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Short communication

Lipophilicity of a series of 1,2-benzisothiazol-3(2H)-ones determined by reversed-phase thin-layer chromatography

Tomasz Sławik[a,*], Cezary Kowalski[b]

[a]*Department of Medicinal Chemistry, Pharmaceutical Faculty, Medical University Lublin, Chodźki 6, 20-093 Lublin, Poland*
[b]*Department of Pharmacology, Veterinary Faculty, Agricultural University Lublin, Akademicka 12, 20-033 Lublin, Poland*

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Abstract

The lipophilicity (R_{Mo}) and specific hydrophobic surface area of seven 1,2-benzisothiazol-3(2H)-ones have been determined by reversed-phase TLC and the effect of different mobile-phase modifiers (acetone, acetonitrile, methanol) on the retention has been studied. The linear correlations between the volume fraction of the organic solvent and the R_M values over a limited range were established for each solute with high values of correlation coefficients (>0.99). The influence of solvent pH on R_M values was investigated.

Keywords: Lipophilicity; R_M values; 1,2-Benzisothiazol-3(2H)-ones

1. Introduction

Lipophilicity is one of the most important physicochemical properties frequently used in QSAR (quantitative structure–activity relationship) analysis [1–3]. The lipophilic character of chemical compounds is expressed by the partition coefficient (or by its decimal logarithm, log *P*) between a non-aqueous and aqueous phase. The lipophilicity can be determined by the traditional, troublesome ''shake-flask'' partition method between *n*-octanol and water (or other organic solvents which are immiscible with water). First, the *n*-octanol–water system for the determination of lipophilicity was proposed by Fujita et al. [4] and Hansch et al. [5]. Other methods used for that reason are more and more popular different chromatographic techniques. Many authors described the application of RP-TLC [6–13] in the adaptation to the chromatographic study of lipophilic properties of different chemical compounds (and drugs). The use of RP-TLC in the investigation of lipophilicity of different organic compounds was started by Biagi and his co-workers [14,15]. Application of RP-TLC in experimental investigations of lipophilicity was also described by Dross et al. [16].

The objectives of the present study were to determine for future QSAR calculations the lipophilicity of some derivatives (substituted in benzene ring) of 1,2-benzisothiazol-3(2H)-one, and to find the relationship between the concentration of organic modifiers of mobile phases and the chromatographic properties of the tested compounds and to find out about the influence of the kind of substituents and their positions on the change in the lipophilicity of the examined 1,2-benzoisothiazol-3(2H)-ones. 1,2-Benzisothiazol-3(2H)-one and its different derivatives substituted in the benzene ring and/or in

*Corresponding author. Fax: +48-81-742-5165.
E-mail address: kzchl@asklepios.am.lublin.pl (T. Sławik).

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position 2 of the heterocyclic ring are compounds with high antimicrobial (mostly antibacterial and antifungal) activity [17–19]. The retention data for these substances have not yet been described in the literature. The study of the retention should provide good information about pharmacologically important physico-chemical parameters.

2. Experimental

2.1. Materials

The chemical structures of the 1,2-benzisothiazol-3(2H)-ones investigated are listed in Table 1. Compounds **1** (BIT) and **7** (5-Cl-BIT) were synthesized according to the method described in the literature [17], compounds **2** (6-F-BIT) and **5** (5-F-BIT) were obtained according to the literature method [20] and compounds **3** (6-F-5-CH_3-BIT), **4** (4-F-7-CH_3-BIT) and **6** (5-Br-BIT) were synthesized by methods elaborated in the Department of Medicinal Chemistry, Medical University Lublin, Poland[1].

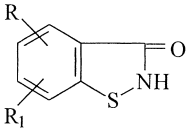
Analytical-grade organic solvents: methanol, acetonitrile were purchased from Merck (Darmstadt, Germany) and acetone from POCH (Lublin, Poland).

The buffers used were: phosphate buffer pH 5.63 and 7.17; borate buffer, pH 11.10; glycine–0.1 *M*/l HCl, pH 2.01 and glycine–0.1 *M*/l NaOH, pH 9.92.

2.2. Chromatographic procedure

Separation was carried out on 10×20 cm precoated RP-TLC plates of RP-$18F_{254S}$ (Merck, Darmstadt, Germany). As mobile phase we used water–acetone, water–acetonitrile and water–methanol mixtures of varying composition. The concentration of organic solvent in the mobile phase ranged from 40 to 80% for acetone and methanol and 20–60% for acetonitrile. The 1,2-benzisothiazol-3(2H)-ones were separately dissolved in acetone at a concentration of 0.5 mg/ml and 5 μl of the solutions were spotted on the plates. The starting points were 10 mm from the bottom edge of the plates. Development was carried out over 9 cm in horizontal DSII chambers (Chromdes, Lublin, Poland) at temperature of 20 °C. Next the plates were dried at room temperature and the spots were localized in UV 254 nm (Fluotest, Hanau, Germany). Each experiment was run in quadruplicate. When the relative standard deviation

Table 1
Chemical structure of 1,2-benzisothiazol-3(2H)-ones



No.	Compound	R	R_1
1	1,2-Benzisothiazol-3(2H)-one (BIT)	H	H
2	6-Fluoro-1,2-benzisothiazol-3(2H)-one (6-F-BIT)	6-F	H
3	6-Fluoro-5-methyl-1,2-benzisothiazol-3(2H)-one (6-F-5-CH_3-BIT)	6-F	5-CH_3
4	4-Fluoro-7-methyl-1,2-benzisothiazol-3(2H)-one (4-F-7-CH_3-BIT)	4-F	7-CH_3
5	5-Fluoro-1,2-benzisothiazol-3(2H)-one (5-F-BIT)	5-F	H
6	5-Bromo-1,2-benzisothiazol-3(2H)-one (5-Br-BIT)	5-Br	H
7	5-Chloro-1,2-benzisothiazol-3(2H)-one (5-Cl-BIT)	5-Cl	H

[1]New synthesis methods; patent pending.

of parallel determinations was >6% the data were omitted from the calculations. The R_M-values of the analytes were calculated separately for each run. For subsequent calculations mean R_M values were used which were calculated from

$$R_M = \log (1/R_F - 1)$$

The calculated R_M values were extrapolated to 0% of organic modifier concentration (R_{Mo}) by using the equation:

$$R_M = R_{Mo} + b \cdot C$$

where C is the concentration in vol% of the organic solvent in the mobile phase and b is the change in R_M caused by unit change of organic modifier concentration in the mobile phase and is related to the specific hydrophobic surface area of the compound.

The influence of mobile phase pH on R_M values was investigated with the constant buffer–acetone (or methanol) ratio 40:60 and buffer–acetonitrile 50:50. Buffer pH was ranging from 2.01 to 11.10.

3. Results and discussion

The relative lipophilicity, expressed by the chromatographic value of R_M of 1,2-benzisothiazol-3(2H)-one (Table 1) was determined by RP-TLC. RP-C_{18} plates were used as non-polar stationary phase and mixtures of water with three organic modifiers (acetone, acetonitrile and methanol) as polar mobile phases. The measured R_M values with different concentrations of the organic solvent, were used for the calculations of R_{Mo} values (extrapolated to zero organic solvent concentration). Table 2 shows

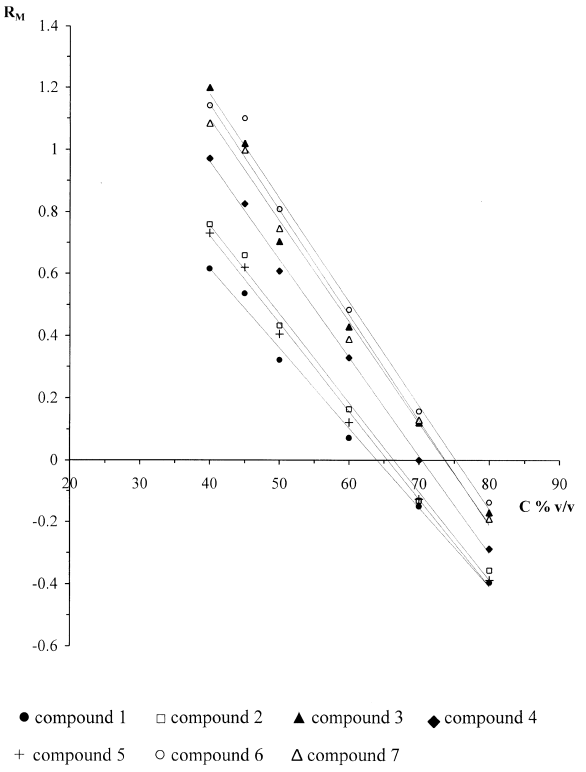


Fig. 1. Relationship between R_M values and methanol concentration in the mobile phase of compounds **1**–**7**.

the R_{Mo} (intercept), b (slope) and r (correlation coefficient) values obtained for the tested compounds. The R_M values of the compounds decreased linearly with increasing concentration of organic modifier in the mobile phase. These results are demonstrated graphically as plots of experimentally obtained R_M values of compounds versus the percentage of methanol (Fig. 1). The calculated R_{Mo}

Table 2
Parameters R_{Mo} (intercept), b (slope) and r (correlation coefficient) of the linear relationship $R_M = R_{Mo} + b \cdot C$

Compound	Acetone			Acetonitrile			Methanol		
	R_{Mo}	$-b$	$-r$	R_{Mo}	$-b$	$-r$	R_{Mo}	$-b$	$-r$
1. BIT	1.309	0.0239	0.9924	1.345	0.0294	0.9965	1.634	0.0255	0.9997
2. 6-F-BIT	1.612	0.0274	0.9985	1.541	0.0313	0.9926	1.902	0.0287	0.9970
3. 6-F-5-CH_3-BIT	1.992	0.0317	0.9999	1.989	0.0352	0.9870	2.530	0.0343	0.9917
4. 4-F-7-CH_3-BIT	1.682	0.0279	0.9974	1.738	0.0333	0.9893	2.225	0.0316	0.9989
5. 5-F-BIT	1.478	0.0255	0.9932	1.469	0.0307	0.9955	1.851	0.0283	0.9978
6. 5-Br-BIT	2.150	0.0331	0.9989	2.029	0.0356	0.9881	2.615	0.0349	0.9955
7. 5-Cl-BIT	1.967	0.0304	0.9983	1.911	0.0349	0.9907	2.415	0.0329	0.9963

(intercept) values are different for each compound and depend only on chemical structures (the kind and the position of substituent in the 1,2-benzisothiazol-3(2H)-one molecules).

The results of the investigations indicate that a linear relationship was obtained in concentration ranges of 40–80% methanol and acetone and 20–60% acetonitrile. The concentration of acetone or methanol in mobile phase less than 40% (and 20% for acetonitrile) caused the compounds to be eluted near the starting line, and with concentration of organic solvents above 80% (acetone, methanol) or 60% (acetonitrile) the compounds were localised near the front of the mobile phase.

The RP-TLC data in Table 2 show that R_{Mo} values from systems with water–acetone and the ones for water–acetonitrile are very similar. The correlation between the R_{Mo} values is described by the equation:

$$R_{\text{Mo acetone}} = 1.11459\,(R_{\text{Mo acetonitrile}}) - 0.174152;\ (r = 0.9830;\ s = 0.0613)$$

The slope and the intercept, very close to 1 and 0, respectively, showed that in this case the nature of organic modifier does not significantly affect parameters R_{Mo} in the RP-TLC equations. The R_{Mo} values obtained using the water–methanol mobile phases are different from these obtained with acetone and acetonitrile as components of mobile phases and are correlated with them by the following equations:

$$R_{\text{Mo methanol}} = 1.21002\,(R_{\text{Mo acetone}}) + 0.0601387;\ (r = 0.9792;\ s = 0.0838)$$

$$R_{\text{Mo methanol}} = 1.39814\,(R_{\text{Mo acetonitrile}}) - 0.235607;\ (r = 0.9978;\ s = 0.0274)$$

The R_{Mo} values for the water–methanol system are higher than those for the water–acetone and water–acetonitrile systems. These differences can be explained by better solubility of the tested compounds in acetone and acetonitrile than in methanol and by the differences in eluting power of organic modifiers used.

The data given in Table 2 show that there is a linear relationship between b values and R_{Mo} of RP-TLC equations (the equations describing such a linear relationship for the 1,2-benzisothiazol-3(2H)-ones are presented in Table 3). The R_{Mo} values are chromatographic parameters which described the partitioning between non-polar stationary phases and polar mobile phases; the slope from equation $R_M = R_{Mo} + b \cdot C$ indicated a change of solubility of tested compounds in mobile phase. The relationship is described by the following equation: $R_{Mo} = B \cdot b + a$, where b is slope and R_{Mo} is intercept from Table 2.

Based on the R_M values (Table 2) the tested compounds can be in accordance with the increasing lipophilicity (for each organic modifier) described as follows: BIT<5-F-BIT<6-BIT<4-F-7-CH_3-BIT<5-Cl-BIT<6-F-5-CH_3-BIT<5-Br-BIT.

The results from Table 2 show differences between the R_M values of investigated compounds. The R_M values for mobile phases containing acetone or acetonitrile are very similar whereas for the water–methanol system R_M values are higher.

The position of the same substituent (isomeric monofluoro derivatives—compounds **2** and **5** and fluoromethyl isomers—compounds **3** and **4**) significantly affect the retention.

Compound **2** (6-F-BIT) R_{Mo} values 1.612 (acetone), 1.541 (acetonitrile), 1.902 (methanol) is more hydrophobic than compound **5** (5-F-BIT) R_{Mo} = 1.478, 1.469 and 1.851, respectively. A methyl group in the molecule increases the lipophilic properties,

Table 3
Relationship between R_{Mo} (intercepts) and b (slopes) of RP-TLC equations (Table 2) $R_{Mo} = B \cdot b + a$

RP-TLC Mobile phase	B	a	r	s
Water–acetone	−90.3219	−0.837693	−0.9953	0.0323
Water–acetonitrile	−108.876	−1.86494	−0.9974	0.0213
Water–methanol	−108.624	−1.16817	−0.9971	0.0316

R_{Mo} for 6-F-5-CH_3-BIT (compound **3**) for each organic modifiers (1.992, 1.989, 2.530, respectively) and they are higher than for 6-F-BIT (compound **2**).

Moreover, the results from Table 2 show considerable differences of the lipophilic properties of isomeric fluoromethyl derivatives (compounds **3** and **4**). The substituents in positions 6- and 5- increase the R_{Mo} values in each tested mobile phases in comparison with 4-F-5-CH_3 substituted isomer. These differences in isomeric compounds can been explained by their different solubility in the mobile phases. For 5-substituted 1,2-benzisothiazol-3(2H)-one derivatives, lipophilicity increases with an increase in molecular mass of substances. The R_{Mo} values obtained for 1,2-benzisothiazol-3(2H)-one (compound **1**) with the water–acetone and water–acetonitrile systems (1.309 and 1.345, respectively) are approximately similar to those described in the literature [21] log $P_{oct/w}$ value (1.29) determined by the ''shake-flask'' method.

The performance investigations showed that the pH of the mobile phase within a pH range 2–11 did not vary in R_M values. These results confirmed the early observations described by Dross et al. [16] that in the case of weak acids there is no need for a pK correction for calculations of R_M values.

4. Conclusion

Reversed-phase thin-layer chromatography method (RP-TLC) proved to be a reliable and accurate method of describing the lipophilic nature of 1,2-benzisothiazol-3(2H)-one derivatives. Good correlations between the retention parameters obtained by RP-TLC, and the concentration of an organic modifier (acetone, acetonitrile, methanol) in the mobile phase were obtained for the studied 1,2-benzisothiazol-3(2H)-one derivatives.

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