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Lipophilicity and antiproliferative activity profiling of 2-benzylidencycloalkanones

Balázs Hallgas[a,c], Zsófia Dobos[a,c], Attila Agócs[b], Miklós Idei[a], György Kéri[a], Tamás Loránd[b], György Mészáros[d,*]

[a] *Hungarian Academy of Sciences, Peptidbiochemical Research Group in Semmelweis University, Department of Medical Chemistry, H-1088 Budapest, Puskin u. 9, Hungary*
[b] *Department of Biochemistry and Medical Chemistry, Faculty of Medicine, University Pécs, H-7624 Pécs, Szigeti út 12, Hungary*
[c] *Co-operative Research Centre, Semmelweis University, Budapest, H-1062 Rippl R. u. 37 Budapest, Hungary*
[d] *Semmelweis University, Department of Medical Chemistry, H-1088 Budapest, Puskin u. 9, Hungary*

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Abstract

High performance liquid chromatographic (HPLC) method has been developed to separate the members of a library including 24 benzylidenecycloalkanone-type structures and to characterize their lipophilicity. The experimental lipophilicity data (k) of the compounds have been compared with their calculated lipophilicity parameters (CLOGP). In general, good correlations between the measured and calculated lipophilicities have been found and these results were in good accordance with our previously data obtained in case of structurally related molecular libraries. In addition, cytotoxicity screening has been performed to determine the antiproliferative activity of these compounds. Some of the investigated compounds possessed noticeable inhibitory potential. Based on the correlation between the antiproliferative activity and experimentally determined lipophilicity of the molecules investigated, limited structural demands to obtain more potent compounds can be exhibited to support the synthetic design.

Keywords: Cycloalkanone; Molecular library; Lipophilicity; Retention factor; CLOGP calculation; Drug-likeness; Antiproliferative screening; QSAR

1. Introduction

During the last decade, the necessity of examining drug-likeness of a novel compound has been arisen because of the enormous increase in the cost of consumables and time demands associated with the development of a new molecular entity. Drug-likeness, considered a multidimensional terrain of chemical concept in which the classical physico-chemical parameters of therapeutically used molecules mostly occur, serves to identify compounds suitable for future development. In the last few years, it has been realized as one of the most important property to be evaluated in the early development phase [1,2]. The most recent drug-likeness definition given by Rishton [2] based on the classical contribution of Lipinsky et al. supplemented the theory with the polar surface area and rotatable bond consideration.

Lipophilicity is not simply one of the five parameters of Lipinsky's Rule of Five but an important and well-known parameter amongst the ADME properties also. Since lipophilicity plays an important role not only in the mechanism of drug molecules pharmacokinetic action but in the pharmacodynamic also, estimation of lipophilic character of a new drug candidate is considered as one of the first parameter to be determined at the earliest possible moment [3].

Lipophilicity of a non-ionic compound whose partition is independent of the pH is commonly characterized by the n-octanol/water (biphasic) partition coefficient (P_{ow}, and $\log P_{ow}$). It has long been recognised that the retention of a compound in reverse-phase-high performance liquid chromatography (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 lipophilicity of a compound [4–6]. Another choice to characterise lipophilicity of a molecule is the computerized

* Corresponding author. Fax: +36 1 2667480.
E-mail address: meszaros@puskin.sote.hu (G. Mészáros).

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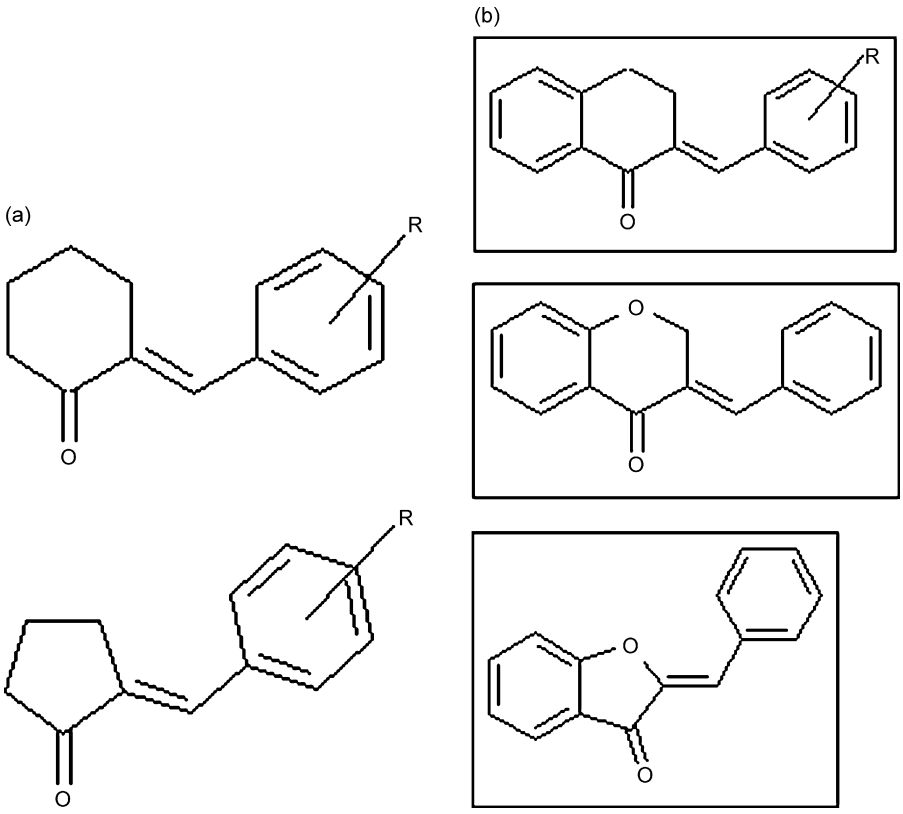


Fig. 1. (a) Basic structure of the compounds investigated, (R: alkyl, alkoxy, halogen). (b) Previously measured structure-related compounds: 2-aryliden-tatralones (R: alkyl, alkoxy, halogen), benzyliden-chromanones, aurones, respectively.

calculation frequently based either on the fragment approach [4,7] or on improved methods derived from the original Hansch–Leo's one [8].

Previously, we have reported the synthesis and bioactivity screening 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 and aurones [9–11]. Several representatives of these classes of compounds showed remarkable antiproliferative and cytotoxic effect—in a cytotoxicity screening against A431 human adenocarcinoma cells. Additional biological effects have been reported also and described previously elsewhere [12–15].

In the present work based on these results, our aim was to design and synthesize a new benzylidene–cycloalkanone library (being in close structural relationship with the previously investigated libraries, Fig. 1a and b) to investigate the effect of the structural, and spherical properties of the cycloalkanone rings and on the other hand that of the substituents of the benzylidene ring on the biological activity values. As it is well known, the electronic, the hydrophobic or the steric properties may have impact on biological activity and additionally, lipophilicity seems to be one of the major property which can control drug-likeness of a compound [3,16]. The size of the cyclic ketone and the substituents on the benzylidene ring was varied and ketones with electron-withdrawing- as well as with electron-donating groups in the benzylidene ring were synthesized. A reliable, fast and accurate high performance liquid chromatographic (HPLC) method was developed to investigate the 24-member molecular library. The lipophilicity of the compounds was evaluated not only experimentally (HPLC) but by in silico calculation based on their chemical structure (CLOGP), too. Antiproliferative effect of the molecules was determined on A431 cells by the methylene blue method [17]. 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 has been investigated.

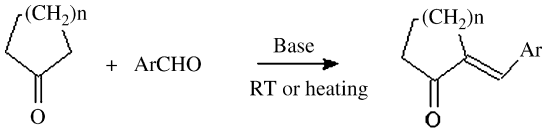
2. Materials and methods

2.1. Materials

Triethylamine (TEA), acetonitrile (ACN), ortho-phosphoric acid, methanol, ethanol, piperidine, methylene blue dye and the parent aldehydes and ketones 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 was designed containing two families of compounds, 2-arylidenecyclopentanones (**CP1–CP14**) and 2-arylidenecyclohexanones (**CH1–CH4, CH6–7, CH10–13**). The substitution pattern enables us to study the effect of the electron-donating- and electron-withdrawing groups. The title compounds have been prepared by base catalyzed aldol



CP1-14, CH1-4, CH6-7, CH10-13
n=1,2; Ar=Ph or substituted phenyl

Fig. 2. Synthesis of substituted cycloalkanones; Ar: phenyl, substituted phenyl (R), heteroaryl.

condensation according to literature methods (Fig. 2) [27–40]. The preparation of 2-arylidenecyclopentanones was carried out at room temperature according to literature methods, while the synthesis of the 2-arylidenecyclohexanones was carried out at 100 °C. The structural characterization of the known compounds is based on the previously published NMR data [15,26]. The new compounds have been prepared in a similar way as mentioned above. The products have been purified by column chromatography, and were recrystallized from a mixture of diethyl ether/hexane. Their structure was verified by NMR and FT-IR spectroscopy. The NMR, FT-IR and melting point data are summarized in the Table 1.

The FT-IR spectra were recorded by an Impact 400 spectrometer (Nicolet) in KBr pellets.

The FT-IR spectra of the new ketones show a very strong νCO maximum about 1670/cm at the cyclohexanone derivatives and at about 1700/cm supporting the structure supposed.

Theoretically, the *E*- and *Z*-geometric isomers can be equally formed in the aldol condensation mentioned above. The *Z*-configuration, however, is highly unfavourable (strong steric interaction between the aryl and carbonyl groups) [26]. In accordance with this, the *E*-configuration unambiguously was verified by the appearance of the "diagnostic" H-2a (H-α) proton signal in the appropriate range (7.4–7.8 ppm) in the ^{1}H NMR spectra [12]. The ^{1}H and ^{13}C assignments of the compounds 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*400WB (400/100 MHz for ^{1}H/^{13}C) spectrometer in $CDCl_3$ solvent. 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 alkylammonium phosphates are used in the eluent for buffering to the free silanol groups [18–21]. Based on the former practice, triethyl-ammonium phosphate was chosen as mobile phase addi-

Table 1
NMR, FT-IR and melting point data of newly synthesized compounds

Compound	^{1}H NMR (400 MHz), δ (ppm)	^{13}C NMR (100 MHz), δ (ppm)	MW	m.p. (°C)	FT-IR (cm^{-1})
CP3	1.98 (m, 1H, H-4), 2.40 (m, 4H, H-5, –CH_3), 2.87 (m, 1H, H-3), 7.22 (m, 3H, H-3′, H-4′, H-5′), 7.42 (m, 1H, H-6′), 7.60 (m, 1H, H-2a).	19.9 (-CH_3), 20.4 (C-4), 29.3 (C-3), 38.0 (C-5), 125.8 (C-4′), 128.6 (C-6′), 129.1 (C-5′), 129.8 (C-2a), 130.5 (C-3′), 134.3 (C-2′), 136.8 (C-2), 138.8 (C-1′), 207.9 (C-1)	186.25	70–72	1705
CP7	1.97 (m, 1H, H-4), 2.35 (m, 1H, H-5), 2.87 (m, 1H, H-3), 3.82 (s, 3H, OMe), 6.44 (s, 1H, H-3′), 6.50 (m, 1H, H-5′), 7.41 (d, 1H, H-6′, $J_{5'6'}$ = 8.4 Hz), 7.75 (m, 1H, H-2a)	20.3 (C-4), 29.5 (C-3), 37.8 (C-5), 55.4 (OCH_3), 98.2 (C-3′), 104.7 (C-5′), 117.6 (C-1′), 126.7 (C-2a), 130.7 (C-6′), 133.6 (C-2), 160.4, 162.1 (C-2′, C-4′), 207.9 (C-1)	232.27	82	1703
CP14	2.05 (m, 1H, H-4), 2.40 (m, 1H, H-5), 2.95 (m, 1H, H-3), 7.10 (m, 2H, H-3′H-5′), 7.35 (m, 1H, H-2a), 7.50 (m, 1H, H-2′, H-6′)	20.1 (C-4), 29.1 (C-3), 37.7 (C-5), 115.8 (C-3′, C-5′, $^{2}J_{CF}$ = 21.7 Hz), 131.0 (C-2a), 131.7 (C-1′), 132.3 (C-6′, C-2′, $^{3}J_{CF}$ = 8.4 Hz), 135.5 (C-2), 163.0 (C-4′, $^{1}J_{CF}$ = 251 Hz), 207.9 (C-1)	190.21	53–54	1712
CH7	1.75 (m, 1H, H-4), 1.90 (m, 1H, H-5), 2.50 (m, 1H, H-3), 3.80 (s, 3H, OMe), 6.45 (m, 2H, H-3′, H-5′), 7.25 (d, 1H, H-6′, $J_{5'6'}$ = 8.4 Hz), 7.65 (s, 1H, H-2a)	23.6, 24.0 (C-4, C-5), 29.3 (C-3), 40.3 (C-6), 55.4 (OCH_3), 98.2 (C-3′), 104.0 (C-5′), 117.6 (C-1′), 131.0, 131.2 (C-2a, C-6′), 134.9 (C-2), 159.9, 161.6 (C-2′, C-4′), 201.6 (C-1)	246.3	70	1669

tive. 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. Retention factors (k) of the samples were calculated from the experimentally determined retention data: $(k = (t_R - t_o)/t_o$, where t_o has been determined by injection of water [22,23]). 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.

2.5. Antiproliferative assay

The A431 human epidermoid carcinoma cell line is well known as epithelial growth factor (EGF) overexpressing system and indicates potential EGFR-related apoptotic effects. Small molecules as inhibitors of protein kinases constitute one of the most major class of the target-selective agents and this system is a straightforward screening setup to test compounds with EGFR inhibitory potential. The investigated compounds were evaluated in this screening system to determine their cytotoxicity features and to examine the impact of the various substituents on the bioactivity regarding to their lipophilicity changing ability. The antiproliferative assay utilized the methylene blue test [17]. This colorimetric assay based on the enzyme activity of various dehydrogenases of the living cells, is suitable for testing the cytotoxic activity of antitumour candidates *in vitro*. Human A431 epidermoid carcinoma cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal calf serum (FCS), 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 paraformaldehyde 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 including 24 structurally related compounds has been investigated. Based on their structural features the library could be divided into 2 subgroups (Table 2.): the group of cyclopentanones (CP) consists of substituted arylmethylene-cyclopentanones (**CP compounds**) and the family of cyclohexanones (CH) contains arylmethylene-cyclohexanones (**CH compounds**).

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 12 min, calculated retention factors (k, k_{rel}) are shown in Table 2. k_{rel} means the ratio of actual compound's k-value and the corresponding parent derivative's k-value (e.g. $k_{rel\ CP2} = k_{CP2}/k$

Table 2
Experimentally measured (k) or calculated (CLOGP) lipophilicities and biological activity values of the investigated compounds

S. no.	Substituents	Benzyliden-									
		Cyclopentanones (CP)					Cyclohexanones (CH)				
		k[a]	log k	Relative k	CLOGP	IC50[b] (μmol/l)	k	log k	Relative k	CLOGP	IC50 (μmol/l)
1	Core	1.21	0.08	1.00	2.65	100.0	1.80	0.25	1.00	3.21	13.8
2	4′-Methyl-	1.89	0.28	1.56	3.15	80.0	2.70	0.43	1.50	3.71	41.2
3	2′-Methyl-	1.70	0.23	1.40	3.15	100.0	2.54	0.40	1.41	3.71	50.0
4	2′-Methoxy-	1.34	0.13	1.11	2.57	13.2	1.85	0.27	1.03	3.13	46.3
5	3′-Methoxy-	1.32	0.12	1.09	2.57	100.0					
6	4′-Methoxy-	1.21	0.08	0.99	2.57	100.0	1.75	0.24	0.97	3.13	29.8
7	2′,4′-Dimethoxy-	1.41	0.15	1.16	2.66	90.0	1.80	0.25	0.99	2.96	21.6
8	3′,4′-Dimethoxy-	0.76	−0.12	0.63	2.66	100.0					
9	3′,4′,5′-Trimethoxy-	0.85	−0.07	0.70	1.95	90.0					
10	3′,4′-Dioxolan-	1.07	0.03	0.88	2.61	75.0	1.56	0.19	0.87	3.12	21.2
11	2′-Chloro-	1.95	0.29	1.61	3.36	49.3	2.63	0.42	1.46	3.92	20.9
12	3′-Chloro-	2.14	0.33	1.76	3.36	100.0	3.04	0.48	1.69	3.92	6.6
13	4′-Chloro-	2.18	0.34	1.80	3.36	95.0	1.78	0.25	0.99	3.92	26.2
14	4′-Fluoro	1.38	0.14	1.14	2.79	64.3					

[a] R.S.D. of the k values was 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.

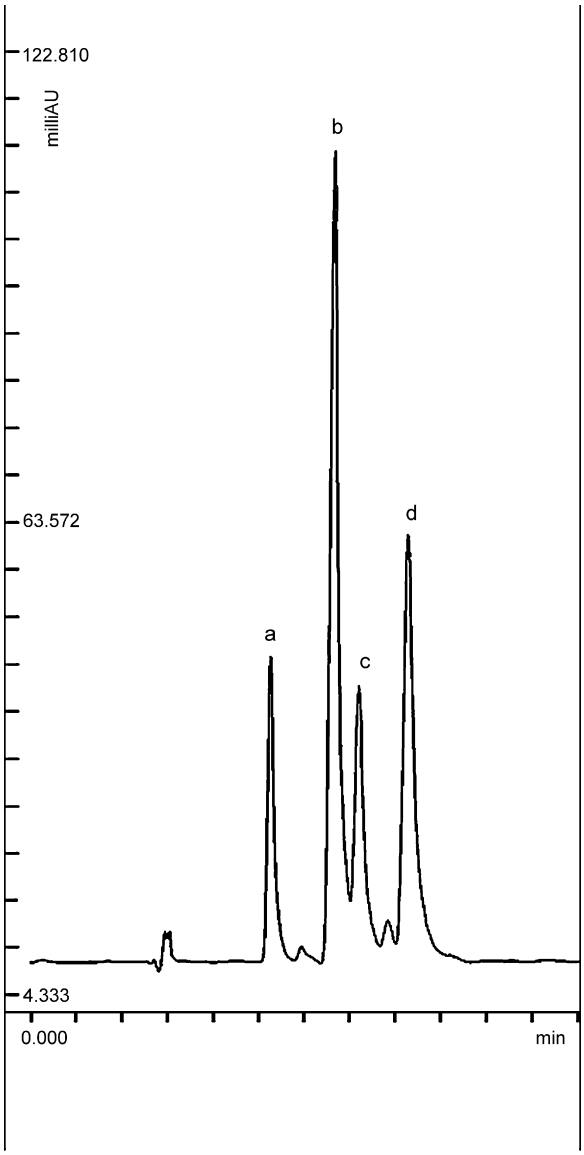


Fig. 3. Representative chromatogram of the mixture of CP-8 (a), CP-1 (b), CP-7 (c) and CH-1 (d) compounds, respectively. Abscissa: retention time (min), ordinate: mAUF units, for experimental parameters, see: Section 2.3.

$_{CP1} = 1890/1212 = 1560$. So k_{rel} should be considered to be the index of lipophilicity in one certain sublibrary. The chromatographic method applied here was able to perceive the small structural differences resulting in a fine alteration of lipophilicity (see in Table 2. e.g.: **CP1/CH1**; **CP2/CP3**; **CP7/CP8** and **CP11/CP12/CP13** pairs in the cyclopentanones, and **CH2/CH3**, **CH4/CH6**, **CH11/CH12/CH13** pairs in the cyclohexanone series). A mixture containing four compounds has been chromatographed for representative purposes (Fig. 3).

In good accordance with the expectations, retention factor of any the CH compounds (containing six-member ring) was greater in every case (except **CH-13**) than that of the respective CP compound (bearing the same substituent,) (Table 2, Fig. 4.) The means of the *k* values were 2.143 and 1.608 for the CH and CP sublibraries, respectively.

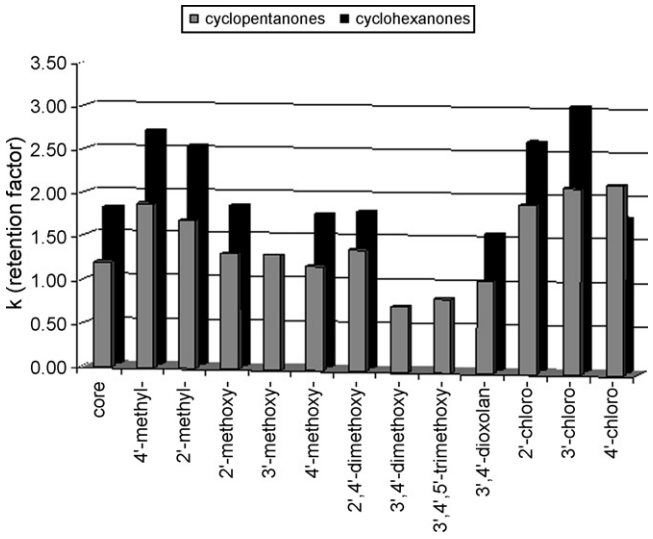


Fig. 4. Lipophilicity of the molecules possessing different substituents in the case of both sublibraries. Abscissa: substituents, ordinate: *k*.

The value of *k* embracing a four-fold difference, ranged from 762 (**CP8**) to 3036 (**CH12**) (Table 1). The effect of the substituents on the retention factor is shown on Fig. 4. There was no remarkable difference between the two sublibraries considering the lipophilicity changing impacts of the various substituents. Almost all of the compounds (except the methoxy and dioxolane containing molecules) showed increased experimental lipophilicity (*k*) comparing to the appropriate parent compound.

The methyl substitution irrespectively of its position caused an increase in the *k*-data both in the CP and CH series (**CH-** and **CP2-3**). In both sublibraries, ortho substitution caused only a slight increase in retention while in case of para substitution greater increase was found (**CH-** and **CP2–3**). As it has been mentioned earlier, the method applied here proved to be able to distinguish these isomers and to separate them based on these slight differences in their lipophilicity.

The methoxy derivatives can cause a slight decrease of the lipophilicity in the para (4′) position while methoxy groups in the ortho- or meta position rather may increase the lipophilicity [11,15]. In full compliance with these earlier findings, now the same results have been found in both sublibraries (**CP6**, **CP4–5**).

The chloro derivatives (**CP-** and **CH11–13**) exhibited relatively high increase in retention compared to the parent molecule. The fluorinated compound (**CP14**) showed just a slightly increased retention. All of the chloro derivatives increased the retention (**CP-**, **CH11–13**) and this fact is in good agreement with our previous results. The only considerable exception is the 4′-chloro-substituted cyclohexanone (**CH13**), because – contrary to the expectations – it eluted with *k*-value very similar to that of the parent molecule. In all other cases the expected and previously proved effects have been found. It is not surprising that one of the 3′-chloro-substituted cyclohexanones had the highest retention (lipophilicity). In agreement with our earlier results, we have found the 3′-chloro derivatives to be the most lipophilic compound [11,15].

3.2. Calculated lipophilicity (CLOGP) values

Lipophilicity profile of the investigated compounds have been characterised by the calculated CLOGP data, also (Table 2). Generally, the CLOGP values increased with the methyl (**CP2–3**) or halogen substitution (**CP11–13**) and decreased slightly with the incorporation of methoxy group (**CP4–6**). In good accordance with the experimentally determined retention factors, the CLOGP values of the CH compounds always were higher than that of the respective CP compound bearing the same substituent (Table 2). The means of the CLOGP values were 2.94 and 3.50 for the CH and CP sublibraries, respectively.

Comparison of the experimentally measured ($\log k$) and computer calculated (CLOGP) lipophilicity parameters revealed a good linear correlation (CLOGP = A $\log k + B$) for the set of the compounds evaluated here ($A = 3.00$, $B = 2.44$, $N = 24$, $R = 0.88$, S.D. = 0.24, $F = 79.82$, $p < 0.0001$, Fig. 5). The highest CLOGP value was calculated in case of the 3′-chloro derivatives (**CP11–13**). In spite of the good linear correlation between the $\log k$ and CLOGP data (Fig. 5), a few discrepancies between the measured and calculated properties have been observed too. Calculations gave the same CLOGP values for structural isomers while the experimentally determined lipophilicity and biological activity of these molecules proved to be different. In certain cases these differences in the experimental lipophilicity were strong enough to separate completely by the HPLC method the structural isomers possessing the same CLOGP value such as in the case of the 3′-chloro derivatives (**CP11–13**) and in the other cases mentioned in the Section 3.1. These observations showed that not only the chemical nature of a substituent but also its position might influence the lipophilicity of a molecule, as it was formerly exhibited for the library of aurones, 2-aryliden-tetralones and Mannich ketones ([24,15,11], respectively).

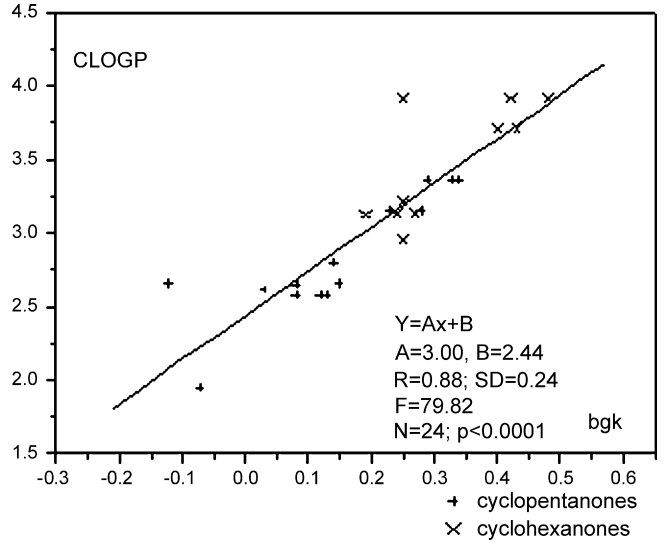


Fig. 5. Relationship between the measured (k) and calculated (CLOGP) lipophilicity data; abscissa: $\log k$, ordinate: CLOGP. For details see Sections 2.3 and 2.4.

3.3. Results of the antiproliferative assay

The A431 human epidermoid carcinoma cell line is well known as epithelial growth factor overexpressing system and indicates potential EGFR-related apoptotic effects (15). Small molecules as inhibitors of protein kinases constitute one of the most major classes of the target-selective agents and the system is a straightforward screening setup to test compounds with EGFR inhibitory potential. Dimmock [25] reported 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 arylidene ring and several physico-chemical properties and biological activity.

In the present work, the relationship between the biological effectiveness and the chemical type of the substituents has been investigated for the set of the compounds applied here. As it is well known, the electronic, the hydrophobic or the steric properties may have impact on biological activity and additionally, lipophilicity seems to be one of the major properties which can control drug-likeness of a compound.

Remarkable differences was detected between the pair of the core compounds (**CP1** and **CH1**), where the cyclohexanone derivative was exhibited to be much more potent than the cyclopentanone compound (Table 2).

In both series, the halogenes and dioxolanes were found to be more potent derivatives than the others. Additionally, amongst the CP compounds the 2′-methoxy, while amongst the CH compounds the 3-chloro showed the highest activity (Table 2).

The detected IC_{50} values were plotted against the measured k data (Fig. 6). In the bioactivity potential, an explicit difference has been detected between the two sublibraries (Table 1 and Fig. 6). The compounds belonging into the CH or CP series segregated from each other. While the CH compounds formed a cluster in the lower right corner (high lipophilicity (k) and high

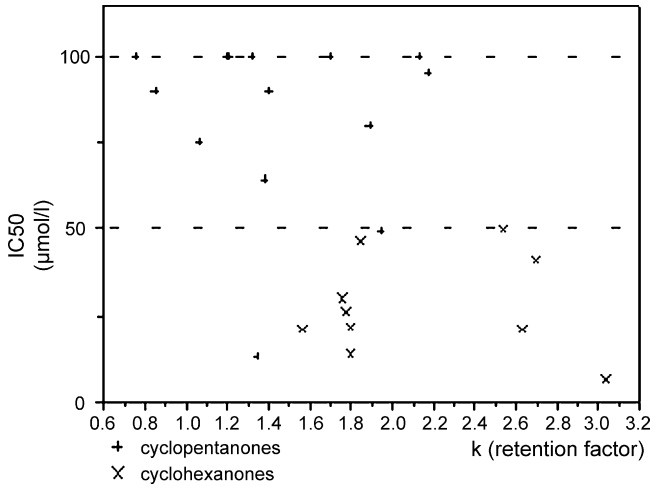


Fig. 6. Relationship between the experimental lipophilicity (k) and antiproliferative activity (IC_{50}), abscissa: k, ordinate: IC_{50} (μmol/l). For details see Sections 2.3 and 2.5.

biological activity [low IC_{50} value]), the CP compounds formed a cluster in the left upper corner (low lipophilicity and low biological activity [high IC_{50}]). Almost in each case, the cyclohexanone derivative showed higher cytotoxic activity than the respective CP derivative with the same substituent. The exception was the 2′-methoxy-cyclopentanone (**CP4**) which is one of the three most potent compounds. The difference is clearly outstanding in case of the 3′-chloro pair where the six-member compound (**CH12**) is highly effective but its CP-pair lacks of effectiveness.

Because these molecules investigated here can be considered as fragments of the previously evaluated 2-arylidene-1-tetralones their cytotoxic potential can be compared with that of the 2-arylidene-1-tetralones. In this case, the following recommendations can be taken regarding the chemical structure to synthesize similar benzylidene–cycloalkanone agents with considerable antiproliferative potential (limited to the A431 biological system): 1, preferred core structure is the cyclohexanone; 2, alkyl substituents are not preferred, they caused really decreased antiproliferative activity; 3, the most preferred substituent is the 3′-chloro one.

In summary, it seems that although these compounds can chemically be considered as fragments of the 2-arylidene-1-tetralones their cytotoxic potential proved to be different from that of the 2-arylidene-1-tetralones. However, some of these recommendations alienated above are in good agreement with our previous considerations [15] obtained for the arylidene-1-tetralones (for example the six-member ring is the preferred core structure); some discrepancies can be found also. While the halogen substitution proved to be detrimental in the case of 2-arylidene-1-tetralones, here, in the case of the CH compounds, the most preferred substituent is the 3′-chloro.

4. Conclusions

An applicable isocratic RP-HPLC method for fast analysis of the members of substituted cycloalkanones was developed. Lipophilicity of the molecules investigated has been characterized both by experimental (k) and computer prediction (CLOGP) data. The good correlation between the experimental- and calculated data has been proved. The RP-HPLC method is suitable to characterize lipophilicity of the investigated molecules with a fast, reliable method of high performance and low demand in respect of sample quantity. The RP-HPLC system – dissimilarly to the calculation method – proved to be able to distinguish between the ortho- and para isomers having different lipophilicity and biological activity. With other words, the experimentally determined physico-chemical parameter (k, $\log k$) may provide reliable and useful data for the pre-selection or pre-screening in various libraries.

Based on the correlation between the antiproliferative activity and experimentally determined lipophilicity of the molecules investigated, limited structural demands to obtain more potent compounds can be exhibited to support the synthetic design. This ability of the chromatographic method may be very advantageous when a pre-selection is needed within molecule libraries containing chemically very similar compounds (e.g. structural isomers).

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