2.64
Lactic acid	A	+0.01	+0.06	3.75	c	3.76	c	0.74	0.66	0.66	0.31	0.66
Leucine (2)	Z:B	−0.31	−0.27	9.74	[30]	9.43	[30]	0.78	0.97	0.92	0.39	1.13
Maleic acid (1)	A	+0.10	+0.08	1.74	c	1.83	c	1.14	0.75	1.16	0.50	0.78
Maleic acid (2)	A	+0.18	−0.01	5.81	c	5.99	c	1.14	0.75	1.16	0.50	0.78
Malic acid (1)	A	+0.01	+0.07	3.25	c	3.26	c	1.14	0.99	1.10	0.47	0.88
Malic acid (2)	A	+0.09	+0.04	4.68	c	4.77	c	1.14	0.99	1.10	0.47	0.88
Malonic acid (1)	A	−0.08	+0.07	2.72	c	2.64	c	1.14	0.69	1.06	0.34	0.68
Malonic acid (2)	A	+0.04	+0.01	5.34	c	5.39	c	1.14	0.69	1.06	0.34	0.68
Maprotiline *	B	−0.27	−0.36	10.22	b	9.95	[47]	0.13	0.68	1.27	1.76	2.33
MES	B	−0.13	−0.20	5.99	c	5.86	c	0.31	1.49	1.76	0.70	1.34
Melphalan (1) ● *	Z:B	−0.21	−0.08	1.62	[53]	1.41	b	0.78	1.37	1.90	1.43	2.22
Melphalan (2) *	Z:A	+0.32	+0.12	2.32	[53]	2.64	b	0.78	1.37	1.90	1.43	2.22
Melphalan (3) ● *	Z:B	+0.10	−0.28	8.93	[53]	9.04	b	0.78	1.37	1.90	1.43	2.22
Methionine (1)	Z:A	+0.01	+0.02	2.11	[27,28]	2.11	[27,28]	0.78	1.06	1.08	0.72	1.15
Methionine (2)	Z:B	−0.28	−0.24	9.12	[27,28]	8.84	[27,28]	0.78	1.06	1.08	0.72	1.15
Methylamine	B	−0.38	−0.39	10.62	[30]	10.24	[30]	0.21	0.57	0.49	0.21	0.35
N,N-Dimethylglycine (1)	Z:A	+0.01	+0.00	2.07	[27,28]	2.07	[27,28]	0.57	0.86	0.80	0.34	0.85
N,N-Dimethylglycine (2)	Z:B	−0.18	−0.29	9.78	[27,28]	9.60	[27,28]	0.57	0.86	0.80	0.34	0.85
Nadolol	B	−0.40	−0.30	9.75	[46]	9.38	[40]	0.83	1.90	1.56	1.68	2.49
Naproxen *	A	+0.05	−0.04	4.09	[13]	4.14	[40]	0.57	0.75	1.49	1.54	1.78
n-Butylamine	B	−0.38	−0.38	10.64	[30]	10.26	[30]	0.21	0.58	0.50	0.20	0.77
Nitrilotriacetic acid (1)	Z:A	+0.01	+0.04	1.83	[27,28]	1.84	[27,28]	1.71	1.52	1.69	0.67	1.28
Nitrilotriacetic acid (2)	Z:A	+0.02	+0.02	2.46	[27,28]	2.48	[27,28]	1.71	1.52	1.69	0.67	1.28
Nitrilotriacetic acid (3)	Z:B	−0.13	−0.25	9.60	[27,28]	9.47	[27,28]	1.71	1.52	1.69	0.67	1.28
N-Me-Iminodiacetic acid (1)	Z:A	+0.03	+0.01	2.23	[27,28]	2.26	[27,28]	1.14	1.19	1.25	0.51	1.06
N-Me-Iminodiacetic acid (2)	Z:B	−0.23	−0.27	9.52	[27,28]	9.29	[27,28]	1.14	1.19	1.25	0.51	1.06
N−Ethylmorpholine	B	−0.26	−0.30	7.67	[30]	7.41	[30]	0.00	0.72	0.61	0.42	1.00
Omeprazole (1) *	X:A	+0.17	+0.07	4.14	[54]	4.31	b	0.35	2.05	3.18	2.67	2.52
Omeprazole (2) ● *	X:B	+0.43	−0.11	8.90	[54]	9.33	b	0.35	2.05	3.18	2.67	2.52
Oxalic acid (1) ●	A	−1.07	+0.10	1.16	c	0.09	c	1.14	0.68	1.06	0.34	0.54
Oxalic acid (2)	A	−0.04	+0.04	3.87	c	3.83	c	1.14	0.68	1.06	0.34	0.54
Oxycodone *	B	−0.21	−0.28	8.94	[47]	8.73	b	0.23	1.80	2.28	2.18	2.26
n-Propylamine	B	−0.38	−0.38	10.57	[30]	10.19	[30]	0.21	0.58	0.50	0.20	0.63
Papavarine *	B	−0.17	−0.20	6.39	[26]	6.22	[40]	0.00	1.47	2.76	2.19	2.59
Pergolide ● *	B	+0.18	−0.33	9.41	[47]	9.62	b	0.31	1.01	1.48	2.22	2.54

Table 1 (*Continued*)

Compounds	Type	ΔpK_a(obs)	ΔpK_a(calc)	pK_a^{25}	Ref[25]	pK_a^{37}	Ref[37]	$\Sigma\alpha_2^H$ (A)	$\Sigma\beta_2^H$ (B)	π_2 (S)	R_2 (E)	McGowan volume (V_x)
Perphenazine (1) ● *	B	+1.64	−0.15	3.72	[47]	5.39	b	0.23	1.84	2.33	2.87	3.02
Perphenazine (2) ● *	B	+0.03	−0.26	8.02	[47]	8.05	b	0.23	1.84	2.33	2.87	3.02
Phenazopyridine *	B	−0.35	−0.22	5.16	[12]	4.80	[24]	0.45	1.09	1.67	2.03	1.64
Phosphoric acid (1)	A	+0.02	+0.10	1.92	c	1.94	c	0.94	1.40	1.02	0.76	0.55
Phosphoric acid (2)	A	−0.01	−0.00	6.70	c	6.69	c	0.94	1.40	1.02	0.76	0.55
Phosphoric acid (3)	A	−0.10	−0.11	11.72	c	11.61	c	0.94	1.40	1.02	0.76	0.55
Phthalic acid (1)	A	+0.01	+0.03	2.72	c	2.73	c	1.14	0.77	1.46	0.94	1.15
Phthalic acid (2)	A	+0.07	−0.02	4.92	c	4.98	c	1.14	0.77	1.46	0.94	1.15
Piperazine (1)	B	−0.18	−0.24	5.55	[30]	5.37	[30]	0.29	0.89	0.63	0.48	0.76
Piperazine (2)	B	−0.25	−0.35	9.79	[30]	9.54	[30]	0.29	0.89	0.63	0.48	0.76
Piperidine	B	−0.37	−0.40	11.12	[4,27,28]	10.73	[4,27,28]	0.13	0.46	0.44	0.36	0.80
PIPES	B	−0.10	−0.16	6.76	[28,29]	6.66	[28,29]	0.63	2.55	2.94	1.11	2.01
Piroxicam (1) ● *	X:B	−0.48	+0.12	2.24	[26,55]	1.76	[40,55]	0.72	2.12	3.12	2.56	2.25
Piroxicam (2) *	X:A	−0.11	+0.01	5.07	[26,55]	4.96	[40,55]	0.72	2.12	3.12	2.56	2.25
Propionic acid	A	+0.01	+0.02	4.70	[27,28]	4.70	[27,28]	0.57	0.36	0.62	0.17	0.61
Propranolol *	B	−0.37	−0.32	9.53	[41,42,44]	9.16	[40]	0.29	1.36	1.44	1.76	2.15
Pyridine	B	−0.13	−0.24	5.22	[4]	5.09	[4]	0.00	0.40	0.82	0.60	0.68
Pyrilamine (1)	B	−0.37	−0.18	4.57	b	4.20	b	0.00	1.45	1.73	1.66	2.39
Pyrilamine (2)	B	−0.27	−0.30	9.12	b	8.85	b	0.00	1.45	1.73	1.66	2.39
Pyrrolidine	B	−0.38	−0.41	11.31	[4]	10.92	[4]	0.13	0.45	0.44	0.36	0.66
Quetiapine (1) ● *	B	+1.29	−0.12	2.27	[47]	3.56	[56]	0.23	2.01	1.93	2.72	2.91
Quetiapine (2) *	B	−0.47	−0.25	7.30	[47]	6.83	[56]	0.23	2.01	1.93	2.72	2.91
Salicylic acid (1)	A	−0.02	0.02	2.84	c	2.82	c	0.70	0.40	1.10	0.91	0.99
Salicylic acid (2)	A	−0.37	−0.21	13.25	c	12.88	c	0.70	0.40	1.10	0.91	0.99
Serine (2)	Z:B	−0.30	−0.29	9.21	[30]	8.91	[30]	1.03	1.30	1.15	0.6	0.76
Sertraline ● *	B	−0.04	−0.32	9.07	[23]	9.03	[47]	0.13	0.67	1.44	1.83	2.26
Succinic acid (1)	A	−0.06	+0.03	3.99	c	3.93	c	0.97	0.69	1.06	0.34	0.82
Succinic acid (2)	A	+0.09	+0.00	5.21	c	5.30	c	0.97	0.69	1.06	0.34	0.82
Sulfuric acid	A	+0.14	+0.10	1.52	c	1.66	c	0.63	1.06	1.58	0.49	0.42
Tamoxifen *	B	−0.12	−0.28	8.48	[39]	8.36	[39]	0.00	1.11	1.85	2.06	3.17
Tartaric acid (1)	A	+0.11	+0.10	2.79	c	2.90	c	1.23	1.30	1.13	0.61	0.94
Tartaric acid (2)	A	+0.13	+0.08	3.90	c	4.03	c	1.23	1.30	1.13	0.61	0.94
Taurine (1)	Z:A	+0.00	+0.03	1.27	c	1.27	c	0.52	1.34	1.64	0.49	0.83
Taurine (2)	Z:B	−0.28	−0.26	8.84	c	8.56	c	0.52	1.34	1.64	0.49	0.83
TES	B	−0.24	−0.21	7.40	[29]	7.16	[29]	1.25	2.3	2.17	1.05	1.57
Tetraethylenepentamine (1)	B	−0.08	−0.12	3.32	[27]	3.24	[27]	0.88	2.44	1.40	0.77	1.73
Tetraethylenepentamine (2)	B	−0.22	−0.16	5.03	[27]	4.81	[27]	0.88	2.44	1.40	0.77	1.73
Tetraethylenepentamine (3)	B	−0.22	−0.25	8.27	[27]	8.05	[27]	0.88	2.44	1.40	0.77	1.73
Tetraethylenepentamine (4)	B	−0.25	−0.28	9.46	[27]	9.21	[27]	0.88	2.44	1.40	0.77	1.73
Tetraethylenepentamine (5)	B	−0.25	−0.28	9.66	[27]	9.40	[27]	0.88	2.44	1.40	0.77	1.73
Thioridazine ● *	B	−0.69	−0.33	9.77	[47]	9.08	b	0.00	1.13	1.93	2.70	2.90
Tolfenamic acid ● *	A	+0.77	−0.04	4.20	[57]	4.97	[39]	0.72	0.64	1.59	1.75	1.90
Triethanolamine	B	−0.24	−0.27	7.76	[30]	7.52	[30]	0.73	1.60	1.04	0.87	1.23
Triethylamine	B	−0.40	−0.38	10.72	[30]	10.32	[30]	0.00	0.53	0.37	0.17	1.05
Trimethylamine	B	−0.26	−0.37	9.80	[30]	9.54	[30]	0.00	0.53	0.36	0.17	0.63
Tris	B	−0.27	−0.28	8.13	c	7.86	c	1.01	1.62	1.16	0.82	0.95
Tris(2-aminoethyl)amine (1)	B	−0.30	−0.26	8.42	[27,28]	8.13	[27,28]	0.68	2.15	1.36	0.71	1.35
Tris(2-aminoethyl)amine (2)	B	−0.37	−0.29	9.50	[27,28]	9.14	[27,28]	0.68	2.15	1.36	0.71	1.35
Tris(2-aminoethyl)amine (3)	B	−0.27	−0.31	10.14	[27,28]	9.87	[27,28]	0.68	2.15	1.36	0.71	1.35
Verapamil *	B	−0.38	−0.22	9.06	[12]	8.68	[50]	0.00	1.89	3.00	1.76	3.79
Vinblastine (1)	B	−0.09	−0.10	5.49	[47]	5.40	b	0.54	4.01	3.72	4.46	6.07
Vinblastine (2)	B	−0.11	−0.15	7.68	[47]	7.57	b	0.54	4.01	3.72	4.46	6.07
Vincristine (1) ●	B	+0.64	−0.07	5.18	[47]	5.82	b	0.54	4.25	4.30	4.59	6.08
Vincristine (2)	B	+0.09	−0.13	7.48	[47]	7.57	b	0.54	4.25	4.30	4.59	6.08
Xipamide (1)	A	−0.17	−0.14	4.75	[52]	4.58	[45]	1.03	1.39	2.75	2.38	2.42
Xipamide (2) ●	A	+0.47	−0.26	10.00	[52]	10.47	[45]	1.03	1.39	2.75	2.38	2.42

(a) A: acid, B: base, X: ordinary ampholyte, Z: zwitterion. Ionization constants not used in model refinement are indicated by (●). Ionization constants determined from cosolvent solutions are indicated by (*). Ionization constants at 0.15 M ionic strength (KCl). (b) This work. (c) From database in Gemini Profiler v3.2 software.

[25–30]. To interpolate the values at 25 and 37 °C, 0.15 M ionic strength, the literature data were fitted to a generic equation in the Gemini Profiler software. For the most common buffers, a built-in feature in the Gemini Profiler software allows for the pK_a values to be generated automatically. The literature values and those determined here are listed in Table 1.

2.4. pK_a temperature effect model equation

The classical treatment of the temperature dependence of the ionization process begins with the Gibbs free energy relationship

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

For a system at equilibrium, the relationship between the free energy and the pK_a is

$$\Delta G^\circ = -RT \ln K_a = 2.303RT pK_a \quad (2)$$

where ΔG° is the free energy change associated with ionization when all the reactants and products are in their standard states. Combining Eqs. (1) and (2) gives

$$pK_a = -\frac{\Delta S^\circ}{2.303R} + \left(\frac{\Delta H^\circ}{2.303R}\right) \times \frac{1}{T} \quad (3)$$

Since ΔS° and ΔH° are usually temperature dependent, the plot of pK_a vs. T^{-1} often shows curvature. For many molecules, ΔS° values depend on temperature linearly. Simple weak acids show the

most negative slopes, while bases show slightly positive slopes [37]. If consideration is confined to a relatively small temperature range, e.g., 25–37 °C, the temperature dependence may be approximated by the linear equations

$$\Delta S^{\circ}(T) = \Delta S^{\circ}{}_{25} + b_0(T - T_1) \quad (4a)$$

$$\Delta H^{\circ}(T) = \Delta H^{\circ}{}_{25} + b_1(T - T_1) \quad (4b)$$

where $T_1 = 298.15$ K (25 °C). Sample values of b_0 and b_1 for well-known molecules may be deduced from thermodynamic constants in the *Handbook of Biochemistry* [37] – propionic acid: $b_0 = -0.527$ J mol^{-1} K^{-2} and $b_1 = -161.3$ J mol^{-1} K^{-1}; piperidine: $b_0 = +0.291$ J mol^{-1} K^{-2} and $b_1 = +89.5$ J mol^{-1} K^{-1}.

With the above linear relationships, Eq. (3) can be expressed at the two temperatures of interest.

$$pK_a^{37} = -\frac{\Delta S^{\circ}{}_{25}}{2.303\,R} + \left(\frac{\Delta H^{\circ}{}_{25}}{2.303\,R}\right) \times \frac{1}{T_2} - \frac{b_0 \Delta T}{2.303RT} + \frac{b_1 \Delta T}{2.303RT_2} \quad (5a)$$

$$pK_a^{25} = -\frac{\Delta S^{\circ}{}_{25}}{2.303\,R} + \left(\frac{\Delta H^{\circ}{}_{25}}{2.303\,R}\right) \times \frac{1}{T_1} \quad (5b)$$

where $T_2 = 310.15$ K (37 °C). The difference between Eqs. (5a) and (5b) produces an expanded form of the van't Hoff equation,

$$\left(\frac{\Delta pK_a}{\Delta T}\right) = -\left(\frac{\Delta H^{\circ}{}_{25}}{2.303RT_1T_2}\right) - \frac{b_0}{2.303R} + \frac{b_1}{2.303RT_2} \quad (6)$$

where $\Delta pK_a = pK_a^{37} - pK_a^{25}$ and $\Delta T = T_2 - T_1$. With the aid of Eq. (3), an entropy-based equation is produced

$$\Delta pK_a = k_0 pK_a^{25} - k_1 \Delta S^{\circ}{}_{25} + g(b_0, b_1) \quad (7)$$

where the theoretical constants, $k_0 = -\Delta T/T_2 = -0.03869$, $k_1 = -\Delta T/2.3RT_2 = -0.00202$, and the gradient function, $g(b_0,b_1) = -b_0\Delta T/2.3R + b_1\Delta T/2.3RT_2 = -0.626b_0 + 0.00202b_1$. For example, propionic acid and piperidine are characterized by $g(b_0,b_1) = +0.0041$ and -0.0014 (dimensionless), respectively [37]. Since $\Delta S^{\circ}{}_{25}$, b_0, and b_1 are not known for new chemical entities (NCE), a strategy was developed to estimate their contribution from the 2D structure of the NCE, using the Abraham linear free energy solvation descriptors [20].

2.5. Abraham LFER descriptors and the design equation

The proton transfer reaction leading to increased ionization (e.g., particularly with simple weak acids) induces substantial rearrangements in hydrogen-bonded water structure surrounding the reactants [6,29,30]. On ionization, entropy usually decreases, with underlying nonlinear heat capacity effects [31–36]. The structure of water becomes more ordered in the presence of the strong electric field arising from charged solute molecules. The molecular volume of charged molecules can affect the temperature dependence, since entropy of hydration of ions decreases with increasing effective ionic hydration radius [2,3]. When the charge is highly delocalized over the surface of the solute, as in some aromatic ions (e.g., rhodamine 123), the solute–solvent interactions are weakened and entropy is affected less. Solute H-bonds and those of the solvent can also lead to a tighter solvation layer surrounding the solute. The weaker van der Waal dispersion forces from the aromatic and/or lone pair electrons can lead to further stabilization of the solvation layer surrounding the solute. Many of these factors are encoded in the Abraham solvation descriptors [20]. It is noteworthy to mention that lots of alternative approaches have been described in the literature, like empirical models [62] and *ab initio* [63] models. However, it is reasonable to use a more practical approach, as with the Abraham descriptors [20], in order to be able to predict large numbers of molecules cost effectively.

Abraham's [20] five LFER solvation descriptors were applied to approximate the second and third terms in Eq. (7), resulted in the design equation:

$$\Delta pK_a = k_0 \times pK_a^{25} + c_0 + c_1 \times \sum \alpha_2^H + c_2 \times \sum \beta_2^H + c_3 \times \pi_2 + c_4 \times R_2 + c_5 \times V_x \quad (8)$$

where $k_0, c_0, c_1, \ldots, c_5$ are the MLR coefficients, and where $\sum \alpha_2^H$ (also called A) and $\sum \beta_2^H$ (also called B) are the solute H-bond acidity and basicity, respectively, π_2 (also called S) is the solute polarity/polarizability due to solute–solvent interactions between bond dipoles and induced dipoles, R_2 (dm^3 mol^{-1}/10; also called E) is the excess molar refraction, which models dispersion force interaction arising from pi- and n-electrons of the solute, and V_x is the McGowan molar volume (dm^3 mol^{-1}/100) of the solute.

The Abraham descriptor calculation and the computational model testing used the Algorithm Builder v1.8 and ADME Boxes v4.9 programs [38] from Advanced Chemistry Development (Toronto, Canada).

2.6. Model validation

The MLR model developed in this study, based on the design Eq. (8), was validated by two variants of the "leave-one-out" (LOO) method, and the "leave-many-out" (LMO) method, using the Algorithm Builder program [38]. The cross-validation strategy was applied to the 187 pairs of measured pK_a^{25} and pK_a^{37} in the training set, and the cross-validated q^2 was used to assess model predictivity. The LOO approach randomly taking out one measurement each time. The LMO approach, randomly excluded 20% of the dependent variables of the measurements in 100 different repeated combinations.

3. Results and discussion

3.1. pK_a determination

Table 1 lists the 207 pK_a values at 25 °C and 37 °C of 143 compounds selected for the study. Included are 34 acids (53 pK_a values), 85 bases (105 pK_a values), and 24 amphoteric molecules (49 pK_a values). Original determinations of pK_a in this study included 9 values at 25 °C and 31 values at 37 °C. Most of the other pK_a values at 37 °C were also determined in our laboratory and have been published elsewhere (Table 1). For compounds determined in aqueous solution in the absence of cosolvent, the estimated standard deviation (SD) was 0.01 in 61% of the cases, with the rest ranging 0.02–0.09. When cosolvent titrations were done, the SD values were somewhat higher: methanol, 1-propanol, and DMSO indicated average SD = 0.04–0.07 (SD range 0.01–0.2).

Fig. 1 a shows the Bjerrum plot for vinblastine, a water-soluble dibasic drug. Three replicate titrations are shown. At $\bar{n}_H = 1.5$, the pH 5.40, which is a good estimate of the value of pK_{a1}. At $\bar{n}_H = 0.5$, the pH 7.57, which corresponds to pK_{a2}. Fig. 1b shows a more complicated Bjerrum plot for chloroquine at three different concentrations. Above pH 9.5, the sparingly soluble compound precipitated, as indicated by the shift of points from the thick solid line in the pH 9.5–11 region. When precipitation occurs, it would be erroneous to equate pH to pK_{a2} at $n_H = 0.5$, and errors as large as a log unit would occur. As a unique capability, the refinement program in the Gemini Profiler instrument can simultaneously determine the solubility constant (81 μg mL^{-1}) as well as the correct $pK_{a2} = 10.10 \pm 0.03$.

Many of the drugs studied were only sparingly soluble, so the cosolvent method was used to estimate the pK_a values. Fig. 2 shows cosolvent plots for an acid (indomethacin) and a base (imipramine), indicating the extrapolated aqueous pK_a at zero cosolvent by two

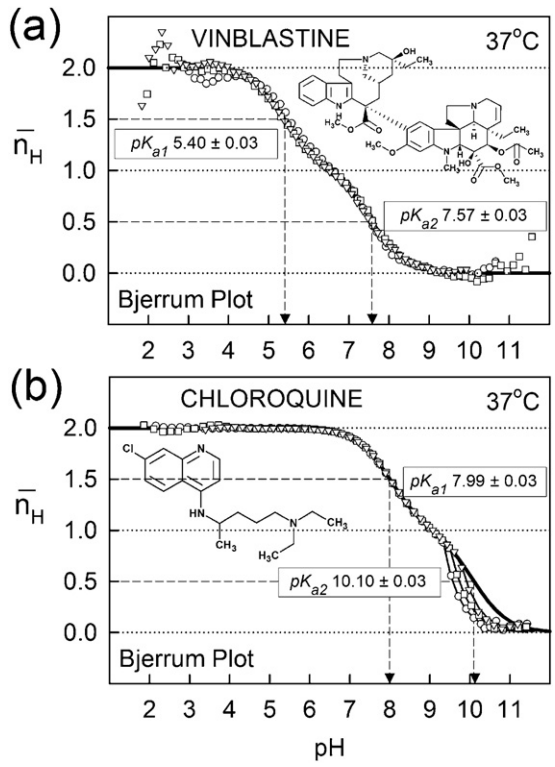

Fig. 1. Bjerrum plots at 37 °C for (a) vinblastine (three titrations, 0.25–0.28 mM) and (b) chloroquine (three titrations, 1.06–1.27 mM). Chloroquine precipitated above pH 9.5.

different popular methods. Usually, acids have positive slopes and bases have negative slopes [6]. The unfilled symbols correspond to the simple extrapolation of p_sK_a vs. wt% cosolvent (upper horizontal scale). This approach appears most suitable for weak bases, as indicated by a comparative study by Völgyi et al. [23]. The filled symbols correspond to the Yasuda–Shedlovsky plots [6,23], $p_sK_a + \log\{[H_2O]/55.51\}$ vs. $(1/\varepsilon - 1/\varepsilon_o)$. This approach appears to show least bias when applied to weak acids [23]. The two types of extrapolation show nearly the same result when the data contain points near zero cosolvent, but can show substantial differences when the extrapolations draw on data far from zero cosolvent, as in Fig. 2d.

3.2. Abraham LFER and the pK_a prediction model

The initial 207 pK_a pairs were separated into three classes: acids (25%), bases (51%) and amphoteric compounds (24%). Each class was separately analyzed according to Eq. (8).

With three outliers (oxalic acid pK_{a1}, xipamide pK_{a2}, tolfenamic acid) removed from the acids class, the MLR converged with the statistics $r^2 = 0.60$, $s = 0.084$, $F = 11$, $n = 50$. The MLR coefficients are listed in Table 2. Due to the negative contribution of the pK_a coefficient, k_0, a high value of pK_a^{25} contributes to a more negative value of ΔpK_a. For example, salicylic acid with a pK_a^{25} of 13.3 contributes −0.29 to the ΔpK_a. At the other end of the range, maleic acid with a pK_{a1}^{25} of 1.7 changes ΔpK_a by only −0.04. The average entropy contribution to ΔpK_a, predicted by MLR coefficients (Table 2) of the Abraham descriptors, is +0.10 (range −0.15 to +0.16). According to the values of the Abraham H-bond descriptors, large amounts of hydrogen bonding cause ΔpK_a to take on more positive values. Also, the bigger the acid molecule, the more positive is ΔpK_a. Dipolarity causes values of ΔpK_a to become more negative. The average ΔpK_a in the acids class is −0.02; the measured values range from −0.37 (salicylic acid pK_{a2}) and −0.34 (indomethacin) to +0.15 (2-naphthoic acid) and +0.18 (maleic acid pK_{a2}).

Out of 105 pK_a pair values for bases, 12 were found to be outliers (astemizole pK_{a1}, carvedilol, dipyridamole, guanabenz, imitanib pK_{a2}, pergolide, perphenazine both pK_a, quetiapine pK_{a1}, sertraline, thioridazine, and vincristine pK_{a1}). When removed from the

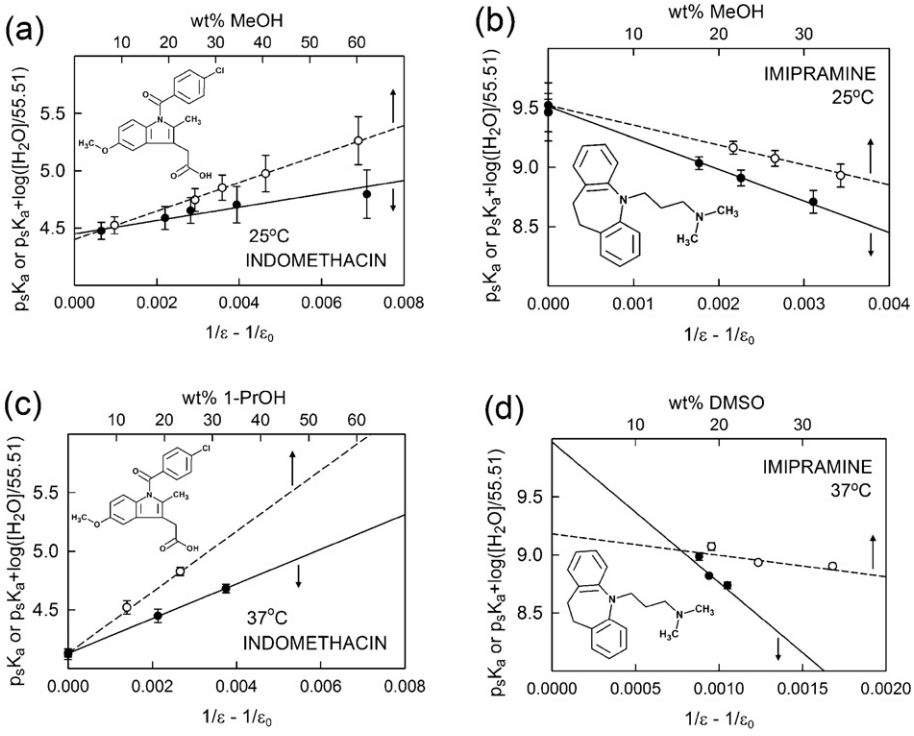

Fig. 2. Cosolvent plots for an acid (indomethacin) and a base (imipramine), indicating the extrapolated aqueous pK_a at zero cosolvent by two different popular methods. The unfilled symbols correspond to the simple extrapolation of p_sK_a vs. wt% cosolvent (upper horizontal scale). The filled symbols correspond to the *origin-shifted* Yasuda–Shedlovsky plots [6,23], $p_sK_a + \log\{[H_2O]/55.51\}$ vs $(1/\varepsilon - 1/\varepsilon_o)$.

Table 2
Abraham solvation descriptor MLR coefficients.[a]

Class	k_0	c_0	$c_1\left(\sum\alpha_2^H\right)$	$c_2\left(\sum\beta_2^H\right)$	$c_3\,(\pi_2)$	$c_4\,(R_2)$	$c_5\,(V_x)$	r^2	s	F	n	Outliers
Acids	−0.022	0.123	0.093	0.045	−0.145	0.004	0.028	0.60	0.084	11	50	3
Bases	−0.026	−0.136	0.008	0.018	0.035	−0.032	0.020	0.55	0.072	17	93	12
Ampholytes	−0.038	0.051	0.011	−0.103	0.060	0.002	0.075	0.74	0.091	18	44	5
Merged								0.80	0.076	750	187	20

[a] Model equation: $\Delta pK_a = k_o \times pK_a^{25} + c_0 + c_1 \times \Sigma\alpha_2^H + c_2 \times \Sigma\beta_2^H + c_3 \times \pi_2 + c_4 \times R_2 + c_5 \times V_x$.

bases class, the MLR converged with the statistics $r^2 = 0.55$, $s = 0.072$, $F = 17$, $n = 93$ (Table 2). As with acids, due to the negative contribution of the pK_a coefficient, k_0, a high value of pK_a^{25} contributes to a more negative value of ΔpK_a. Many amines with a $pK_a^{25} > 10$ decrease the ΔpK_a by at least −0.22. At the other end of the range, 2-nitroaniline with a pK_a^{25} of −0.26 changes ΔpK_a by +0.01. The average entropy contribution, predicted by Abraham descriptors, is −0.06 (range −0.12 to +0.07), a decrease of 0.16 units from the acids values. According to the values of the MLR coefficients of the Abraham H-bond descriptors, large amounts of hydrogen bonding cause ΔpK_a to take on more positive values, just as with acids. Also, the larger the acid, the more positive is ΔpK_a. Increased dipolarity causes values of ΔpK_a to become more positive. Dispersion forces lead to a negative contribution. The average ΔpK_a in the bases class is −0.28; the values range from −0.47 (chloroquine pK_{a2}) to +0.09 (vincristine pK_{a2}).

Out of 49 pK_a pair values for ampholytes, five were found to be outliers (domperidone pK_{a1}, melphalan pK_{a1} and pK_{a3}, omeprazole pK_{a2}, piroxicam pK_{a1}). When removed from the ampholytes class, the MLR converged with the statistics $r^2 = 0.74$, $s = 0.091$, $F = 18$, $n = 44$ (Table 2). The average entropy contribution to ΔpK_a, predicted by Abraham descriptors, is +0.12 (range +0.05 to +0.30), similar to the value found with acids. Large H-bond acceptor strength causes ΔpK_a to take on more negative value, an effect opposite of that in the other two classes. Also, the larger the acid, the more positive is ΔpK_a, a contribution more than three times larger than in the other two classes. Dipolarity causes values of ΔpK_a to become more positive. Dispersion forces also lead to a positive contribution. The average ΔpK_a in the acids class is −0.11; the values range from −0.49 (gabapentin pK_{a2}) to +0.32 (melphalan pK_{a2}). With both acid and base functionality, ampholytes have ΔpK_a values spread across a larger range of values (Fig. 3). Partly because of this, the r^2 value is the highest of the three classes. Also, since most of the ampholytes are zwitterionic buffers or amino acids, whose pK_a values are known to a very high precision, r^2 is higher than those from the other two classes containing a higher proportion of drug molecules, whose pK_a values are not known to the same level of precision.

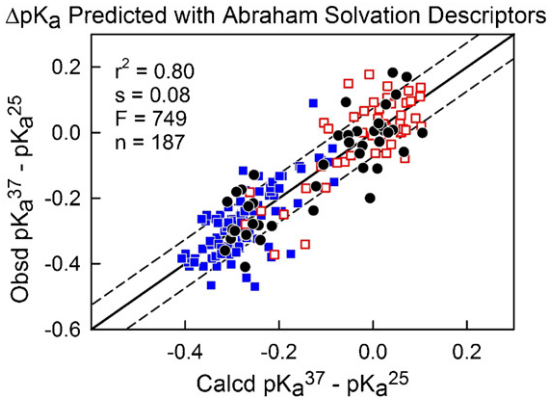

Fig. 3. The predicted vs. experimental pK_a difference between 37 °C and 25 °C values ($\Delta pK_a = pK_a^{37} - pK_a^{25}$) for 187 p$K_a$ values. The individual class type analyses (acids, bases, ampholytes) using Abraham solvation descriptors (cf., Table 2) were merged in the plot. The statistics correspond to the merged sets (cf., Table 2). The filled square symbols correspond to bases; the unfilled square symbols refer to acids, and the filled circle symbols represent amphoteric compounds.

Fig. 3 shows a plot of ΔpK_a observed vs. calculated by the individual classes. When the results are merged, the statistics are $r^2 = 0.80$, $s = 0.076$, $F = 749$, $n = 187$. The bases tend to cluster around −0.3, the acids tend to cluster around 0.0, while ampholytes spread over the entire range of values.

3.3. Cross validation

The multiple linear regression model developed in this study, based on Eq. (8), was validated by two variants of the LOO method, using the Algorithm Builder V1.8 program [38]. The traditional LOO approach, with repetitive MLR calculation, each time randomly taking out one measured ΔpK_a, produced the $q^2 = 0.798$. The LMO approach, where 20% of the dependent variables were randomly removed, with the MLR repeated 100 times, produced nearly the same $q^2 = 0.795$, with the q^2 standard deviation of 0.049. These values are only slightly less than the value of r^2 (0.80) determined by normal MLR analysis, suggesting internal robustness of the model.

3.4. Outliers

Table 1 labels the outliers with the dot symbol after the compound name. Several of the compounds were rejected from consideration due to very large experimental ΔpK_a shifts. For example, for astemizole, dipyridamole, perphenazine pK_{a1}, quetiapine pK_{a1}, thioridazine, tolfenamic acid, $\Delta pK_a = -0.69$, −1.26, +1.64, +1.29, −0.69, +0.77, respectively. The Abraham model could not predict these high values. All of the compounds are sparingly soluble, where in several cases, oligomeric aggregates appear to form in aqueous solution [60,61]. The formation of aggregates is highly temperature sensitive, and often, the apparent pK_a value is altered by the formation of aggregates [60]. Some of the outliers, like carvedilol, had a ΔpK_a with the "wrong" sign. A very careful re-examination of the original titration data indicates high quality. It is not clear why this effect is observed; the formation of aggregates cannot be ruled out. Since many of the drugs studied are practically insoluble, highest precision determination of the pK_a values by the cosolvent method was a challenge in some instances; some of these drug molecules were labeled as outliers for this reason. Other factors may have to do with the formation of stable five and/or six-membered ring intramolecular hydrogen bonds [64] in the proximity of the ionizable groups, as perhaps in the structures of oxalic, oxipamide, and tolfenamic acid, which may not follow the classical temperature dependence. Out of 207 pK_a values collected, 20 outliers still leaves enough measurements to develop a reasonably useful model for predicting pK_a values at 37 °C from known values at 25 °C.

4. Conclusion

We have developed a very simple model for predicting pK_a values at the biologically relevant temperature of 37 °C (0.15 M

ionic strength) from knowledge of the value at 25 °C, using the 2D structure of the drug-like molecule to calculate an approximate entropy contribution in the classical temperature-dependent pK_a equation (Eq. (7)). This prediction model resulted in the statistics $r^2 = 0.80$, $s = 0.076$, $n = 187$. This investigation is expected to be a useful contribution, since pK_a determinations are scarcely reported at 37 °C, and the use of 25 °C values in biological applications, such as pH-dependent cell-based permeability measurements, or critical dissolution studies (usually performed at 37 °C), can potentially lead to somewhat biased in vivo–in vitro correlations.

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