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Journal of Chromatography B, 801 (2004) 229–235
JOURNAL OF CHROMATOGRAPHY B
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# Comparison of measured and calculated lipophilicity of substituted aurones and related compounds

B. Hallgas [a], T. Patonay [b], A. Kiss-Szikszai [b], Zs. Dobos [a], F. Hollósy [a], D. Erős [c], L.Órfi [c], Gy. Kéri [a], M. Idei [a,*]

[a] *Peptidbiochemical Research Group, Department of Medical Chemistry, Hungarian Academy of Sciences, Semmelweis University, Puskin u. 9, H-1088 Budapest, Hungary*
[b] *Department of Organic Chemistry, University of Debrecen, Egyetem tér 1, H-4032, Debrecen, Hungary*
[c] *Institute of Pharmaceutical Chemistry, Semmelweis University, Hőgyes u. 9, H-1088 Budapest, Hungary*

Received 1 July 2003; received in revised form 4 November 2003; accepted 17 November 2003

**Abstract**

A molecule library containing 55 aurone- and thioaurone-type structures has been designed and synthesised. Reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed to separate these compounds and to characterise their lipophilicity by experimental method ($k'$). The experimental lipophilicity data have been compared with the computer calculated lipophilicity parameters (CLOGPs) of the same molecules. In general, good correlations between the measured and calculated lipophilicities have been found with the exception of structure isomers and compounds capable for hydrogen bonding. The chromatographic method was suitable to separate the structure (*ortho* and *para*) isomers of aurone and thioaurones and was sensitive enough to differentiate their lipophilicities. Our findings suggest the usefulness of the chromatographic method in fast characterisation of the lipophilicity of structurally closely related molecules.
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*Keywords:* Lipophilicity; Aurones

## 1. Introduction

In the last decade there was a huge rise in the number of de novo synthesised molecules due to the appearance of combinatorial chemistry both in random and focused molecule libraries [1]. Increasing number of compounds tested in biological and/or biochemical assays of increasing cost induced a serious need for invention of rational methods in drug design [2]. The so-called "drug-likeness" has been recognised as one of the most important parameter influencing the fate of a molecule in the preclinical phase. It means that some selected physico-chemical parameters (e.g. log $P$, p$K_a$, hydrogen-bonding ability) of most of the therapeutically used compounds fall into the same range. A new compound with one parameter out of these ranges still could be useful in therapy but a compound having two or three deviant parameters is hardly expected to become a drug [3]. This situation enhanced the necessity of characterising new compounds by physico-chemical methods. One of the current approaches in rational drug design is to estimate lipophilic character of the new drug candidates as this property plays an important role in the mechanism of their biological action [3–5].

Lipophilicity of a non-ionic compound whose partition is independent of pH is commonly characterised by the *n*-octanol/water (biphasic) partition coefficient ($P_{ow}$, and log $P_{ow}$). The method used most conventionally to determine log $P_{ow}$ is the shake flask method. However, there are serious technical difficulties to measure compounds with very high log $P_{ow}$ ($\geq$6.5) [6,7]. A simple choice to substitute this method is the use of RP-HPLC to characterize lipophilic properties of a compound [8–10]. Another possibility to characterise lipophilicity is the computerised estimation frequently based on fragment approach [8,25]. In the former case the properties of the molecules can be characterised directly by the chromatographic retention derived from their distribution between the stationary and mobile phases [11]. On the basis of retention factor ($k'$) determined by a highly effective, fast and well-automated experimental method

* Corresponding author. Fax: +361-266-7480.
*E-mail address:* miki@puskin.sote.hu (M. Idei).

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doi:10.1016/j.jchromb.2003.11.021

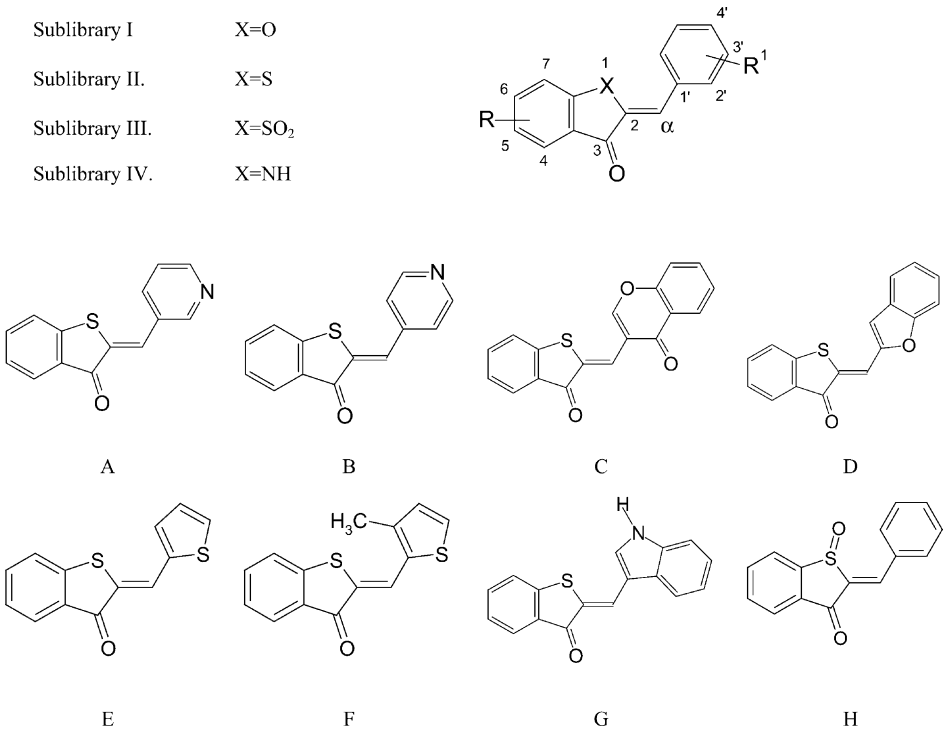


Fig. 1. Basic structure of aurone and its analogues.

biological activity of the members of a molecule library can be predicted [3] making unnecessary to process all members of the library in time-, sample- and labour-consuming biological tests [12].

Aurones and their derivatives belong to the large and diverse family of flavonoids. During the past decades numerous biological activities have been published such as the inhibition of platelet aggregation, analgetic, antiasthmatic, anti-inflammatory, antiallergic, antihyperlipidemic and coronary dilation effects [13–18]. Recently, limited cytotoxic effect of various aurones has also been reported [19].

The aim of the present work was to separate the members of aurone and aurone derivative libraries by suitable RP-HPLC method. Our intention was to characterise their lipophilicities by experimentally determined values ($\log k'$) and to correlate these experimental lipophilicities with the calculated ones (CLOGP).

## 2. Materials and methods

### 2.1. Synthesis of aurones and related compounds

Aurones (sublibrary **I**) were prepared from the corresponding 2′-hydroxychalcones by treating with mercury(II) acetate [20] or trimethylsilyl azide [21] (for structure of synthesized aurone derivatives and sublibraries see Fig. 1). 1-Thioaurones (sublibrary **II**) and related analogues (**A**–**G**) 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 [22] Sulphones (sublibrary **III**) were prepared by oxidation of thioaurones (**II**) with dimethyldioxirane [22] or hot hydrogen peroxide/acetic acid [23]. 2-Arylidenindol-3-(2*H*)-ones (sublibrary **IV**) were synthesized by piperidine-catalyzed condensation of indoxyl acetate and the corresponding aldehyde [24]. 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).

### 2.2. Chemicals

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

### 2.3. HPLC measurements

For chromatographic analysis stock solutions (0.5 mg/ml) of the samples in acetonitrile:water (4:1) 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: Hypersil 5 MOS 5 μm, 300 mm × 4.6 mm (BST, Hungary); injector: Rheodyne. Eluent A: 0.083 M triethyl ammonium phosphate

Table 1
Calculated CLOGP and measured log $k'$ values of 55 compounds

| | Substituents | | Sublibrary **I** (X = O) | | Sublibrary **II** (X = S) | | Sublibrary **III** (X = $SO_2$) | | Sublibrary **IV** (X = NH) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R | R1 | log $k'$[a] | CLOGP | log $k'$ | CLOGP | log $k'$ | CLOGP | log $k'$ | CLOGP |
| a | H | H | 0.050 | 4.291 | 0.210 | 4.642 | −0.291 | 2.801 | −0.335 | 3.851 |
| b | H | 2′-Me | 0.144 | 4.790 | 0.315 | 5.141 | −0.201 | 3.300 | −0.228 | 4.350 |
| c | H | 3′-Me | – | – | 0.393 | 5.141 | – | – | – | – |
| d | H | 4′-Me | 0.187 | 4.790 | 0.374 | 5.141 | −0.101 | 3.300 | −0.203 | 4.350 |
| e | 5-Me | H | 0.200 | 4.790 | – | – | – | – | – | – |
| f | H | 4′-iPr | 0.526 | 5.718 | | | – | – | 0.111 | 5.278 |
| g | H | 2′-MeO | 0.163 | 4.210 | – | – | – | – | – | – |
| h | H | 4′-MeO | 0.021 | 4.210 | 0.199 | 4.561 | −0.232 | 2.720 | – | – |
| i | H | 3′,4′-$(MeO)_2$ | – | – | −0.035 | 4.300 | −0.394 | 2.459 | – | – |
| j | H | 3′,4′-$OCH_2O$ | −0.053 | 3.856 | 0.099 | 4.207 | −0.300 | 2.366 | – | – |
| k | H | 3′,4′-$OCH_2CH_2O$ | −0.036 | 4.215 | – | – | – | – | – | – |
| l | H | 4′-BnO | 0.496 | 5.978 | – | – | – | – | – | – |
| m | 6-BnO | H | 0.495 | 6.087 | – | – | – | – | – | – |
| n | 6,7-$(BnO)_2$ | H | 0.787 | 7.460 | – | – | – | – | – | – |
| o | H | 4′-F | 0.060 | 4.434 | 0.190 | 4.785 | −0.317 | 2.944 | – | – |
| p | 5-F | H | 0.093 | 4.476 | – | – | – | – | – | – |
| r | H | 4′-Cl | 0.269 | 5.004 | 0.400 | 5.355 | −0.060 | 3.514 | – | – |
| s | 5-Cl | H | 0.265 | 5.046 | – | – | – | – | – | – |
| t | H | 3′,4′-$Cl_2$ | – | – | 0.621 | 5.948 | – | – | – | – |
| u | H | 4′-Br | 0.313 | 5.154 | 0.444 | 5.505 | 0.006 | 3.664 | – | – |
| v | H | 4′-CN | −0.008 | 3.724 | 0.067 | 4.075 | – | – | −0.333 | 3.284 |
| w | H | 4-$NO_2$ | – | – | 0.162 | 4.385 | – | – | – | – |
| z | H | 4-$Me_2N$ | – | – | 0.212 | 4.807 | – | – | – | – |
| x | H | 5-Tet | – | – | – | – | – | – | −0.971 | 2.704 |
| A | see Fig. 1 | | | | −0.213 | 3.145 | | | | |
| B | see Fig. 1 | | | | −0.252 | 3.145 | | | | |
| C | see Fig. 1 | | | | −0.031 | 3.882 | | | | |
| D | see Fig. 1 | | | | 0.357 | 5.202 | | | | |
| E | see Fig. 1 | | | | 0.11 | 4.288 | | | | |
| F | see Fig. 1 | | | | 0.194 | 4.787 | | | | |
| G | see Fig. 1 | | | | −0.126 | 4.632 | | | | |
| H | see Fig. 1 | | | | −0.634 | 2.527 | | | | |

Abbreviations used: Me: methyl, Bn: benzyl, iPr: isopropyl, 5-Tet: 5-tetrazolyl.
[a] Values are means of three parallel measurements, where R.S.D. was less than 2%.

(TEAP), pH 2.25; eluent B: 95% ACN + 5.0% A. Parameters of the gradient elution: 0 min: 100% A, from 100% A to 100% B within 20 min, 22 min: 100% B, 23 min: 100% A. Isocratic runs were performed in an eluent of 52% (v/v) ACN in eluent A. Flow rate: 1 ml/min; temperature: 20 °C. Injected volume 20 μl, four injections have been performed for each sample to control repeatability. Retention factors ($k'$) of the samples were calculated from the experimentally determined retention data: $k' = (t_R - t_0)/t_0$. Correlation between the $k'$ and software predicted lipophilicity (CLOGP) has been investigated, parameters of the CLOGP = $A \log k' + B$ equation has been determined.

### 2.4. Calculation of CLOGP data

Software-predicted lipophilicity of the compounds was calculated with the program CLOGP accessible via Internet (http://www.daylight.com/daycgi/clogp) working with the Hansch-Leo's "fragment constant" method on the basis of the chemical structure of the compound processed [6,25].

## 3. Results and discussion

### 3.1. Results of the HPLC measurements

A library consisting of 55 structurally related compounds have been investigated. Based on their structural features the library could be further divided into four sublibraries. Sublibrary **I** contains the aurone (**I**/**a**) and its derivatives substituted in various positions (Table 1, sublibrary **I**). The sublibraries **II** and **III** are the so-called thioaurones and their sulphones where X = S or X = $SO_2$ (Table 1, sublibraries **II** and **III**, see Fig. 1) while in the sublibrary **IV** nitrogen (X = NH) is incorporated in ring B. The compounds **A**–**G** belonging structurally to sublibrary **II** had to be signed individually because the ring C was replaced by different heterocycles.

There are several methods and stationary phases to study the correlation between the chromatographic retention and $P_{ow}$ [26]. The limits of applicability of silica based phases promoted the application polymer based stationary phases [27,28]. Important development in this field is the

appearance of the universally applicable poly(vinyl alcohol) based octadecyl (ODP) stationary phase which made possible the application of short column and fast methanol/water gradient [28]. This ODP column applicable in the pH range of 2–13 allowing the determination of log *P* for the neutral form of strong bases [28]. The former investigations on molecular libraries possessing chemical structures related to the aurones had been performed by our group on silica based reversed phase columns [29–31]. In favour of the prospective comparability of the results, notwithstanding the excellent properties of the ODP column, silica based reversed phase column was applied in the present work, too.

Suitable RP-HPLC method has been developed to separate all of the members of the sublibraries applying the Hypersil 5 MOS column. Isocratic separation was performed within 24 min, calculated retention factors ($k'$) of the compounds are shown in Table 1. Representative chromatogram of the **II/a**, **d**; **III/d** compounds is shown on Fig. 2.

The effect of the different substituents and that of the different heteroatoms on the retention was studied (compounds **I/b**, **d–f**). The retention factor increased with the incorporation of an apolar methyl group and its change for isopropyl group caused further increase in $k'$ value (Table 1). Among the derivatives substituted with halogen atoms the retention factor also increased in the F < Cl < Br order (compounds **I/o–u** and **II/o–u**). Incorporation of halogen atoms Cl, Br but not F increased the retention factor in comparison with the parent compound. $k'$ values of fluorine-containing compounds were usually close to the unsubstituted derivatives. On the contrary, the pseudohalogen cyano group decreased the retention (**I/v**, **II/v**). In general, the retention factor was increased within a sublibrary by non-polar substituents while groups such as methoxy- and dioxolanyl functionalities (**I/h–k**) capable to create hydrogen bonding with water molecules decreased it, except **I/g** where two-methyl group hindered the H-bonding ability of oxygen atoms increasing the retention in this way. The benzyloxy groups (having ability to create H-bonds) increased the retention factor because of their large apolar part (compounds **I/l–m**).

The isocratic method applied here worked well for the separation of the structure isomers, too. Impact of the spherical factor on the $k'$ value was shown by the structure isomers, where the methyl groups were positioned at *ortho*- or *para*-position. Thus, significant differences were detected between the 2′- and 4′-methylaurones (**I/b**, **d**) and -thioaurones (**II/b**, **d**). The chromatographic method applied was able to percieve the small structural differences resulting in a fine alteration of lipophilicity (**I/b**, **d**). Incorporation of the methyl group in 2′ position (**I/b**) affected the shape of the molecule in its central part making the molecule more spherical and resulted in smaller $k'$ values in comparison with the derivatives with methyl groups in positions 3′ or 4′. The significant difference in the $k'$ values of 2′- and 4′-methyl derivatives have also been observed in the sublibraries **II–IV**. In accordance with this tendency,

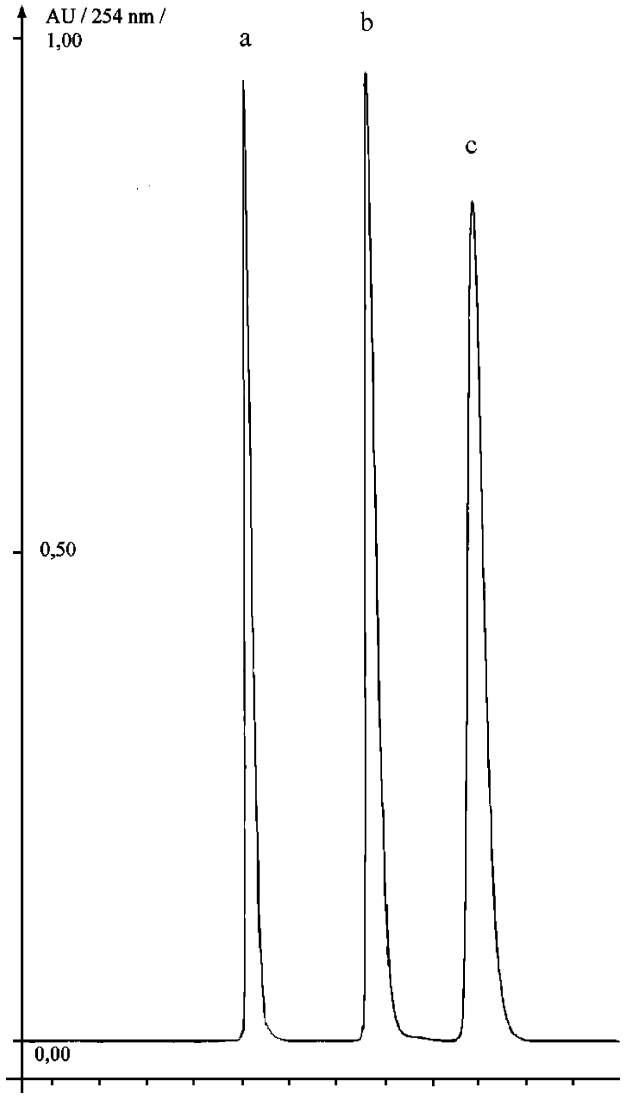


Fig. 2. Chromatogram of the compounds **III/d**, **II/a** and **II/d** (peaks a, b and c, respectively). Abscissa: retention time (min), ordinate: detector response at 254 nm (mAUFs) (for experimental details see Section 2.3).

no marked differences were found between the $k'$ values of the aurones having methyl group in position 5 (**I/e**) or 4′ (**I/d**). Similarly, there was no difference found in retention parameters of compounds **I/l** and **I/m** where benzyloxy substituents connected to the opposite sites of the molecule but the length and the shape of the molecules remained nearly the same.

The effect of the change of the oxygen heteroatom for sulphur, sulphone or nitrogen in the ring B on the measured $k'$ values have been investigated, too (Table 1). In each case the change of O for S resulted in an increase of the $k'$ value (see pairs **I/b** and **II/b**, **I/d** and **II/d**, **I/h** and **II/h**, **I/o** and **II/o**, **I/r** and **II/r**). These results are in full accordance with our earlier results obtained in the case of parallel carboxamide libraries: the substitution of oxygen atom for sulphur resulted in an increase both in lipophilicity and cytotoxic activity [29].

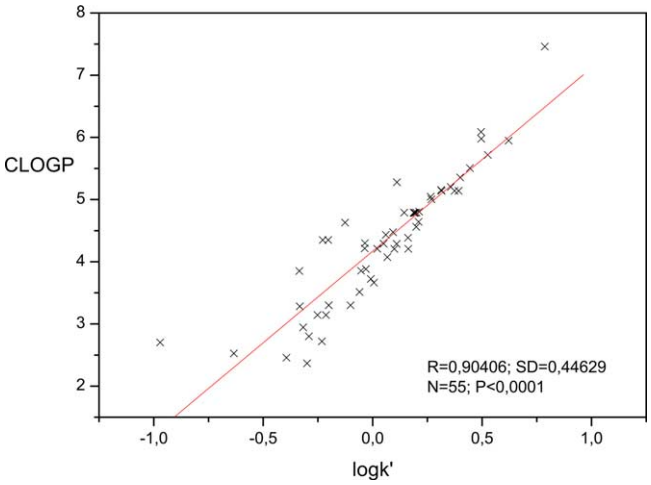


Fig. 3. Calculated (CLOGP) vs. measured ($\log k'$) lipophilicity values of the 55 members of the whole library (Table 1). Abscissa: $\log k'$; ordinate: CLOGP.

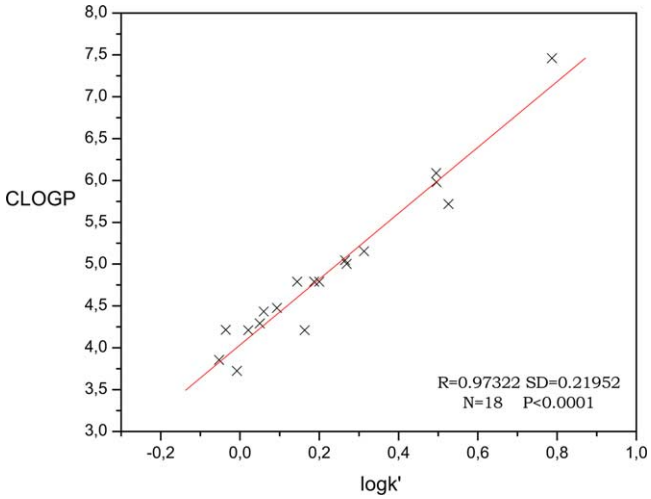


Fig. 4. Calculated (CLOGP) vs. measured ($\log k'$) lipophilicity values of the 18 members of the aurone sublibrary (sublibrary **I**, see Table 1). Abscissa: $\log k'$; ordinate: CLOGP.

Replacement of oxygen for the strongly polar sulphinyl group resulted in a strong decline in retention factor (see **I/a** and **H**). Replacement of oxygen by sulphonyl group showed a similar but less marked effect (see **I/a**, **H** and **III/a**). These results can be explained in terms of the highly symmetric structure and the decreased polarisability of the sulphonyl group. The oxygen–sulphone replacement can also be observed in the pairs **I/a–III/a**, **I/h–III/h**, **I/o–III/o** and **I/r–III/r**.

Replacement of the heteroatom oxygen by NH resulted in a decrease in retention factors due to the higher polarity and the likely formation of hydrogen bonding between this functionality and the water of the eluent (see **I/a–IV/a**, **I/d–IV/d** and **I/v–IV/v**).

### 3.2. The results of CLOGP calculations

Lipophilicity values of the investigated compounds have been characterised by the calculated CLOGP data, too. A good correlation between the measured ($\log k'$) and calculated (CLOGP) lipophilicity values was found (CLOGP = $A \log k' + B$) for the whole library ($A = 2.946$, $B = 4.170$, $n = 55$, $R = 0.9041$, S.D. = 0.4463, $F = 237, 139$, $P < 0.0001$, Fig. 3). The correlation was stronger for the aurone sublibrary ($A = 3.934$, $B = 4.034$, $n = 18$, $R = 0.9732$, S.D. = 0.2195, $F = 286, 753$; $P < 0.0001$, Fig. 4) and for the thioaurone sublibrary ($A = 3.298$, $B = 3.962$, $n = 31$, $R = 0.9504$, S.D. = 0.3197, $F = 270, 569$, $P < 0.0001$ Fig. 5). The fitting parameters of the aurone and thioaurone sublibraries have been compared. 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 [29]. In accordance with that, the slope of the aurone sublibrary proved to be higher than that of the thioaurone one, while the intercept values did not differ from each other.

The CLOGP values (Table 1) increased with the methyl or halogen substitution (compounds **b–e** and compounds **o–u**, respectively) and decreased with the incorporation of methoxy groups (compounds **g**, **h**). The effect of change of the heteroatom on calculated lipophilicity data also correlated well with the measured $k'$ values. In all cases CLOGP data increased with the change of the oxygen for sulphur (see pairs **I/b–II/b**, **I/d–II/d**; **I/h–II/h**, **I/o–II/o**; **I/r–II/r**). The CLOGP values decreased remarkably by changing the oxygen for sulphinyl or NH (**I/a–H**; **I/a–IV/a**; **I/d–IV/d**; **I/v–IV/v**), while this effect was smaller in the case of by sulphonyl group (**I/a–III/a**), in accordance with the experimental facts.

In spite of the good linear correlation between the $k'$ and CLOGP data (Figs. 3–5), a few serious discrepancies have also been observed comparing the measured and

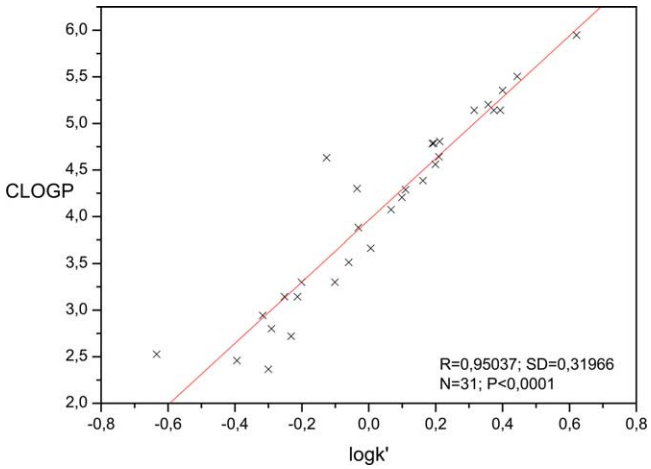


Fig. 5. Calculated (CLOGP) vs. measured ($\log k'$) lipophilicity values of the 31 members of the thioaurone sublibrary (sublibrary **II**, see Table 1). Abscissa: $\log k'$; ordinate: CLOGP.

calculated values. Due to the logic of the fragment approach applied here, calculations gave the same CLOGP values for *ortho*- and *para*-isomers while experimentally determined lipophilicity of these molecules proved to be different (see **I/b**, **d**, **e**; **I/g**, **h** and **II/b–d**).

CLOGP calculation predicted a decreased lipophilicity for the methoxy-substituted derivatives comparing to the parent molecule. In fact, a decrease of $k'$ was observed in the case of the 4′-methoxyaurone (**I/h**) while a slight increase of the $k'$ was detected for the 2′-methoxy derivative (**I/g**). These observations showed that not only the chemical nature of the substituents but also their position might be able to influence the lipophilicity of the molecules. In conclusion, software calculation applied in some cases was "blind" for the description of lipophilicity differences of the isomers being important in the prediction of their biological activity. The same phenomenon was also proved in the case of Mannich ketones with different cytotoxicity but similar CLOGP data [30,31].

Noteworthy, that $\log k'$ values measured for the members of the indolone series (**IV/a**, **b**, **d**, **g**, **w**, **x**) located out of the regression line (see regression for the whole library, Fig. 3). The existence of hydrogen bonding may be the reason of the anomaly. The same effect is assumed in the case of three outlier of thioaurone series (**G**, **II/i** and **H**). While no outlier molecule has been found in the case of the aurone sublibrary by cross validation performed with the "leave one out" method of Allen [32], the cross validation pointed out the presence of three outliers in the case of the thioaurone sublibrary. Eliminating any of these three molecules resulted in better correlation between the $\log k'$ and CLOGP. One of the outliers bears a N heteroatom in its ring C (**G**), the second one contains two methoxy groups (**II/i**) and the third is the sulphoxide (compound **H**, Table 1). A much better correlation between the $\log k'$ and CLOGP has been obtained for the remaining 28 members of the thioaurone sublibrary after removing the three outliners ($A = 3.653$, $B = 3.859$, $n = 28$, $R = 0.9869$, S.D. $= 0.1665$, $F = 977, 275$, $P < 0.0001$). The dissociable hydrogen, the hydrogen-bonding capability of compound **G** and **II/i**, respectively, or the high electron density on the sulphoxide oxygen of compound **H** allowing a strong interaction with the water molecules may decrease the retention of the otherwise lipophilic outlier molecules on the reversed phase column.

## 4. Conclusions

Separation of 55 aurone derivatives possessing similar chemical structure could be obtained within 24 min. Beyond the check of the chromatographic purity an experimental physico-chemical parameter characterising the lipophilicity of the compounds (retention factor, $k'$) can be obtained during the course of the analysis. Good linear correlation was found between the experimentally determined ($\log k'$) and the computer calculated (CLOGP) lipophilicity parameters. In the case of isomers (e.g. *ortho*- and *para*-isomers) the software calculation method was found to give the same result for different molecules. This means that this CLOGP program did not recognise the fine structural lipophilicity affecting alterations having pivotal role in biological activity. Contrary to it, the RP-HPLC system proved to be able to make differences among the *ortho*- and *para*-isomers having different lipophilicity but the same CLOGP data. With other words, the experimentally determined physico-chemical parameter ($\log k'$) may provide real and useful data for the preselection or pre-screening in various libraries. This ability of the chromatographic method may be very advantageous when a preselection is needed within molecule libraries containing chemically very similar compounds (e.g. structure isomers).

## Acknowledgements

This work was supported by the following grants: OTKA 37188, 32415; ETT 232/2001; NKFP–1/041 (MEDICHEM), NKFP–1/A/0020/2002 (MOLDIAG), Hungarian State Eötvös Foundation.

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