

Correlation between different lipophilicity parameters and antimycobacterial activities of 2-hydroxyacetamides

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ABSTRACT

Background: Several di-substituted 2-hydroxyacetamides have shown promising antimycobacterial activity. In an attempt for further development of these compounds which are easily synthesized, investigation of the relationship between their physicochemical properties and inhibitory activities appeared of interest. Since lipophilicity as a physicochemical parameter plays an important role on biological activity and drug design, the relationship between partition coefficient as a measure of lipophilicity and antimycobacterial activity of the studied compounds was investigated.

Methods: Partition coefficients of 2-hydroxyacetamides **a-n** measured by the shake flask ($\log P$), reversed-phase high-performance liquid chromatography (RP-HPLC $\log k$) methods and by theoretical calculation (ClogP) were compared and the relationship between the resulting values and antimycobacterial activities of the studied compounds determined by Alamar Blue Assay was investigated.

Results: When all compounds were included in the correlation analyses, no significant relationship between MICs (minimum inhibitory concentrations) and lipophilicity parameters was observed, but HPLC values showed significant correlation with ClogP. The best correlation between MICs and lipophilicity parameters for di-substituted amides **g-n** which were more active than mono-substituted amides were RP-HPLC based $\log k$ and for mono-substituted amides were shake flask $\log P$ values which in both cases were not significant. When deviating compounds **m** and **n** were excluded from the correlation analyses, for di-substituted amides, correlation between MICs and $\log k$ as well as correlation between values of partition coefficient by different methods became significant.

Conclusion: Results of this investigation indicates that both ClogP and $\log k$ are equally suitable for prediction of partition coefficient.

Keywords: 2-Hydroxyacetamides, Shake flask method, ClogP, RP-HPLC, Di-substituted amides, Mono-substituted amides

INTRODUCTION

In the previous paper the synthesis of several 2-hydroxyacetamides **a-n** which were designed on the basis of similarities with n-glycolylmuramic acid residues of mycobacterium tuberculosis cell wall and their antibacterial activity in comparison with ethambutol as the reference drug determined by Alamar Blue Assay was described (1). Of the prepared compounds mostly di-substituted amides **g-n** bearing hydrophilic groups showed higher activity. For further development of these compounds which are easily synthesized and some of them showed promising antimycobacterial activity, investigation of the relationship between their physicochemical

properties and inhibitory activities appeared of interest. Since lipophilicity as a physicochemical parameter plays an important role on biological activity and drug design, and there are many reports on the relationship between antimicrobial activity and lipophilicity of antimicrobial agents of different classes (2), the partition coefficient of the studied compounds as a measure of lipophilicity was investigated by different methods.

One of the most widely used methods for determination of partition coefficient which has good correlation with biological activity is the shake flask method. However this method is laborious, time-consuming, costly, and has

limitation to log P values between -2 and +4 (3). In addition, impurities may adversely affect the results. Computational methods have also been found useful for estimation of log P values (4) provided that all values for all fragments of the molecule are available and connectivity pattern are included in the data base. Otherwise there will be large differences between the values obtained by this method and experimental values. Reversed-phase HPLC techniques (RP-HPLC) which are simple, rapid and do not require pure compound for analyses (5) have been the method of choice for determination of log P values because the results are of good precision and reproducible. In this method, log k which are obtained by extrapolation of values from the binary phases to 100% water, reflects polar-non-polar partitioning of the shake flask method. In this investigation, partition coefficients of the compounds **a-n** were determined by the shake flask (6), reversed-phase high-performance liquid chromatography (7), and calculation methods (8-9) and correlation of each method with others as well as with antimycobacterial activity of the tested compounds (1) were investigated.

EXPERIMENTAL

Instrumentation

The HPLC system was Hitachi-Merck, model HSM 7000 consisting of a pump model L-7100, a Rheodyne manual injector and a Hitachi-Merck UV detector model L7420. The stationary phases were a RP-C18 (5 μ M) column, 250 mm \times 4 mm I.D. A Shimadzu UV spectrophotometer model 160 A was used to measure the concentrations of the solutes in the shake flask method.

Chemicals

Compounds **a-n** were synthesized according to the methods previously described (1) and their identity and purity were checked by Mass Spectra, 1 H NMR, IR, and TLC (Silica gel). Methanol (HPLC grade) and other chemicals were purchased for Merck (Darmstadt, Germany). All chemical and reagents were analytical grades. Purified water was prepared by Water's LQ10.

Measurement of Log P by the Shake-flask Method (6):

A mixture of equal volumes of n-octanol and water was shaken at 25 \pm 1 $^\circ$ C for 24 hours on a mechanical shaker in order to saturate each phase with the other phase. The mixture was then allowed to stand for 24 hrs at 25 \pm 1 $^\circ$ C for separation of phases.

For each compound, a stock solution with a concentration of 0.01 mol per liter in water pre-

saturated with n-octanol was prepared. Duplicate test vessels containing a mixture of equal volume of stock solution and n-octanol (pre-saturated with water) were shaken on a vortex shaker for 5 minutes. Phase separation was then carried out by centrifugation of the vessels for 3 minutes at 4000 g (25 \pm 0.5 $^\circ$ C) and concentration of the solute in water phase (C_{water}) was measured from their absorbencies at 205 nm by a UV spectrophotometer. The partition coefficient was calculated using the following equation:

$$\text{Log } P_{o/w} = \text{Log}(C_{\text{n-octanol}}/C_{\text{water}})$$

All the measurements were carried out at room temperature (25 \pm 1 $^\circ$ C). In order to check the performance of the method, aniline was used as a reference substance.

Measurement of log k by the HPLC method (7)

The mobile phases were made by mixing methanol with water in proportions of 50:50, 30:70 and 10:90 (v/v). The flow rate was 1ml/min.

An aqueous solution of urea was used for measurement of the column dead time (t_0). Each chromatographic run was repeated at least three times. The retention times (t_r), which measured at room temperature in triplicate, had a relative SD less than 1.5. For each compound, capacity factors (k) was calculated at three compositions of methanol:water (50:50, 30:70, 10:90) using the following equation:

$k = (t_r - t_0)/t_0$. The log k Values were plotted against the volume percent of methanol in the eluent and based on the established linear relationship (in each case, the correlation coefficient was above 0.99), the values of log k corresponding to 100% water were obtained by extrapolation for each compound (Table 1).

Theoretical calculation of log P (ClogP)

Theoretical calculations of log P values were carried out using an online version of ALOGPS 2.1 software (10).

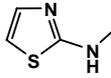
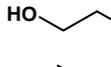
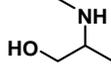
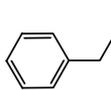
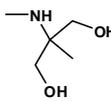
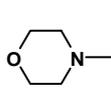
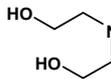
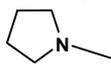
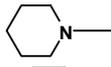
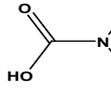
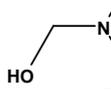
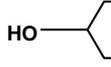
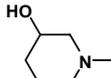
Statistical Analyses

Spearman's rank correlation test was used to examine the significance of correlation between partition coefficients of the studied compounds derived by different methods as well as with their MIC values. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The values of partition coefficient of the studied compounds derived by the shake-flask (log P), and RP-HPLC (log k) methods, and calculation (Clog P) and their MIC values are listed in table 1.

Table 1. Structures, inhibitory activities (MIC), and lipophilicity parameters of 2-hydroxyacetamides.

		MIC ^a (μg/mL)	Shake flask log P ^b	ClogP ^c	log k ^d
<i>a</i>		100	0.13	-0.27	1.09
<i>b</i>		87.5	-0.98	-1.57	-0.63
<i>c</i>		29.5	0.02	-0.73	0.37
<i>d</i>		74	0.98	0.86	1.98
<i>e</i>		67.5	-1.29	-1.06	-0.08
<i>f</i>		57	-1.47	-0.66	-0.06
<i>g</i>		12.5	-1.22	-1.17	-0.48
<i>h</i>		25.5	-0.99	-0.36	1.00
<i>i</i>		38.75	-0.14	0.20	1.17
<i>j</i>		13.75	-1.09	-0.56	0.45
<i>k</i>		15.75	-1.11	-0.80	0.00
<i>l</i>		14.25	-0.35	-0.61	n.m*
<i>m</i>		33.25	-0.40	-1.89	0.25
<i>n</i>		60	-1.82	-0.69	0.51

^a Minimum inhibitory concentration, ^b Logarithm of octanol-water partition coefficient measured by shale-flask method
^c Theoretically calculated log P by ALOGPS software, ^d logarithm of chromatography capacity factor, *not measured

Table 2. correlation of lipophilic parameters and MIC of 2-hydroxyacetamides.

correlation	Compounds a-n		Compounds a-f		Compounds g-n		Compounds g-n excluding i & m	
	ρ ^e	P-value	ρ	P-value	ρ	P-value	ρ	P-value
MIC ^a & Shake flask log P ^b	0.36	0.19	0.52	0.23	0.14	0.74	0.71	0.11
MIC & Clog P ^c	0.27	0.34	0.34	0.45	0.19	0.65	0.77	0.07
MIC & log k	-0.03	0.92	-0.18	0.67	0.68	0.09	0.90	0.03
Shake flask log P & Clog P	0.55	0.04	0.75	0.05	0.45	0.26	0.83	0.04
Shake flask log P & log k ^d	0.32	0.27	0.43	0.34	0.50	0.25	1.00	-
Clog P & log k	0.64	0.01	0.61	0.15	0.86	0.01	1.00	-

^a Minimum inhibitory concentration, ^b Logarithm of octanol-water partition coefficient, ^c Theoretically calculated log P by ALOGPS software, ^d logarithm of chromatography capacity factor, ^e Spearman correlation coefficient

An examination of data revealed that in general there were not any correlation between different partition coefficient values and antimycobacterial activity of compounds and MIC values of disubstituted amides **g-n** are higher than those of mono substituted amides **a-f**. Therefore compounds were grouped into di- and mono-substituted amides and in each group correlation of lipophilicity parameters by different methods with each other and with MICs were investigated (table 2). For mono- substituted amides, no significant correlation was found between partition coefficient values and MIC of compounds. In the case of di-substituted amides, a rather good correlation was observed between MICs and log *k* values ($\rho=0.68$), but it could be considered significant only at $p < 0.1$. While there were good and significant correlation between log *k* and log *P* values obtained by calculation when all or di-substituted amides with and without compounds *j* and *m* were included in correlation analyses, the correlation between log *k* and shake-flask log *P* could be considered significant only for di-substituted amides without deviating compounds *j* and *m*. For mono-substituted amides **a-f** only lipophilicity parameters derived by the shake-flask method and calculation showed a good correlation.

While higher activities of di-substituted amides **g**, and **j-1** than those of **h** and **i** could be related to their higher hydrophilicities, compounds **m** and **n** which were more hydrophile than the compound **i** showed less and comparables activities, respectively. When these deviating compounds were not included in the correlation, all correlations related to di-substituted amides improved and correlation between activity and log *k* as well as correlations between different lipophilicity parameters could be considered statistically significant.

CONCLUSION

Of three octanol-water partition coefficient values measured directly by the shake flask method, indirectly by the reversed-phase high-pressure liquid chromatography (RP-HPLC), and by

calculation (Clog *P*), there was only significant correlation between log *k* obtained by HPLC and MIC when di-substituted amides with exception of compounds *j* and *m* were included in correlation analyses. This finding suggests factors other than lipophilicity such as steric and electronic might have influences on the activity. In agreement with results of reports on; high correlation between Clog*P* and log *k* values of 2-benzylthioderivatives as potential antituberculous drugs(7), several pharmaceutical substances in suppository base-phosphate buffer system(11), and antimycotic 2-substituted benzothiazoles (12), there was significant correlation between log *k* and Clog*P* for all as well as di-substituted amides *g-n* of this study with and without deviating compounds *j* and *m*. However, in contrast to results of investigations in which high correlation between log *k* and shake flask log *P* values for; a high-throughput hydrophobicity determination (5), pyrimidinic nucleoside derivatives(13) and several herbicides(14) have been reported, no such correlation was found in this investigation which could be due to the difficulties inherent in the shake-flask method of log *P* determination which have resulted in different values for a particular analyte in the literature. A variation of 0.3 in the reported log $P_{\text{octanol/water}}$ equates to a two fold difference in the partition coefficient. Shake-flask method suffers inaccuracies from several sources (5). Impurities may adversely affect the results and necessity of assurance that concentrations of the analytes are well below the critical micelle concentration (CMC) which for some compounds may be as low as 10^{-5} M. In addition to these problems, for some compounds other errors such as adsorption onto glass walls may interfere with determination.

To conclude, results of this investigation indicate that both Clog *P* and log *k* are equally suitable for prediction of partition coefficient.

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REFERENCES

1. Daryae F, Kobarfard F, Khalaj A, Farina P, Larijani Z. Synthesis and antimycobacterial activity of 2-hydroxyacetamides. DARU 2005; 13: 94-99.
2. Klimešová V, Svoboda M, Waisser K, Kaustová J, Buchta V, Králová K. Synthesis of 2-benzylthiopyridine-4-carbothioamide derivatives and their antimycobacterial, antifungal and photosynthesis-inhibiting activity. Eur J Med Chem 1999; 34: 433-440.
3. Van De Waterbeemd H, Lennernäs H, Artursson P. Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability. Wiley-VCH, Weinheim, Germany, 2004, p 26.
4. Leo A. Calculating log P_{oct} from structures. Chem Rev. 1993; 93: 1281-1305.

5. Donovan SF, Pescatore M C. Method for measuring the logarithm of the octanol-water partition coefficient by using short octadecyl-poly(vinyl alcohol) high-performance liquid chromatography columns. *J Chromatogr A* 2002; 952: 47-61.
6. Fugita T, Iwasa J, Hansch C. A new substituent constant, π , derived from partition coefficients. *J Am Chem Soc* 1964; 86: 5175-5180.
7. Kastner P, Klimes J, Velenovska P, Klimešová V, Lipophilicity characterization by reversed-phase HPLC of potential antituberculotics. *J Liq Chromatogr Rel Technol* 2002; 25: 2849-2856.
8. Tetko I V, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko EV, Zefirov N S, Makarenko AS, Tanchuk V.Y, Prokopenko VV. Virtual computational chemistry laboratory-design and description. *J Comput Aid Mol Des* 2005; 19: 453-456.
9. Tetko IV. Computing chemistry on the web. *Drug Discov Today* 2005; 10: 1497-1500.
10. VCCLAB. Virtual Computational Chemistry Laboratory, <http://www.vcclab.org>, 20/03/2005
11. Dimitrova B, Doytchinova I, Zelatkova, M., Reversed-phase high performance liquid chromatography for evaluating the distribution of pharmaceutical substances in suppository base-phosphate buffer system, *J Pharm and Biomed Anal* 2000 ;23 :955-964
12. Matysiak J, Niewialdony A, Senczyna B, Zabinska A, Rozylo JK. Relationships between LC retention, octanol-water partition coefficient, and fungistatic properties of 2-(2,4-dihydroxyphenyl) benzothiazoles. *AOAC Int* 2004; 87: 579-586.
13. Teijeiro SA, Moroni GN, Motura M I, Brinon MC, Lipophilic character of pyrimidinic nucleoside derivatives: correlation between shake flask, chromatographic (RP-TLC and RP-HPLC) and theoretical methods. *J Liq Chromatogr Rel Technol* 2000; 23:855
14. Paschke A, Neitzel PL, Walther W, Schürman G. Octanol/ water partition coefficient selected herbicides: determination using shake-flask method and reversed-phase high-performance liquid chromatography. *J Chem Eng Data* 2004; 49: 1639