ORIGINAL ARTICLE

HETEROCYCLES 40. THE LIPOPHILICITY EVALUATION OF SOME NEW PYRIDIN-3/4-YL-THIAZOLO[3,2-B][1,2,4]TRIAZOLE COMPOUNDS WITH ANTI-INFLAMMATORY ACTIVITY BY RP-TLC AND COMPUTATIONAL METHODS

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Abstract

A series of new pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazole compounds and their corresponding thioetheric intermediates with anti-inflammatory activity were evaluated in order to establish their lipophilic profile by reversed phase thin layer chromatography (RP-TLC). The lipophilicity parameters were determined experimentally, based on the chromatographic data ($\mathbf{R_M}$, $\mathbf{R_{M0}}$, **b**, $\mathbf{mR_M}$, $\mathbf{\varphi_0}$, $\mathbf{PC1/R_M}$). A series of theoretical lipophilicity indices were estimated with different computational methods. A good correlation was observed between the experimental lipophilicity parameters and the computed logP values ($R^2 > \pm 0.83$). The scores obtained by applying the principal components analysis (PCA) classify the investigated compounds into three groups, their repartition being correlated with the chemical structure and the anti-inflammatory activity.

Rezumat

O serie de noi derivaţi de piridin-3/4-il-tiazolo[3,2-b][1,2,4]triazol şi intermediarii tioeterici corespunzători cu activitate antiinflamatoare au fost evaluaţi în vederea stabilirii profilului lor de lipofilie prin cromatografie pe strat subţire cu fază inversă (RP-TLC). Parametrii de lipofilie au fost determinaţi experimental, pe baza datelor cromatografice ($\mathbf{R_M}$, $\mathbf{R_{M0}}$, **b**, $\mathbf{mR_M}$, $\mathbf{\varphi_0}$, $\mathbf{PC1/R_M}$). O serie de indici de lipofilie teoretici au fost estimaţi prin diferite metode computaţionale. S-a observat o bună corelaţie între parametrii de lipofilie experimentali şi valorile logP determinate computaţional ($R^2 > \pm 0{,}83$). Scorurile obţinute prin aplicarea analizei componentelor principale (PCA) clasifică compuşii investigaţi în trei grupuri de lipofilie, repartiţia lor fiind corelată cu structura chimică şi cu activitatea antiinflamatoare.

Keywords: lipophilicity, thiazolo[3,2-b][1,2,4]triazole, anti-inflammatory activity

Introduction

Lipophilicity has a key role in the passive diffusion of bioactive compounds through biological membranes, thus influencing their pharmacological and pharmacokinetic properties (absorption, distribution, toxicity, elimination). Several reported studies revealed significant predictive relationships between the determined lipophilicity parameters and the chemical structure, bioactivity and pharmacokinetic properties of bioactive compounds [12, 13, 14].

In practice, lipophilicity is estimated by the logarithm of the partition coefficient *P* (logP) in a *n*-octanol-water two-phase system and it can be determined by various methods. The oldest method - the shake-flask test - is no longer used because of several limitations: it requires large amounts of solvents and time consuming, the degree of purity of the compounds must be very high and the solubility of the organic compounds is often very low in water. In order to avoid the drawbacks of the classical procedure, several alternative methods have already been performed and they are today available. The most applied method for the evaluation of lipophilicity is based on reversed-phase thin-layer chromatography (RP-TLC) [1, 5-7, 16] and reversed-phase high performance liquid chromatography (RP-HPLC) [9]. Nowadays, a series of computed lipophilicity indices can be estimated using different software and internet modules [1, 5-7, 9, 16] but it is necessary to compare them with the experimental data.

Thiazole 1,2,4-triazole and thiazolo[3,2-b][1,2,4]-triazole derivatives are known for their biological potential, the most studied being the antimicrobial and anti-inflammatory activities [2, 3, 10, 15, 18]. Our researches have already highlighted a good and moderate anti-inflammatory activity of a series of pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazole derivatives [17], which presented different pharmacokinetic profiles, depending on their adsorption, diffusion and metabolisation rates. Because lipophilicity is found in strong relationship with the pharmacokinetic and pharmacological properties of compounds, the aim of this study was to evaluate the lipophilicity of the

previously synthesized thiazolo[3,2-b][1,2,4]triazole derivatives bearing pyridin-3/4-yl moiety, in order to correlate it with their chemical structure and biological activity. The lipophilicity parameters determined experimentally by RP-TLC were correlated with a series of computed lipophilicity indices.

Materials and Methods

Pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazoles and their S-alkylated precursors (Figure 1) used in the present study were previously synthesized in our laboratory and evaluated for their anti-inflammatory activity [17].

The lipophilicity evaluation of all tested compounds was performed using standard C-18 modified silica gel plates 20x10 cm RP-18-F245s (Merck, Darmstadt, Germany) as stationary phase and five methanol-water binary mixtures as mobile phase, with different methanol content: 55%, 60%, 65%, 70% and respectively 75% methanol. The evaluated compounds were dissolved in acetone (1 mg/mL) and 2 µL of each solution was applied manually on the chromatographic plates. The elution of compounds was realised in a developing chamber previously saturated for 30 min with the mobile phase, in normal conditions (atmospheric pressure and room temperature). The developing distance was 8 cm in all cases. After the ascending elution, the spots were visualized under UV light at $\lambda = 254$ nm. Analytical-grade methanol used in the present experiment was purchased from Merck (Darmstadt, Germany).

A series of computed logP values were determined using different software and internet modules: Chem 3D Ultra 8.0 (uses fragmental and atomistic methods) [8], ALOGPS 2.1 vcclab (uses electro-topological-state, fragmental, atomistic and reductionist methods) [19] and Marvin, Calculator Plugin and Chemical Terms module (Marvin Sketch 5.3.2) [11]. The geometry of each previously drawn structure was optimized using Chem 3D Ultra 8.0 in order to calculate three logP values: ClogP, $LogP^C$ – Crippen and $LogP^V$ – Viswanadhan with the same software. Seven logP values (ALOGPs, AClogP, ALOGP, MLOGP, KOWWIN, XLOGP2 and XLOGP3) were computed with ALOGPS 2.1 internet website and two surface area descriptors were obtained with Marvin Sketch 5.3.2 module (MSA – molecular surface area, PSA – polar surface area).

Results and Discussion

The chemical structures of the investigated compounds are presented in Figure 1.

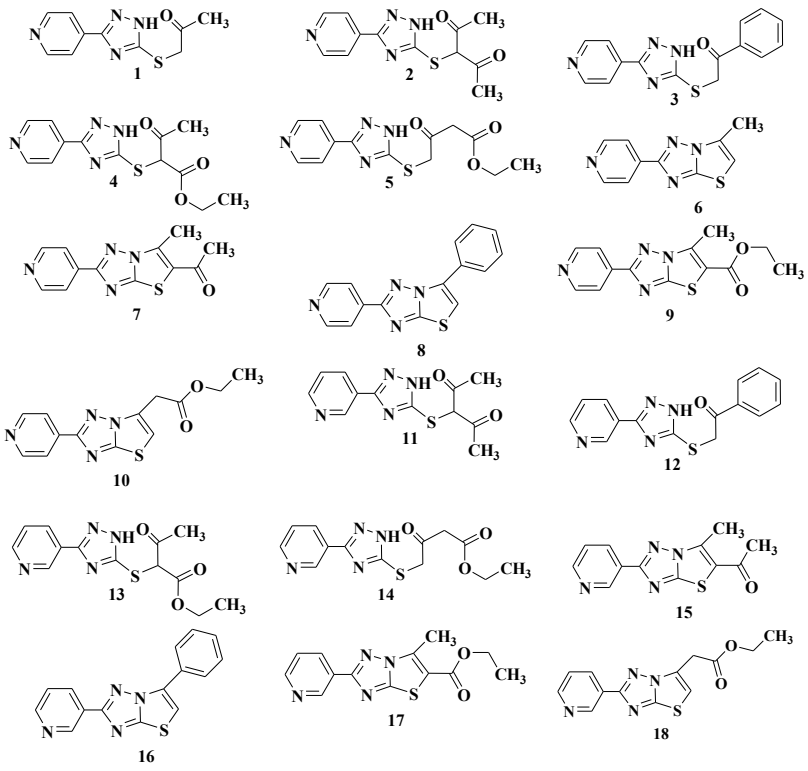

Figure 1.
Pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazole compounds and the corresponding thioether intermediates (1-18)

The retention factor (**Rf**) values obtained by RP-TLC were used to calculate the $\mathbf{R_M}$ parameter (isocratic retention factor) with Bate-Smith and Westall equation [4]:

$$R_M = \log[(1/R_f) - 1]$$

Based on the linear relationship between R_M values and the concentration of the organic solvent in the mobile phase (Sosczewinski-Wachtmeister equation), three lipophilicity parameters were calculated: R_{M0} (which corresponds to 0% methanol in the mobile phase), **b** (which represents the slope) and **C** (which represents the volume fraction of methanol in the mobile phase):

$$R_M = R_{M0} + bC$$

The chromatography hydrophobic index, φ_0, was calculated using the following equation:

$$\varphi_0 = R_{M0}/b$$

Based on other reported studies [6], another two important lipophilicity parameters were evaluated: the arithmetic average (mR_M) and PC_1/R_M (which represents the score of the first principal component obtained by applying the principal component analysis directly on the retention parameter).

All the above mentioned lipophilicity parameters (R_M, R_{M0}, **b**, mR_M, φ_0, $PC1/R_M$) derived from the retention data are presented in Table I.

All lipophilicity parameters determined experimentally were correlated with different computed logP values (Table II). Statistical analysis in presented in Table III.

Table I

Lipophilicity parameters determined experimentally by RP-TLC for the polyheterocyclic compounds **1-18**

Compound	mR_M	R_{M0}	b	φ_0	$PC1/R_M$
1	-0.078	1.938	-0.031	-62.506	1.651
2	0.318	2.233	-0.029	-75.708	0.663
3	0.492	3.419	-0.045	-75.980	0.201
4	0.416	2.654	-0.034	-77.140	0.409
5	0.141	2.423	-0.035	-69.037	1.097
6	0.598	2.983	-0.037	-81.272	-0.049
7	0.688	3.356	-0.041	-81.851	-0.283
8	1.310	4.392	-0.047	-92.667	-1.849
9	1.181	4.216	-0.047	-90.272	-1.523
10	0.596	3.499	-0.045	-78.275	-0.057
11	0.312	2.247	-0.030	-75.409	0.677
12	0.479	3.537	-0.047	-75.247	0.232
13	0.356	2.338	-0.030	-76.643	0.566
14	0.113	2.567	-0.038	-68.080	1.162
15	0.653	3.374	-0.042	-80.532	-0.195
16	1.198	4.038	-0.044	-92.398	-1.563
17	1.121	4.057	-0.045	-89.759	-1.372
18	0.482	3.099	-0.040	-76.903	0.234

Table II

Lipophilicity indices determined by computational methods for the polyheterocyclic compounds **1-18**

Compound	Computed lipophilicity parameters											
	ALOGPs	**AC logP**	**ALOGP**	**MLOGP**	**KOWWIN**	**$XLOGP_2$**	**$XLOGP_3$**	**$LogP^C$**	**$LogP^V$**	**ClogP**	**PSA**	**MSA**
1	1.00	1.06	0.80	1.55	-0.01	1.08	1.18	0.77	1.45	1.32	72.53	308.38
2	1.30	0.52	0.48	1.27	0.10	1.09	1.13	0.52	1.07	0.99	88.60	363.24
3	2.44	2.22	2.61	2.95	1.80	2.76	2.83	2.56	3.26	3.15	71.53	384.72
4	1.43	0.69	1.10	1.60	-0.15	1.57	1.66	1.31	1.82	1.68	97.83	411.43
5	1.23	1.06	1.18	1.60	-0.08	1.61	1.56	1.31	1.78	1.55	97.83	416.79
6	1.65	2.24	1.58	2.07	1.88	1.71	1.84	1.41	3.22	3.39	43.08	272.71
7	1.68	2.29	1.61	2.05	1.56	1.14	1.86	1.04	2.58	2.92	60.15	329.45
8	3.42	3.56	3.24	3.47	3.10	3.43	3.10	3.01	4.61	4.82	43.08	347.75
9	2.04	2.78	2.08	2.36	2.21	1.66	2.39	1.91	3.43	3.69	69.38	379.47
10	1.72	2.13	1.97	2.09	1.92	1.66	1.63	1.12	3.04	3.14	69.38	378.26
11	1.31	0.52	0.48	1.27	0.10	1.09	1.13	0.52	1.07	0.99	88.60	363.24
12	2.49	2.22	2.61	2.95	1.80	2.76	2.83	2.56	3.26	3.15	71.53	384.72
13	1.38	0.69	1.10	1.60	-0.15	1.57	1.66	1.31	1.82	1.68	97.83	411.55
14	1.22	1.06	1.18	1.60	-0.08	1.61	1.56	1.31	1.78	1.55	97.83	417.11
15	1.68	2.29	1.61	2.05	1.56	1.14	1.86	1.04	2.58	2.92	60.15	329.46
16	3.36	3.56	3.24	3.47	3.10	3.43	3.10	3.01	4.61	4.82	43.08	348.07
17	2.03	2.78	2.08	2.36	2.21	1.66	2.39	1.91	3.43	3.69	69.38	379.55
18	1.71	2.13	1.97	2.09	1.92	1.66	1.63	1.12	3.04	3.14	69.38	378.46

Table III

Correlation between experimental and computed lipophilicity parameters for all studied compounds (bolded values are statistical significant)

Experimental/computed lipophilicity parameters	Experimental lipophilicity parameters				
	mR_M	R_{M0}	b	φ_0	$PC1/R_M$
mR_M	1.000	**0.926**	-0.728	**-0.983**	-1.000
R_{M0}	**0.926**	1.000	**-0.933**	**-0.858**	**-0.928**
b	-0.728	**-0.933**	1.000	0.622	0.732
φ_0	**-0.983**	**-0.858**	0.622	1.000	**0.982**
$PC1/R_M$	-1.000	**-0.928**	0.732	**0.982**	1.000
ALOGPs	0.799	**0.833**	-0.751	-0.748	**-0.800**
AC logP	**0.863**	**0.937**	**-0.878**	-0.786	**-0.865**
ALOGP	0.752	**0.884**	**-0.887**	-0.669	-0.755
MLOGP	0.741	**0.850**	**-0.836**	-0.662	-0.744
KOWWIN	**0.863**	**0.925**	**-0.858**	**-0.805**	**-0.865**
XLOGP2	0.558	0.640	-0.630	-0.498	-0.560
XLOGP3	0.752	**0.850**	**-0.826**	-0.682	-0.754
$LogP^C$	0.662	0.758	-0.743	-0.589	-0.665
$LogP^V$	**0.845**	**0.921**	**-0.865**	-0.779	**-0.848**
ClogP	**0.881**	**0.937**	**-0.862**	**-0.822**	**-0.883**
PSA	-0.684	-0.690	0.600	0.645	0.685
MSA	-0.175	-0.094	0.005	0.183	0.174

A similar retention mechanism was observed for all studied compounds and no secondary interactions with the stationary phase were revealed. Figure 2 represents the profile of the chromatographic lipophilicity parameters **R_M** and **mR_M**. It can be observed that compounds **1, 5, 14** and **18** are less lipophilic, while compounds **8, 9, 16** and **17** are the most lipophilic ones (Figure 2). A strong correlation was observed between **R_{M0}** and **b** values ($R^2 > 0.98$) (Table I) which highlighted only a congeneric class within all studied compounds.

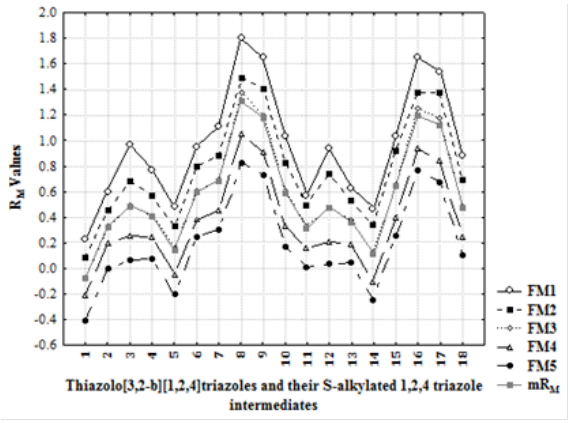

Figure 2.

Profile of chromatographic retention parameters (R_M) for all five mobile phases (FM1: 55% methanol; FM2: 60% methanol; FM3: 65% methanol; FM4: 70% methanol; FM5: 75% methanol)

Using the first two principal scores (**PC1/ R_M** and **PC2/ R_M**) determined by applying PCA analysis on the retention parameter allowed us to obtain the lipophilicity chart (Figures 3 and 4) which divides all studied compounds into three different groups, that can be correlated with several structural particularities.

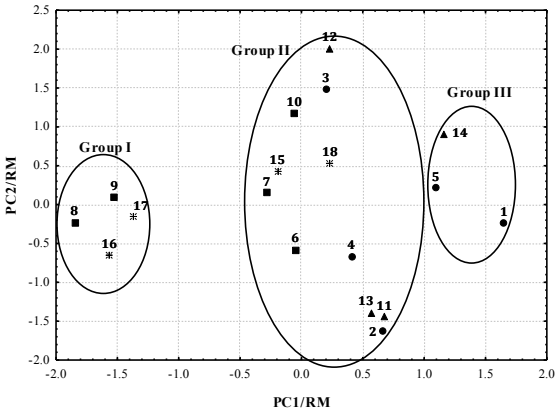

Figure 3.

The lipophilicity chart of compounds **1-18**. Representation with different symbols depending on the chemical structure: **circle** (•) - thioethers derived from pyridin-4-yl-1,2,4-triazole; **square** (■) - pyridin-4-yl-thiazolo[3,2-b][1,2,4]-triazole derivatives; **triangle** (▲) - thioethers derived from pyridin-3-yl-1,2,4-triazole; **star** (*) - pyridin-3-yl-thiazolo[3,2-b][1,2,4]-triazole derivatives

The first group of the lipophilicity chart contains four pyridin-3/4-yl-thiazolo-triazole compounds with either an aromatic ring (**8** and **16**) or a methyl and ethylcarboxylate rest (**9** and **17**). The second group contains eleven compounds: three S-alkylated pyridin-4-yl-1,2,4-triazoles (**2, 3, 4**), three S-alkylated pyridin-3-yl-1,2,4-triazoles (**11, 12, 13**), three pyridin-4-yl-thiazolo-triazoles (**6, 7, 10**) and two pyridin-3-yl-thiazolo-triazoles (**15, 18**). The last group has three thioethers derived from

pyridin-3/4-yl-1,2,4-triazole: **1, 5** and **14**. It is important to note that all four compounds from Group I are presenting good or moderate anti-inflammatory activities (Figure 4) and they are also the most lipophilic compounds, according to Figure 2. On the other hand, compounds placed in the last group from the lipophilicity chart (Group III) are the less lipophilic ones and their anti-inflammatory activity is poor (Figure 4).

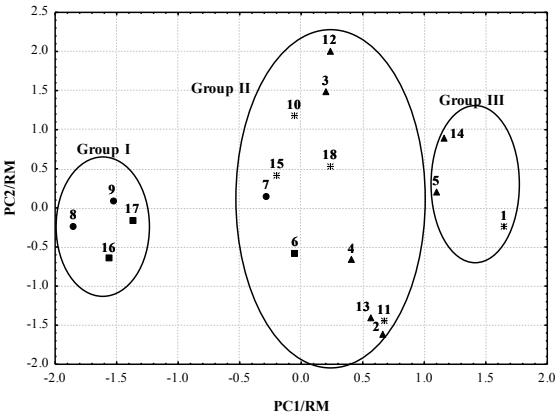

Figure 4.

The lipophilicity chart. Representation with different symbols depending on the anti-inflammatory activity: **circle** (•) - good anti-inflammatory activity, **square** (■) - moderate anti-inflammatory activity, **triangle** (▲) - rapid, but short anti-inflammatory activity, **star** (*) - no anti-inflammatory activity

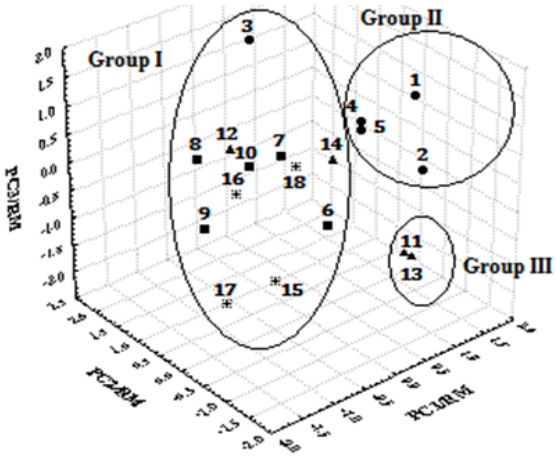

Figure 5.

The lipophilicity space of compounds **1-18**. Representation with different symbols depending on the chemical structure: **circle** (•) - thioethers derived from pyridin-4-yl-1,2,4-triazole; **square** (■) - pyridin-4-yl-thiazolo[3,2-b][1,2,4]-triazole derivatives; **triangle** (▲) - thioethers derived from pyridin-3-yl-1,2,4-triazole; **star** (*) - pyridin-4-yl-thiazolo[3,2-b][1,2,4]-triazole derivatives

The lipophilicity space represented in Figures 5 and 6, obtained by using the first three principal scores (**PC1/R_M**, **PC2/R_M** and **PC3/R_M**), classify the tested compounds into three groups. The most part of the tested compounds are found in group I (compounds **3, 6-10, 12, 14-18**). In group II, are found four thioethers which have in common the pyridin-4-yl-1,2,4-triazole ring system (compounds **1, 2, 4** and **5**). Group III contains only two compounds, **11** and **13**, both of them being thioethers derived from pyridin-3-yl-1,2,4-triazole with high structural similarity (Figure 1). All compounds possessing good and moderate anti-inflammatory activities are found in the first group of the lipophilicity space (Figure 6).

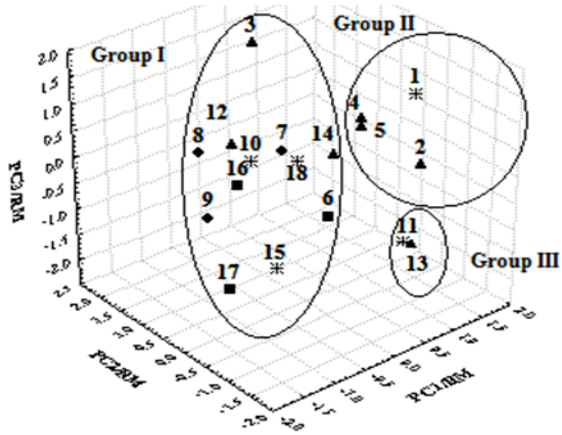

Figure 6.

The lipophilicity space. Representation with different symbols depending on the anti-inflammatory activity: **circle** (•) - good anti-inflammatory activity; **square** (■) - moderate anti-inflammatory activity; **triangle** (▲) - rapid, but short anti-inflammatory activity; **star** (*) - no anti-inflammatory activity

As shown in Table III, a good correlation has been found between the lipophilicity parameters obtained experimentally (**R_{M0}, b**) and the computed lipophilicity indices **AC logP, ALOGP, MLOGP, KOWWIN, $XLOGP_3$, $LogP^C$** and **$LogP^V$** ($R^2 > \pm 0.83$)**.** The statistical analysis (Table III) revealed also a strong correlation between all lipophilicity parameters: **mR_M, R_{M0}, b, φ_0, PC1/R_M** ($R^2 > \pm 0.92$).

Conclusions

A new class of polyheterocyclic compounds bearing thiazolo-triazole and pyridin-3/4-yl moieties was studied by RP-TLC in order to evaluate their lipophilicity. Several lipophilicity parameters were determined experimentally from the retention data obtained using five methanol-water mobile phases of different concentrations.

The lipophilicity parameters determined experimentally were correlated with various computed logP values and good correlations were observed ($R^2 > \pm 0.83$).

By applying the principal components analysis directly on the retention parameter **R_M**, the first two/three

principal scores were obtained. The first two principal scores were used to plot the lipophilicity chart, while the lipophilicity space was determined with the first three principal scores. Both lipophilicity chart and lipophilicity space place all newly synthesized compounds into three groups. The repartition of compounds in different groups of lipophilicity was correlated with their chemical structures and with their anti-inflammatory activity.

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