Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 3 pp. 379–383, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

REVERSED-PHASE TLC STUDY OF SOME LONG CHAIN ARYLPIPERAZINE OF IMIDAZOLIDINE-2,4-DIONE AND IMIDAZO[2,1-*f*]PURINE-2,4-DIONE DERIVATIVES

AGNIESZKA ZAGÓRSKA[1], ANNA CZOPEK[1], KAROLINA PEŁKA[1], KRYSTYNA STANISZ-WALLIS[2] and MACIEJ PAWŁOWSKI[1]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacokinetics and Physical Pharmacy, Pharmaceutical Faculty, Jagiellonian University Medical College, 9 Medyczna St., 30-688 Kraków, Poland

Abstract: The chromatographic parameters of arylpiperazinylpropyl derivatives of imidazolidine-2,4-dione and imidazo[2,1-*f*]purine-2,4-dione were investigated using reversed-phase thin layer chromatography method. The results revealed that R_{M0} of investigated compounds depended on substituent in arylpiperazinyl fragment as well as on a nature of (cycloalkyl)aromatic ring at 5 position of imidazolidine-2,4-dione and at 7 position of imidazo[2,1-*f*]theophylline. The R_{M0} parameters were compared with computationally calculated partition coefficients values by principal component analysis (PCA). To verify the influence of lipophilic parameter of investigated compounds on their biological activity the statistical analysis of Mann-Whitney was performed.

Keywords: imidazolidine-2,4-diones, imidazo[2,1-*f*]purine-2,4-diones, lipophilicity

The monoamine hypothesis of depression postulates that a functional deficiency of 5-hydroxytryptamine (5-HT, serotonin) or noradrenaline and/or dopamine in the brain is a key to the pathology and/or behavioral manifestations associated with depression. An arylpiperazine moiety is one of the most universal templates used for designing agents active on serotonin (5-HT) receptors. Simple arylpiperazines are classified as non-selective 5-HT and other receptor ligands but long chain arylpiperazines (LCAPs) have been found as serotonin receptor ligands in particular 5-HT_{1A} and 5-HT_{2A} ones. Their general chemical structure contains: an alkyl chain (2–4 methylene units) attached to the N4 atom of the piperazine moiety, and a terminal fragment: amide or imide. The significance of the terminal part in ligand-receptor interaction has been the subject of many structure-activity relationships studies (1–3).

Lipophilicity is one of the most important physicochemical properties frequently used in QSAR (quantitative structure–activity relationship) analysis. This parameter, expressed as a partition coefficient or its decimal logarithm (log*P*), can be determined experimentally by various analytical methods (RP-HPLC, spectrophotometry, MEEKC, cyclic voltametry, titrimetry); however, the reversed-phase thin-layer chromatography (RP-TLC) is often used technique in recent years in this case (4–8). In this report, we describe the use of RP-TLC to determine the lipophilicity of some LCAPs derivatives of imidazolidine-2,4-dione (**1**-**10**) and imidazo[2,1-*f*]purine-2,4-dione (**11**-**22**). The investigated compounds have interesting biological properties and have been tested as a potential antidepressant or antipsychotic agents (9, 10). The relationship between the concentration of the organic modifier in the mobile phase and the chromatographic properties of the investigated compounds, as well as the influence of substituent on the lipophilicity of compounds **1**–**22**, was also studied. The lipophilicity values determined chromatographically were compared with the theoretically calculated partition coefficients values obtained by the use of computational methods. To verify the influence of lipophilic parameters of investigated compounds on their biological activity, the statistical analysis was performed.

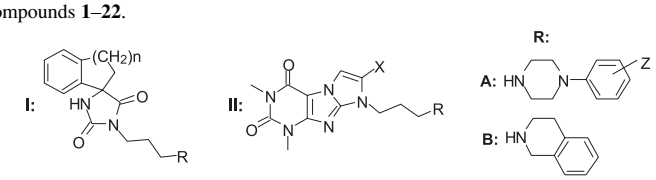
* Corresponding author: e-mail: azagorsk@cm-uj.krakow.pl; phone: + 4812 6205456, fax: +4812 6205405

EXPERIMENTAL

The investigated LCAPs derivatives of imidazolidine-2,4-dione and imidazo[2,1-*f*]purine-2,4-dione were synthesized according to the methods described in the literature (9, 10). The chemical structures of these compounds are presented in Table 1. Methanol used was HPLC grade from E. Merck (Darmstadt, Germany). Water was deionized by use of a Millipore system. TLC was performed on precoated C18 F_{254} plates (10 × 20 cm, E. Merck) in horizontal DSII chambers (Chromdes, Lublin, Poland) under unsaturated (sandwich) conditions at room temperature. Mixtures of methanol content ranging between 50 and 80% (v/v) in 5% increments and water were used as seven mobile phases. The investigated compounds were separately dissolved in methanol (1 mg/mL) and applied on the plates (10 μL) as spots. The starting points were 10 mm from the bottom edge of the plates and development was carried out over 9.0 cm. After the development (30–60 min), the plates were air-dried at room temperature (22°C) and examined under a 254-nm UV lamp (CM-10, Spectroline, New York, USA). Each experiment was run in triplicate.

All graphs and statistical procedures were performed using the computer program Statistica for Windows (v.10.0, Statsoft Inc., 2011).

Table 1. The structures of compounds **1**–**22**.



Comp.	Core	X/n	R	Z	Comp.	Core	X/n	R	Z
1	I	1	A	H	**12**	II	H	A	2-OCH_3
2	I	1	A	2-OCH_3	**13**	II	H	A	3-Cl
3	I	1	A	3-Cl	**14**	II	H	B	–
4	I	1	A	3-CF_3	**15**	II	CH_3	A	H
5	I	1	B	–	**16**	II	CH_3	A	2-OCH_3
6	I	2	A	H	**17**	II	CH_3	A	3-Cl
7	I	2	A	2-OCH_3	**18**	II	CH_3	B	–
8	I	2	A	3-Cl	**19**	II	C_6H_5	A	H
9	I	2	A	3-CF_3	**20**	II	C_6H_5	A	2-OCH_3
10	I	2	B	–	**21**	II	C_6H_5	A	3-Cl
11	II	H	A	H	**22**	II	C_6H_5	B	–

Table 2. R_{M0} (intercept), and r (correlation coefficient) values for linear relationship $R_M = R_{M0} + bC$.

Compound	R_{M0}	r	Compound	R_{M0}	r
1	3.261	0.9919	**12**	3.194	0.9970
2	2.943	0.9912	**13**	3.315	0.9965
3	3.354	0.9835	**14**	3.096	0.9990
4	3.702	0.9931	**15**	3.946	0.9874
5	3.541	0.9895	**16**	3.185	0.9925
6	3.020	0.9654	**17**	3.944	0.9950
7	3.516	0.9960	**18**	2.799	0.9874
8	3.665	0.9857	**19**	3.468	0.9915
9	3.947	0.9970	**20**	3.867	0.9925
10	2.678	0.9745	**21**	3.002	0.9931
11	3.729	0.9874	**22**	3.391	0.9825

Table 3. Log*P* values obtained by the use of computational methods.

Compound	*A*log*P*$_s$	milog*P*	logP$_{KOWIN}$	*X*log*P*2	logP$_{PALLAS}$	logP$_{CAChe}$
1	2.79	2.98	3.58	2.63	2.40	3.09
2	2.96	2.98	3.5	2.63	2.34	2.84
3	3.56	3.63	4.14	3.34	3.04	3.61
4	3.81	3.85	4.46	3.64	3.28	3.98
5	2.75	2.78	3.70	2.46	2.86	2.90
6	3.34	3.50	3.99	3.08	2.73	3.49
7	2.99	3.51	4.07	3.20	2.68	3.24
8	3.92	4.15	4.63	3.91	3.36	4.01
9	4.23	4.37	4.95	4.21	3.62	4.37
10	3.24	3.30	4.19	3.03	3.40	3.29
11	2.36	2.32	2.65	2.02	1.50	1.96
12	2.43	2.33	2.73	1.93	1.57	1.70
13	2.95	2.97	3.29	2.64	2.24	2.48
14	2.03	2.12	2.85	1.76	0.77	1.76
15	2.73	2.54	3.20	2.48	0.99	1.52
16	2.80	2.55	3.28	2.39	1.05	1.27
17	3.26	3.19	3.84	3.10	1.73	2.04
18	2.41	2.34	2.40	2.22	1.28	1.33
19	3.65	3.99	4.41	3.83	2.93	2.96
20	3.65	4.00	4.49	3.75	3.00	2.71
21	4.13	4.64	5.06	4.45	3.67	3.48
22	3.42	3.72	-	3.70	2.71	2.76

RESULTS

The relative lipophilicity (R_{M0}) of the investigated compounds was determined with the use of nonpolar RP-C18 plates. On the basis of literature data (11, 12), methanol was selected as the organic modifier of the mobile phases. For all the compounds and each seven mobile phase, the R_M values were calculated by the use of the well known formula $R_M = \log ([1 - R_F]/R_F)$. The calculated R_M values were then used for the calculation of R_{M0} values extrapolated to zero percent of methanol concentration with the equation $R_M = R_{M0} + bC$, where C is the concentration in volume percent of methanol in the mobile phase and b is the change in R_M caused by unit methanol concentration in the mobile phase. The obtained R_{M0} values for investigated compounds were also compared with the theoretical values of partition coefficients. The coefficients *A*log*Ps*, milog*P*, logP_{KOWIN} and *X*log*P*2 were calculated from the Virtual Computational Laboratory website (13), logP_{Pallas} by Pallas 3.1 and Xlog*P* by CAChe 7.75. To verify the influence of parameter R_{M0} of investigated compounds on their biological activity, the statistical analysis was performed.

DISCUSSION AND CONCLUSION

It was found that the R_M values decreased linearly with increasing concentration of organic modifier in the eluent. The relationships between the relative lipophilicity expressed as R_{M0} values and the concentration of methanol in the mobile phase showed good linearity for all seven mobile phase systems ($r > 0.97$), as shown in Table 2. For investigated compounds, the relationship between the structure and values of R_{M0} was observed. The extension of the ring in position 5 of hydantoin and the introduction of phenyl moiety at position 7 of imidazo[2,1-*f*]theophylline caused a significant increase of R_{M0} values. The introduction of electron withdrawing substituents (Cl, CF_3) into the phenylpiperazine system resulted in a significant increase of R_{M0} values for imidazolidine-2,4-dione derivatives. The

Table 4. The eigenvalues and the ratios of the variance explained by the seven components using covariance matrix.

Component	Eingenvalue	Variance explained (%)	Total variance explained (%)
1	5.556159	79.37370	79.3737
2	0.994110	14.20157	93.5753
3	0.312365	4.46236	98.0376
4	0.057631	0.82330	98.8609
5	0.048352	0.69075	99.5517
6	0.027833	0.39761	99.9493
7	0.003550	0.05072	100.0000

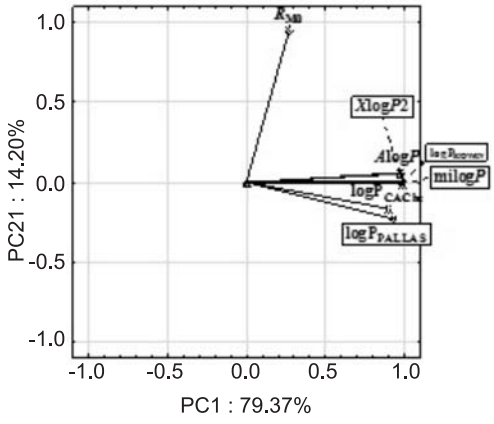


Figure 1. Comparison of the obtained R_{M0} values with the calculated coefficients by PCA

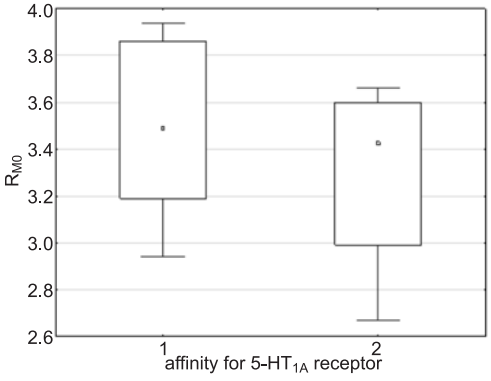


Figure 2. R_{M0} ranges and mean lipophilicity for all compounds, except **14**, **18**, **21** and **22** ($K_{i\ 5\text{-}HT1A}$: 1 < 100 nM, 2 > 100 nM)

substituents at position 2 or 3 of aromatic ring can be arranged in series, according to obtained R_{M0} values, namely: CF_3 > Cl > OCH_3. Furthermore, replacement of the arylpiperazine fragment with

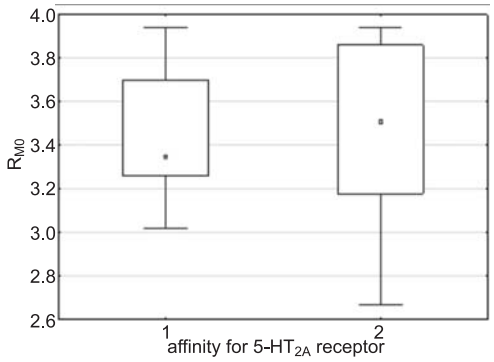


Figure 3. R_{M0} ranges and mean lipophilicity for all compounds, except **14**, **18, 21** and **22** ($K_{i\ 5\text{-}HT2A}$: 1 < 100 nM, 2 > 100 nM)

tetrahydroisoquinoline moiety caused a decrease of adjusted relative lipophilicity parameter. Surprisingly, in the 7-phenyl-imidazo[2,1-*f*]purine-2,4-dione group, the exchange of tetrahydroisoquinoline moiety into phenylpiperazine derivatives as well as the introduction of methoxy group caused an increase of experimental R_{M0} values, most likely due to electron interactions of individual fragments. The obtained results revealed that substituent in arylpiperazinyl fragment as well as a nature of (cycloalkyl)aromatic ring at 5 position of imidazolidine-2,4-dione and at 7 position of imidazo[2,1-*f*]theophylline had an impact on R_{M0} values. Moreover, the experimental coefficients differ from the computationally calculated partition coefficients (Table 3); only the *A*log*P*s coefficient can be slightly compared with the obtained R_{M0} values.

The multivariate comparison of the experimentally obtained values and the coefficients calculated by the computational methods was made by principal component analysis (PCA). This technique decorrelates the variables and converts them into the linear combinations called "principal components." The graphical interpretation of the multidimension-

al space of the data set used by the PCA transformation is obtained with a smaller number of new dimensions of space. The second column of Table 4 explained the percent of observed variance. The first component (PC1) of the index explains 79.4% of the total variance. The second component (PC2) explains 14.2%, while the third component (PC3) explains only 4.46% of the total variance. Together, the first two principal components contain 93.58% of the total variance. The first component is determined by the theoretical variables computationally calculated and are closely related, therefore, constitute a homogeneous group. The second component represents the empirical variable R_{M0}. Both components are mutually orthogonal, that is not depend on each other (Fig. 1).

The differences between the computationally calculated partition coefficients confirm the legitimacy of the determination of lipophilicity for the investigated compounds by the use of the experimental (RP-TLC) method. It can be anticipated that the experimental results would give a better correlation with the biological activity than the theoretical ones. For investigated compounds, the impact of lipophilicity on the affinity for 5-HT receptors in the Mann-Whitney test was statistically not significant (Figs. 2 and 3). It suggested that lipophilicity is one of the many factors, which could influence and modify the activity of the investigated compounds for 5-HT receptors.

Acknowledgment

We are pleased to acknowledge the generous financial support of this work by the Medical College of Jagiellonian University (grant No. K/ZDS/001909).

REFERENCES

1. Badarau E., Suzenet F., Bojarski A. J., Finaru A-L., Guillaumet G.: Bioorg. Med. Chem. 19, 1600 (2009).
2. Volk B., Gacsalyi I., Pallagi K., Poszavacz L., Gyonos I., Szabo E., Bako T., Spedding M., Simig G., Szenasi G.: J. Med. Chem. 54, 6657 (2011).
3. Chłoń-Rzepa G., Żmudzki P., Zajdel P., Bojarski A.J., Duszyńska B., Nikiforuk A., Tatarczyńska E., Pawłowski M.: Bioorg. Med. Chem. 15, 5239 (2007).
4. Morak-Młodawska B., Pluta K.: J. Liq. Chromatogr. Relat. Technol. 31, 611 (2008).
5. Waksmundzka-Hajnos M., Matosiuk D., Petruczynik A., Kijkowska-Murak U.: Acta Chromatogr. 20, 563 (2008).
6. Inglot T., Gumieniczek A., Komsta Ł., Kasińska A.: Chromatographia 68, 977 (2008).
7. Pękala E., Marona H.: Biomed. Chromatogr. 23, 543 (2009).
8. Kulig K., Malawska B.: J. Planar Chromatogr. 22, 141 (2009).
9. Czopek A., Byrtus H., Kołaczkowski M., Pawłowski M., Dybała M., Nowak G., Tatarczyńska E., Wesołowska A., Chojnacka-Wójcik E.: Eur. J. Med. Chem. 45, 1295 (2010).
10. Zagórska A., Jurczyk S., Pawłowski M., Dybała M., Nowak G., Tatarczyńska E., Nikiforuk A., Chojnacka-Wójcik E.: Eur. J. Med. Chem. 44, 4288 (2009).
11. Komsta Ł., Skibiński R., Berecka A., Gumieniczek A., Radkiewicz B., Radoń M.: J. Pharm. Biomed. Anal. 53, 911 (2010).
12. Rutkowska E., Pająk K., Jóźwiak K.: Acta Pol. Pharm. Drug Res. 70, 3 (2013).
13. Tetko I.V., Tanchuk V.J.: VCC-Lab 2002, www.vcclab.org/lab/alogps\

Received: 30. 07. 2013