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Study of hydrophobic properties of biologically active open analogues of flavonoids

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ABSTRACT

Hydrophobicity can either be determined experimentally or predicted by means of commercially available programs. In the studies concerning biological activities of pyrazine analogues of chalcones, 3-(2-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones were more potent than the corresponding 3-(4-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones. As the difference in lipophilicity may be a factor responsible for the difference in the potency, R_M values of the compounds were determined by RP-TLC and compared with log *P* values calculated by various commercially available programs. Important discrepancies were found between experimental and computational lipophilicity data. Therefore, we have tried to find a reliable method for calculating R_M values from *in silico* derived molecular parameters. The R_M values obtained with the chromatographic system consisting of Silufol UV 254 plates impregnated with silicon oil as the stationary phase and acetone–citrate buffer (pH = 3) 50:50 (v/v) as the mobile phase correlated well with van der Waals volumes (V_W) and hydration energies (ΔG_{H_2O}) derived of molecular models calculated on RHF/AM1 level.

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1. Introduction

The discovery and development of a new drug is a long, difficult and expensive process. Industry's R&D investment has increased by 12% on overage year-on-year since 1970. It takes almost 1.8 billion US$ over a period of 13.5 years to develop a new drug [1]. Theoretical models for predicting absorption, distribution, metabolism and excretion (ADME) properties and also safety and toxicity of potential drugs play important roles in the drug development process [2–5].

Lipophilicity is the key factor in many ADME processes involving membrane transport, but it also influences the interaction with metabolizing enzymes and transporters [6,7,8]. The logarithm of the partition coefficient between *n*-octanol and water (log *P*) has often been used to represent molecular lipophilicity. The dominant role of octanol–water partition coefficients in the pharmacokinetic processes, as well as in ligand–macromolecule interactions triggered further research on the development of rapid methods for their experimental assessment, among them chromatographic techniques [9–12], as well as of suitable calculation methods [13–18].

Chalcones (1,3-diphenylprop-2-en-1-ones) are open analogues of flavonoids in which the two aromatic rings are joined by a three carbon, α,β-unsaturated carbonyl system. Biological effects of both naturally occurring chalcones and their synthetic congeners have recently been reviewed [19]. As a part of studies aimed at finding novel biologically active pyrazine derivatives a number of ring substituted 3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones was prepared in our laboratory. The compounds (see Fig. 1) were studied as potential antifungal [20–22], antimycobacterial [20–22] and platelet-antiaggregatory agents [23]. An influence of these compounds on photosynthetic processes was studied as well [20–22]. In all types of bioassays, 3-(2-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones (**1a**–**1e**) were more potent than the corresponding 3-(4-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones (**2a**–**2e**). As the difference in lipophilicity could be responsible for the higher potency of 2-OH derivatives, log *P* values of the compounds were calculated by means of commercially available programs. However, the results obtained with various programs differed significantly. Therefore we tried to find a more reliable method for the evaluation of lipophilicity, and the results of these efforts are reported in the present paper.

2. Materials and methods

2.1. Synthesis of model compounds

Model 3-(2-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones **1a**–**1e** and 3-(4-hydroxyphenyl)-1-(pyrazin-2-yl)prop-2-en-1-ones **2a**–**2e** were prepared by the Claisen-Schmidt

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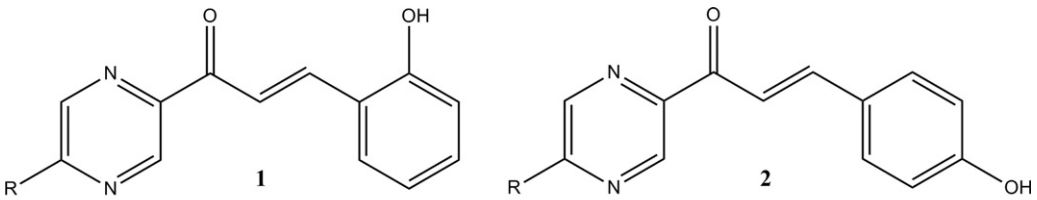


a: R = H, b: R = *tert*-butyl, c: R = isobutyl, d: R = butyl, e: R = propyl

Fig. 1. Studied compounds.

condensation of the corresponding acetylpyrazines with 2-hydroxybenzaldehyde and 4-hydroxybenzaldehyde, respectively [20].

2.2. Calculations of log P by means of commercially available programs

ACD/logP v. 1.0 (Advanced Chemistry Development Inc., Toronto, Kanada), *HyperChem v. 6.03* (HyperCube Inc., Gainesville, USA) and *ChemBioDraw Ultra v. 11.0* (CambridgeSoft Corporation, Cambridge, USA) that automatically generate log *P* from the structure were used. log *P* calculated with *ACD/logP v. 11.02* were found in Chemical Abstracts [24]. The results are given in Table 1.

2.3. Evaluation of lipophilicity by RP-TLC

Two chromatographic systems were used to determine hR_M values ($hR_M = R_M \times 100$ and R_M can be calculated as: $R_M = \log(1/R_F - 1)$):

A: *stationary phase* – Celufol plates (Kavalier, Votice) impregnated by development in 5% solution of octan-1-ol in diethyl ether; *mobile phase* – acetone + citrate buffer (pH = 3) 50:50 (v/v) saturated with octan-1-ol.
B: *stationary phase* – Silufol plates UV 254 (Kavalier, Votice) impregnated by immersion in 5% solution of silicone oil (Lukoil M 200, Lučební závody, Kolín) in diethyl ether; *mobile phase* – acetone + citrate buffer (pH = 3) 50:50 (v/v)

Studied compounds were dissolved in methanol (1 mg/ml) and 2 μl was applied on the plate with an interval 1.3–1.4 cm between circular spots using micropipette. The starting line was 1.5 cm from the lower edge of the plate. The plates were developed over a path of 12.0 cm in a normal chamber (16 cm × 16 cm × 7 cm) at room temperature, previously equilibrated for 2 h. After development the plates were dried in a gentle stream of air and the spots were visualized in λ = 254 nm UV light by means of a Camag UV lamp. An arithmetic average was calculated from three independent TLC measurements.

2.4. Generating hR_M values from quantum-chemical calculations

Quantum-chemical calculations were run on a PC computer using software *HyperChem v. 6.03* (HyperCube Inc., Gainesville, USA). The models of compounds were formed on RHF/AM1 level (Austin Model 1 [25–27]). The most stable conformations of unsubstituted compounds **1a** and **2a** were optimized by conformational analysis using random variation of dihedral angles. The other derivatives were designed on the basis of these models. Van der Waals volume (V_W) and surface (S_W) and solvent accessible volume (V_{SA}) and surface (S_{SA}) were calculated using the grid method described by Bodor et al. [28] using the atomic radii of Gavezotti [29] and solvent probe radius 1.4 Å. Hydration energy (ΔG_{H_2O}) was calculated by the method published by Ooi et al. [30].

Correlation and regression analyses of the QSAR study were run on a PC computer using the Microsoft Excel program. Multiple regression analyses, which involve finding the best fit of dependent variable (experimental hR_M value) to a linear combination of independent variables (descriptors), were performed by the least squares method. In the equations, the figures in the parentheses are the standard errors of the regression coefficients, *n* is the number of compounds, *r* is the multiple correlation coefficient, *F* is the significance test (*F*-test), and *s* is the standard error of estimate. *F* test values are for all equations statistically significant at the 1% level of probability of error.

3. Results and discussion

Commercially available programs generating log *P* from the structure have been widely used recently [16–18]. However, the results are dependent on the size of the training set and the algorithm used for calculation and may not be reliable, especially in the case of *ortho*-substituted compounds where possible intramolecular interactions affecting lipophilicity must be taken in account [31–36]. In the present paper, *ACD/logP v. 1.0* and *v. 11.02* (Advanced Chemistry Development Inc., Toronto, Canada), *HyperChem v. 6.03* (HyperCube Inc., Gainesville, USA) and *ChemBioDraw Ultra v. 11.0* (CambridgeSoft Corporation, Cambridge, USA) were used to calculate log *P* values of hydroxylated 3-phenyl-1-(pyrazin-2-yl)prop-2-en-1-ones. Table 1 shows that according to *ACD/logP v. 1.0*, 2-OH derivatives (**1a**–**1e**) are less lipophilic than the corresponding 4-OH derivatives (**2a**–**2e**), whilst the version 11.02 indicated that 2-OH chalcones (**1b**–**1e**) are more lipophilic than their 4-OH counterparts (**2b**–**2e**). Suprisingly, this does not apply for compound **1a** and **2a**. *HyperChem* and *ChemBioDraw Ultra* showed no differences in the lipophilicity of the two series. *ChemBioDraw Ultra* enables to calculate *C* log *P*, *i.e.* the *n*-octanol/water partition coefficient based on established chemical interactions. However, based on this value no difference in lipophilicity of the two series was observed either.

We have therefore decided to verify the results experimentally by measuring of hR_M values which characterize the molecular lipophilicity [37] on reversed phase thin-layer chromatography (RP-TLC). Chromatographic systems were chosen on the basis of literature data [32,38]. Citrate buffer (pH = 3) [39] was used to suppress dissociation of the phenolic groups. The results (hR_MA and hR_MB in Table 1) clearly show that 2-OH derivatives (**1a**–**1e)** are more lipophilic than the corresponding 4-OH derivatives (**2a**–**2e**). Hence, some intramolecular interactions that are not reflected by commercially available programmes must occur in the molecules of the studied compounds, and the definite conclusions about their lipophilicity should be preferably done on experimental data.

Although RP-TLC is relatively inexpensive, it still requires a great deal of highly accurate laboratory work. The expression of lipophilicity by properties of molecules that can be obtained

Table 1
Molecular parameters and lipophilicity of compounds **1a**–**1e** and **2a**–**2e**.

Compound	log *P* ACD 1.0	log *P* ACD 11.02	log *P* HyperChem	log *P* ChemBio Draw	*C* log *P* ChemBio Draw	hR_MA	hR_MB	ΔG_{H_2O} [kcal/mol]	V_W [Å^3]	V_{SA} [Å^3]	S_W [Å^2]	S_{SA} [Å^2]	$hR_MCalc.$
1a	1.61	1.102	2.56	0.94	1.30066	−86	−33	−10.9	205.08	688.1	242.99	435.45	−32
2a	2.36	1.153	2.56	0.94	1.30066	−79	−37	−12.84	204.98	690.25	243.03	440.67	−38
1b	3.30	3.793	4.12	3.07	3.12667	19	12	−7.41	271.76	880.24	320.52	535.53	12
2b	4.05	3.240	4.12	3.07	3.12667	−10	9	−9.35	271.77	883.57	320.86	539.24	5
1c	3.48	3.975	3.94	2.88	3.25666	−2	9	−7.6	272.23	888.69	321.35	545.39	11
2c	4.23	3.422	3.94	2.88	3.25666	−16	2	−9.53	272.52	892.00	322.59	545.98	5
1d	3.66	4.159	4.01	2.97	3.38667	7	12	−7.56	272.43	900.08	324.00	552.77	11
2d	4.41	3.605	4.01	2.97	3.38667	−10	5	−9.5	272.73	904.26	325.34	557.46	5
1e	3.13	3.628	3.61	2.55	2.85767	−31	3	−8	255.67	846.42	303.25	523.42	2
2e	3.88	3.074	3.61	2.55	2.85767	−52	−9	−9.94	255.86	849.46	304.85	530.05	−5

from models calculated by quantum-chemical methods is another possibility. In the present study, experimental hR_M values were correlated with van der Waals volume (V_W), solvent accessible volume (V_{SA}), van der Waals surface (S_W), solvent accessible surface (S_{SA}) and hydration energy (ΔG_{H_2O}). The models of the studied compounds were formed on the semi-empirical level, which should be sufficiently accurate for this purpose, and the volumes or the surfaces of the molecules were calculated from the most stable conformation with the lowest energy. The best fit was found between hR_MB and van der Waals volume (V_W), especially in the combination with the hydration energy (ΔG_{H_2O}). The correlation coefficient for this combination of parameters is 0.991. Slightly worse results were obtained when the solvent accessible volume V_{SA} (with the solvent probe radius 1.4 Å) or surfaces (S_W, S_{SA}) of the calculated molecules were used as independent variables.

Estimating lipophilicity of chalcones using the relationship expressed by Eq. (1) thus represents a suitable and even better alternative to some common algorithms based on fragment addition methods.

The following relationships were derived:

$$hR_MB = 0.47(\pm0.06)V_W + 3.4(\pm0.9)\Delta G_{H_2O} - 91(\pm21)$$
$$n = 10 \quad s = 2.735 \quad r = 0.991 \quad F = 94 \tag{1}$$

$$hR_MB = 0.64(\pm0.06)V_W - 170(\pm10)$$
$$n = 10 \quad s = 4.570 \quad r = 0.971 \quad F = 133 \tag{2}$$

$$hR_MA = 1.2(\pm0.2)V_W - 330.0(\pm50)$$
$$n = 10 \quad s = 14.800 \quad r = 0.920 \quad F = 44 \tag{3}$$

$$hR_MB = 0.15(\pm0.02)V_{SA} + 3.8(\pm1.0)\Delta G_{H_2O} - 92(\pm25)$$
$$n = 10 \quad s = 3.216 \quad r = 0.988 \quad F = 139 \tag{4}$$

$$hR_MB = 0.39(\pm0.05)S_W + 3.6(\pm0.9)\Delta G_{H_2O} - 86(\pm22)$$
$$n = 10 \quad s = 2.928 \quad r = 0.990 \quad F = 169 \tag{5}$$

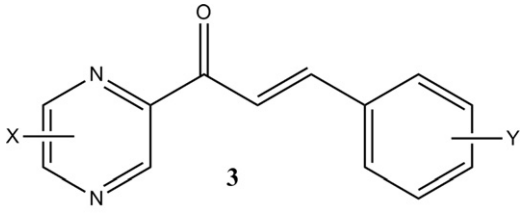
$$hR_MB = 0.54(\pm0.05)S_W - 170(\pm20)$$
$$n = 10 \quad s = 4.900 \quad r = 0.967 \quad F = 115 \tag{6}$$

$$hR_MB = 0.25(\pm0.05)S_{SA} + 4.4(\pm1.2)\Delta G_{H_2O} - 95(\pm32)$$
$$n = 10 \quad s = 3.87511 \quad r = 0.98205 \quad F = 95 \tag{7}$$

$$hR_MB = 0.38(\pm0.05)S_{SA} - 200(\pm20)$$
$$n = 10 \quad s = 6.295 \quad r = 0.945 \quad F = 67 \tag{8}$$

The equation with the highest correlation coefficient (1) was then used to generate hR_MCalc. For the results see Table 1.

In the end of our study we tried to validate our best model (Eq. (1)). We measured hR_M values for three novel compounds with the similar structure (Fig. 2).



a: X = H; Y = 3-hydroxy
b: X = H; Y = 4-methoxy
c: X = 4-(n-butyl); Y = 4-methoxy

Fig. 2. Compounds used for the validation.

Table 2
Validation of the regression equation (1).

Compound	ΔG_{H_2O} [kcal/mol]	V_W [Å^3]	hR_MCalc	hR_MB
3a	−12.66	205.30	−38	−37
3b	−7.62	223.17	−12	−14
3c	−4.47	290.37	30	32

Then we calculated their *in silico* models and obtained values of hydration energy (ΔG_{H_2O}) and van der Waals volume (V_W). These parameters were used for predicting of hR_MCalc values which are in good conformity with numbers (hR_MB) obtained from the reversed phase thin-layer chromatography (Table 2).

4. Conclusions

Our paper shows different methods for the description of hydrophobic properties of compounds by alternative ways where we used calculated parameters from computed models of molecules on the semi-empirical level which is possible to obtain in relatively short time on present-day PC computers. In our calculation we succeeded the best result (Eq. (1)) with the combination of van der Waals volume (V_W) and the hydration energy (ΔG_{H_2O}). But in our series of compounds also one parameter equations (2), (3), (6) and (8) give good correlation with relatively exact prediction of lipophilic parameters of possible new compounds. In these equations van der Waals volume (V_W) or molecular surfaces (either van der Waals or solvent accessible) were used as the computed parameter which describe the molecule.

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