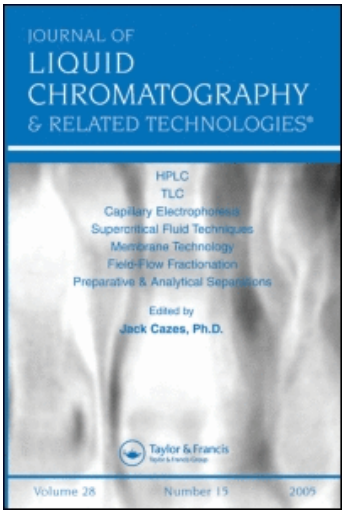


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# EFFECT OF ORGANIC MODIFIER ON THE LIPOPHILICITY OF ANTIPROLIFERATIVE ACTIVE 4-(5-AMINO-1,3,4-THIADIAZOL-2-YL)BENZENE-1,3-DIOLS BY REVERSED-PHASE OVERPRESSURED LAYER CHROMATOGRAPHY

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# EFFECT OF ORGANIC MODIFIER ON THE LIPOPHILICITY OF ANTIPROLIFERATIVE ACTIVE 4-(5-AMINO-1,3,4-THIADIAZOL-2-YL)BENZENE-1,3-DIOLS BY REVERSED-PHASE OVERPRESSURED LAYER CHROMATOGRAPHY

**Andrzej Niewiadomy, Alicja Skrzypek, Joanna Matysiak, and Marek Studziński**

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□ *The retention behavior of compounds of antiproliferative activity from the 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols group by OPLC was investigated. RP-18 stationary phase and five different organic modifiers were used. In the case of ethanol as an organic solvent the highest concentration of water in the eluent can be applied. On the base of the linear relationship between the retention and the concentration of organic solvent, various lipophilicity descriptors were determined. Moderate correlations between the chromatographic parameters and the calculated log P values according to the Villar approach were found. The $R_{Mw}$ parameters were compared with the antiproliferative activity of compounds against human cancer cell T47D (breast cancer) to find QSAR equations. The best results for ethanol as the organic solvent were found.*

**Keywords** 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols, lipophilicity, OPLC, organic modifier, QSAR, RP-18

## INTRODUCTION

Lipophilicity is the descriptor of the molecule applied in numerous QSAR studies. This parameter can be used for modeling of the permeation process through biological membranes and to reach active sites in bioreceptors.[1] Relative lipophilicity values determined in a set of congeneric compounds may help explain differences in bioactivity and design new, potentially more effective analogues.

Reversed phase high performance liquid chromatography (RP-HPLC) is commonly applied for this purpose.[2] Lipophilicity descriptors have been calculated by assuming the linear TLC dependence of $R_M$ on $\varphi$,

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expressed by the Soczewiński-Wachtmeister equation (1):[3]

$$R_M = R_{Mw} + S\varphi \quad (1)$$

$R_{Mw}$ is the theoretical value of $R_M$ for pure water as the mobile phase, widely used as a measure of lipophilicity in the structural activities studies. S is the slope of the regression curve and reflects the difference between the $R_M$ values for pure water and the pure modifier ($\varphi = 0$ and $\varphi = 1$, respectively) indicating the mechanism of retention and eluting strength.

One of the decisions is in choice of the chromatographic system is which mobile phase is appropriate. Methanol is, by far, the most commonly used organic modifier in the determination of partition coefficient by RP chromatography. It results from the most water-like structure of all that is used for RP-HPLC solvents. It is capable of hydrogen bond acceptance and donation, and its solubility parameter is most similar to water in comparison to acetonitrile or tetrahydrofuran. The effect of the stationary phase is also smaller with methanol.[4] McCornic & Karger have shown that adsorption of acetonitrile and tetrahydrofuran to alkyl bonded silica occurs to a greater extent than with methanol.[5] Other studies show that the plots of log k versus $\varphi$ of methanol generally has significantly less curvature than tetrahydrofuran or acetonitrile. It is consistent with the solubility parameters for these solvents. Thus, the use of methanol minimizes errors in extrapolation to 0% organic. Other investigators have found a superior octanol-water log P correlation for the studied set of compounds using methanol rather than acetonitrile or tetrahydrofuran as the modifiers.[6]

The aims of the present work were investigations of influence of the type of organic modifier on the lipophilicity and specific hydrophobic surface area of 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols set that posses antiproliferative activity against cancer cells.[7,8] The chromatographic descriptors obtained in this way were correlated with their biological activity. Reversed Phase Overpressure Layer Chromatography (RP OPLC) was applied for these purposes.[9–11]

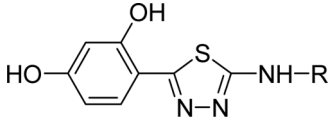


## EXPERIMENTAL

### Chemicals

The N-substituted 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols used (Table 1) were synthesized in the Laboratory of Chemistry Department at University of Life Sciences in Lublin, Poland and reported previously.[7,8] The organic modifiers and other chemical reagents were of analytical reagent grade (Merck, Germany).

**TABLE 1** Structure, Antiproliferative Activity (log $1/ID_{50}$) of N-substituted 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols and Parameters $R_{Mw}$ (intercept), S (slope) of the Linear Dependences in Eq. (1) for Methanol and Ethanol as the Organic Modifiers




| | | | | Methanol | | | | | Ethanol | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | R | log $1/ID_{50}$ | log P | $R_{Mw}$ | −S | n | r | s | $R_{Mw}$ | −S | n | r | s |
| 1. | $CH_3$- | −2.166 | −0.12 | 2.2483 | 3.345 | 6 | 0.9746 | 0.0108 | 1.7310 | 3.799 | 5 | 0.8828 | 0.0521 |
| 2. | $CH_3(CH_2)_3$- | –[a] | 0.87 | 3.3422 | 4.245 | 6 | 0.9946 | 0.0108 | 2.2122 | 3.513 | 5 | 0.8125 | 0.0412 |
| 3. | $(CH_3)_3C$- | −2.215 | 0.58 | 3.1112 | 3.959 | 5 | 0.9924 | 0.0112 | 2.1620 | 3.676 | 6 | 0.8954 | 0.0465 |
| 4. | $CH_3(CH_2)_5$- | –[a] | 1.53 | 4.0360 | 4.683 | 6 | 0.9846 | 0.0183 | 3.0596 | 4.392 | 6 | 0.9321 | 0.0378 |
| 5. | cyclohexyl- | −1.165 | 0.94 | 3.8660 | 4.893 | 6 | 0.9959 | 0.0094 | 2.9894 | 4.828 | 5 | 0.9259 | 0.0395 |
| 6. | $CH_2=CHCH_2$- | −2.039 | 0.43 | 2.8859 | 3.929 | 6 | 0.9822 | 0.0196 | 2.1428 | 4.100 | 6 | 0.8931 | 0.0470 |
| 7. | $C_6H_5$- | −1.127 | 1.75 | 4.0878 | 5.114 | 6 | 0.9692 | 0.0257 | 2.7779 | 4.582 | 7 | 0.9311 | 0.0431 |
| 8. | 2-$CH_3$-$C_6H_4$- | −1.303 | 1.95 | 4.0914 | 5.143 | 6 | 0.9647 | 0.0275 | 2.7806 | 4.550 | 7 | 0.9348 | 0.0420 |
| 9. | 2,6-di-$CH_3$-$C_6H_3$- | −1.731 | 2.19 | 4.2032 | 5.312 | 6 | 0.9725 | 0.0243 | 2.8398 | 4.585 | 7 | 0.9354 | 0.0418 |
| 10. | 2-$C_2H_5$-$C_6H_4$- | –[a] | 2.28 | 4.2212 | 5.222 | 6 | 0.9718 | 0.0246 | 2.9589 | 4.594 | 6 | 0.9368 | 0.0365 |
| 11. | 2-F-$C_6H_4$- | −1.280 | 1.55 | 3.8273 | 4.742 | 6 | 0.9759 | 0.0227 | 2.7481 | 4.528 | 7 | 0.9367 | 0.0414 |
| 12. | 3-F-$C_6H_4$- | −1.232 | 1.83 | 4.0872 | 4.954 | 6 | 0.9828 | 0.0193 | 2.9186 | 4.614 | 7 | 0.9398 | 0.0404 |
| 13. | 4-F-$C_6H_4$- | −1.141 | 1.52 | 4.0912 | 5.046 | 6 | 0.9740 | 0.0236 | 2.9908 | 4.838 | 7 | 0.9370 | 0.0413 |
| 14. | 2-Cl-$C_6H_4$- | −1.240 | 1.87 | 3.9275 | 4.718 | 6 | 0.9676 | 0.0264 | 2.9780 | 4.809 | 7 | 0.9259 | 0.0446 |
| 15. | 3-Cl-$C_6H_4$- | −1.086 | 2.12 | 4.2444 | 4.873 | 6 | 0.9772 | 0.0221 | 2.9837 | 4.347 | 7 | 0.9364 | 0.0415 |
| 16. | 4-Cl-$C_6H_4$- | −1.109 | 2.24 | 4.3115 | 4.936 | 6 | 0.9858 | 0.0175 | 3.0604 | 4.456 | 7 | 0.9324 | 0.0427 |
| 17. | 2,4-di-Cl-$C_6H_3$- | −0.624 | 2.40 | 4.7479 | 5.295 | 6 | 0.9697 | 0.0255 | 3.6880 | 5.234 | 5 | 0.9964 | 0.0093 |
| 18. | 2,5-di-Cl-$C_6H_3$- | −1.006 | 2.69 | 4.6910 | 5.418 | 5 | 0.9978 | 0.0052 | 3.2086 | 4.551 | 6 | 0.9394 | 0.0358 |
| 19. | 4-Br-$C_6H_4$- | −1.097 | –[b] | 4.0691 | 4.670 | 6 | 0.9853 | 0.0134 | 3.0344 | 4.296 | 6 | 0.9211 | 0.0407 |
| 20. | 4-$CH_3O$-$C_6H_4$- | –[a] | 1.78 | 3.2732 | 3.757 | 5 | 0.9881 | 0.0140 | 2.7532 | 4.148 | 6 | 0.9162 | 0.0418 |
| 21. | 4-$CH_3CH_2O(C=O)$-$C_6H_4$- | –[a] | 1.64 | 3.2690 | 3.547 | 6 | 0.9765 | 0.0225 | 3.0431 | 4.702 | 6 | 0.9183 | 0.0413 |
| 22. | $C_6H_5$-$CH_2$- | –[a] | 1.54 | 3.3090 | 4.070 | 5 | 0.9884 | 0.0138 | 2.5294 | 4.290 | 6 | 0.9198 | 0.0410 |

[a]Experiment was not performed.
[b]Descriptor is not obtained at AM1 level of calculation.

## Chromatography

OPLC studies were performed on the precoated plates of RP-$18_{254S}$ ($10 \times 20$) (Merck, Darmstadt, Germany). One μL samples of the solutes ($0.5\,mg \cdot cm^{-3}$ in methanol) were spotted with the Linomat 5 applicator (Camag). The chromatograms were developed over a distance of 7.0 cm in the automatic Personal OPLC BS-50 chromatograph (OPLC-NIT, Budapest, Hungary). Water-organic modifiers were applied as the mobile phases in the following concentration range: methanol: (MET) 0.50–0.75; acetone (ACT): 0.40–0.65; ethanol (ETH) 0.30–0.60; acetonitrile (ACN): 0.35–0.55; and tetrahydrofuran (THF): 0.35–0.60 v/v of organic solvent at 0.05 intervals. OPLC determinations were performed at 21°C, external pressure 50 bar, volume of rapid flow 150 μL, flow rate 150 μL/min, volume of mobile phase 800 μL. Two independent runs were carried out in all experiments. All developed plates were dried at room temperature. Compounds were detected with the Camag VideoStore 2 system (Camag, Switzerland) consisting of the Reprostar 3 UV/Vis analysis lamp with the cabinet cover or by a Shimadzu CS-9000 duul-wavelength TLC scanner (Shimadzu Europe, Duisburg, Germany).



## Calculations

The log P values according to Villar were determined by PC Spartan Pro Ver 1 using the semi empirical AM1 type of calculation.[12]

The coefficients in the regression equations were calculated by the multiple regression analysis Statistica Program, Version 7.1.[13] Statistical significance of the regression equation was tested by the correlation coefficient (r), the standard error of estimate (s), and the variance ratio (F) at specified degrees of freedom (df), n – number of compounds.

## Antiproliferative Activity Assay

The compounds were tested *in vitro* against a cell line T47D (breast cancer) from the American Type Culture Collection (Rockville, Maryland, USA). The SRB test measuring the cell proliferation inhibition of *in vitro* culture was applied.[14] The cytotoxic activity *in vitro* was expressed as log $1/ID_{50}$ (Table 1).[7,8] $ID_{50}$ expresses the concentration (μM) of the compound that inhibits proliferation rate of the tumor cells by 50% as compared to the control untreated cells.

## RESULTS AND DISCUSSION

The structure of N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles studied is presented in Table 1. The panel substitution includes N-alkyl and aryl derivatives.

To calculate solute lipophilicity the capacity factors ($R_M$) have been determined for different concentrations of the following organic modifiers: methanol, ethanol, acetone, acetonitrile, and tetrahydrofuran on the octadecyl stationary phase. The experimental data obtained by the RP-OPLC method as $R_M$ plots versus the percentage composition of different organic modifiers in the mobile phase for some compounds are presented in Figure 1. The lipophilicity parameters determined for five different organic modifiers are presented in Tables 1 and 2.

The absolute values of slopes of 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles are the highest for the water-THF mobile phase and the lowest for the water-ACN one. It is in accordance with the increasing eluting power of organic modifier of reversed phases expressed by the $1/E_0$ parameter.[15,16] At the same time, high selectivity for methanol and acetone, and the lowest one for ethanol as the organic modifier were found. This refers to both binary and theoretical aqueous mobile phases (Tables 1 and 2).

Biagi et al. found that for closely congeneric compounds, the ratio of the intercept ($R_{Mw}$) to the slope (S) of Eq. 1 is constant.[17] Table 3 depicts the relationships between these parameters of 2-amino-1,3,4-thiadiazoles in five different organic modifiers. The best results for THF (Eq. (6)) and the poorest ones for ethanol (Eq. (3), Table 3) were obtained. However, in all cases, congenerity of compounds was confirmed. Significantly different slopes and intercepts of these equations for tetrahydrofuran (Eq. (6)) as the organic modifier in relation to the others were found. Comparable



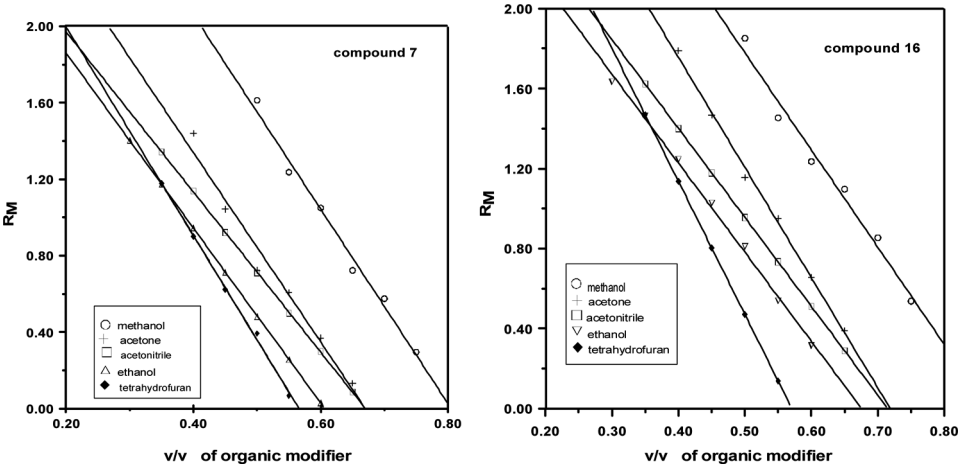



**FIGURE 1** The influence of organic modifiers concentration on the retentions of compounds **7** and **16** by OPLC RP-18 chromatography.

**TABLE 2** Parameters $R_{Mw}$ (intercept) and S (slope) of the Linear Dependences in Eq. (1) for Acetonitrile, Acetone, and Tetrahydrofuran as the Organic Modifiers

| | Acetonitrile | | | | | Acetone | | | | | Tetrahydrofuran | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | $R_{Mw}$ | −S | n | r | s | $R_{Mw}$ | −S | n | r | s | $R_{Mw}$ | −S | n | r | s |
| 1. | 1.6418 | 3.171 | 6 | 0.8971 | 0.0462 | 1.4262 | 2.811 | 5 | 0.9623 | 0.0247 | 1.8409 | 4.256 | 4 | 0.9986 | 0.0036 |
| 2. | 2.3975 | 3.453 | 7 | 0.9609 | 0.0327 | 2.7587 | 4.162 | 5 | 0.9965 | 0.0092 | 2.9529 | 5.465 | 4 | 0.9981 | 0.0065 |
| 3. | 2.5517 | 3.961 | 5 | 0.9090 | 0.0380 | 2.5870 | 3.821 | 5 | 0.9914 | 0.0119 | 2.3963 | 4.002 | 5 | 0.9882 | 0.0120 |
| 4. | 2.5517 | 3.072 | 7 | 0.9833 | 0.0214 | 3.5888 | 4.902 | 5 | 0.9958 | 0.0101 | 3.8594 | 6.717 | 5 | 0.9879 | 0.0122 |
| 5. | 2.9300 | 4.319 | 6 | 0.9931 | 0.0122 | 3.1838 | 4.738 | 5 | 0.9949 | 0.0111 | 3.2099 | 5.708 | 5 | 0.9881 | 0.0121 |
| 6. | 1.6690 | 2.689 | 6 | 0.8989 | 0.0529 | 2.2169 | 3.757 | 6 | 0.9908 | 0.0141 | 2.1821 | 4.229 | 5 | 0.9925 | 0.0096 |
| 7. | 2.8194 | 4.202 | 7 | 0.9666 | 0.0303 | 3.3214 | 4.956 | 6 | 0.9868 | 0.0169 | 3.1239 | 5.557 | 5 | 0.9898 | 0.0112 |
| 8. | 2.8770 | 4.256 | 7 | 0.9695 | 0.0290 | 3.2163 | 4.778 | 6 | 0.9893 | 0.0152 | 3.3540 | 6.197 | 5 | 0.9998 | 0.0010 |
| 9. | 2.4294 | 3.183 | 7 | 0.8458 | 0.0631 | 3.3204 | 5.005 | 6 | 0.9890 | 0.0154 | 2.7939 | 4.914 | 5 | 0.9906 | 0.0107 |
| 10. | 2.8844 | 3.958 | 7 | 0.9815 | 0.0226 | 3.3930 | 4.842 | 5 | 0.9954 | 0.0107 | 3.6460 | 6.493 | 5 | 0.9917 | 0.0101 |
| 11. | 2.8323 | 4.140 | 7 | 0.9701 | 0.0286 | 3.3313 | 4.863 | 6 | 0.9929 | 0.0124 | 3.2578 | 5.657 | 5 | 0.9858 | 0.0133 |
| 12. | 3.1252 | 4.615 | 7 | 0.9763 | 0.0255 | 3.8315 | 5.574 | 6 | 0.9872 | 0.0167 | 3.4305 | 5.999 | 5 | 0.9888 | 0.0117 |
| 13. | 2.7902 | 3.992 | 7 | 0.9538 | 0.0355 | 3.4256 | 4.966 | 6 | 0.9951 | 0.0102 | 3.3924 | 5.899 | 4 | 0.9863 | 0.0130 |
| 14. | 3.2779 | 4.836 | 7 | 0.9907 | 0.0288 | 3.5353 | 5.194 | 6 | 0.99074 | 0.0141 | 3.5226 | 6.202 | 5 | 0.9802 | 0.0156 |
| 15. | 3.1794 | 4.447 | 7 | 0.9327 | 0.0426 | 3.9237 | 5.465 | 6 | 0.9936 | 0.0117 | 3.7989 | 6.656 | 5 | 0.9799 | 0.0157 |
| 16. | 3.1733 | 4.270 | 7. | 0.9619 | 0.0323 | 3.9577 | 5.527 | 6 | 0.9958 | 0.0095 | 3.8955 | 6.759 | 5 | 0.9776 | 0.0166 |
| 17. | 3.4860 | 4.664 | 5 | 0.9891 | 0.0163 | 3.9463 | 5.178 | 5 | 0.9983 | 0.0069 | 3.8318 | 6.266 | 5 | 0.9811 | 0.0152 |
| 18. | 3.4420 | 4.478 | 5 | 0.9815 | 0.0175 | 3.9860 | 5.226 | 5 | 0.9905 | 0.0152 | 4.2405 | 7.312 | 4 | 0.9804 | 0.0139 |
| 19. | 3.1950 | 4.305 | 5 | 0.9765 | 0.0299 | 4.1128 | 5.652 | 5 | 0.9885 | 0.0167 | 4.2090 | 7.361 | 5 | 0.9710 | 0.0168 |
| 20. | 3.1328 | 4.463 | 7 | 0.9567 | 0.0344 | 3.5489 | 5.081 | 5 | 0.9930 | 0.0132 | 3.5920 | 6.401 | 5 | 0.9683 | 0.0176 |
| 21. | 2.9066 | 3.991 | 7 | 0.9806 | 0.0231 | 3.2627 | 4.284 | 5 | 0.9766 | 0.0239 | 3.7587 | 6.904 | 5 | 0.9886 | 0.0118 |
| 22. | 2.5304 | 3.792 | 7 | 0.9835 | 0.0213 | 4.1665 | 6.571 | 5 | 0.94930 | 0.0349 | 3.6761 | 7.037 | 5 | 0.9849 | 0.0136 |

**TABLE 3** The Linear Relationships: $R_{Mw} = a\ (-S) + b$ between $R_{Mw}$ (intercept) and S (slope) of RP-TLC Eq. (1) for Various Organic Modifiers in the Mobile Phase

| Mobile Phase | a (±SE) | b (±SE) | n | r | s | F | Eq. |
|---|---|---|---|---|---|---|---|
| Methanol-water | 0.9204 (±0.0802) | −0.4462 (±0.3744) | 22 | 0.9318 | 0.2252 | 131.72 | (2) |
| Ethanol-water | 0.8900 (±0.1284) | −1.1420 (±0.5709) | 22 | 0.8402 | 0.2360 | 48.03 | (3) |
| ACN-water | 0.8432 (±0.0783) | −0.5860 (±0.3201) | 22 | 0.9230 | 0.1902 | 116.07 | (4) |
| Acetone-water | 0.7996 (±0.0617) | −0.5363 (±0.3046) | 22 | 0.9453 | 0.2193 | 168.05 | (5) |
| THF-water | 0.6186 (±0.0384) | −0.3494 (±0.2333) | 22 | 0.9635 | 0.1700 | 259.39 | (6) |

slopes for methanol and ethanol were found (Eqs. (2) and (3)). It can suggest similar retention mechanism of the studied compounds in these chromatographic systems (Figure 1, Table 3).

The data in Tables 1 and 2 shows that the extrapolated $R_{Mw}$ values from RP-18 OPLC chromatography with ACN and ethanol or acetone and THF as the organic modifier are very similar. The relationships between these parameters are described by the following equations:

$$R_{Mw(ACN)} = 1.0051(\pm 0.1202)R_{Mw(ETH)} + 0.0209(\pm 0.3403)$$
$$n = 22, r = 0.8817,\ s = 0.2341,\ F = 69.87$$

$$R_{Mw(ACT)} = 0.9683(\pm 0.0955)R_{Mw(THF)} + 0.1099(\pm 0.3264)$$
$$n = 22,\ r = 0.9849,\ s = 0.2715,\ F = 102.68$$

The slopes and intercepts, very close to 1 and 0, respectively, showed that in the cases of both pairs of the organic modifier their nature does not significantly affect the parameters $R_{Mw}$ in the OPLC equations. The $R_{Mw}$ values for other organic modifiers are different. The highest correlation between $R_{Mw}$ obtained from methanol as the commonly applied organic modifier and $R_{Mw}$ parameters obtained from ethanol was found. This relationship can be expressed by the following equation:

$$R_{Mw(ETH)} = 0.6151(\pm 0.0754)R_{Mw(MET)} + 0.4524(\pm 0.2912)$$
$$n = 22,\ r = 0.8768,\ s = 0.2983,\ F = 66.51 \quad (9)$$

It can be the effect of analogical structure of both organic solvents and identical types of interactions with the analyte and the octadecyl stationary phase. However, different eluting power of the retentions of all compounds changes, probably in proportion for both modifiers.

Log P values were applied for comparison determined by the computational method using the Villar approach.[18] The results are presented in Table 1. The best relationships found between the log P values and

**TABLE 4** The Linear Relationships: log $1/ID_{50} = a\ R_{Mw} + b$ between Antiproliferative Activity of Compounds against the Cells of Human Breast Cancer Line T47D and Lipophilicity $R_{Mw}$

| mobile phase | A (±SE) | b (±SE) | n | r | s | F | |
|---|---|---|---|---|---|---|---|
| methanol-water | 0.6107 (±0.0883) | −3.7331 (±0.3494) | 16 | 0.8795 | 0.2214 | 47.82 | (12) |
| ethanol-water | 0.9073 (±0.0925) | −3.9012 (±0.2638) | 16 | 0.9343 | 0.1659 | 96.10 | (13) |
| ACN-water | 0.7171 (±0.1078) | −3.3831 (±0.3114) | 16 | 0.8715 | 0.2281 | 44.21 | (14) |
| acetone-water | 0.5317 (±0.0844) | −3.1190 (±0.2877) | 16 | 0.8596 | 0.2377 | 39.63 | (15) |
| THF-water | 0.5798 (±0.0788) | −3.2495 (±0.2638) | 16 | 0.8913 | 0.2109 | 54.13 | (16) |

chromatographic lipophilicity from methanol and acetone are described by the following equations:

$$\log P = 1.0040(\pm 0.1255)R_{Mw(MET)} - 2.2196(\pm 0.4834)$$
$$n = 21, R = 0.8781, s = 0.3467, F = 63.97 \quad (10)$$

$$\log P = 0.9333(\pm 0.1274)R_{Mw(ACT)} - 1.5086(\pm 0.4319)$$
$$n = 21, R = 0.8593, s = 0.3412, F = 53.64 \quad (11)$$

Similar results to methanol for ethanol as the organic modifier were obtained.

To determine the influence of lipophilicity on the antiproliferative activity and to find the QSAR equations human breast cancer cell line T47D was used as the bioindicator. The results of assays were expressed as $ID_{50}$ values. The logarithmically transformed $1/ID_{50}$ values—lipophilicity dependences are presented in Table 4. In all cases, the liner equations were obtained with stronger activity of compounds of high lipophilicity (Eqs. (12)–(16)). The best results for ethanol as the organic modifier were obtained (Eq. 13, Figure 2). This may result from the possibility of applying greater water concentration for the mobile phase. Thus, the use of ethanol minimizes errors in extrapolation to 0% of organic.

Hollósy and coworkers[19,20] also reported a good prediction of cytotoxic activity of carboxamide derivatives by means of their log k data determined on the Hypersil 5 MOS column under isocratic conditions (24% v/v ACN in the aqueous phase). The strongest antiproliferative effect against A431 cells determined by the MTT method also showed analogs of high lipophilicity expressed in this way.

The obtained results show explicitly the synthesis directions for new analogs of high antiproliferative activity from the 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diol set. High lipophilicity compounds are preferable. This can be achieved by the design and synthesis of derivatives with halogen atoms of high atomic mass or with the alkyl substituents in the N-phenyl ring or in the resorcinol moiety.

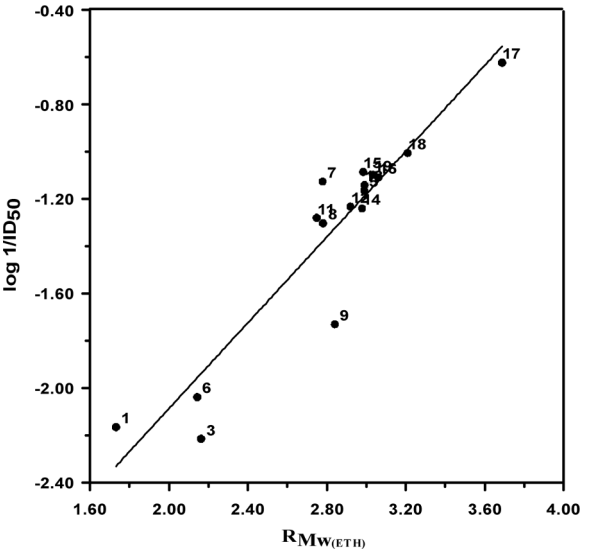



**FIGURE 2** The relationship between the antiproliferative activity expressed by log $1/ID_{50}$ against a human cancer cell line T47D and the $R_{Mw}$ values obtained for ethanol as the organic modifier as described by Eq. (13).



## CONCLUSIONS

The $R_{Mw}$ values obtained by RP-18 OPLC chromatography can be applied to predict antiproliferative activity of N-substituted 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diol set and they show the directions for the new synthesis. The best results of the antiproliferative activity-lipophilicity analysis for ethanol as the organic modifier were obtained. It can result from higher water content in the mobile phases used for extrapolation. At the same time methanol-water is the most selective eluent for the tested series of compounds with respect to the substituent effect on retention. High efficiency, very short time of development of chromatograms, and increasing number of samples analyzed on one chromatoplate for the OPLC method permits the ues of OPLC for a quick analysis of a large number of biologically active compounds in the QSAR studies.

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