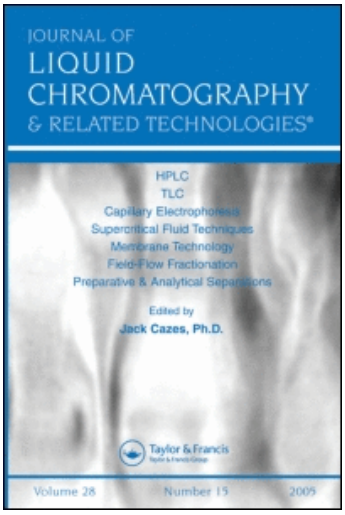

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Huszár, Monika , Hallgas, Balázs , Idei, Miklós , Kiss-Szikszai, Attila , Horváth, Anikó and Patonay, Tamás(2008) 'Lipophilicity of Substituted Aurones and Related Compounds Measured on Immobilized Artificial Membrane (IAM) and Conventional C_8 (MOS) Columns', Journal of Liquid Chromatography & Related Technologies, 31: 20, 3143 — 3158
To link to this Article: DOI: 10.1080/10826070802480115
URL: http://dx.doi.org/10.1080/10826070802480115

Journal of Liquid Chromatography & Related Technologies®, 31: 3143–3158, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802480115

Lipophilicity of Substituted Aurones and Related Compounds Measured on Immobilized Artificial Membrane (IAM) and Conventional C_8 (MOS) Columns

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Abstract: A high performance liquid chromatographic method utilizing an immobilized artificial membrane (IAM HPLC) column has been developed to separate the members of a library containing 38 aurone and thioaurone type structures and to characterise their lipophilicity. The experimental lipophilicity data (k'_{IAM}) have been compared with the previously determined ones C_8(MOS) column (k'_{MOS}) and with their calculated lipophilicity parameters (CLOGP). In general, good correlations between the measured and calculated lipophilicities have been found both for the IAM and MOS column. The IAM column showed higher efficiency in separation of isomeric aurones or thioaurones than the MOS one and, consequently, it showed higher potential in differentiation of their lipophilicities. Our findings proved the usefulness of the HPLC method in fast characterisation of the lipophilicity of drug candidates closely related in structure.

Keywords: Aurone, Immobilized Artificial membrane (IAM) column, Lipophilicity, Molecular library, Retention factor, Thioaurones

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INTRODUCTION

Previous studies revealed that poor physico-chemical properties caused serious attrition in the course of the drug development and the enforcement of rigorous criteria during candidate selection failed to avoid these pitfalls.[1] Henceforward, in the last decade studies of drug databases revealed that clinically successful drugs tend to have drug like properties. Druglikeness as a complex set of *in vivo* properties is mainly determined by physico-chemical features and structural parameters such as solubility, lipophilicity, etc., and also by stability and metabolic behaviour. The emerging importance of druglikeness has led to test these properties in an early phase using high throughput (HT) methods of which the process is often called pharmaceutical profiling.[2] Lipophilicity[1,3,4] is a tendency of a compound to partition to non-polar versus aqueous environments and is usually characterised by the n-octanol/water (biphasic) partition coefficient (P_{ow} and $\log P_{ow}$) in cases of neutral compounds.[5–7]

The shake flask method used most traditionally to determine $\log P_{ow}$ meets serious technical difficulties in the measurement of compounds with very high $\log P_{ow}$ (≥ 6.5).[6] Moreover, $\log P_{ow}$ is not the most appropriate parameter to estimate all features associated with lipophilicity, especially in biological partition processes.[9] The second approach to characterize lipophilicity is membrane partitioning because drug molecules typically should be able to pass through at least one biological membrane to reach the site of action. Nowadays, different methods as the Caco-2 cell permeability system, and several chromatographic techniques such as reversed phase liquid chromatography, micellar electrokinetic chromatography, micellar liquid chromatography, immobilised micellar chromatography, have been developed to examine the lipophilicity profile of a molecule.[8,10,11–14] Simple alkyl bonded standard reversed phases (RP) retain analytes barely on the basis of hydrophobicity, in contrast with the immobilized artificial membrane (IAM) column where the combination of interactions such as hydrophobic feature, ion pairing, and hydrogen bonding (all of which are important in real membrane permeability) are possible.[15] This combination of interactions has been described as phospholipophilicity.[16,17] The interaction between the analytes and phosphatidylcholine derivatives bonded on the silica surface of the IAM columns might be a much more reliable model of passing through biomembranes than that of measured on C_8 or C_{18} alkyl phases. In our work, the IAM-PC DD column was used where the IAM phase is prepared by covalently immobilizing monolayers of a phospholipid analogue containing only the single chain phosphatidylcholine ligand that lacks the glycerol backbone. This stationary phase is C_3 and C_{10} end-capped and has anionic properties under physiological conditions because of the phosphoesther linkage.[18,19]

Aurones and their derivatives belong to the large and diverse family of flavonoids. Few decades ago numerous biological effects such as analgesic, antiasthmatic, antihyperlipidemic[20–24] activity have been patented. Recently, antiviral,[25] antileishmanicid, and limited cytotoxic effect[26] have also been published and the chemistry of thioaurones has been reviewed.[27] The resemblance of aurone, chalcone, and flavone structures, as well as their similarity to combretastatin-A4P (CS-A4P - tumour vascular targeting agent), can be recognized by comparing their scaffolds.[27,28]

In this work, as a continuation of our previous study, where a similar library was analyzed using a C_8 (MOS) column,[7] a reliable, fast, and accurate HPLC method to separate the 38 members of an aurone library on a IAM column,[19] and to characterise their phospholipophilicity has been developed. Experimental retention factor (k'_{IAM}) measured on the IAM column has been compared with the previously calculated CLOGP as well k'_{MOS} values measured earlier.

EXPERIMENTAL

Materials

Triethylamine (TEA), acetonitrile (ACN), ortho-phosphoric acid, and methanol were purchased from Fluka (Buchs, Switzerland). Solutions were prepared of deionised, bacteria free water made by Elgastat UHP system (Elga Ltd. Bucks, England).

Synthesis

Preparation of compounds investigated here (Figure 1 and Table 1) have been described elsewhere.[7] Aurones (sublibrary I, Table 1) were prepared from the corresponding 2′-hydroxychalcones upon treatment

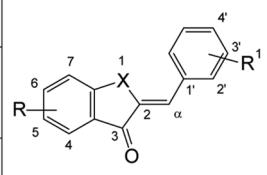
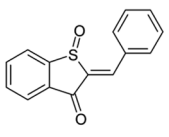
Sublibrary I.	X=O		
Sublibrary II.	X=S		
Sublibrary III.	$X=SO_2$		H

Figure 1. Structure of the investigated compounds.

Table 1. Calculated and experimentally determined lipophilicity values of the investigated compounds

Substituents			Sublibrary I. (X = O)					Sublibrary II. (X = S)					Sublibrary III. (X = SO2)				
			IAM	MOS*				IAM	MOS*				IAM	MOS*			
	R	R1	k′	k'_{rel}	k′	k'_{rel}	CLOGP	k′	k'_{rel}	k′	k'_{rel}	CLOGP	k′	k'_{rel}	k′	k'_{rel}	CLOGP
a	H	H	2.243	1.00	1.122	1.00	4.291	5.243	1.00	1.621	1.00	4.642	0.939	1.00	0.512	1.00	2.801
b	H	2′-Me	2.266	1.01	1.393	1.24	4.790	5.593	1.07	2.065	1.27	5.141	1.086		0.63	1.23	3.300
c	H	3′-Me	—	—			—	8.069	1.54	2.472	1.52	5.141	—				—
d	H	4′-Me	3.273	1.46	1.538	1.37	4.790	6.643	1.27	2.366	1.46	5.141	1.307	1.39	0.793	1.55	3.300
e	5-Me	H	3.197	1.43	1.585	1.41	4.790	—				—	—				—
f	H	4′-iPr	6.944	3.10	3.357	2.99	5.718						—				—
g	H	2′-MeO	2.868	1.28	1.455	1.30	4.210	—				—	—				—
h	H	4′-MeO	2.176	0.97	1.05	0.94	4.210	5.034	0.96	1.581	0.97	4.561	0.943	1.00	0.586	1.14	2.720
i	H	3′,4′-$(MeO)_2$	—	—			—	3.216	0.61	0.923	0.57	4.300	0.720	0.77	0.404	0.79	2.459
j	H	3′,4′-OCH_2O-	2.005	0.89	0.885	0.79	3.856	5.01	0.96	1.256	0.77	4.207	1.086	1.16	0.501	0.98	2.366
k	H	3′,4′-OCH_2 CH_2O-	3.48	1.55	0.92	0.82	4.215	—				—	—				—
l	H	4′-BnO	10.612	4.73	3.133	2.79	5.978	—				—	—				—
m	6-BnO	H	9.416	4.20	3.126	2.79	6.087	—				—	—				—
n	6,7-$(BnO)_2$	H			6.124	5.46	7.460	—				—	—				—
o	H	4′-F	2.413	1.08	1.148	1.02	4.434	4.434	0.85	1.549	0.95	4.785			0.482	0.94	2.944
p	5-F	H	2.207	0.98	1.239	1.10	4.476	—				—	—				—
r	H	4′-Cl	5.113	2.28	1.858	1.66	5.004	9.083	1.73	2.512	1.55	5.355			0.871	1.70	3.514
s	5-Cl	H	2.149	0.96	1.841	1.64	5.046	—				—	—				—
t	H	3′,4′-Cl_2	—	—			—	—	—4.178		2.58	5.948	—				—
u	H	4′-Br	5.61	2.50	2.056	1.83	5.154	13.069	2.49	2.780	1.71	5.505			1.014	1.98	3.664
v	H	4′-CN	1.766	0.79	0.982	0.88	3.724	3.085	0.59	1.167	0.72	4.075	—				—
w	H	4-NO_2	—	—			—	4.524	0.86	1.452	0.90	4.385	—				—
z	H	4-Me_2N	—	—			—	7.586	1.45	1.629	1.00	4.807	—			—	—
y	5-Br	H	5.236	2.33			5.154										
H	see Figure I.						0.405		0.232			2.527					

*: MOS data presented here is measured and detailed in ref [7], ** k'_{rel} means the ratio of the k′ value of the actually examined molecule and that of the parent molecule of the sublibrary, (e.g. $k'_{I/b,rel,MOS} = k'_{I/b,MOS}/k'_{I/a,MOS} = 1.24$).

*** values are means of three parallel measurements, where RSD was less than 2%.

by mercury(II) acetate[29] or trimethylsilyl azide.[30] 1-Thioaurones (sublibrary II) were synthesized by the piperidine-catalyzed condensation of 1-thiocoumaran-3-one and the corresponding aldehyde in hot methanol solution. Sulphoxide (H) was obtained from thioaurone (II/a, Table 1) by dimethyldioxirane oxidation.[31] Sulphones (sublibrary III) were prepared by oxidation of thioaurones (II) with dimethyldioxirane[31] or hot hydrogen peroxide/acetic acid.[32] Products have been identified by comparison of literature data or on the basis of their IR, ^{1}H NMR spectra and elemental analyses (C, H, and N).

HPLC Measurements

For chromatographic analysis stock solutions (0.5 mg/mL) of the samples in acetonitrile: water (V/V 3:1) were prepared and filtered through a 0.2 μm Millipore filter unit. These solutions were kept in Eppendorf tubes at −20°C. HPLC analysis of the samples were performed with Varian (Basel, Switzerland) 9012 Solvent Delivery System, Varian 9065 Polychrom Diode Array Detector; column: IAM-PC DD 150 × 4.6 mm 12 μm/0.3 μm (Regis Technologies Inc., IL, USA); injector: Rheodyne. Eluents: A: 0,083 M triethyl ammonium phosphate (TEAP), pH 7.40. Isocratic runs were performed in an eluent of 33 V/V% ACN in eluent A. Flow rate: 1 mL/min; temperature: 20°C. Injected volumes 20 μL, three parallel injections were analyzed. Retention factors (k′) of the samples were calculated from the experimentally determined retention data: ($k' = (t_R - t_o)/t_o$). Correlation between the k'_{IAM} and software predicted lipophilicity (CLOGP) or k'_{MOS} values of the same compounds has been investigated. The technique and method applied for the previous determination of retention factor on MOS column (k'_{MOS})are detailed in ref.[7]

Calculation of CLOGP Data

Software predicted lipophilicity of the compounds was calculated with the program CLOGP as it was cited in ref.[4]

RESULTS AND DISCUSSION

Results of the HPLC Measurements

A library consisting of 38 structurally related compounds shown by Figure 1 has been investigated. Based on their structural features the library could be further divided into 3 sublibraries. Sublibrary I contains

the aurone (I/a) and its derivatives substituted in various positions while sublibraries II and III include thioaurones and their sulphones (X = S or $X = SO_2$), respectively.

A suitable RP-HPLC method has been developed applying the IAM-PC DD column to separate each member of the sublibraries. Isocratic separation was performed within 24 minutes, calculated retention factors (k'_{IAM}) of the compounds are shown in Table 1.

The effect of the substituents and that of the heteroatoms on the retention measured both on the IAM (k'_{IAM}) and MOS (k'_{MOS}) columns was studied. Also, the retention ability of the IAM and MOS columns has been compared based on the ratio of the k′ value of the actually examined molecule and that of the parent molecule of the sublibrary (e.g., $k'_{I/b,rel,MOS} = k'_{I/b,MOS}/k'_{I/a,MOS} = 1.24$, Table 1). All of the invoked data measured on MOS column is detailed in ref.[7]

The value of k′ observed on the IAM column embracing a 32-fold difference, ranged from 0.405 (compound H, Table 1) to 13.069 (compound II/u) depending on the chemical structure. Halogen substituted thioaurones (II/r, II/u) exhibited the highest retention and the 4′-bromo derivative (II/u) was the uppermost. The lowest retentions were associated to the 1-thioaurone-sulphoxide and sulphone derivatives (H, III/a). In the case of the MOS column the same parameter showed 14-fold difference from 0.232 (compound H) to 3.357 (compound I/f). Comparison of the data of the next highest/lowest pairs (i.e., I/l and III/i for IAM column, I/f and III/i for MOS column) reveal the same tendency, a 15-fold difference in the case of the former and only 8-fold difference in the case of the latter one.

It means that the retention factors of the molecules distributed in a much wider range on the IAM than on the MOS column.

The retention factor on the IAM column increased with the incorporation of an apolar methyl group and its change for an isopropyl group caused further increase in k′ value compared to the parent compound (i.e., I/a,d,f, II/a,d).

The IAM column (similarly to the MOS one) proved to be suitable for separation of the structure isomers, too, and the method applied here was able to perceive the small structural differences resulting in a fine alteration of lipophilicity (I/b–d or I/g–h). While the fragment constant method could not make a difference between the structural isomers (like I/b,d,e or II/b–d), the IAM column was able to differentiate them. Impact of the spherical factor on the k′ value was shown by the structure isomers, where a methyl group was attached at position *ortho* or *para*. Thus, significant differences were detected between the k'_{rel} values 2′- and 4′-methylaurones (I/b,d) and -thioaurones (II/b,d). Incorporation of the methyl group in position 2′ (I/b) modified the shape of the molecule in its central part making the molecule more spherical. This change

in the shape resulted in smaller k'_{rel} values in comparison with the derivatives with methyl groups in positions 3′ or 4′ both on the IAM and MOS column. However, the difference between position 2′ and the other positions was more pronounced in the case of the IAM column, again. The significant difference in the k'_{rel} values of 2′- and 4′-methyl derivatives have also been observed in the sublibraries II–III. This observation confirms our previous findings reported in[7] that in terms of structural isomers the shape of the molecule may strongly influence its chromatographic behaviour or lipophilicity profile.

Calculations optimizing the geometry of substituted aurones and 1-thioaurones by the MM2 method[35] revealed that substituents attached to position 2′ increase the C-2–C_α–C-1′–C-2′ dihedral angle, moving the molecule from the flattened to the spherical shape (see Figure 4, Table 4). No marked differences in the dihedral angles could be observed when the compounds carrying the same substituent in position 3′ and 4′ were compared.

In accordance with this tendency, no marked differences were found between the k′ values of the aurones with the methyl group in position 5 (I/e) or 4′ (I/d), both compounds had higher retention than the parent molecule due to their more elongated shape.

Similarly, just a slight difference was found between the retention data of compounds I/l and I/m where benzyloxy substituents were connected to the opposite sites of the molecule but the length and the shape of the molecules remained nearly the same. Comparing the two phases, the IAM was able to separate these two isomers (I/m,l) while they eluted together on the MOS phase. The IAM phase was more sensitive for the benzyloxy substitution than the MOS column and a stronger increase in the retention has been observed (I/l and I/m).

Incorporation of the methoxy group in position *para* decreased, while in position *orto* increased the retention on the IAM column (compare I,II/h and I,II/g); a similar effect was observed on the MOS column. This divergence shows again the special character of position 2′ located at the central part of the molecule. This finding is in full accordance with our previous results reported in.[7]

Incorporation of the dioxolane moiety decreased the retention both on the IAM and MOS columns either in the aurone or the thioaurone sublibrary (I–II/j). However, this effect was much less than in the case of the analogous 3′,4′-dimethoxy substitution (compare I/i and I/j). At the same time, the effect of dioxolane and dioxane substitution resulted in different changes on the two columns; the fused dioxolane ring caused a decrease on the IAM column, while in the case of the dioxane substitution a marked rise has been observed in the retention. On the MOS stationary phase both moieties decreased the retention compared to the parent molecule (I/j,k).

Among the halo substituted aurone and thioaurone derivatives the retention factor increased in the F < Cl < Br order on the IAM column (compounds I/o,r,u and II/o,r,u). A parallel change was observed in the MOS data but the differentiation on the IAM phase was more pronounced again. Incorporation of halogen atoms Cl or Br, but not the F, increased the retention factor in comparison with the parent compound, the k′ values of fluoro compounds were usually close to the unsubstituted derivatives. Taking the small radius of fluoro atom into consideration, this phenomenon underlines again the determining role of the shape of the molecule. Bromo containing isomers (I/u,y) with the halogen atom carried on the opposite ends of the molecule were also separated on the IAM column. In accordance with the observations on chlorine derivatives, the 4′-Br compound showed higher retention than the 5-Br isomer. However, the difference between the retention factors proved to be smaller than in case of the chlorine.

Incorporation of cyano or nitro groups into both parent compounds (Ia and IIa) resulted in the decrease of the retention on the IAM column ($k'_{rel,IAM} = 0.79$ (I/v), 0.59 (II/v), and 0.86 (II/w), respectively), a result similar to that of observed on the MOS column. This trend could be interpreted in terms of highly polar character of these substituents.

The Influence of the Heteroatom on the Retention

The effect of the change of the oxygen heteroatom for sulphur atom or sulphone unit on the k′ values measured on the IAM column has also been investigated, results are given in Table 2.

The change of oxygen for sulphur resulted in a marked increase of the k′ value on the IAM column in each case, The average increase of the k′ was 3.37 while the increase of the CLOGP was 0.351 for each aurone/thioaurone pair. A similar effect has been previously observed on the MOS phase, the average difference was 0.541 for the O-S pairs (Table 2). These data show again the higher differentiation capability of the IAM phase compared to the MOS one and the limited differentiating power of the CLOGP due to its calculation procedure based on the fragment method. At O-S exchange on the IAM column the greatest retention increase was observed in the case of the 4′-Br substitution (I–II/u) while the lowest one in the case of the 4′-CN compound (I–II/v).

In accordance with the highly polarized S–O bonds, the S/SO_2 exchange resulted in a considerable decrease of the retention (Table 2). These results are in full accordance with those obtained on the MOS column for the same molecules[7] but more marked differences were observed by using the IAM column again. These findings support our earlier results obtained in the case of parallel carboxamide libraries, aurones, and oxidised thioaurones; the substitution of oxygen atom for

Table 2. Influence of the heteroatom exchange in position X on the retention on different columns or on the CLOGP values

	O-S change at position X			S-SO_2 change at position X		
molecules; (substituents)	$\Delta k'_{IAM}$*	$\Delta k'_{MOS}$*	ΔCLOGP*	$\Delta k'_{IAM}$**	$\Delta k'_{MOS}$**	ΔCLOGP**
a (parent)	3.000	0.500	0.351	−4.989	−1.11	−1.841
b (2′-Me)	3.327	0.672	0.351	−4.507	−1.435	−1.841
d (4′-Me)	3.370	0.828	0.351	−5.336	−1.573	−1.841
h (4′-MeO)	2.858	0.531	0.351	−4.091	−0.995	−1.841
j (3′4′-OCH_2O-)	3.005	0.371	0.351	−3.924	−0.755	−1.841
o (4′-F)	2.021	0.401	0.351			
r (4′-Cl)	3.970	0.654	0.351			
u (4′-Br)	7.459	0.724	0.351			
v (4′-CN)	1.319	0.185	0.351			
mean:	3.370	0.541	0.351	−4.5694	−1.174	−1.841

*difference between the aurone-thioaurone pair substituted with the same group, i.e. $k'_{II/a}$-$k'_{I/a}$ and measured on IAM or MOS phase.

**difference between the thioaurone S,S-dioxide-thioaurone pair substituted with the same group, i.e. $k'_{III/a}$-$k'_{II/a}$ and measured on IAM or MOS phase.

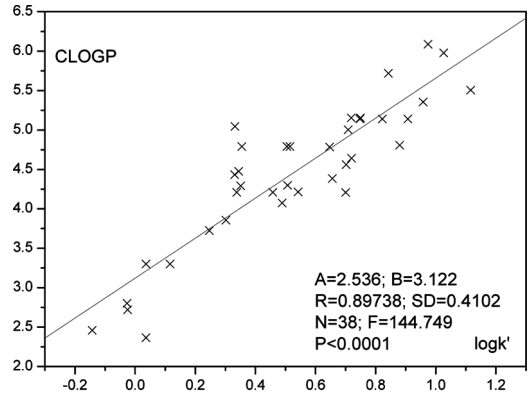
sulphur resulted in an increase both in lipophilicity and cytotoxic activity.[7,33]

Comparison of the Measured Retention Data and the Calculated CLOGP Values

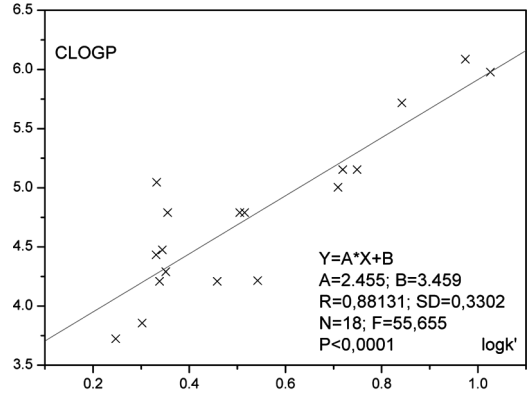
Lipophilicity values of the investigated compounds have been previously characterised by the calculated CLOGP data.[7] The structure-CLOGP relationship has been investigated in a detailed way also.

Good correlation was found between the measured ($\log k'_{IAM}$) and calculated (CLOGP) lipophilicity values ($CLOGP = A x \log k' + B$) for the whole library ($A = 2.536$, $B = 3.122$, $n = 38$, $R = 0.8974$, $SD = 0.4102$, $F = 144.749$, $p < 0.0001$, Figure 2a), and although, the whole compound set was structurally diverse, the linear correlation proved to be good.

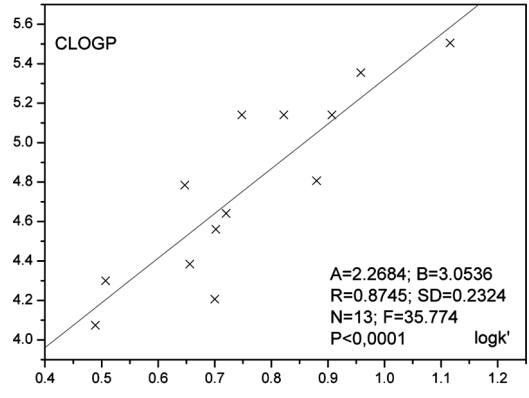
On the basis of structural differences the sublibraries are separated from each other. The fitting parameters of the separated aurone and thioaurone sublibraries have been compared ($N_{aurones} = 18$, $A = 3.459$, $B = 2.455$, $R = 0.8813$, $SD = 0.3302$; $F = 55.655$; $p < 0.0001$; $N_{thioaurone} = 13$;



(a)



(b)



(c)

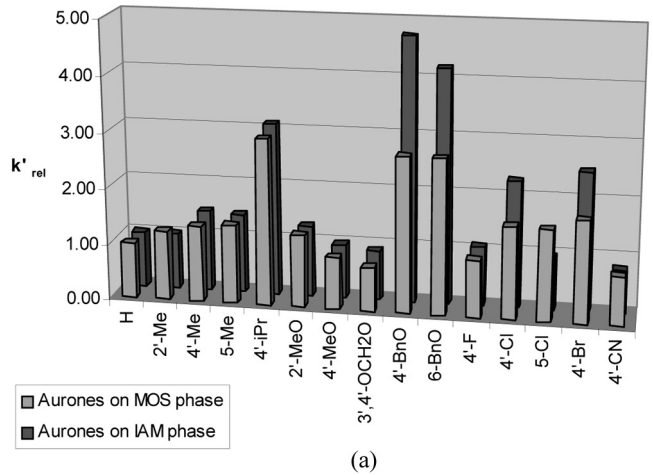
Table 3. Linear regression of the logk′-CLOGP data for each investigated compounds on the IAM and MOS columns. (CLOGP = A*logk′ + B, Figure 3.a and ref.[26])

	MOS	IAM
A	2.946	2.536
B	4.170	3.122
R	0.9041	0.8974
SD	0.4463	0.4102
N	55	38
F	237.139	144.749
P	<0.0001	<0.0001

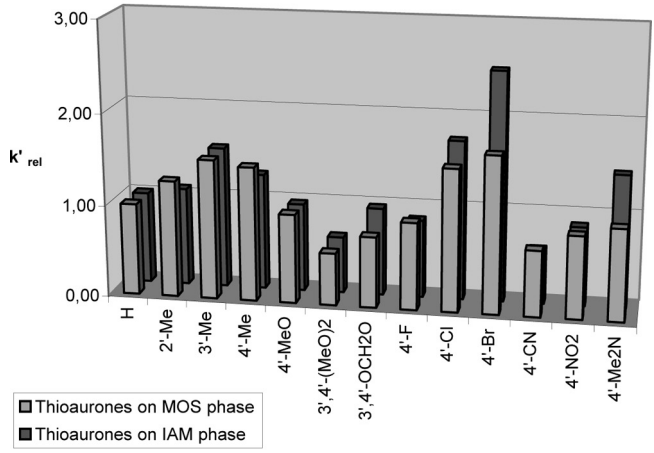
A = 2.268, B = 3.054, R = 0.8745; SD = 0.2324; F = 35.774; p < 0.0001; Figures 2b and 2c, respectively). As it has been pointed out earlier, substitution of oxygen heteroatom by sulphur increases the lipophilicity of the molecule resulting in increased retention factor (k′) on reversed stationary phase.[7,33] This fact is demonstrated on the IAM column in the case of the aurone/thioaurone pairs (see the $k'_{rel,IAM}$ values of the respective aurone/thioaurone pairs in Table 1).

While no outlier molecule has been found in the case of the thioaurone sublibrary by cross validation performed with the "leave one out" method of Allen,[34] the cross validation pointed out the presence of 2 outliers in the case of the aurone and 1-thioaurone sulphone sublibraries. Elimination of any of these 2 molecules resulted in better correlation between the logk′ and CLOGP. One of the outliers is 3′,4′-methylenedioxy-1-thioaurone (III/j) and the second is the 5-chloroaurone (I/s). A much better correlation between the logk′ and CLOGP has been obtained for the remaining 36 members of the whole library after removing the 2 outliers (A = 2.478, B = 3.147, n = 36, R = 0.91837, SD = 0.3476, F = 177.738, p < 0.0001). Correlation data (CLOGP versus logk′) obtained on the two columns has been compared, regression (R) proved to be similar on the two columns (Table 3).

Figure 2. (a) Calculated (CLOGP) *vs.* measured (logk′) lipophilicity values of the 38 members investigated on the IAM column of the whole library (Table 1). Abscissa: logk′; ordinate: CLOGP. (b) Calculated (CLOGP) *vs.* measured (logk′) lipophilicity values of the 18 members of the aurone sublibrary on the IAM column (sublibrary I., see Table 1). Abscissa: logk′; ordinate: CLOGP. (c) Calculated (CLOGP) *vs.* measured (logk′) lipophilicity values of the 13 members of the thioaurone sublibrary (sublibrary II., see Table 1). Abscissa: logk′; ordinate: CLOGP.



(a)



(b)

Figure 3. Comparison of the retention behaviour of the two phase based on the k'_{rel} values of the selected representative molecules of both libraries. Abscissa: representative derivatives with different substituents Ordinate: k'_{rel} values.

Comparison of the IAM and MOS Columns

As it was mentioned earlier, comparison of the data revealed the IAM column to respond in a more sensitive way for the structural changes of the analytes and allowed a finer differentiation than the MOS column did. The most important differences between the two columns are as follows.

Table 4. Comparison of dihedral angles of some selected substituted aurones

R	Compound	Dihedral angle[a] (degree)	Steric energy (kcal/mol)	Compound	Dihedral angle[a] (degree)	Steric energy (kcal/mol)
2′-Me	I/b (Conformer A[b])	55.2	12.3	II/b (Conformer A[b])	60.2	12.3
2′-Me	I/b (Conformer B[c])	38.7	11.0	II/b (Conformer B[c])	47.4	11.5
3′-Me				II/c (Conformer A[b])	42.4	10.0
3′-Me				II/c (Conformer B[c])	40.7	9.9
4′-Me	I/d	23.6	9.3	II/d	40.8	9.8

[a]Dihedral angle of atoms C-2–C_{α}–C-1′–C-2′.
[b]R substituent and O/S-1 atom on the same side.
[c]R substituent and O/S-1 atom on the opposite side.

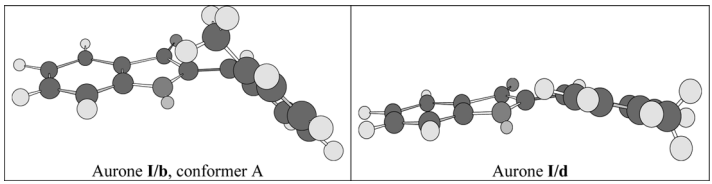


Figure 4. Optimized geometry (MM2) of aurones I/b and I/d (see Table 4).

1. The retention factors of the molecules distributed in a wider range on the IAM than on the MOS column (Tables 1 and 2).
2. The IAM column proved to be able to separate the benzyloxy derivatives.
3. Comparison of the IAM and MOS columns revealed, that the O/S replacement in each case resulted in higher increase, while the S/SO_2 exchange resulted in a higher decrease of the retention factor on the IAM column than on the MOS one (Table 2).
4. As it is shown by the diagrams of Figure 3, the tendencies are generally the same and in many cases the k'_{rel} data are similar in both columns. At the same time, the effect of the incorporated substituent proved to be greater on the IAM than on the MOS column in the case of the benzyloxy and halo derivatives and for the good H-bonding acceptor dimethylamino group.

The higher sensitivity of the IAM column can advantageously be applied when comparison of the lipophilicity of molecules, determination of structure lipophilicity, or any other experimental (biological) data lipophilicity relationship is needed.

CONCLUSIONS

Separation of 38 aurone and thioaurone derivatives possessing similar chemical structure could be obtained both on a immobilized artificial membrane (IAM) and a traditional C_8 (MOS) column.

Good linear correlation was found between the experimentally determined (logk′) and the computer calculated (CLOGP) lipophilicity parameters on both columns.

Both columns proved to be suitable to separate the aurones and thioaurones, and to characterize their lipophilicity. Both the IAM and C_8 (MOS) system proved to be able to make differences among the ortho- and para isomers having different lipophilicity but the same CLOGP data. Consequently, the experimental physico-chemical parameter (logk′) determined either on the IAM or the C_8 (MOS) column may provide real

and useful data for the preselection or prescreening in various libraries, and seem to be a better parameter than the predicted value based on the fragment method. The retention factors of the molecules investigated here distributed in a wider range on the IAM column than on the MOS one, allowing a finer differentiation on the first column. The O/S replacement in each case resulted in higher change in retention factor on the IAM column than on the MOS one. This ability of the IAM column may be very advantageous when a preselection is needed within molecule libraries including chemically very similar compounds (e.g., structure isomers).

The IAM column showed higher efficiency in separation of isomeric aurones or thioaurones and, consequently, in differentiation of their lipophilicities, than the MOS one. Our findings proved the usefulness of the HPLC method in fast characterisation of the lipophilicity of structurally closely related drug candidates.

ACKNOWLEDGMENTS

This work was supported by the following grants: Hungarian Science Research Fund (OTKA 60197, ETT 448/2006, GVOP 3.2.1.2004/04/0043/3.0.

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Received April 1, 2008
Accepted June 16, 2008
Manuscript 6308