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Characterization of lipophilicity and antiproliferative activity of *E*-2-arylmethylene-1-tetralones and their heteroanalogues

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Abstract

A molecular library based on *E*-2-arylmethylene-1-tetralone has been designed and synthesized. A reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed and applied to separate them and to characterize their lipophilicity. The chromatographic method applied here was suitable to separate the structural (*ortho* and *para*) isomers of compounds and was sensitive enough to differentiate their lipophilicities. The measured (k') and computer calculated (CLOGP) lipophilicity values has been compared. Good linear correlation has been found in the case of these structurally related molecules. In vitro biological assay has been performed with Methylene blue dying to investigate the antiproliferative potency of the compounds synthesized in this work. The measured (k') and calculated (CLOGP) lipophilicities of the compounds were compared with the antiproliferative activities and an optimum value of lipophilicity has been found for these compounds.

Keywords: Tetralones; Molecular library; Lipophilicity; Retention factor and CLOGP calculation; Antiproliferative activity

1. Introduction

Living organism as raw material has been proven to be the richest source of possible medicinal remedies. There is hardly a group of diseases or pathological states in which an effective chemical agent or the core structure of it is not derived from a natural source. An analysis of the number of chemotherapeutic agents and their sources indicates that over 60% of approved drugs are derived from natural compounds [1]. These molecules originating from natural sources may have a wide spectra of pharmacological properties, e.g. the nutritional properties of lectins [2] or the Chinese and Indian traditional herbal plants used for improvement of memory and cognitive function can be mentioned [3], certain phytosterols possess hormone-like pharmacological effects [4] or certain alkaloids can be used in symptomatic pharmacotherapy of migraine [5]. In addition, it should be noted that the class of flavonoids with more than 4500 different known representatives is an enormous class of plant secondary metabolites having so different pharmacological effects as inhibition of nitric oxide synthase [6], cancer preventive effects [7] or potential impact on the etiology of certain vascular disease [8].

In the last decades, a large number of plant extracts have been found containing secondary metabolites able to inhibit various protein tyrosine kinases. Recently, Hollósy and Kéri reviewing the major groups of this plant derived protein tyrosine kinase (PTK) inhibitors and candidates [9] assumed that the natural products are suitable for further exploration to obtain novel leads for new molecular entities as thera-

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peutic agents [10] because of their enormous chemodiversity.

E-2-Arylmethylene-1-tetralones and *E*-3-phenylmethylenechroman-4-ones and their derivatives closely related to flavonoids belong to the plant secondary metabolites most investigated recently. Numerous biological activity have been attributed to the tetralones mentioned. So, we have reported the synthesis and antifungal properties of some bicyclic α,β-unsaturated ketones–homoisoflavones—as *E*-2-arylmethylene-1-tetralones, *E*-3-arylmethylenechroman-4-ones, *E*-3-arylmethylene-1-thiochroman-4-ones [11,12]. Several representants of these classes of compounds showed remarkable antifungal activity. Their minimum inhibitory concentration (MIC) values ranged from 1.5 to 6.0 μg/mL in an antimycotic screening against human pathogenic yeasts (*Candida albicans, C. parapsilosis, C. glabrata, Cryptococcus neoformans* and *Trichosporon cutaneum*). Their effects proved to be better than those of the six commercial antimycotic agents similarly tested in the same study [11]. Shih et al. [13] reported antitumor activity of closely related phenylmethylene-1-tetralones on Jurkat cell line and revealed strong structure-activity relationship.

Because of the enormous increase in the cost of consumables and time demands, the necessity of examining druglikeness of a novel compound has emerged [15]. Druglikeness, being a multidimensional terrain of chemical properties in which the classical physico-chemical parameters of therapeutically used molecules occur mostly, serves to identify compounds suitable for drug development [15,16]. The most recent druglikeness definition by Gilbert M. Rishton based on the classical contribution of Lipinsky et al. supplemented the theory with the polar surface area and rotatable bond consideration [16].

All benefits of pre-screening can be gained by exploration of physico-chemical properties in advance and early attrition of the useless compounds (before the expensive biological assays) could be achieved. One of the most important parameters to decide the status achievable by a new molecule in the pharmacological development process is lipophilicity. Since this property plays an important role not only in the mechanism of its pharmacokinetic action but in the pharmacodynamic also, estimation of the lipophilic character of a new drug candidate is proved to be one of the first parameter to be determined at the earliest possible moment [28].

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 shake flask method is the most conventionally used one to determine $\log P_{ow}$. However, there are serious technical difficulties to measure compounds with very high $\log P_{ow}$ ($\geq$6.5) [17,18]. Another choice to characterise lipophilicity of a molecule is the computerised estimation of it, frequently based on the fragment approach [19,22], but there are many improved method derived from the original Hansch-Leo's one [17]. Another possibility is to investigate the influence of lipophilicity on biological activity instead of fragment approach either atom-based (e.g. Crippen's, Viswanadhan's, Broto's method) [23] or molecular lipophilicity potential (MLP) developed by Testa and co-workers [24].

The HPLC method turned to be a valuable detection tool to determine octanol–water partition coefficient directly by measuring the concentration of the compound in the two partitioning solvents one by one. It has long been recognised that the retention of a compound in RP-HPLC is governed by its lipophilicity and thus it correlates with the log *P* measured in *n*-octanol–water system. Therefore, it is a plausible alternative to use RP-HPLC as a substitute for the classical slow and uncomfortable shake-flask method to characterize lipophilic properties of a compound [19–21]. A great number of publications on the efforts made to adjust HPLC methods and to improve stationary phases to substitute $P_{o/w}$ measurements are well reviewed by Testa and co-workers [27] or Valkó [28].

In this work, based on the results obtained with the bicyclic unsaturated ketones, we have been prompted to design and synthesize new type of antiproliferative compound library of bicyclic α, β-unsaturated ketones based on the *E*-2-arylmethylene-1-tetralones or on their homologues and heterocyclic analogues (Fig. 1). The size of the bicyclic ketone and the heteroatom itself in the ketone ring was varied. In addition α,β-unsaturated ketones both with electron withdrawing and electron donating group in the aromatic ring were synthesized.

A reliable, fast and accurate HPLC method was developed to investigate the 26 membered molecular library of *E*-2-arylmethylene-1-tetralones and their heterocyclic analogues. Our intention was to characterize the lipophilicity and antiproliferative activity of these compounds. The lipophilicity of the compounds was evaluated not only experimentally (HPLC) but by in silico calculation based on their chemical structure (CLOGP), too. Antiproliferativ effect of the molecules was determined on A431 cells by the Methylene blue method [26]. Both the relationship between the measured (k') and calculated (CLOGP) lipophilicity data and that of between the biological activity and the lipophilicity data obtained by HPLC or by calculation have been investigated.

The former experimental work on molecular libraries possessing chemical structures related to the compounds investigated here had been performed on silica based reversed phase columns by our group [29–31]. In favour of the prospective comparability of the results silica based reversed phase column was applied in the present work, too.

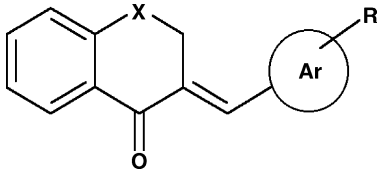


Fig. 1. Basic structure of the compounds investigated; X = $-CH_2$, $(CH_2)_2$, O, S, SO, SO_2; Ar = phenyl, substituted (R) phenyl, heteroaryl.

2. Materials and methods

2.1. Materials

Triethylamine (TEA), acetonitrile (ACN), *ortho*-phosphoric acid, methanol, ethanol, piperidine, Methylene blue dye and the parent aldehydes utilised in the synthesis were purchased from Fluka (Buchs, Switzerland). Solutions were prepared of deionised, bacteria-free water made by Elgastat UHP system (Elga Ltd. Bucks, England).

2.2. Synthesis

A library consisting of 26 structurally related compounds have been investigated. Based on their structural features the library could be further divided into two subgroups. Group I (Table 1) is consisting of substituted arylmethylene–tetralones (**1**–**10**, the bold numbers refer to the numbers of Table 1), heteroaryl–methylene–tetralones

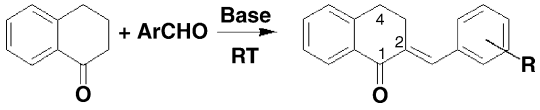
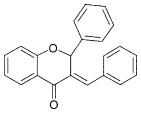
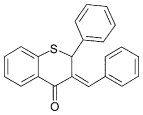


Fig. 2. Synthesis of aryliden-tetralones; Ar = phenyl, substituted phenyl (R), heteroaryl.

(**11**–**18**) and the indanone (**20**) and benzosuberone (**19**) derivatives. Group II contains the chromanones and their analogues (**21**–**26**). The compounds investigated here have been prepared by base catalysed aldol condensation (Fig. 2) [11,12]. With the *E*-2-arylmethylene-1-tetralones (**1**–**18**,), *E*-2-phenylmethylene-1-benzosuberone (**19**) and *E*-2-phenylmethylene-1-indanone (**20**) the reaction was carried out at room temperature in ethanol. The synthesis of the *E*-3-arylmethylenechroman-4-ones and *E*-3-arylmethylene-1-thiochroman-4-ones (**21**–**22**) was performed at 140 °C with piperidine as a catalyst without solvent. The 4-thiochromanone 1-oxide (**23**) and 1,1-dioxide (**24**) was pre-

Table 1
Experimentally measured (k'; $\log k'$) or calculated (CLOGP) lipophilicities and biological activity values of the investigated compounds

	X	Ar	R	k'[a]	$\log k'$	CLOGP	IC_{50}[b] (μmol/l)
Tetralones and their analogues							
1	$-CH_2-$	Phenyl	H	4.534	0.656	4.398	11.780
2	$-CH_2-$	Phenyl	4′-Me	5.894	0.770	4.897	20.990
3	$-CH_2-$	Phenyl	4′-OMe	3.644	0.562	4.317	7.313
4	$-CH_2-$	Phenyl	4′-fluor	4.233	0.627	4.541	15.830
5	$-CH_2-$	phenyl	2′-chlor	6.068	0.783	4.871	20.630
6	$-CH_2-$	Phenyl	4′-chlor	6.968	0.843	4.871	15.500
7	$-CH_2-$	Phenyl	3′, 4′-chlor	11.761	1.070	5.824	20.780
8	$-CH_2-$	Phenyl	2′, 4′-chlor	11.417	1.058	5.824	17.470
9	$-CH_2-$	Phenyl	2′, 6′-chlor	9.214	0.964	5.824	24.560
10	$-CH_2-$	Phenyl	4′-brom	7.938	0.900	5.261	100.000
11	$-CH_2-$	2-Furyl	H	2.408	0.382	3.574	46.720
12	$-CH_2-$	2-Pyrrolyl	H	1.425	0.154	3.004	100.000
13	$-CH_2-$	*N*-Methyl-2-pyrrolyl	H	2.223	0.347	3.470	63.750
14	$-CH_2-$	2-Thienyl	H	3.190	0.504	4.044	100.000
15	$-CH_2-$	2-Pyridyl	H	0.788	−0.103	2.901	60.610
16	$-CH_2-$	3-Pyridyl	H	0.390	−0.409	2.901	8.240
17	$-CH_2-$	4-Pyridyl	H	0.571	−0.243	2.901	7.105
18	$-CH_2-$	3-Indolyl	H	2.235	0.349	4.388	100.000
19	$-(CH_2)_2-$	Phenyl	H	5.205	0.716	4.957	62.100
20	–	Phenyl	H	2.239	0.350	3.839	58.470
Chromanones and related compounds							
21	O	Phenyl	H	3.175	0.502	3.750	4.580
22	S	Phenyl	H	4.961	0.696	4.191	3.980
23	SO	Phenyl	H	0.670	−0.174	2.413	17.110
24	SO_2	Phenyl	H	0.134	−0.873	2.359	10.800
25		Phenyl	H	8.328	0.921	5.508	3.970
26		Phenyl	H	11.479	1.060	5.749	6.890

[a] R.S.D. of the k' values were less than 2%, the compounds investigated here were injected individually, number of intra-day repetitions was 3 ($n = 3$) for each of the compounds.
[b] R.S.D. of the IC_{50} values were less than 10%, three replicates has been made for each of the compounds.

pared according to ref. [25]. All of the compounds were purified by recrystallisation from methanol and with column chromatography. Their structural characterisation is based on FT-IR methods and previously published NMR data [14,32–43]. The FT-IR spectra were recorded by an Impact 400 (Nicolet) spectrometer in KBr pellets. Theoretically, the *E*- and *Z*- geometric isomers can be equally formed in the reaction mentioned above. The *Z*-configuration, however, is highly unfavourable because of strong steric interaction between the aryl and carbonyl groups [14]. In full accordance with this, the *E*-configuration unambiguously was verified by the appearance of the H-α proton signal in the appropriate range in the ^{1}H-NMR spectra [11]. The ^{1}H and ^{13}C assignments (not known from the literature) of compound (**18**) were based on simple ^{1}H and ^{13}C measurements and corroborated by ^{1}H–^{1}H COSY, gradient enhanced ^{13}C–^{1}H HSQC as well as ^{13}C–^{1}H HMBC experiments executed using standard Varian software. NMR spectra were recorded with Varian ^{UNITY}*INOVA* 400 WB (400/100 MHz for ^{1}H/^{13}C) spectrometer. Chemical shifts are referenced to Me_4Si (^{1}H) or to the residual solvent signals (^{13}C). Measurements were run at 298 K probe temperature.

2.3. HPLC measurements

For chromatographic analysis stock solutions (0.5 mg/ml) of the samples in acetonitrile:water (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: Hypersil 5 MOS 5 μm, 250 mm × 4.6 mm (BST, Hungary); injector: Rheodyne. In many cases different alkyl-ammonium phosphates are used in the eluent for buffering when the determination of the interaction between the analyte and the hydrophobic alkyl chains of the stationary phase is intended. Based on these former practice triethyl-ammonium phosphate was chosen as mobile phase additive [44–48]. Eluents: A: 0.083 M triethyl ammonium phosphate (TEAP, made by weighing the calculated quantities of triethyl amine and phosphoric acid), pH 2.25; B: 95% ACN + 5% A (TEAP).

Isocratic runs were performed in an eluent of 40 v/v% ACN in A eluent; flow-rate: 1 ml/min; temperature: 20 °C. Injected volume 20 μl, the compounds investigated here were injected individually, number of intra-day repetitions was 3 ($n = 3$) for each of the compounds (a mixture containing 12 compounds has been chromatographed for representative purposes, see: Fig. 3). Retention factors (k') of the samples were calculated from the experimentally determined retention data ($k' = (t_R - t_0)/t_0$, where t_0 has been determined by injection of water [54,55]). Correlation between the k' and software predicted lipophilicity (CLOGP) has been investigated, parameters of the CLOGP = $A \log k' + B$ equation has been determined.

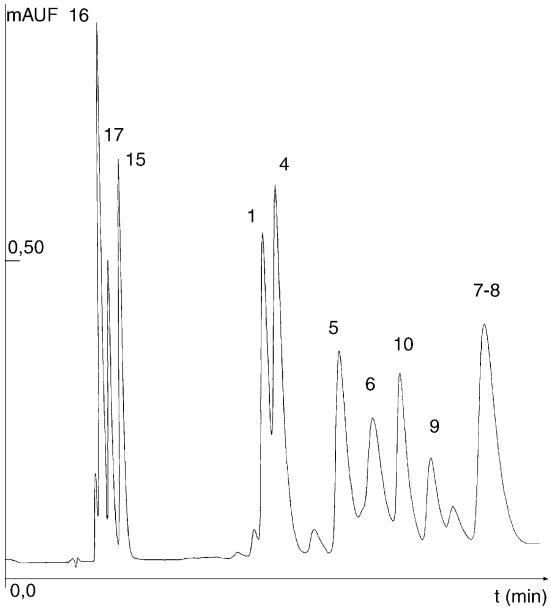


Fig. 3. Chromatogram of a representative mixture containing the following compounds **16**, **17**, **15**, **1**, **4**, **5**, **6**, **10**, **9**, and **7**–**8** (Table 1). Abscissa: retention time (min), ordinate: detector response at 254 nm (mAUFs). For experimental details see Section 2.3.

2.4. Calculation of CLOGP data

Software-predicted lipophilicity of the compounds was calculated with the program CLOGP accessible via Internet (www.daylight.com/daycgi/clogp) working with the Hansch-Leo's, "fragment constant" method.

2.5. Antiproliferative assay

Proliferation assays were based on the Methylene blue test [26]. This colorimetric assay based on the enzyme activity of various dehydrogenases of the living cells is suitable for testing the cytotoxic activity of anti-tumour candidates in vitro. Human A431 epidermoid carcinoma cells were cultured in DMEM (Dulbecco's Mod Eagle Medium) supplemented with 10% FCS (foetal calf serum), 200 mM L-glutamine, 10,000 U/ml penicillin and 10 mg/ml streptomycin (Gibco Life Sci) at 37 °C and 5% CO_2. Cells were seeded into 96-well plates and incubated for 16 h before serial dilutions of compounds were added (three replicates has been made for each of the compounds investigated). Antiproliferative efficacy was assessed after 48 h: cells were fixed by 10% buffered paraformaledhyde in 0.9% NaCl. Wells were then stained by 1% methylene blue, followed by thorough washing. Both apoptotic and necrotic cells previously detached from the surface of wells are thus removed. Methylene blue stain from cells entrapped on the plate surface was dissolved by ethanol (100%): 0.1 M HCl 1:1 and optical densities measured by a microtiter plate photometric reader at 650 nm.

3. Results and discussion

3.1. Results of the HPLC measurements

A library consisting of 26 structurally related compounds have been investigated. Based on their structural features the library could be further divided into two subgroups. Group I (Table 1) is consisting of substituted arylmethylene–tetralones (**1**–**10**), heteroaryl–methylene–tetralones (**11**–**18**) and the indanone (**20**) and benzosuberone (**19**) derivatives. Group II contains the chromanones and their analogues (**21**–**26**).

The RP-HPLC method applied in this work proved to be applicable for fast analysis of the molecular library investigated. Isocratic separation was performed within 38 min, calculated retention factors (k', $\log k'$) are shown in Table 1. Representative chromatogram of the **16**, **17**, **15**, **1**, **4**, **5**, **6**, **10**, **9**, and **7**–**8** compounds, respectively is shown on Fig. 3. The chromatographic method applied here was able to percieve the small structural differences resulting in a fine alteration of lipophilicity (e.g. chlor substituted derivatives like **7**–**8**–**9**).

The value of k' embracing a 30-fold difference, ranged from 0.39 to 11.76 depending on the substituted aryl side chain in group I. (see Table 1, compound **16** and **7**, respectively). Obviously, the dichlor derivatives (**7**–**8**–**9**) exhibited the highest retention and amongst the three structurally different isomers the 3′, 4′-chlor (**7**) derivative is the uppermost. The lowest retention is associated to the heteroaryl–pyridyl-derivatives (**15**–**16**–**17**), in particular, to the 3-pyridyl one (**16**). The molecules with heteroaryl moieties (**11**–**18**) can be categorized as a subgroup not only by structural differences but on the score of measured values too. None of them reached the lipophilicity value of the parent molecule (**1**), not even the compound (**14**) with 2-thienyl moiety.

The benzosuberone compound (**19**) has just showed a slight increase in k' comparing to the parent molecule (**1**). However, in the case of the indanone derivative (**20**) less than half of the basic tetralone's value (**1**) has been detected. Possibly it caused by not only the absence of the methylene fragment but by the different 3D structure of the two molecules. The indanone have been stated to be present in completely planar conformation but in case of tetralones and benzosuberones the enone fragment slightly and the aryl ring much more deviate from the planar skeleton [14]. Similar relation between the planarity of the analyte and its retention has been found by Larkins and Olesik [53].

In the subgroup of compounds bearing substituted aryl ring not only the methoxy-(**3**) but the fluoro derivative (**4**) also have exhibited lower k' as the basic tetralone. Methyl substitution (**2**) has caused just a slight, while other halogen substitution at various position (**4**–**5**–**6** and **10**) has caused considerable rise in lipophilicity and retention. Impact of the spherical factor on the k' value was shown by the structural isomers, where the chlor- or dichlor substituents were incorporated into *ortho*, *para* positions and in case of dichlor compounds into combined positions (Fig. 3). Thus, significant differences were detected between the 2′- and 4′-chlor derivatives (**5**–**6**), while the compound with *ortho*, *ortho*′-dichlor substituents (**9**) has been completely separated from the other two dichlor-derivatives having chlor atoms either in *para*-position and in addition *meta*- or *ortho*-position (**7**–**8**). Incorporation of the chlor atom in 2′ position (**5**) affected the shape of the molecule in its inner part turning the molecule more spherical and resulted in smaller k' values in comparison with the derivative of chlor atom in positions 4′ (**6**). The same effect on lipophilicity could be observed being associated with spatial features of the dichlor derivatives because both compound having chlor atom in *para*-position (**7**–**8**) caused stronger rise in retention factor than the compound **9** bearing two chlor substituent in *ortho* position. Similar observation has been reported in our earlier paper in case of structural isomers of methyl-aurone, thio-aurones and Mannich ketones [30,31].

Good separation has been achieved in the sub-group of heteroaryl derivatives (**11**–**18**). The molecules with heteroatom built-in the five-membered ring (**11**–**14**) showed higher retention than those with heteroatom built-in the 6-membered pyridyl ring (**15**–**17**). The lower retention of the pyridyl compounds was caused by the slight basic character of the pyridyl ring.

The chromatographic method applied here proved to be able to separate (Fig. 3) the three structural isomer pyridyl derivatives (**15**–**17**). As it can be seen the 3-pyridyl (**16**) compound has the lowest retention and their separation was probably governed by the likely difference in their basicity. An approximate estimation on the basicity of the isomer pyridyl compounds can be obtained with the help of the isomer methylpiridines. The three different methylpyridine molecules have the following experimental pK_b values 8.0; 8.37; 8.02 (*ortho*, *meta*, *para*, respectively). [Data from SRC PhysProp Database: http://www.syrres.com/esc/physdemo.htm]. It can be seen that basicity of the *ortho*, and the *para* compounds is almost the same, but the *meta* weakly differs. It can be explained by the electron donating effect of the methyl group being the weakest at *meta*-position and stronger equally in the other two positions. In the case of the structural isomer pyridyl molecules (**15**–**17**) the same effect may explain the nice separation (Fig. 3). Because the electron distribution of these compounds not as simple as that of methylpyridines, perhaps the extension of delocalisation and the distortion of aromatic electron distribution by methylene group could explain the presumably different basicity of each structural isomers.

The impact of the O, S, SO, and SO_2 exchange in position X on chromatographic behaviour and lipophilicity of the molecules has been investigated in the group II (chromanone and its analogues; **21**–**26**). As it was expected, the chromanone's retention (**21**) proved to be lower than that of the tetralone (**1**) because of the polarisability and capability for hydrogen bond. It had previously been reported that lipophilicity could be increased by exchanging the carbon atom to sulphur [49]. In full accordance with it, now

the same effect could be obtained with thiochromanon: the k' data of thiochromanon (**22**) was significantly higher than that of the tetralone (Table 1). The compound with sulphinyl moiety (**23**) showed much decreased retention parameter as it was expected and incorporation of the sulphonyl derivative (**24**) was found to cause further decrease in k'. Both effect can be explained the stronger polarisability of sulphonyl and sulphinyl group and was demonstrated earlier by our group in reference to aurones and oxidised thioaurones [49].

The *E*-3-phenylmethylene-4-flavanone (**25**) and its thio derivative (**26**) bear one more aryl group at position 2. An increase in retention factor could be expected as a result of incorporation of an apolar aryl group or sulphur atom. In full accordance with this expectation, the former compound (**25**) showed an increased retention comparing to its parent chromanone molecule (**21**) and incorporation of sulphur instead of carbon atom caused further rise of k' data (Table 1).

3.2. Calculated lipophilicity (CLOGP) values

Lipophilicity profile of the investigated compounds have been characterised by calculated CLOGP data also. Comparison of the experimentally measured ($\log k'$) and computer estimated (CLOGP) lipophilicity parameters revealed a good linear correlation (CLOGP = $A \log k' + B$) for the set of the compound evaluated here ($A = 3.2789$, $B = 2.0405$, $n = 26$, $R = 0.93187$, S.D. = 0.40289, $F = 158.33468$, $p < 0.0001$, Fig. 4). Similarly, treating the different sub-groups separately, good linear correlation has been obtained for the tetralones ($A = 2.6746$, $B = 2.9390$, $n = 12$, $R = 0.96739$, S.D. = 0.17011, $F = 145.86742$, $p < 0.0001$) and chromanones ($A = 3.3513$, $B = 1.8117$, $n = 6$, $R = 0.91794$, S.D. = 0.6403, $F = 21.96074$, $p = 0.0094$), while weaker correlation has been obtained for the tetralones containing hetero atoms (compounds 11–18, $A = 3.2290$, $B = 1.3776$, $n = 8$, $R = 0.79838$, SD = 0.37523, $F = 10.54736$, $p = 0.01752$).

Generally, the CLOGP values (Table 1) increased with the methyl (**2**) or halogen substitution (**4**–**10**) and decreased slightly with the incorporation of methoxy group (**3**). Usually the molecules with five- or six-membered rings containing heteroatom (in the arylidene moiety) had lower computed values as the parent tetralone molecule had. In accordance with the experimentally determined values, the lowest CLOGP data amongst the arylmethylene-1-tetralones associated with pyridyl derivatives (**15**–**17**) but the far lowest value calculated for the thiochromanone- 1,1-dioxide (**24**).

The highest calculated CLOGP value was obtained in the case of the three dichlor derivatives (**7**–**9**) and the thioflavanone (**26**) almost reached that value. Investigating the influence of the size of the alicyclic ring fused to the aryl ring on the CLOGP values, in accordance with the experimentally determined log k' data (**20**–**1**–**19**, respectively) a gradual rise in the calculated parameter could be observed (in order of indanone < tetralone < benzosuberone).

The impact of the heteroatom on calculated lipophilicity data also correlated well with the experimental values. CLOGP data increased with the exchange of oxygen for sulphur (pairs **11**–**14**, **21**–**22**) and the CLOGP values decreased remarkably by exchanging the oxygen for sulphinyl or sulphonyl moieties (**21**–**23**–**24**). It is not unexpected result because previously the same effect have been revealed in the case of CLOGP profiling of aurone–thioaurone molecules and parallel carboxamide library, too [29,49].

In spite of the good linear correlation between the k' and CLOGP data (Fig. 5), a few discrepancies between the measured and calculated properties have also been observed. Calculations gave the same CLOGP values for structural isomers while the experimentally determined lipophilicity of these molecules proved to be different (**7**–**8**–**9** and **15**–**16**–**17**). In certain cases these differences were strong enough to separate completely the structural isomers such as in the case of the three pyridyl derivatives mentioned before or in the case of the chloro derivatives (pair of **5**–**6** and **7**–**9**). These observations showed that not only the chemical nature of the substituents but also their position might influence the lipophilicity of the molecules, as it was formerly

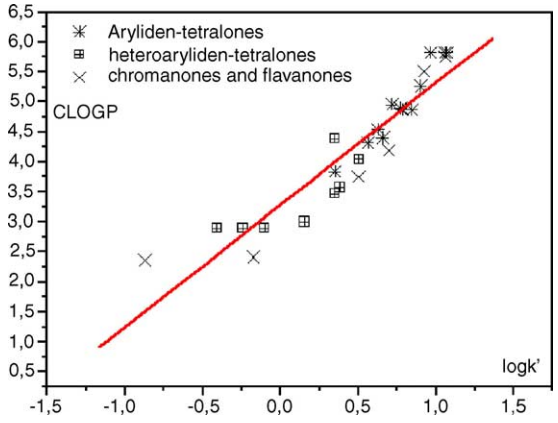


Fig. 4. Relationship between the measured ($\log k'$) and calculated (CLOGP) lipophilicity data; Abscissa: $\log k'$, ordinate: CLOGP. For details see Sections 2.3 and 2.4.

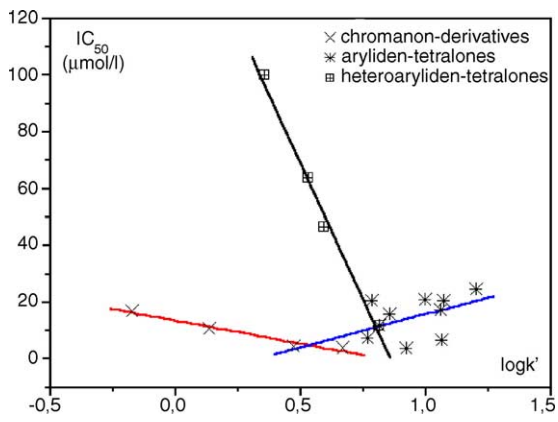


Fig. 5. Relationship between the experimental lipophilicity ($\log k'$) and antiproliferative activity (IC_{50}), Abscissa: $\log k'$, ordinate: IC_{50} (μmol/l). For details see Sections 2.3 and 2.5.

exhibited for the library of aurones and Mannich ketones [29,49].

Structural variations of the molecules of Table 1 were limited to the type of the heteroatom incorporated in the alicyclic ring and to the substitution on the aryl ring (R) or the type of the aryl ring itself (**9**–**17**). These variations allowed studying the influence of the substitution not only on the biological activity but on lipophilicity also.

3.3. Results of the antiproliferative assay

The A431 human epidermoid carcinoma cell line is well known as epithelial growth factor (EGF) overexpressing system and indicate potential EGFR-related apopototic effects. Small molecules as inhibitors of protein kinases constitute one of the most major class of the target-selective agents and the system is a straightforward screening setup to test compounds with EGFR inhibitory potential. The first small molecule EGF-RTK inhibitor already approved as for the treatment cancer is ZD1839 (gefitinib, Iressa), which proved to be "the" selective, non toxic treatment for a subset of non-small cell lung cancer (NSCLC) patients as monotherapy [50]. There are additional new drug candidates currently undergoing clinical phase III development, e.g. OSI-774 (erlotinib, Tarceva) and GW 2016 as reversible inhibitors of EGF-RTK or CI 1033 and EKD 569 as irreversible inhibitor. Other types of molecules with RTK (receptor tyrosine kinase) inhibitory activity were characterized and categorized by Nakaya and Miyasaka [51] and the second group in that classification was the group of flavones structurally related to the molecules investigated in the present work.

Dimmock et al. reported in 1999 on the cytotoxic activity and quantitative structure activity relationship (QSAR) studies of molecules with similar structure to the molecules investigated here and had revealed strong correlations between the spatial arrangement of aryliden ring and several physico-chemical properties and biological activity. In that work they found *E*-2-arylmethylene-1-benzosuberones to be more potent scaffold [52].

In the present work almost only *E*-2-arylmethylene-1-tetralones and its six-membered alicyclic ring containing derivatives have been evaluated. The strongest antiproliferative effect associated with the *E*-3-phenylmethylene-4-flavanon (**25**) and the, *E*-3-arylmethylene-1-thiochroman-4-one (**22**) ($IC_{50} \sim 3.97$ μmol/l for both). Remarkable activity ($IC_{50} < 10$ μmol/l) were found in the case of the 3-pyridyl, 4-pyridyl, 4′-methoxy and *E*-3-phenylmethylene-4-thioflavanone compounds (**16**, **17**, **3**, **26**, respectively), while just modest biological effectiveness were found related to five molecules as **1**, **4**, **8**, **23**, **24** and high IC_{50} (>20 μmol/l) at the remaining compounds.

The detected IC_{50} values are plotted against the measured $\log k'$ data of the three different sub-groups investigated (Fig. 5). It can be pointed out that all the derivatives incorporating heteroatom in the aryliden ring in position 2 (**11**–**15**) have no impact on the proliferation of A431 cells at pharmacological doses. It is noteworthy because of the remarkable activity of other pyridyl derivatives (**16**–**17**). These differences have been obtained in the bioactivity of the three derivatives also could be achieved in the separation. The three isomers could been completely separated from each other (see compounds **15**–**17** on Fig. 3).

As for the halogen substitution, a slight decrease in the biological potency can be observed for chlor and fluoro derivatives (**4**–**9**) but complete loss of activity can be found for 4′-bromo compound (**10**). Usually, all the structural changes affecting the type of the aryliden substitution or skeleton of the fused ring system worsened the effectiveness of the compound, except the 4′-methoxy group (**3**) and the pyridyl groups mentioned before.

Both the electronic, the hydrophobic or the steric properties may have impact on biological activity, but amongst the aryl derivatives all compounds (except the methoxy containing molecule) showed increased experimental lipophilicity. In the case of heteroaryl derivatives perhaps the chemical nature and position of the heteroatom and its lipophilic properties together are responsible for the biological effects. Considering only the scaffolds with different carbon number in the alicylic ring of the fused system, Perjési et al. found 3D structural difference between these derivatives containing five to seven member alkanone ring [14]. These steric deviations may cause the observed changes of activity.

The relationship between the biological effectiveness and the experimental (k') lipophilicity profile has been investigated for compounds showing at least minimal inhibitory potencial ($IC_{50} < 25$ μm/l) on Fig. 6. In this set of compounds regardless of structural features a non-linear parabolic relationship could be obtained. Analysis of the selected structurally slightly related molecules pointed out the minimum score the curve has. Based on the parabolic curve it can be stated that in the set of compounds studied in this work an optimum value of experimental lipophilicity (k') could be determined from the point of view of bioactivity. This optimal value of proved to be: $k'_{opt} = 0.51$ for the compounds inves-

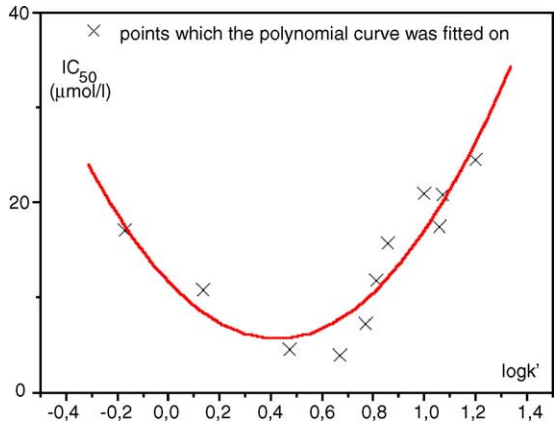


Fig. 6. Combined IC_{50}–$\log k'$ plot of the compounds possessing significant antiproliferative activity ($IC_{50} < 25$ μmol/l). Abscissa: $\log k'$, ordinate: IC_{50} (μmol/l). For details see Sections 2, 2.5 and 3.3.

tigated here. Delineation of the IC_{50} versus CLOGP resulted in a similar non-linear minimum curve (not shown) with an optimal value of $CLOGP_{opt} = 3.5$. This optimal value of the calculated lipophilicity (CLOGP) was confirmed by the linear equation between $\log k'$ and CLOGP reported above.

Taken all together the following considerations (concerning to the A431 biological system) can be made to synthesize agents of considerable antiproliferative potential:

Beneficial elements in the structure: six-membered alicyclic ring fused to an aromatic ring as chromanone or thiochromanone scaffold, in case of aryliden ring the preferred substitution has lipophilicity lowering potency.
Detrimental elements in the structure: heteroaromatic rings containing the heteroatom in position 2′, halogen substitution, substituted aromatic rings with high steric demand.

4. Conclusions

An applicable isocratic RP-HPLC method for fast analysis of the members of tetralone and chromanone-derivatives were developed. Lipophilicity of the molecules investigated has been characterized by both experimental (k') and computer prediction (CLOGP) data. The good correlation between the experimental- and calculated data has been proved. 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. Contrary to it, the RP-HPLC system proved to be able to make differences among the *ortho*- and *para*-isomers having different lipophilicity and biological activity 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.

Based on the good correlation between the antiproliferative activity and experimentally determined lipophilicity of the molecules investigated an optimum value of lipophilicity and structural demands to obtain good antiproliferative activity can be determined. 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. structural isomers).

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