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DETERMINATION OF LIPOPHILICITY PARAMETERS OF NEW DERIVATIVES OF N^3-SUBSTITUTED AMIDRAZONES BY REVERSED PHASE THIN LAYER CHROMATOGRAPHY

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Abstract: The retention behavior of 23 derivatives of N^3-substituted amidrazones with potential pharmacological activity were evaluated by reverse-phase thin layer chromatography. Examined compounds were divided into three groups depending on molecular structure and analyzed using methanol/water and methanol/water/acetic acid mobile phases on RP18 HPTLC plates. The linear relationship between retention parameter R_M and the percentage composition of methanol has been obtained within all groups in whole examined concentration range, which permitted determination of lipophilicity parameters: R_{M0} by extrapolation and φ_0 by interpolation. R_{M0} values obtained within groups were compared with log P values obtained by eight computational algorithms (KOWWIN, XLOGP2, XLOGP3, ALOGPS, MLOGP, ALOGP, AC logP, miLogP).

Keywords: amidrazones, triazoles, thin-layer chromatography, lipophilicity

Amidrazones – hydrazones of acid amides – are useful precursors in the synthesis of many chemical compounds (1-3). In recent years, some derivatives of N^3-substituted amidrazones showed diverse biological activity: antiviral, antibacterial, immunomodulatory, anti-inflammatory, analgesic, antitumor and others (4-7).

Growing number of close-related amidrazone derivatives cause need to find a criteria which enable preliminary estimation of biological activity of obtained compounds and optimization of lead structures in the future. Lipophilicity is one of the crucial physicochemical properties that comprise influence of various molecular parameters of compounds and is highly correlated with biological effects of potential drugs (8, 9). It significantly affects both transport of compounds through membranes in a biological system and the formation of compound-receptor complex. Chromatographic methods using RP-18 phases are especially useful for lipophilicity analyses regarding similarity of stationary phase to biological membranes. RP-TLC is a rapid, easy to perform technique which requires small quantities of the sample and enables simultaneous analysis of several compounds. It is reliable method for bioavailability prediction of potential drugs, which provide useful informations to QSAR studies (10, 11).

In present work, we evaluated chromatographic lipophilicity parameters of 23 potentially active amidrazone derivatives using RP-TLC method and compared them with computed log P values.

EXPERIMENTAL

Amidrazones derivatives **1-23** synthesized in Department of Organic Chemistry in Nicolaus Copernicus University in Bydgoszcz were initially divided into 3 groups (triazoles **1-10**, linear **11-16** and **17-23**) basing on their chemical structure (Table 1). Compounds were dissolved in methanol (3 mg/mL), samples (10 μL) of each class were applied on individual plates, then dried on air. Five mixtures of methanol and water (40, 45, 50, 55, 60% v/v) and the same mixtures with 0.2% acetic acid addition were used as mobile phases.

Chromatography was performed on HPTLC RP-18W nano-silica gel aluminium plates (60 Å medium pore diameter, F_{254} aluminium sheets, Fluka, Germany, 0.150 mm thick layer). The plates (10 × 10 cm) were developed in horizontal DS-chamber (Chromdes, Lublin, Poland) using saturat-

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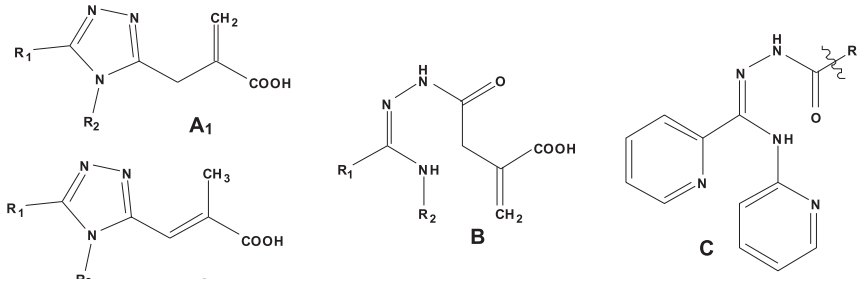
ed conditions (face-down, 30 min of saturation in ambient temperature). The developing distance was 8 cm. Developed plates were air dried and observed under 254 nm ultraviolet lamp. Compounds were localized by quenching the plate fluorescence. If multiple spots appeared, the plate was sprayed with 1% copper (II) sulfas solution in methanol/water 50/50% v/v mixture to visualize proper spots. All solvents and reagents were of analytical grade, and water freshly distilled. All experiments were performed in a room with stable temperature of 22°C.

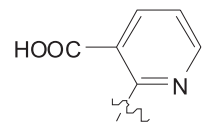
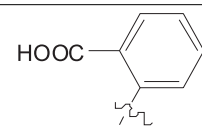
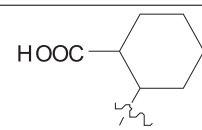
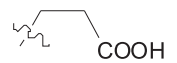
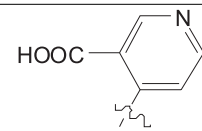
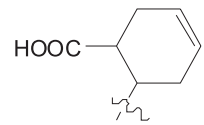
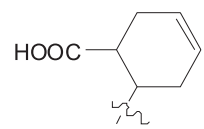
Regression and correlation analyses were performed by Statistica 10 software.

RESULTS

In reverse-phased system, triazoles **1-10** showed single spots without the presence of impu-

Table 1. The structures of compounds **1-23**.



Comp.	Core	R_1	R_2	Comp.	Core	R
1	A_1	2-C_5H_4N	C_6H_5	**17**	C	
2	A_2	2-C_5H_4N	C_6H_5			
3	A_1	2-C_5H_4N	2-C_5H_4N	**18**	C	
4	A_2	2-C_5H_4N	2-C_5H_4N			
5	A_1	2-C_5H_4N	4-NO_2-C_6H_4	**19**	C	
6	A_1	4-C_5H_4N	4-CH_3-C_6H_4			
7	A_1	C_6H_5	C_6H_5	**20**	C	
8	A_1	4-C_5H_4N	C_6H_5			
9	A_1	2-C_5H_4N	4-CH_3-C_6H_4	**21**	C	
10	A_1	C_6H_5	4-NO_2-C_6H_4			
11	B	2-C_5H_4N	C_6H_5	**22**	C	
12	B	2-C_5H_4N	2-C_5H_4N			
13	B	2-C_5H_4N	4-NO_2-C_6H_4			
14	B	4-C_5H_4N	C_6H_5			
15	B	2-C_5H_4N	4-CH_3-C_6H_4	**23**	C	
16	B	C_6H_5	4-NO_2-C_6H_4			

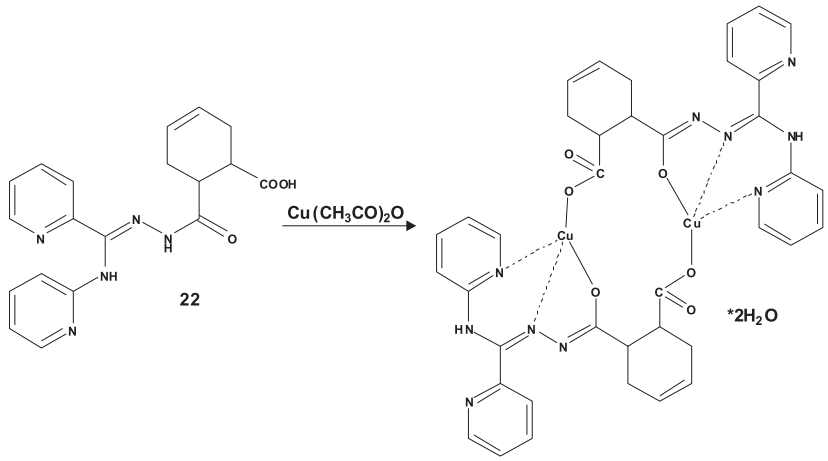


Figure 1. The exemplary reaction of **22** with Cu^{2+} ions leading to formation of copper(II) complex (12)

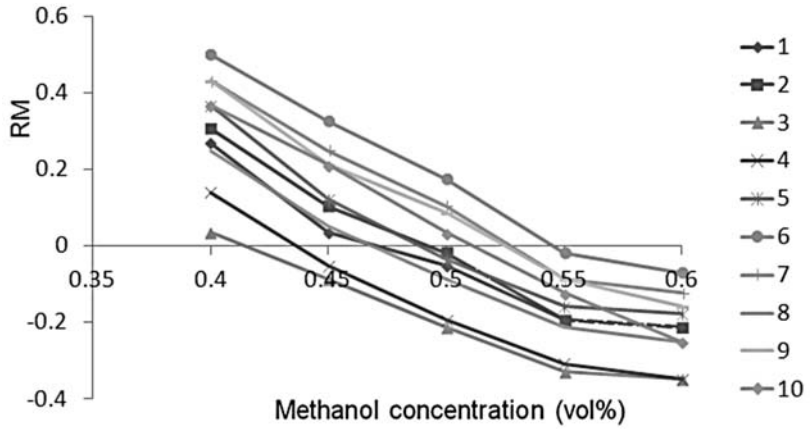


Figure 2. The relationship between R_M values and methanol concentration in the mobile phase of compounds **1-10**

rities and degradation products for all used mobile phases. Compounds **11-23** revealed limited stability in acidic solution - multiple spots occurred on plates after developing in mobile phase with acetic acid addition. Furthermore, chromatograms of compounds **15**, **16** and **23** showed additional spots in methanol-water mobile phases even for fresh dissolved samples. In these cases, unchanged compound was revealed by reaction with copper (II) ions resulting with yellowish spots of created complex on pale blue background. Detection reagent (1% $CuSO_4$ solution in methanol/water mixture) was used basing on reaction described previously (Fig. 2) (12). All linear derivatives **11-23** created color copper(II) complexes in these conditions, while additional spots remained unchanged.

The relative lipophilicity R_M values for five methanol-water mobile phases and compounds **1-23** were calculated by formula $R_M = \log([1-R_f]/R_f)$. The R_{M0} values were calculated from Soczewiński-Wachtmeister equation: $R_M = R_{M0} - S\varphi$, where φ was the volume fraction of organic modifier in an aqueous-organic solvent mixture, S was the slope of the regression curve and R_{M0} (lipophilicity index) is the retention parameter for pure water as the eluent. Methanol was chosen as the most recomended organic modifier of the mobile phases for lipophilicity estimation (15).

Results are presented as R_M plots *versus* the percentage composition of methanol in mobile phase (Figs. 2-4). Obtained lipophilicity parameters (R_{M0}, S, φ_0) are presented in Table 2. The R_{M0} values obtained were compared with the theoretical values of partition coefficient ($\log P_{calc}$) calculated by eight available on-line programs (KOWWIN, XLOGP2, XLOGP3, ALOGPS, MLOGP, ALOGP, AC logP, miLogP) (14) (Table 3).

Table 2. The lipophilicity parameters obtained from the linear equation $R_M = R_{M0} - S\varphi$ and correlation coefficients for the investigated compounds.

Comp.	R_{M0}	S	φ_0	R^2
1	1.17	-4.768	5.584	-0.9596
2	1.34	-5.063	6.768	-0.9748
3	0.82	-4.393	3.588	-0.9793
4	1.08	-4.845	5.225	-0.9744
5	1.39	-5.121	7.133	-0.9567
6	1.67	-5.354	8.934	-0.9885
7	1.56	-5.275	8.236	-0.9837
8	1.22	-4.912	5.968	-0.9750
9	1.58	-5.343	8.427	-0.9874
10	1.62	-5.525	8.947	-0.9986
11	1.29	-2.688	3.474	-0.9959
12	1.42	-2.528	3.601	-0.9949
13	1.57	-3.010	4.721	-0.9897
14	1.18	-2.427	2.866	-0.9842
15	1.63	-3.118	5.090	-0.9923
16	2.17	-3.859	8.376	-0.9910
17	1.12	-2.427	2.725	-0.9803
18	1.43	-2.523	3.618	-0.9708
19	2.45	-3.654	8.961	-0.9942
20	1.18	-2.206	2.600	-0.9708
21	0.96	-2.208	2.111	-0.9704
22	1.90	-2.918	5.546	-0.9851
23	1.13	-2.339	2.649	-0.9777

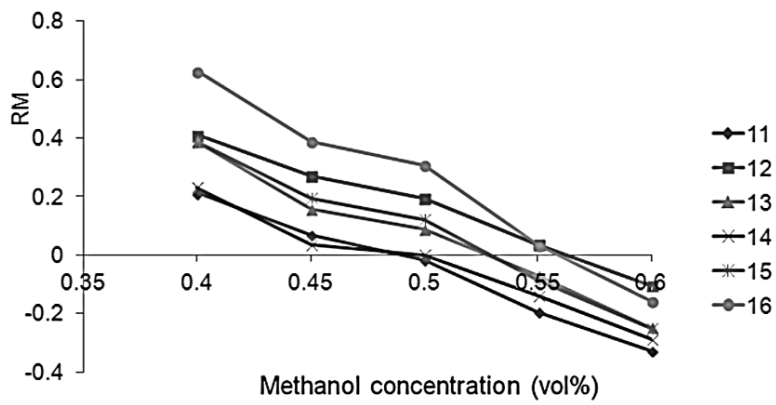


Figure 3. The relationship between R_M values and methanol concentration in the mobile phase of compounds **11-16**

DISCUSSION AND CONCLUSION

The linear dependence $R_M = R_{M0} - S\varphi$ with high values of correlation coefficients (R^2 = 0.97-0.99) was observed for all three groups of compounds in wide range of methanol concentration in mobile phase (40-60%, v/v) which permitted determination of lipophilicity parameters: R_{M0} by extrapolation and φ_0 by interpolation. Within all analyzed groups were also observed linear relationships of $R_{M0} = f(S)$ with

Table 3. The comparison R_{M0} values of compounds **1-23** with log P values calculated by eight computational programs.

Comp.	R_{M0}	KOWWIN	XLOGP2	XLOGP3	ALOGPS	MLOGP	ALOGP	AC logP	miLogP
1	1.17	2.28	3.58	2.14	1.46	3.41	2.74	1.55	1.82
2	1.34	1.70	3.75	2.07	2.20	3.41	3.11	1.71	2.15
3	0.82	1.09	3.06	1.40	0.85	3.22	2.13	1.76	1.34
4	1.08	1.01	3.23	1.34	1.09	3.22	2.49	1.92	1.67
5	1.39	2.10	3.47	1.97	1.91	3.53	2.63	1.42	1.78
6	1.67	2.83	3.93	2.47	1.36	3.38	2.80	1.81	2.12
7	1.56	3.47	4.74	3.17	2.15	4.15	3.46	2.57	2.96
8	1.22	2.28	3.49	2.10	1.28	3.14	2.31	1.49	1.67
9	1.58	2.83	4.02	2.50	1.60	3.65	3.22	1.87	2.27
10	1.62	3.29	4.64	3.00	2.71	4.22	3.35	2.44	2.92
11	1.29	1.31	2.29	1.87	1.97	1.98	2.09	2.00	0.65
12	1.42	0.12	1.76	1.14	1.34	1.80	1.48	1.40	-0.25
13	1.57	1.12	2.18	1.70	1.84	2.13	1.99	1.87	0.61
14	1.18	1.31	2.20	1.54	1.76	1.98	1.66	1.90	0.53
15	1.63	1.85	2.73	2.24	2.17	2.22	2.58	2.32	1.10
16	2.17	2.31	3.34	2.44	2.70	3.49	2.71	2.84	1.78
17	1.12	0.21	2.30	1.52	1.59	1.93	1.73	1.67	0.10
18	1.43	1.40	3.46	2.26	2.07	2.90	2.45	2.64	0.89
19	2.45	1.41	2.88	2.14	1.76	2.59	2.47	1.71	1.05
20	1.18	-0.29	1.50	0.50	1.01	1.62	1.00	1.22	-0.56
21	0.96	0.21	2.21	1.19	1.41	1.93	1.30	1.57	-0.37
22	1.90	1.20	2.36	1.73	1.73	2.51	2.02	1.61	0.56
23	1.13	0.52	1.79	0.84	1.28	1.55	1.40	0.86	-0.58

Table 4. Correlation matrix for various log P_{calc} *versus* R_{M0} relationships within groups ($p < 0.05$, ns – not significant).

Comp.	KOWWIN	XLOGP2	XLOGP3	ALOGPS	MLOGP	ALOGP	AC logP	miLogP
1-10	0.86	0.82	0.85	0.67	0.67	0.80	ns	0.80
11-16	ns	0.82	ns	ns	0.92	ns	ns	ns
17-23	0.76	ns	ns	ns	ns	ns	ns	0.80

high values of regression ≥ 0.95 which are characteristic for closely related compounds (Fig. 5). This plot confirmed also high structural resemblance between compounds **11-16** and **17-23**.

Molecular mechanism of chromatographic retention is similar for compounds **11-23** and their donor-acceptor properties are also similar to each other. Since retention mechanism in chromatography on reversed phases could be regarded as similar to the ability of diffusion through cell membranes, this suggests their similar bioavailability. The same properties should also be expected between triazoles **1-10**.

Depending on R_{M0} values, compounds could be classified in groups by growing lipophilicity. Since an increase in lipophilicity can be usually connected with the increased biological activity, therefore compounds **19**, **16**, **22** should be expected the most active. Amidst the second group of compounds (**11-16**), there is unexpected R_{M0} value of compound **12** higher than isomers **11** and **14**. For comparison, in the first group, derivatives disubstituted with 2-pyridine (**3**, **4**) showed lower value of lipophilicity index than derivatives possessing 2-pyridine and phenyl rings (**1**, **2**). This phenomenon could be generated by creating intermolecular hydrogen bonds within compound **12** which disguise its hydrophilic groups. On the other hand, compounds **12** and **18** differing in lateral sub-

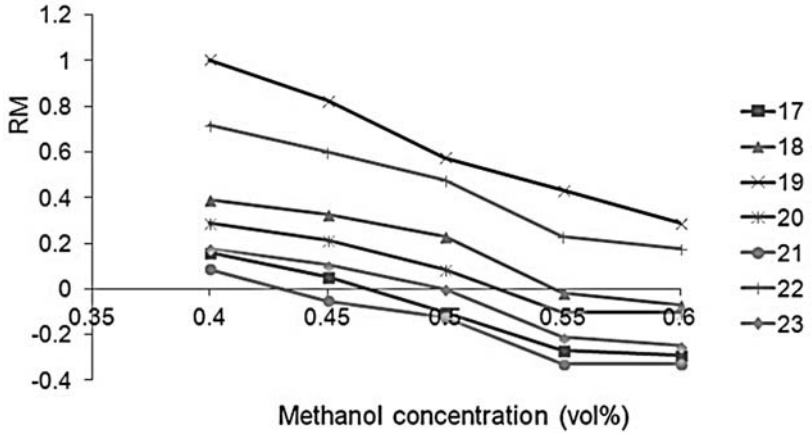


Figure 4. The relationship between R_M values and methanol concentration in the mobile phase of compounds **17-23**

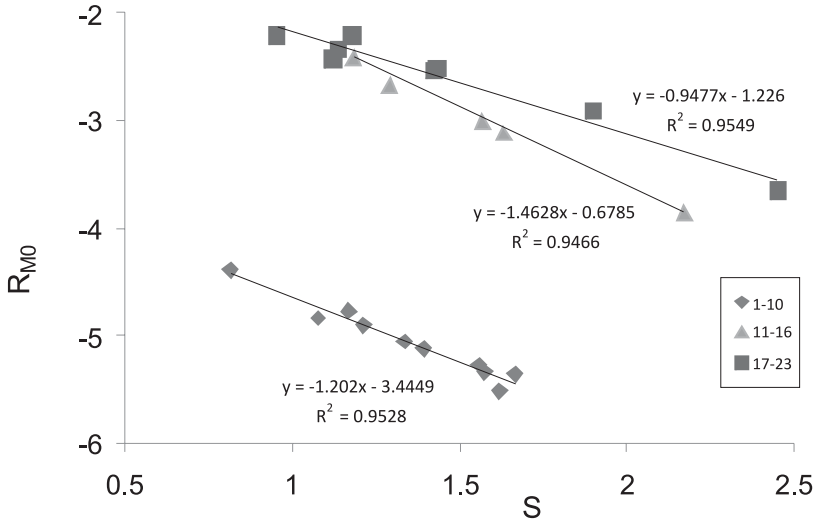


Figure 5. Structural similarity of the analyzed compounds within groups: **1-10** (diamonds), **11-16** (triangles), and **17-23** (squares)

stituent near carboxylic group showed very similar R_{M0} values although all computational methods predicted higher lipophilicity for derivative **18**.

Relative lipophilicity R_{M0} values were compared with log P_{calc} values calculated by computational methods (Table 4). Strong correlations ($R^2 > 0.8$) between log P_{calc} and R_{M0} of compounds **1-10** was found with KOWWIN, XLOGP2, XLOGP3, ALOGP, miLogP programs and moderate ($R^2 \sim 0.67$) with ALOGP and MLOGP. In the second group of compounds (**11-16**), the strongest correlation was obtained by MLOGP, good result was also obtainded by XLOGP2. Only KOWWIN and miLog showed strong significant correlations between calculated and experimental values within third group of compounds (**17-23**).

Computer programs basing on fragmentation method don't consider intramolecular bonds and well-known among amidrazone derivatives tautomerism (15, 16). Five strong correlations were found between log P values calculated by eight programs and lipophilicity indexes of triazole derivatives **1-10**, however only four significant correlations were found for two other groups of acyclic compounds. None of the examined programs showed significant correlations with the values of lipophilicity indexes for three groups of compounds. Therefore, experimentally found lipophilicity

parameters should better reflect the real physicochemical properties of linear N^3-substituted amidrazone derivatives than theoretical calculations.

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