Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmaceutical Sciences 25 (2005) 417–425
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

A modification of the Hammett equation for predicting ionisation constants of *p*-vinyl phenols

Julius Sipilä [1], Harri Nurmi [1], Ann Marie Kaukonen, Jouni Hirvonen, Jyrki Taskinen, Jari Yli-Kauhaluoma *

Viikki Drug Discovery Technology Center, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland

Received 22 February 2005; accepted 6 April 2005
Available online 29 April 2005

Abstract

Currently there are several compounds used as drugs or studied as new chemical entities, which have an electron withdrawing group connected to a vinylic double bond in a phenolic or catecholic core structure. These compounds share a common feature – current computational methods utilizing the Hammett type equation for the prediction of ionisation constants fail to give accurate prediction of pK_a's for compounds containing the vinylic moiety. The hypothesis was that the effect of electron-withdrawing substituents on the pK_a of *p*-vinyl phenols is due to the delocalized electronic structure of these compounds. Thus, this effect should be additive for multiple substituents attached to the vinylic double bond and quantifiable by LFER-based methods. The aim of this study was to produce an improved equation with a reduced tendency to underestimate the effect of the double bond on the ionisation of the phenolic hydroxyl. To this end a set of 19 *para*-substituted vinyl phenols was used. The ionisation constants were measured potentiometrically, and a training set of 10 compounds was selected to build a regression model ($r^2 = 0.987$ and S.E. = 0.09). The average error with an external test set of six compounds was 0.19 for our model and 1.27 for the ACD-labs 7.0. Thus, we have been able to significantly improve the existing model for prediction of the ionisation constants of substituted *p*-vinyl phenols.

Keywords: pK_a prediction; LFER; Hammett equation; *para*-substituted vinyl phenols

1. Introduction

Approximately 30% of all drug candidates are discarded due to their poor pharmacokinetic performance (Avdeef, 2001, 2003). This shadows early stage drug development with a need to improve and speed up the physico-chemical profiling of new chemical entities (NCEs). Physico-chemical properties include pK_a, log *P*, log *D* and solubility, among others. The pH-dependence of log *D* and solubility in relation to their pK_a makes the ionisation constant of a drug molecule a very important parameter when assessing the pharmacokinetic behaviour of NCEs. It is worth remembering that approximately two-thirds of all drugs are ionisable (Maurin et al., 2002). Therefore, it is important to be able to predict how the molecule behaves in the different environments of the gastro-intestinal tract (GIT). Depending on the charge-state of the molecule, it shows varying solubility and lipophilicity that control the molecule's permeation, distribution and affinity towards target systems (Avdeef, 2001; Avdeef and Testa, 2002). Recently, it has been reported that the serum albumin binding of a drug would also be largely dependent on its ionisation state (Ermondi et al., 2004).

Physico-chemical profiling forms an integral part of the pharmacokinetic assessment of NCEs during the early absorption, distribution, metabolism and excretion (ADME) phase of drug discovery and development (van de Waterbeemd and Gifford, 2003). During early stage physico-chemical profiling e.g. pK_a, log *P* and solubility are measured or computationally estimated. The computational approach, even prior to synthesis, would be the preferable

* Corresponding author. Tel.: +358 9 191 59170; fax: +358 9 191 59556.
E-mail address: jari.yli-kauhaluoma@helsinki.fi (J. Yli-Kauhaluoma).
[1] Contributed equally to the article.

0928-0987/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejps.2005.04.002

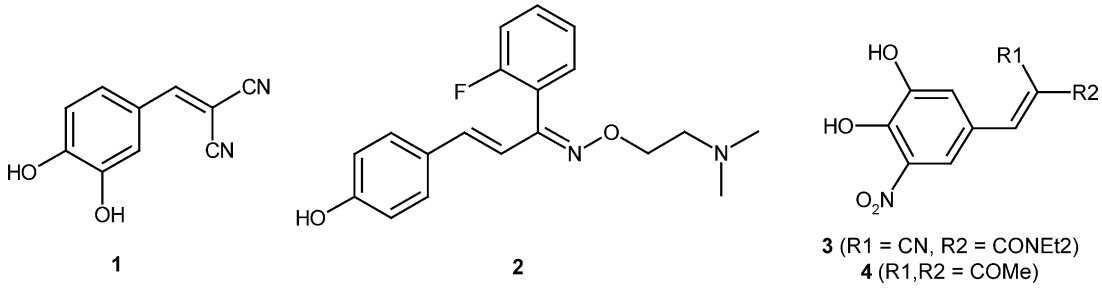


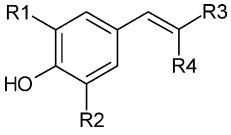
Fig. 1. Examples of *p*-vinyl substituted phenolic drugs Tyrphostin 23 (**1**, a tyrosine kinase inhibitor), eplivanserin (**2**, a $5HT_{2A}$ receptor antagonist), entacapone and nitecapone (**3** and **4**, COMT inhibitors).

method for physico-chemical profiling, but currently the in silico methods do not possess satisfactory accuracy. Problems arise especially during the calculation of the ionisation constants for complex drug molecules with several functional groups capable of intermolecular and intramolecular interactions. As poor pharmacokinetics is one of the main reasons for drug candidate attrition (Kennedy, 1997), fast computer-aided tools for the prediction of pK_a values based on the molecular structure are needed to avoid expensive failures at later stages of the drug development process.

There are several software systems available for the prediction of pK_a values. These programs are based on Hammett type linear free energy relationships (LFER) (e.g. ACD/Labs pK_a DB and Pallas pKalc), ab initio quantum mechanical calculations (Jaguar pK_a module) and a combination of LFER, structure-activity relationships and perturbed molecular orbital theory (SPARC On-Line Calculator). The accuracy and reliability of the current pK_a prediction software has recently been reviewed (Livingstone, 2003). The results obtained with the most widely used LFER methods rely on the quality and extent of the experimental database used in the parameterisation. Despite the considerable size of the databases implemented in the commercial pK_a predicting software, there are classes of compounds for which the predictions are not satisfactory. QSAR studies of catechol *O*-methyltransferase (COMT) inhibition have shown that the pK_a of the substrate is a key factor determining the affinity (Lautala et al., 2001; Lotta et al., 1992; Taskinen et al., 1989). It was found that the most widely used programs gave surprisingly inaccurate pK_a predictions for potent COMT-inhibitors, e.g. entacapone and related *p*-vinyl substituted phenols. Even with current LFER based methods the calculated pK_a value of entacapone deviates by almost two units (1.67) producing a completely erroneous estimation of the charge state across the physiological pH-range. Accurate prediction of ionisation constants of *p*-vinyl substituted phenols would be very important as compounds with this structural moiety have shown a variety of biological activity, e.g. catechol *O*-methyltransferase (COMT) inhibition (Kaakkola et al., 1994), 5-HT_2 receptor antagonism (Congy et al., 1990; Rinaldi-Carmona et al., 1992), melanin inhibition (Hori et al., 1987, 1988), antiviral activity (Higa and Sakai, 1988), tyrosine kinase inhibition (Isshiki et al., 1987; Lyall et al., 1989) and anti-inflammatory activity (Flynn et al., 1991). Examples of *p*-vinyl substituted phenolic drugs are given in Fig. 1.

This study is based on the assumption that the long-range effect of electron-withdrawing substituents on the pK_a of *p*-vinyl phenols is due to the strongly delocalized electronic structure of these compounds. This effect should be additive for multiple substituents attached to the vinylic double bond and quantifiable by LFER-based methods. The purpose is to improve the accuracy of the computational methods using Hammett type equations to predict ionisation constants of *p*-vinyl phenols. To this end, experimental data on a total of 15 *p*-vinyl phenols (Table 1) were determined, six of which were synthesized and nine were purchased from commercial sources. The ionisation constants were determined using the potentiometric method. In the case of water-insoluble compounds, co-solvent was used to dissolve the sample and the apparent values were extrapolated to zero co-solvent content by means of the Yasuda–Shedlovsky procedure (Yasuda,

Table 1
Chemical structures of the *p*-vinyl phenols



Compound	R_1	R_2	R_3	R_4
3	OH	NO_2	$CON(C_2H_5)_2$	CN
4	OH	NO_2	$COCH_3$	$COCH_3$
5	H	H	$COOCH_3$	H
6	H	H	CN	CN
7	H	H	$CONH_2$	H
8	H	H	$COCH_3$	H
9	H	H	NO_2	H
10	H	H	$C_6H_4NO_2$-*p*	H
11	H	H	COC_6H_5	H
12	H	H	COOH	H
13	OH	H	COOH	H
14	OH	H	$COOC_2H_5$	CN
15	H	H	COOH	CN
16	H	H	$COOCH_3$	CN
17	H	H	CH_3	H
18	OCH_3	H	CH_3	H
19	H	H	$C_5H_4N^+CH_3I^-$	H
20	H	H	$C_5H_4N^+CH_3I^-$	H
21	H	H	COC_6H_4F-*p*	H

Table 2
Comparison of experimental pK_a values and predictions obtained with different software products for compounds **3**–**21**

Compound number	pK_a	pK_a SPARC	Dif.	pK_a ACD 4	Dif.	pK_a ACD 7	Dif.	pK_a Pallas pKalc 3.1	Dif.
3	4.40[a]	4.13	0.27	6.07	1.67	6.07	1.67	5.87	1.47
4	4.60[a]	4.68	0.08	6.93	2.33	6.93	2.33	5.39	0.79
5	8.51	8.43	0.08	8.59	0.08	8.82	0.31	9.43	0.92
6	6.94	7.14	0.20	7.37	0.43	7.37	0.43	8.36	1.42
7	8.61	8.44	0.17	9.62	1.01	9.79	1.18	9.82	1.21
8	8.28	8.56	0.28	9.73	1.45	9.88	1.60	9.94	1.66
9	7.90	7.31	0.59	8.04	0.14	8.04	0.14	9.34	1.44
10	9.13	8.34	0.79	9.41	0.28	9.32	0.19	9.63	0.50
11	8.23	8.59	0.36	9.75	1.52	9.76	1.53	9.81	1.58
12	8.97	9.16	0.19	9.82	0.85	10.16	1.19	9.77	0.80
13	8.66	8.66	0.00	9.69	1.03	9.97	1.31	9.57	0.88
14	7.09	6.76	0.33	8.97	1.88	8.99	1.90	8.77	1.68
15	7.93	7.66	0.27	9.07	1.14	9.59	1.66	8.98	1.05
16	7.25	7.23	0.02	9.10	1.85	9.12	1.87	8.91	1.66
17	9.82[a]	9.70	0.12	10.11	0.29	10.05	0.23	9.88	0.06
18	9.89[a]	9.59	0.30	10.15	0.26	10.10	0.21	–[b]	–[b]
19	8.40	7.28	1.12	9.68	1.28	9.71	1.31	–[b]	–[b]
20	8.39	7.23	1.16	9.43	1.04	9.47	1.08	–[b]	–[b]
21	8.20	8.51	0.31	9.71	1.51	9.62	1.42	–[b]	–[b]
Mean			0.35		1.05		1.13		1.14
S.D.			0.34		0.67		0.68		0.48

[a] Experimental pK_a values were taken from literature for compounds **3** (Wikberg et al., 1993), **4** (Wikberg et al., 1993), **17** (Brauer et al., 1964) and **18** (Brauer et al., 1964).
[b] The demo version of Pallas p*K*alc was used. The demo license allows prediction of only 15 pK_a values.

1959; Shedlovsky, 1962; Avdeef, 1993). The set of molecules was divided into two groups, one for building the new model (training set) and the other one for validating the model (external test set). To complement and broaden the external test set, pK_a values for four additional compounds were retrieved from the literature (Table 1). The experimental results across all studied compounds were also compared to the computational values obtained with ACD/Labs pK_a DB, Pallas pKalc and SPARC On-Line Calculator (Table 2).

2. Experimental

2.1. Materials

All chemicals were used as received without further purification. Solvents for synthesis work were purchased from Riedel-de Haën, Rathburn and J.T. Baker, and reagents from Aldrich Chemical Company and Fluka Ag. In the pK_a measurements chemicals were of analytical grade unless otherwise stated and solvents were used as ionic strength adjusted (0.15 M KCl) mixtures of solvent and Milli-Q-water as described in the manual for the apparatus (Anonymous, 1999). Ionic strength adjusted water (ISA) was prepared from potassium chloride (KCl) (Riedel-de Haën) at 0.15 M using Milli-Q-water (Millipore Corporation, Bedford Massachusetts, USA). A solution of 0.5 mM KOH was prepared using a Titrisole ampoule (Merck, Darmstadt, Germany) and its exact concentration determined by titration of potassium phthalate samples ($n = 3$). The 0.5 mM HCl solution was purchased from Oy FF-Chemicals Ab (Yli-Ii, Finland). Methanol (gradient grade) and dimethyl sulfoxide (DMSO) were acquired from Fluka AG.

The chemical structures of all studied *p*-vinyl phenols are given in Table 1. Compounds **5**–**9** and **16** were synthesized in our laboratory. Commercial sources of compounds **10**–**15** and **19**–**21** are 4-[2-(4-nitrophenyl)vinyl]phenol (**10**) Chembridge (San Diego, USA), 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (**11**) Chembridge, *p*-coumaric acid (4-hydroxycinnamic acid) (**12**) Fluka AG, caffeic acid (3,4-dihydroxycinnamic acid) (**13**) Aldrich, 2-cyano-3-(3,4-dihydroxyphenyl)acrylic acid ethyl ester (**14**) ICN Biomedicals, 2-cyano-3-(4-hydroxyphenyl)acrylic acid (**15**) Aldrich, 4-[2-(4-hydroxyphenyl)vinyl]-1-methylpyridinium iodide (**19**) IBS (Moscow, Russia), 2-[2-(4-hydroxyphenyl)vinyl]-1-methylpyridinium iodide (**20**) IBS, 1-(4-fluorophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**21**) Maybridge (Cornwall, UK).

2.2. Analysis of synthesized compounds

Melting points were determined twice from each substance on an Electrothermal IA 9100 digital melting point apparatus and are uncorrected. TLC was performed on 0.25 mm thick aluminium sheets precoated with Kieselgel 60 (Merck, Darmstadt). Flash chromatography was carried out on silica gel [Kieselgel 60, 230–400 ASTM, Merck, Darmstadt] (Still et al., 1978). Infrared spectra for the phenols **5**–**9** were recorded on a Pye Unicam SP3-200 infrared spectrophotometer (Pye Unicam Ltd., Cambridge, UK). IR spectrum of **16** was recorded on a Perkin Elmer FT-IR spectrometer 1725X. The sample was prepared as a

KBr tablet. Relative intensities are indicated as s, strong; m, medium; w, weak. ^{1}H NMR spectra for **5**–**9** were obtained in $CDCl_3$ or DMSO-d_6 solutions on a Varian Unity 500 spectrometer (500 MHz). Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane (TMS) as an internal reference at 0.00 ppm and referenced to the residual solvent. For values (**16**) obtained with Varian Mercury 300 Plus spectrometers, the chemical shifts (δ) are given in ppm relative to NMR solvent signals. The multiplicity of the signal is indicated as: s, singlet; t, triplet; br, broad; m, multiplet. Coupling constants (J) are quoted in Hertz.

The low resolution mass spectra were obtained on a GC–MSD (HP5890A), equipped with HP-5970 mass selective detector. The capillary column used was HP5-MS (12 m × 0.25 mm), carrier gas was He, 0.3 MPa and the oven temperature program was 100 °C (0 min) + 20 °C/min until at 300 °C for 5 min. The injection and detection temperatures were 250 and 280 °C, respectively. LC–MS spectra (**5**–**9**) were recorded on an API 300 Sciex mass spectrometer with APP-ionisation using a Perkin Elmer 200 LC and Chromolith SpeedROD RP-18e 50–4.6 mm column. The flow rate in LC was 2 ml min^{-1} and it was split 1/10 to gain 200 μl min^{-1} for the mass spectrometer. Water (A) and acetonitrile (B) were used as eluents to give a gradient of A = 85% to 10% during 10 min and then at A = 10% for 2 min. Injection volume was 80 μl, and compounds were identified with negative polarity. LC–MS spectrum (**16**) was recorded on an API 3000 Sciex mass spectrometer with TurbolonSpray-ionsource using a Hewlett Packard 1100 LC and XTerra MS C-18 30–4.6 mm (2.5 μm) column. The flow rate in LC was 0.7 ml min^{-1} and it was split 1/100 for the mass spectrometer. Water/NH_4OAc-buffer pH 4 (9:1, A) and acetonitrile/NH_4OAc-buffer pH 4 (9:1, B) were used as eluents to give a gradient of B = 2% to 60% during 13 min and then B = 60% to 90% during 2 min. Injection volume was 10 μl, and phenols were identified with positive polarity. Microanalyses (CHN) were obtained from Robertson Microlit Laboratories, Inc., Madison, NJ, USA.

2.3. Synthesis of p-vinyl substituted phenols

2.3.1. Methyl 3-(4-hydroxyphenyl)acrylate (5)

A mixture of *p*-coumaric acid (4.00 g, 24.4 mmol), methanol (150 ml), and sulphuric acid (1.00 ml, 18.8 mmol) was refluxed for 5.5 h. Methanol was evaporated in vacuo. The crude product was partitioned between ethyl acetate (110 ml) and water (20 ml). Sodium hydrogencarbonate was added until solution became slightly basic. Ethyl acetate layer was separated, washed with brine (2 × 30 ml), dried with anhydrous sodium sulphate, filtered and evaporated in vacuo. The crude product was triturated with hexane (1 × 20 ml, 1 × 10 ml) to give **5** as a white crystalline solid (3.54 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.81 (s, 3 H), 5.44 (s, 1 H), 6.32 (d, 1 H, *J* 16.5), 6.86 (dd, 2 H, *J* 7.0 and 2.0), 7.44 (dd, 2 H, *J* 9.5 and 2.3), 7.65 (d, 1 H, J 15.5); mp 137–138 °C [lit. 134–138 °C (Nishioka et al., 1997)]; LC–MS: 177 [$M-1$] at 2.56 min; IR (KBr, cm^{-1}): 3340 m, 2990 w, 2900 w, 1890 w, 1680 s, 1630 m, 1600 s, 1500 m, 1430 m, 1275 m, 1200 m, 1170 m, 980 m, 830 s; R_f 0.67 (1:1, EtOAc–hexane); Found C, 67.3; H, 5.7. $C_{10}H_{10}O_3$ requires C, 67.4; H, 5.7%.

2.3.2. 2-(4-Hydroxybenzylidene)malononitrile (6) (Ertel and Friedrich, 1977)

A solution of malononitrile (2.71 g, 41.0 mmol) and *p*-hydroxybenzaldehyde (2.01 g, 16.4 mmol) in methanol (20 ml) was refluxed for 2 h to give a red mixture. Upon cooling to room temperature, a pink precipitate was formed. It was filtered and washed with hexane–diethyl ether 5:1 (2 × 6 ml), water (1 × 10 ml) and diethyl ether (3 × 5 ml). The crude product was precipitated from a solution of water (80 ml) and ethanol (55 ml) to give an orange solid, which was dissolved into a boiling solution of ethyl acetate (65 ml) and methanol (32 ml). The solvent mixture was allowed to evaporate slowly, and the precipitated solids were removed. The remaining solvents (30 ml) were evaporated in vacuo to give **6** as a yellow solid (0.93 g, 33%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.98 (d, 2 H, *J* 8.5), 7.89 (d, 2 H, *J* 9.0), 8.31 (s, 1 H), 11.07 (br s, 1 H); mp 186–188 °C [lit. 183–186 °C (Ertel and Friedrich, 1977)]; LC–MS: 169 [$M-1$] at 2.91 min; IR: 3310 m, 2170 s, 1605 s, 1575 s, 1555 s, 1505 m, 1430 s, 1370 s, 1320 m, 1295 s, 1220 s, 1170 s, 935 m, 835 s, 800 m; R_f 0.50 (1:1, EtOAc–hexane); Found C, 70.4; H, 3.5; N, 16.6. $C_{10}H_6N_2O$ requires C, 70.6; H, 3.6; N 16.5%.

2.3.3. 3-(4-Hydroxyphenyl)acrylamide (7) (Cevasco and Thea, 1994)

5 (1.01 g, 5.66 mmol) was dissolved into a 2 M solution of ammonia in methanol (15.0 ml). The resulting mixture was heated in a threaded pyrex tube for 5 days. Methanol was evaporated in vacuo, and ammonium hydroxide (25.2%, 30 ml) was added to the reaction mixture. The flask was shaken for 7 days. The resulting brown solution was filtered, and the solids were washed with water (3 × 15 ml). The clear filtrate was made acidic with 3 M hydrochloric acid, and the resulting solution was extracted with ethyl acetate (4 × 50 ml). The organic layer was washed with brine (3 × 30 ml), dried with anhydrous sodium sulphate and evaporated in vacuo. The crude product was dissolved in tetrahydrofuran (5 ml), and filtered through a short pad of silica gel using chloroform–methanol (4:1). Finally, the product was chromatographed on silica gel (8:1, $CHCl_3$–MeOH) to give **7** as a white solid (0.62 g, 67%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.38 (d, 1 H, *J* 15.5), 6.78 (d, 2 H, *J* 8.5), 6.95 (br s, 1 H), 7.31 (d, 1 H, *J* 16), 7.38 (d, 2 H, *J* 8.5), 7.41 (br s, 1 H), 9.83 (s, 1 H); mp 190–192 °C [lit. 192–194 °C (Nishioka et al., 1997)]; LC–MS: 164 [$M-1$] at 2.61 min; IR (KBr, cm^{-1}): 3340 m, 3240 m, 1650 s, 1550 s, 1490 m, 1370 s, 1220 s, 1160 w, 1100 w, 980 s, 820 s, 785 m, 685 w; R_f 0.46 (4:1, $CHCl_3$–MeOH); Found C, 66.1; H, 5.4; N, 8.3. $C_9H_9NO_2$ requires C, 66.2; H, 5.6; N 8.6%.

2.3.4. 4-(4′-Hydroxyphenyl)but-3-en-2-one (**8**) (Kad et al., 1998)

p-Hydroxybenzaldehyde (2.5 g, 20.4 mmol) was dissolved in NaOH (10%, 7 ml). A mixture of acetone (8.85 ml, 120.5 mmol) and NaOH (10%, 10 ml) was added to the solution. The beaker was covered with a watch glass and the mixture was radiated with microwaves (150 W) for 15 min giving a deep red mixture. The solution was acified using 3 M HCl and extracted with diethyl ether and evaporated in vacuo. The crude product was recrystallized from benzene. Finally, the product was chromatographed on silica gel (1:1, EtOAc–pentane) to give **8** as a yellow solid (0.310 g, 9%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (s, 3 H), 6.61 (d, 1 H, *J* 16), 6.86 (d, 2 H, *J* 9.0), 7.46 (d, 2 H, *J* 8.5), 7.47 (d, 1 H, *J* 16); mp 102–106 °C [lit. 102–103 °C (Kad et al., 1998)]; GC–MS: 162 [*M*] at 8.63 min; FT-IR (KBr, cm^{-1}): 3279 s, 1673 s, 1573 s, 1513 m, 1436 m, 1363 m, 1281 m, 1167 m, 1105 s, 981 m, 817 s, 699 m; R_f 0.60 (2:1, EtOAc–hexane); Found C, 73.6; H, 6.1. $C_{10}H_{10}O_2$ requires C, 74.0; H, 6.2%.

2.3.5. 4-(2-Nitrovinyl)phenol (**9**) (Schiefer and Kindl, 1971)

A mixture of nitromethane (0.45 ml, 8.38 mmol), *p*-hydroxybenzaldehyde (3.19 g, 26.1 mmol) and ammonium acetate (0.35 g, 4.58 mmol) in acetic acid (6.0 ml) was stirred at 95 °C for 4 h. The cooled solution was filtered and evaporated in vacuo. The crude product was purified with SiO_2 column chromatography (200:1, $CHCl_3$–AcOH) and evaporated in vacuo to afford **9** as a yellow solid (0.17 g, 13%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.85 (d, 2 H, *J* 8.5), 7.72 (d, 2 H, *J* 8.5), 8.05 (obsc., 2 H), 10.4 (s, 1 H); mp 158–163 °C [lit. 154–160 °C (Rosenmund, 1913)]; LC–MS: 162 [*M* − 1] at 0.73 min; IR (KBr, cm^{-1}): 3430 w, 3130 m, 1585 s, 1470 s, 1430 m, 1320 s, 1255 s, 1195 w, 1155 m, 960 m, 810 m, 720 w; R_f 0.71 (2:1, EtOAc–hexane); Found C, 58.0; H, 4.1; N, 8.3. $C_8H_7NO_3$ requires C, 58.2; H, 4.3; N 8.5%.

2.3.6. Methyl 2-cyano-3-(4-hydroxyphenyl)acrylate (**16**) (Bäckström et al., 1989)

A mixture of *p*-hydroxybenzaldehyde (500 mg, 4.09 mmol), methyl cyanoacetate (487 mg, 4.91 mmol) and pyridine (38.9 mg, 0.49 mmol) were mixed and sonicated for 1 h and stirred on a water bath for 1.5 h. The crude product was washed with acetone, filtrated and washed with 95% EtOH. To give crude **16** as a light yellow solid. The product was recrystallized from 95% EtOH to yield 123 mg (15%) of **16**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.84 (s, 3 H), 6.96 (d, 2 H, *J* 9), 8.01 (d, 2 H, *J* 9), 8.25 (s, 1 H); mp 206–209 °C [lit. 208–210 °C (Kaupp et al., 2003)]; GC–MS: 203 [*M*] at 9.27 min; FT-IR (KBr, cm^{-1}): 3338 s, 3024 m, 2225 m, 1725 s, 1590 s, 1520 m, 1438 s, 1268 s, 1208 s, 1174 s, 1091 m, 852 m, 814 s, 634 m; R_f 0.68 (Et_2O).

2.4. Potentiometric titrations

pK_a measurements were performed with a computerised Sirius GLpKa-potentiometric titrator (firmware v.1.114, Sirius Analytical Instruments Ltd., Forest Row, UK) at controlled temperature (25 ± 1 °C) under inert gas (nitrogen/argon) flow. The results were processed using the pK_a log *P* V5.2a or Refinement Pro software. Blank titrations were performed at the start of each day to standardize electrode performance. Standardization was carried out using Four-Plus^{TM} blank refinement script (acquisition of HCl concentration factor and the Four-Plus^{TM} parameters α, *S*, j_H and j_{OH} described in detail by Avdeef's group) (Avdeef et al., 1993). When experimental data is refined, goodness-of-fit (GOF) indicates how well the calculated titration curve correlates with the experimental data. Refinement can be seen as very successful when $0.5 \leq GOF \leq 1.5$ (Avdeef, 1993).

p-Vinyl phenols **5**–**16** and **19**–**21** were titrated starting with an initial sample concentration of 0.5–4.0 mM (Table 3). All titrations were performed using methanol as co-solvent unless otherwise stated. When the compound was water-insoluble, methanol was used provided that the substances dissolved sufficiently into the methanol–water mixtures. Methanol was the preferred co-solvent due to its very low capacity to dissolve carbon dioxide and low tendency to cause pH-metric errors, hence having a large extrapolation range (0–60% co-solvent) (Avdeef et al., 1993). The apparent pK_a values (p$_s K_a$) from the non-aqueous titrations were used to obtain extrapolated aqueous pK_a values by the Yasuda–Shedlovsky procedure, where (p$_s K_a$ + log[H_2O]) is plotted against $1/\varepsilon$ and ε is the dielectric constant of the mixture (Avdeef et al., 1993, 1999; Takács-Novák et al., 1997). In case of co-solvent titrations, the r^2 values for the extrapolation as well as the slope of the extrapolation curve (Table 3) are reported.

Titrations were carried out in the pH range of 2–12, using the most suitable pH area for the particular phenol (Table 3). All experiments were carried out by titrating from alkaline to acidic pH to ensure dissolution of sample at the start of the experiment and to minimize undesired precipitation of the phenols. To ensure correct results while using organic solvents, a minimum of six titrations was used with the widest possible range of co-solvent ratios (see Table 3) (Takács-Novák et al., 1997). When considered necessary, extrapolated pK_a values were confirmed by performing 'aqueous' titrations in ISA water containing 4% (v/v) of DMSO, which was considered a good estimate of aqueous pK_a due to its near aqueous dielectric properties (Hänninen et al., 2003).

Experimental pK_a values were taken from literature for compounds **3** (Wikberg et al., 1993) (4.40), **4** (Wikberg et al., 1993) (4.60), **17** (Brauer et al., 1964) (9.82) and **18** (Brauer et al., 1964) (9.89).

2.5. Regression analysis and prediction of pK_a values

All Hammett σ constants for the substituents were calculated using the ACD/Labs pK_a DB program. The regression analysis was performed with the statistical software package SPSS (version 11.5.1, SPSS Inc., Chicago, IL, USA).

Table 3
Experimental conditions and results of experiments

Compound number	Number of titrations	Co-solvent[a] range (wt.%)	*c* (sample) (mmol/l)	pH range	pK_a exp.	GOF/r^2[b]	*a*[c]
5	9	14–54	0.68–2.00	11.0–3.5	8.51 ± 0.04	GOF = 2.33/r^2 = 0.9542	100.0
6	9	10–50	0.64–1.50	11.0–3.0	6.94 ± 0.02	GOF = 1.23/r^2 = 0.2154	−7.6
7	12	9–48	1.08–2.15	12.0–3.0	8.61 ± 0.06	GOF = 2.93/r^2 = 0.9340	118.4
8	12	10–49	0.36–2.03	11-5-3.0	8.28 ± 0.02	GOF = 2.66/r^2 = 0.9616	106.6
9	12	10–50	0.52–1.01	11.0–3.0	7.90 ± 0.05	GOF = 1.80/r^2 = 0.5914	45.9
10	8	31–46	0.50–0.63	11.5–3.0	9.13 ± 0.05	GOF = 0.95/r^2 = 0.6493	98.8
11	9	10–51	0.25–0.65	11.5–3.5	8.29 ± 0.06	GOF = 1.51/r^2 = 0.6661	56.0
12	11	9–48	0.87–2.01	11.5–3.0	4.43 ± 0.02 (—COOH)	GOF = 1.34/r^2 = 0.9831	133.5
					8.97 ± 0.05 (—OH)	GOF = 2.37/r^2 = 0.9822	151.3
13	9	9–49	0.80–2.00	11.5–3.0	4.41 ± 0.04 (—COOH)	GOF = 1.21/r^2 = 0.9838	132.2
					8.66 ± 0.07 (—OH)	GOF = 2.94/r^2 = 0.9255	145.3
14	18	9–49	0.53–2.07	11.5–3.5	7.09 ± 0.05 (*p*-OH)	GOF = 3.98/r^2 = 0.0665	5.4
15	15	9–56	1.46–4.02	12.0–1.8	2.62 ± 0.06 (—COOH)	GOF = 4.00/r^2 = 0.8941	81.5
					7.93 ± 0.05 (—OH)	GOF = 5.21/r^2 = 0.9130	111.3
16	6	4% DMSO (aqueous)	0.55–0.94	11–4	7.253 ± 0.002	GOF = 0.47	
16	6	34–59	0.53–1.09	11–4	7.22 ± 0.04	GOF = 2.21/r^2 = 0.5537	15.0
19	6	Aqueous	0.53–0.61	11.0–3.5	8.395 ± 0.006[d]	GOF = 0.54	
20	6	Aqueous	0.52–0.58	11.0–3.5	8.393 ± 0.007[d]	GOF = 0.65	
21	6	25–50	0.28–0.50	11.0–3.5	8.20 ± 0.04[d]	GOF = 1.25/r^2 = 0.7742	78.7

[a] The co-solvent is methanol unless otherwise stated.
[b] GOF is goodness-of-fit.
[c] Parameter *a* is the slope of the Yasuda–Shedlovsky extrapolation.
[d] For acids the value is normally positive, i.e. higher pK_a values are obtained at higher solvent concentration. Low slope values (<100) often cause low r^2 values (<0.9000), as can be radically seen with compounds **6** and **14**.

The computer programs used for comparing experimental and computational values were ACD/Labs pK_a DB (v. 7.07, Advanced Chemistry Development Inc., Toronto, Ont., Canada), Pallas pKalc (demoversion 3.1, Compudrug International Inc., Sedona, AZ, USA) and SPARC On-Line Calculator (Hilal et al., 1994).

3. Results and discussion

3.1. Experimental pK_a values

Experimental methods for the determination of ionisation constants, especially potentiometric titration, have lately been under intensive commercial development (Avdeef and Testa, 2002). This has improved and accelerated acquisition of experimental data used in the development of computational methods for the prediction of ionisation constants. Potentiometric titration is favoured since it is fast, accurate and enables the measurement of the pK_a of water insoluble compounds by means of the co-solvent approach (Albert and Serjeant, 1984).

A model can be only as good as the experimental data used to build it. Thus, a minimum of six experiments were carried out either in aqueous environment or in co-solvent systems (Table 3). With water-insoluble compounds, the co-solvent approach was used with methanol as first choice. The widest possible methanol range, solubility allowing, was used and the change in methanol content between experiments was selected to be approximately 5% to minimize the potential error in extrapolated pK_a values (Takács-Novák et al., 1997). This approach enabled the production of experimental ionisation constants with a maximum error estimate of 0.07 pK_a units.

3.2. Modification of the Hammett equation

The commonly used Hammett equation [Eq. (1)] for the prediction of pK_a values of substituted phenols is of the type

$$\mathrm{p}K_\mathrm{a} = \mathrm{p}K_\mathrm{a0} + \Delta\mathrm{p}K_\mathrm{a} = \mathrm{p}K_\mathrm{a0} + A\sum\sigma, \quad (1)$$

where $\sum\sigma$ is the sum of appropriate Hammett σ constants for substituents attached to the benzene ring. Constants pK_{a0} and A vary in different implementations. Hammett σ_{para-} -constants, which should be used for *p*-substituted phenols, are only available for a limited set of substituents. To predict the pK_a values of *p*-vinyl phenols using tabulated σ_{para-} -values we need to find out the relationship between the substituent constants of the groups attached to the vinylic carbon atom and the total effect on the dissociation constant of the phenolic hydroxyl in the *p*-position. To achieve this, the experimental pK_a data for the training set (compounds **5**–**12**, **15** and **16**) was fitted in the equation:

$$\mathrm{p}K_\mathrm{a} = \mathrm{p}K_\mathrm{a0} + \Delta\mathrm{p}K_\mathrm{a\_vin} = \mathrm{p}K_\mathrm{a0} + B\sum\sigma_{para-(\mathrm{vin})}, \quad (2)$$

where $\sum\sigma_{para\text{-}(\mathrm{vin})}$ is the sum of Hammett σ_{para-} -constants of substituents in the *p*-position connected to the aromatic ring through the vinylic group –CH=C<. Hammett σ constants, experimental pK_a values and predicted pK_a values for the training set are given in Table 4. The derived regression model showed a good fit (R^2 = 0.987) with no outliers

Table 4
Experimental pK_a values, Hammett σ_{para}-constants, predicted pK_a values and residuals for the training set

Compound number	pK_a exp.	σ_{para}-(vin)_1	σ_{para}-(vin)_2	$\sum \sigma_{para}$-(vin)	pK_a pred.	Residual
5	8.51	0.75	0.00	0.75	8.48	0.03
6	6.94	1.00	1.00	2.00	6.97	0.03
7	8.61	0.61	0.00	0.61	8.65	0.04
8	8.28	0.84	0.00	0.84	8.37	0.09
9	7.90	1.27	0.00	1.27	7.85	0.05
10	9.13	0.31	0.00	0.31	9.01	0.12
11	8.23	0.83	0.00	0.83	8.38	0.09
12	8.97	0.31	0.00	0.31	9.01	0.07
15	7.93	1.00	0.31	1.31	7.80	0.13
16	7.25	1.00	0.75	1.75	7.27	0.02

(Fig. 2a). The values of constants pK_{a0} and B with 95% confidence intervals were 9.38 ± 0.13 and -1.20 ± 0.12 pK_a units, respectively. The standard error of the estimate was 0.09 and the maximum residual 0.13 pK_a units.

The final extended Hammett equation for *p*-vinyl phenols was derived by adding the correction term ΔpK_a, obtained from the work by Biggs and Robinson (Biggs and Robinson, 1961) for non-vinylic substituents to the regression equation:

$$\text{p}K_a = \text{p}K_{a0} + \Delta\text{p}K_a + \Delta\text{p}K_{a\_vin} \quad (3)$$

$$\text{p}K_a = \text{p}K_{a0} + A\sum\sigma + B\sum\sigma_{para\text{-(vin)}} \quad (4)$$

$$\text{p}K_a = 9.38 - 2.23\sum\sigma - 1.20\sum\sigma_{para\text{-(vin)}} \quad (5)$$

Constants A and B can be used to estimate the attenuation factor by which the double bond dampens the electron withdrawing effect of substituents connected to the aromatic ring. Using values from the final equation [Eq. (5)] we get a value of 0.54 for this attenuation factor (B/A).

3.3. Validation of the modified Hammett equation

The derived Hammett equation [Eq. (5)] was validated with an external test set of six *p*-vinyl substituted phenols and catechols (compounds **3**, **4**, **13**, **14**, **17** and **18**) and the predictions were compared with results obtained with ACD/pK_a DB and SPARC On-Line Calculator (Table 5). The pK_a values of the compounds in the test set varied from 4.4 to 9.9 and good predictions were obtained over this wide pK_a range (Fig. 2b). The difference between experimental pK_a values and predictions calculated with Eq. (5) ranged from 0.01 (compound **14**) to 0.31 (compound **18**) pK_a units. The mean error of pK_a predictions was smaller than 0.2 pK_a units for both SPARC On-Line Calculator and the method derived in this study while a mean error of nearly 1.3 pK_a units was obtained with ACD/pK_a DB. With these programs the same trend is observed throughout the *p*-vinyl substituted phenol series listed in Table 2.

A correlation between the substituent constants (σ_{para}-) of groups connected to the vinylic carbon and the pK_a lowering effect of the whole vinylic moiety on the phenolic hydroxyl in the *para* position was found and quantified. It is interesting to compare the electronic attenuation factor for the double bond connected to the benzene ring calculated here (0.54) with the previously published ones. A value of 0.47 was reported based on the ionisation constants of a substituted cinnamic acid series (Jaffe, 1953). Comparison of the ester hydrolysis reactions of substituted cinnamates and benzoates yielded a

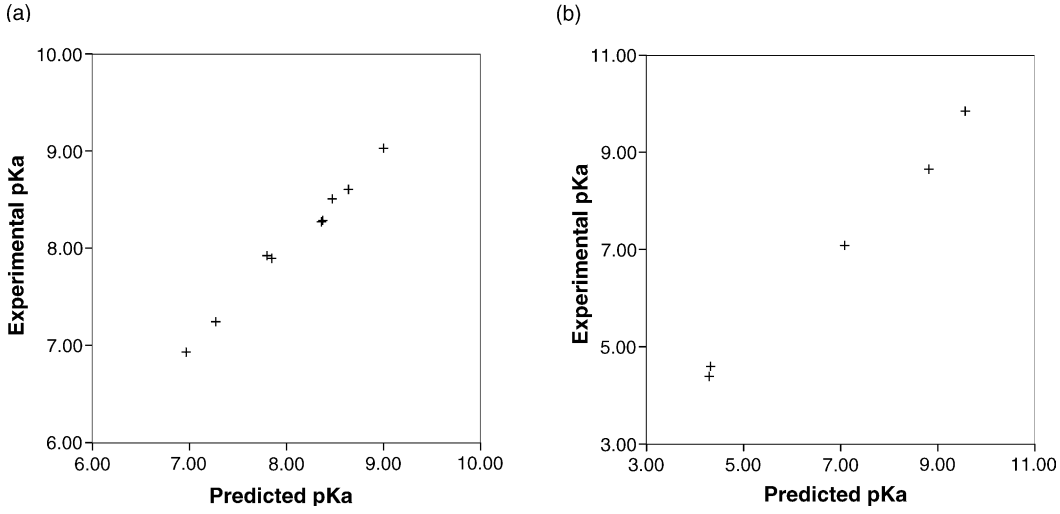


Fig. 2. Predicted pK_a values from the regression model vs. experimental pK_a values are plotted in (a) for the training set and (b) for the external test set.

Table 5
Comparison of experimental pK_a values and predictions obtained with the extended Hammett equation derived in this work, SPARC On-Line Calculator and ACD/pK_a DB 7 for the external test set

Compound number	pK_a exp.	pK_a pred.	Dif.	pK_a SPARC	Dif.	pK_a ACD 7	Dif.
3	4.40	4.31	0.09	4.13	0.27	6.07	1.67
4	4.60	4.33	0.27	4.68	0.08	6.93	2.33
13	8.66	8.83	0.17	8.66	0.00	9.97	1.31
14	7.09	7.10	0.01	6.76	0.33	8.99	1.90
17	9.82	9.58	0.24	9.70	0.12	10.05	0.23
18	9.89	9.58	0.31	9.59	0.30	10.10	0.21
Mean			0.19		0.18		1.27
S.D.			0.11		0.13		0.88

value of 0.54 (Jaffe, 1953; Williams, 1984, 2003). In this work we measured the effect of electron withdrawing vinylic substituents on the pK_a of the phenolic hydroxyl in the *para* position, while the previously published values measure the electronic effect that a *para* substituent has on the properties of the moiety connected to the benzene ring by the vinylic bond. The attenuation appears to be similar in both directions. The derived extended Hammett equation was used to predict the pK_a values of an external test set, and the results were satisfactory: all six predictions were correct to a few tenths of pK_a units.

3.4. Calculation of $\sigma_{para\text{-}}$ -values

Phenols **19**–**21** were initially included because they were used to evaluate the accuracy of current computational approaches for prediction of pK_a values. Currently there are no $\sigma_{para\text{-}}$ -constants for these three phenols and, thus, they could not be included in the regression model or the test set. The ionisation constants for compounds **19**–**21** (Table 2) were used to calculate $\sigma_{para\text{-}}$ -constants with the derived Hammett Equation. The calculated $\sigma_{para\text{-}}$ -values are 0.81, 0.82 and 0.98 for the vinylic substituents of compounds **19**–**21**, respectively.

4. Conclusions

With the Hammett equation presented in this paper, it is straightforward to get reliable pK_a predictions of *p*-vinyl substituted phenols if the $\sigma_{para\text{-}}$ -constants of the substituents connected to the benzene ring by a double bond are available. Despite the fairly narrow pK_a range and substitution spectrum of the training set, accurate predictions were obtained also for the multi-substituted low-pK_a compounds of the external test set. The pK_a lowering effect of *p*-vinyl substituents quantified in this work appears to be additive and extrapolation to a wide pK_a range yielded accurate predictions. The observed linearity and additivity of the electron withdrawing effect over the delocalized electron structure of *p*-vinyl phenols makes it possible to obtain accurate pK_a predictions for these compounds with linear free energy-based methods. The more complicated SPARC On-Line Calculator, which combines linear free energy calculations with perturbation theory and structure–activity relationships, seems to predict this long-range electronic effect well. The approach used here could be implemented in the widely used LFER-based pK_a software packages to improve the predictions for this type of compounds to the same level.

Acknowledgments

The authors wish to thank the Finnish Graduate School in Pharmaceutical Research (JS) and The National Technology Agency TEKES (HN) for financial support.

References

Anonymous, 1999. Sirius GLpKa Instruction Manual. Sirius Analyticals Ltd., East Sussex.

Albert, A., Serjeant, E.P., 1984. The Determination of Ionization Constants—A Laboratory Manual. Chapman & Hall, Cambridge.

Avdeef, A., 1993. pH-metric log *P*. II. Refinement of partition coefficients and ionization constants of multiprotic substances. J. Pharm. Sci. 82 (2), 183–190.

Avdeef, A., 2001. Physicochemical profiling (solubility permeability and charge state). Curr. Top. Med. Chem. 1 (4), 277–351.

Avdeef, A., 2003. Absorption and Drug Development: Solubility, Permeability and Charge State. John Wiley & Sons Inc., New Jersey.

Avdeef, A., Box, K.J., Comer, J.E.A., Gilges, M., Hadley, M., Hibbert, C., Patterson, W., Tam, K.Y., 1999. pH-metric log *P* 11. pK_a determination of water-insoluble drugs in organic solvent–water mixtures. J. Pharm. Biomed. Anal. 20 (4), 631–641.

Avdeef, A., Comer, J.E.A., Thomson, S.J., 1993. pH-Metric log *P*. 3. Glass electrode calibration in methanol–water, applied to pK_a determination of water-insoluble substances. Anal. Chem. 65 (1), 42–49.

Avdeef, A., Testa, B., 2002. Physicochemical profiling in drug research: a brief survey of the state-of-the-art of experimental techniques. Cell. Mol. Life Sci. 59, 1681–1689.

Bäckström, R., Honkanen, E., Pippuri, A., Kairisalo, P., Pystynen, J., Heinola, K., Nissinen, E., Linden, I.-B., Männistö, P.T., Kaakkola, S., Pohto, P., 1989. Synthesis of some novel potent and selective catechol-*O*-methyltransferase inhibitors. J. Med. Chem. 32 (4), 841–846.

Biggs, A.I., Robinson, R.A., 1961. Ionisation constants of some substituted anilines and phenols—a test of Hammett relation. J. Chem. Soc., 388–393.

Brauer, G.M., Argentar, H., Durany, G., 1964. Ionization constants and reactivity of isomers of eugenol. J. Res. Natl. Bur. Std. 68A (6), 619–624.

Cevasco, G., Thea, S., 1994. Participation of an extended *p*-oxo ketene intermediate in the dissociative alkaline hydrolysis of aryl 4-hydroxycinnamates. J. Org. Chem. 59 (21), 6274–6278.

Congy, C., Labeeuw, B., Gueule, P., Rinaldi, M., 1990. Preparation of chalcone oxime ethers as 5-HT2 receptor antagonists and platelet antiaggregants, EP 373998.

Ermondi, G., Lorenti, M., Caron, G., 2004. Contribution of ionization and lipophilicity to drug binding to albumin: a preliminary step toward biodistribution prediction. J. Med. Chem. 47 (16), 3949–3961.

Ertel, W., Friedrich, K., 1977. Umsetzung von Aromaten mit Chlormethylenmalonsäure-Derivaten. Eine neue Synthese aromatischer Aldehyde. Chem. Ber. 110, 86–95.

Flynn, D.L., Belliotti, T.R., Boctor, A.M., Connor, D.T., Kostlan, C.R., Nies, D.E., Ortwine, D.F., Schrier, D.J., Sircar, J.C., 1991. Styrylpyrazoles, styrylisoxazoles, and styrylisothiazoles. Novel 5-lipoxygenase and cyclooxygenase inhibitors. J. Med. Chem. 34 (2), 518–525.

Hänninen, K., Kaukonen, A.M., Kankkunen, T., Hirvonen, J., 2003. Rate and extent of ion-exchange process: the effect of physico-chemical characteristics of salicylate anions. J. Contr. Release 91 (3), 449–463.

Higa, T., Sakai, R., 1988. Antiviral guanidine derivative compositions and their methods of use, WO 8800181 EP 271571.

Hilal, S.H., Carreira, L.A., Karickhoff, S.W., 1994. Sparc—a computer-program for estimation of physicochemical properties. Abstr. Pap. Am. Chem. Soc. 208, 189-COMP Part 1.

Hori, K., Nakamura, K., Kawai, M., Mogi, I., Imokawa, G., Takaishi, N., 1987. Preparation of *N*,*N*-dialkyl-*p*-hydroxycinnamamides as melanin inhibitors, JP 62056459.

Hori, K., Nakamura, K., Kawai, M., Motegi, I., Imokawa, G., Takaishi, N., 1988. Preparation of (2-hydroxyethyl)cinnamamides and skin lighteners containing them, EP 255695.

Isshiki, K., Imoto, M., Sawa, T., Umezawa, K., Takeuchi, T., Umezawa, H., Tsuchida, T., Yoshioka, T., Tatsuta, K., 1987. Inhibition of tyrosine protein kinase by synthetic erbstatin analogs. J. Antibiot. 40 (8), 1209–1210.

Jaffe, H.H., 1953. A reexamination of the Hammett equation. Chem. Rev. 53 (2), 191–261.

Kaakkola, S., Gordin, A., Männistö, P.T., 1994. General-properties and clinical possibilities of new selective inhibitors of catechol *O*-methyltransferase. Gen. Pharmacol. 25 (5), 813–824.

Kad, G.L., Singh, V., Khurana, A., Singh, J., 1998. The synthesis of physocopsisenone, a new secondary metabolite from the sponge phycopsis sp. J. Nat. Prod. 61 (2), 297–298.

Kaupp, G., Reza Naimi-Jamal, M., Schmeyers, J., 2003. Solvent-free Knoevenagel condensations and Michael additions in the solid state and in the melt with quantitative yield. Tetrahedron 59 (21), 3753–3760.

Kennedy, T., 1997. Managing the drug discovery/development interface. Drug Discov. Today 2 (10), 436–444.

Lautala, P., Ulmanen, I., Taskinen, J., 2001. Molecular mechanisms controlling the rate and specificity of catechol *O*-methylation by human soluble catechol *O*-methyltransferase 59 (2), 393–402.

Livingstone, D.J., 2003. Theoretical property predictions. Curr. Top. Med. Chem. 3 (10), 1171–1192.

Lotta, T., Taskinen, J., Bäckström, R., Nissinen, E., 1992. Pls modeling of structure–activity-relationships of catechol *O*-methyltransferase inhibitors. J. Comput. Aid. Mol. Des. 6 (3), 253–272.

Lyall, R.M., Zilberstein, A., Gazit, A., Gilon, C., Levitzki, A., Schlessinger, J., 1989. Tyrphostins inhibit epidermal growth factor (EGF)-receptor tyrosine kinase activity in living cells and EGF-stimulated cell proliferation. J. Biol. Chem. 264 (24), 14503–14509.

Maurin, B., Grant, J.W., Stahl, P.H., 2002. In: Stahl, P.H., Wermuth, C.G. (Eds.), Handbook of Pharmaceutical Salts—Properties, Selection, Use. Verlag Helvetica Chimica Acta, Zürich, pp. 9–17.

Nishioka, T., Watanabe, J., Kawabata, J., Niki, R., 1997. Isolation and activity of *N*-*p*-coumaroyltyramine, an α-glucosidase inhibitor in Welsh onion (*Allium fistulosum*). Biosci. Biotechnol. Biochem. 61 (7), 1138–1141.

Rinaldi-Carmona, M., Congy, C., Santucci, V., Simiand, J., Gautret, B., Neliat, G., Labeeuw, B., Le Fur, G., Soubrie, P., Breliere, J.C., 1992. Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine2 receptor antagonist. J. Pharmacol. Exp. Ther. 262 (2), 759–768.

Rosenmund, K.W., 1913. Über Phenyl-äthanol-amine und Phenyl-nitro-äthanole und ihre Oxy Derivative. Ber. Deutsch. Chem. Ges. 46, 1034–1050.

Schiefer, S., Kindl, H., 1971. ^{14}C-Synthesen biologisch interessanter *p*-substituierter Aryläthan-Körper. J. Labelled Compd. Radiopharm. 7 (3), 291–297.

Shedlovsky, T. (Ed.), 1962. The Behaviour of Carboxylic Acids in Mixed Solvents. Pergamon Press, Oxford.

Still, W.C., Kahn, M., Mitra, K., 1978. Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. 43, 2923–2925.

Takács-Novák, K., Box, K.J., Avdeef, A., 1997. Potentiometric pK_a determination of water-insoluble compounds: validation study in methanol/water mixtures. Int. J. Pharm. 151 (2), 235–248.

Taskinen, J., Vidgren, J., Ovaska, M., Bäckström, R., Pippuri, A., Nissinen, E., 1989. Qsar and binding model for inhibition of rat-liver catechol-*O*-methyl-transferase by 1,5-substituted-3,4-dihydroxybenzenes. Quant. Struct. Act. Rel. 8 (3), 210–213.

van de Waterbeemd, H., Gifford, E., 2003. ADMET in silico modelling: towards prediction paradise? Nat. Rev. Drug Discov. 2 (3), 192–204.

Wikberg, T., Ottoila, P., Taskinen, J., 1993. Identification of major urinary metabolites of the catechol-*O*-methyltransferase inhibitor entacapone in the dog. Eur. J. Drug Metabol. Pharmacokinet. 18 (4), 359–367.

Williams, A., 1984. In: Page, M.I. (Ed.), The Chemistry of Enzyme Action, vol. 6. Elsevier Science Publishers B.V., Amsterdam, pp. 127–202.

Williams, A., 2003. Free Energy Relationships in Organic and Bio-organic Chemistry. The Royal Society of Chemistry, Cambridge.

Yasuda, M., 1959. Dissociation constants of some carboxylic acids in mixed aqueous solvents. Bull. Chem. Soc. Jpn. 32 (5), 429–432.