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Research Article

The Determination of Partition Coefficient of 6-Mercaptopurine Derivatives by Thin Layer Chromatography

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Mercaptopurine and its derivatives are used in the treatment of leukemia. To estimate their lipophilicity, a simple and novel thin layer chromatography method was developed. The mobile phase was the mixture of acetonitrile and water. The acetonitrile content varied by 5% from 50% to 80%. The linear relationship between $\log P$ and R_{m0} for substances with known lipophilicity was found. The lipophilicity of purine derivatives was worked out from the calibration curve. The most lipohilic compound was methylazathioprine (0.64).

1. Introduction

Lipophilicity describes the affinity of the compound to organic phase in a two-phase system of two immiscible liquids. The organic phase is a nonpolar solvent. The polar phase is water. Many biochemical, pharmacological, and toxicological processes involving the drug action strongly depend on lipophilicity [1, 2]. The partition coefficient can be estimated by a classical shake-flask method. However, it has many disadvantages. For instance, it is time-consuming and requires relatively large amounts of solutes and solvents. Besides, the $\log P$ is limited to the range between −2 and +4. It implies that this method cannot be applied to either very hydrophilic or very hydrophobic compounds [2–4].

In 1965, Boyce and Milborrow proved that retention factor R_m is useful for determining the lipophilicity of the substances [5]. The shake-flask method was successfully replaced by the reversed-phase high-performance liquid chromatography (RP-HPLC) and the high-performance thin layer chromatography (RP-HPTLC). The TLC technique has many significant advantages. It is simple and less laborious. The amount of the sample of a substance is very small and it does not have to be very pure [5]. We established the linear relationship between the $\log P$ and R_{m0} value for the compounds with known lipophilicity [6]. This relationship exists whenever the separation mechanism is the partition of the analyte between mobile and stationary phases. Having determined the R_{m0} for the investigated compounds, we calculated the $\log P$ from the linear relationship for the compounds with known lipophilicity [7].

The analysed substances are 6-mercaptopurine (6-MP) and its derivatives, that is azathioprine (AZA), methylazathioprine (MAZA), and 6-metylmercaptopurine (6-MMP) (Figure 1). Azathioprine and 6-mercaptopurine are prodrugs widely used in the treatment of various diseases including acute lymphoblastic leukemia [8]. Azathioprine can be administered simultaneously with diclofenac in the treatment of patients with rheumatoid arthritis [9]. 6-methylmercaptopurine is a methylation product of 6-mercaptopurine and it is inactive [8].

2. Experimental

2.1. Thin Layer Chromatography. The chromatographic separation of 6-MP, 6-MMP, AZA, and MAZA was carried out on HPTLC RP-18, F254s glass plates (10 × 10 cm, Merck, Germany). The mixture of acetonitrile-water (v/v) was used as the mobile phase. The content of acetonitrile (POCH, Gliwice, Poland) varied in 5% increments from 50% to

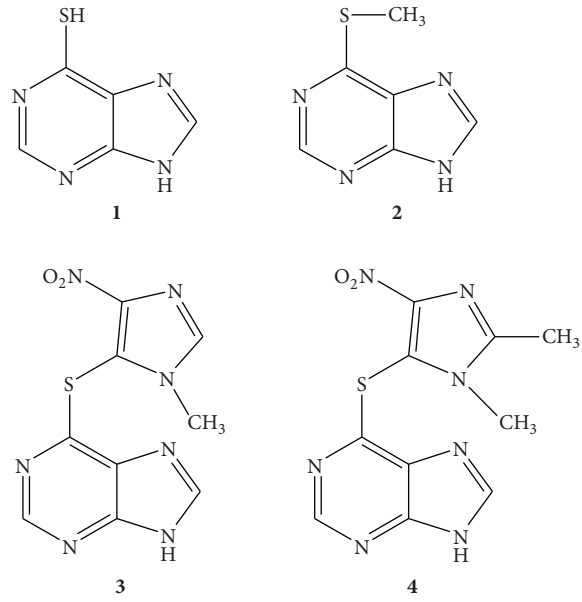

Figure 1: The structures of investigated compounds (6-MP **1**, 6-MMP **2**, AZA **3**, and MAZA **4**).

80% [6]. All experiments were performed in the ambient temperature (22 ± 1°C).

The 1% solutions (m/v) of investigated compounds in methanol (POCH, Gliwice, Poland) were applied at the start line with a Hamilton syringe (10 μL). The developed chromatograms were dried and the spots were visualized with UV_{254} light. The R_f values were calculated by use of

$$R_f = \frac{a}{b}, \tag{1}$$

where "a" is the distance from baseline travelled by solute and "b" is the distance from baseline travelled by solvent.

Their R_f values were used to calculate the parameter R_m according to Bate-Smith and Westal equation (2) [10]:

$$R_m = \log\left(\frac{1 - R_f}{R_f}\right). \tag{2}$$

The R_m values were extrapolated to an acetonitrile concentration of zero (R_{m0}) [11] by

$$R_m = R_{m0} + aC, \tag{3}$$

where C is the concentration of acetonitrile (%, v/v) in the mobile phase. The value of the slope (a) indicates the rate at which the solubility of the compound increases in the mobile phase [12].

2.2. The Calculation of Lipophilicity. The lipophilicity of the investigated compounds, expressed as the $\log P$, was calculated from

$$\log P = B + aR_{m0}, \tag{4}$$

Table 1: The R_{m0} values for investigated compounds.

Compound	R_{m0} ± SD	RSD [%]
MAZA	0.506 ± 0.008	1.58
AZA	0.263 ± 0.008	3.04
6-MP	0.433 ± 0.037	8.54
6-MMP	0.452 ± 0.020	4.42

where R_{m0} is the R_{m0} value for a substance with known $\log P$; "a" and "B" are the slope and intercept, respectively. The regression equation was $\log P = 1.265R_{m0}$. The S_a value was 0.176; the intercept was not statistically significant.

All measurements were performed in triplicate. The precision was within the range 1,56–9,42%. The method was linear (r = 0.998-0.999).

The compounds with known lipophilicity were isatin (Fluka, Germany), N-(2,6-dichlorophenyl)-acetamide m.p. 190-191°C (Medical University of Lublin, Poland), N-(2,4-dichlorophenyl)-acetamide m.p. 141-142°C (Medical University of Lublin, Poland), 3,4-dichloroaniline (Aldrich, UK), 2,6-dichloroaniline (Aldrich, UK), and p-nitrophenol (POCH, Gliwice, Poland) [13].

The R_{m0} for the above 6-mercaptopurine derivatives is calculated from (3). The R_{m0} and $\log P$ values are listed in Tables 1 and 2, respectively.

3. Results and Discussion

The main aim of this study was to determine the $\log P$ (logarithm of partition coefficient) values by the novel and simple thin layer chromatography method.

The experimental data revealed a linear relationship between R_m and the concentration of the organic modifier (acetonitrile). The R_m value decreases with the increase in

Table 2: The log P value for investigated compounds.

Compound	Log P	Milog P[a]	Alog P[b]
MAZA	0.64	0.56	0.71
AZA	0.33	0.50	0.22
6-MP	0.55	0.34	0.52
6-MMP	0.57	0.54	0.64

[a] [17], [b] [18].

concentration of modifier in the mobile phase. In all cases, the TLC equations were linear. The intercepts (R_{m0}) in these equations can be considered as a measure of the partitioning compounds between the stationary phase and the mobile phase in 0% organic solvent. It can be considered as the balance between the interactions with the nonpolar phase and the interactions with the polar phase [12].

The methylated derivatives (MAZA and 6-MMP) are more lipophilic than AZA and 6-MP. The log P value for AZA is lower than the one for 6-MP (Table 2). It is caused by $-NO_2$ group which is an electron-donor substituent. It decreases the lipophilicity of the compound [14]. It is caused by the presence of three additional nitrogen atoms and two oxygen atoms in AZA molecule. The AZA is a prodrug of 6-MP. AZA within two hours is transformed into 6-MP [15]. The removal of nitroimidazole ring from the AZA molecule results in the increase in the lipophilicity.

The introduction of the methyl group to AZA molecule results in the increase in the lipophilicity of MAZA. The increase in lipophilicity of MAZA makes it a new potential anticancer agent. *In vitro* studies on human cancer cells and *in vivo* studies in mice with transplanted cancer cells have confirmed this activity [16].

The log P values calculated by online software [17, 18] are similar to the ones determined empirically. However, some differences can be observed (Table 2). The experimental log P values are of the same order as the values of log P determined by software. The major difference is observed for AZA and 6-MP between the Milog P (calculated log P based on group contribution) and the experimental data (Table 2). The Alog P (the calculated log P based on atoms contribution) for 6-MP value is similar to the experimental one. However, Lucangioli et al. also observed similar situation for AZA. The experimental values of log P estimated by different techniques differed significantly from the one determined by the software (0.10–0.68 versus −0.54) [19]. The differences between computational and experimental values for AZA, MAZA, and 6-MP were also observed by Hoffmann et al. [20].

4. Conclusion

The elaborated HP-TLC method is a novel one that enables us to determine the lipophilicity of the 6-MP derivatives. The results of the present study are in accordance with the values obtained from the software. However, the computational data cannot replace the experimental ones.

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