PHYSICOCHEMICAL PROPERTIES OF 9-(ALKYLSULFANYL)- AND 9-(ARYLSULFANYL)ACRIDINE DERIVATIVES AND THEIR INTERACTION WITH (2-HYDROXYPROPYL)CYCLODEXTRINS

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Dedicated to Professor Jaroslav Podlaha on the occasion of his 70th birthday.

Acid-base properties and aggregations of 9-(alkylsulfanyl)- and 9-(arylsulfanyl)acridine derivatives were studied as a part of systematic study of physicochemical properties of these compounds synthesized as potential drugs. The effect of the (2-hydroxypropyl)cyclodextrins on these properties and their association with the acridines was also followed.

Keywords: Cyclodextrins; Sulfanylacridines; Dissociation constants; Aggregation; Inclusion complexes; Association constants; Dissolution; NMR spectroscopy.

In the frame of the study of basic properties of newly synthesized 9-(alkylsulfanyl)- and 9-(arylsulfanyl)acridines, we have published the effect of substituents on the electron-donor properties[1] of these compounds. We also reported electrochemical oxidation of these compounds as the model of their possible biotransformation[2]. Chromatographic methods for determination of these compounds were also proposed[3,4].

Sulfanylacridine derivatives[5] are pharmacologically active mainly against microbial[6,7] and protozoal infections[8,9]. Recently, these compounds were also tested in treatment of Alzheimer[10] and Creutzfeld–Jacob[11] diseases. Based on the analogy to other polycyclic aromatic compounds with heterocyclic nitrogen, they could also be active in the reversal of multidrug resistance[12] (by inhibition of transmembrane transport[13]).

Cyclodextrins[14] are cyclic oligosacharides consisting of six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucose units connected by α-1,4-glycoside bonds. The cavity of cyclodextrins is weakly hydrophobic

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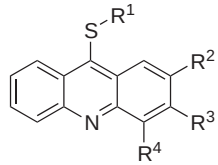
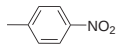
relative to the hydrophilic exterior with primary and secondary hydroxy groups. Cyclodextrins form inclusion complexes based on interactions of noncovalent nature (electrostatic interactions, van der Waals forces, π–π interactions) and steric effects[15]. These complexes are often used in analytical chemistry[16]. In pharmaceutical preparations they improve the solubility and stability of pharmaceuticals[17], and are used as carriers of active substances in biological systems and to retard the release of active substances from the pharmaceutical matrix[18].

We have studied acid-base properties, aggregation, and solubility of newly synthesized 9-sulfanylacridine derivatives (Table I) and the effect of cyclodextrins on these properties. The association constants of the formed inclusion complexes were also determined. The (2-hydroxypropyl)cyclodextrins were used as they are recommended as more soluble and less toxic than the unsubstituted ones[19] and they are widely used in pharmaceutical research[20,21].

TABLE I
Structures of studied 9-sulfanylacridines



Compound	R^1	R^2	R^3	R^4
BG 51	CH_3	H	H	H
BG 138	CH_3	OCH_3	H	H
BG 55	C_2H_5	H	H	H
BG 180	C_2H_5	H	H	OCH_3
BG 980	*n*-C_3H_7	OCH_3	H	H
BG 375	*n*-C_3H_7	H	H	OCH_3
BG 376	*n*-C_6H_{13}	H	H	OCH_3
BG 238	$CH_2CH_2N(CH_3)_2$	H	NH_2	H
BG 186	$CH_2CH_2N(CH_2CH_3)_2$	H	H	OCH_3
BG 979		OCH_3	H	H
BG 463		H	NH_2	H

EXPERIMENTAL

Instruments and Operating Conditions

Determination of dissociation constants was performed by potentiometric titration using an automatic microburette ABU 80 (Radiometer) and pH-meter pHM 64 (Jenway). The compounds ($c = 1 \times 10^{-4}$ mol l^{-1} in 9% ethanol) were titrated with 0.1 M HCl; the constant ionic strength of 0.1 was maintained with KCl. The pK_a values were evaluated from the titration curves obtained. Aggregation and determination of the association constants were studied by UV spectrophotometry using a HP-8455 diode-array spectrophotometer (Hewlett–Packard) with quartz cuvettes with 1-cm absorption layer. As it is known that presence of inorganic salts supports the aggregation, it was not possible to adjust the pH of solution by buffers. Therefore, the pH was maintained by addition of appropriate volume of 0.1 M HCl or 0.1 M NaOH. The ionic strength of solutions was not set. ^{1}H NMR spectra were measured by Varian^{UNITY} Inova 400 at 399.95 MHz in deuterium oxide at 25 and 50 °C. 2-Methylpropan-2-ol (δ 1.25 ppm) was used as a standard. The COSY spectra were measured using standard double pulsed sequence in the absolute mode. The NOESY and ROESY spectra were measured in the standard double pulsed sequence as phase-sensitive experiments (mixing time 0.3 s for NOESY and 0.1 s for ROESY). All 2D experiments were made in the spectral window 5000 Hz for proton signals. The solubility measurements were performed using an Erweka Dissolution Tester DT 6R and a Shimadzu UV-160 HIM 226 spectrophotometer. The inclusion complexes were prepared[22] by precipitation and by mixing (which was found to be more effective) with the ratio of compound:cyclodextrin 1:3.

Chemicals and Solutions

The studied compounds were synthesized by the previously described method[23], their identity was checked by elemental analysis and NMR and their purity by HPLC and GC-MS. Stock solutions of the studied derivatives were prepared with a concentration of 1×10^{-3} mol l^{-1} in 90% ethanol. The (2-hydroxypropyl)cyclodextrins (Sigma Aldrich) were used as supplied. Their stock solutions with a concentration of 0.25 mol l^{-1} were prepared in distilled water. All other used chemicals were of analytical grade.

RESULTS AND DISCUSSION

Acid-Base Properties of 9-Sulfanylacridines

Acid-base properties of biological active compounds are very important characteristics both from the theoretical and practical viewpoints. The effect of molecular structure on acid-base equilibria is an important topic in modern organic chemistry. Moreover, the knowledge of dissociation constants is necessary in the prediction of biological activity using the QSAR method. From the pharmaceutical viewpoint, acid-base properties are very important for drug interactions with biological systems.

The determined pK_a values of studied 9-sulfanylacridines are summarized in Table II. We have also calculated the pK_a values using the Pallas 3.0 program (CompuDrug Chemistry); their values are also given in Table II. In the case of compounds with more nitrogen atoms, the calculated lower pK_a values refer to heterocyclic nitrogens. Only one pK_a value is obtained (both experimentally and by calculation) in the acid range for compounds with NH_2 group near to heterocyclic nitrogen atom because of the strong N–N interaction. It was impossible to obtain the pK_a value for the strongly basic group $–CH_2CH_2N(CH_2CH_3)_2$. The determined and calculated pK_a values correlate satisfactorily (Fig. 1); therefore, the program Pallas can be used for the prediction of pK_a values of compounds in this structure group. In the presence of (2-hydroxypropyl)cyclodextrins (up to concentrations of 0.01 mol l^{-1}) the values of dissociation constants decrease slightly (in tenths of unit). The potential presence of (2-hydroxypropyl)cyclodextrins as drug carriers would not thus affect the acid-base equilibria of studied compounds.

Aggregation of 9-Sulfanylacridines

As it follows from the previous part that the pK_a values of all the studied compounds are in the range of 5–7, all the following studies were performed at pH 3 (protonized forms of the compounds) and pH 8 (free bases),

TABLE II
Experimental and calculated dissociation constants of studied 9-sulfanylacridines

Compound	pK_a	
	experimental[a]	calculated
BG 51	5.34	5.61
BG 138	6.40	6.51
BG 55	5.68	5.45
BG 980	6.23	6.56
BG 238	7.10	7.20; 8.45
BG 186	6.13	6.88; 9.15
BG 979	6.13	6.73
BG 463	5.51	6.23

[a] Relative standard deviations are lower than 2.5%.

which is near the physiological pH. We have found that the free bases of some 9-sulfanylacridines aggregate at very low concentrations in aqueous solution, in analogy to the aggregation of other acridine derivatives[24]. New absorption bands with absorption maxima at longer wavelengths are formed in the absorption spectra of the studied compounds. Generally[25],

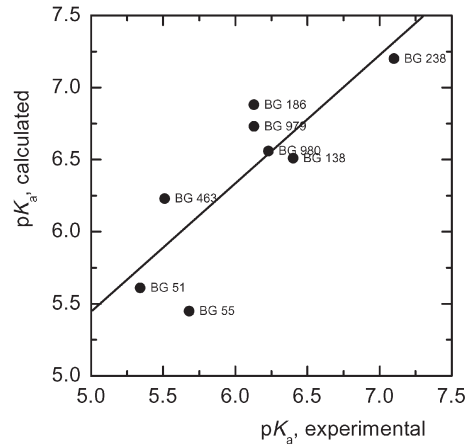


FIG. 1
The plot of calculated vs measured values of dissociation constant for studied 9-sulfanylacridines (r = 0.8307)

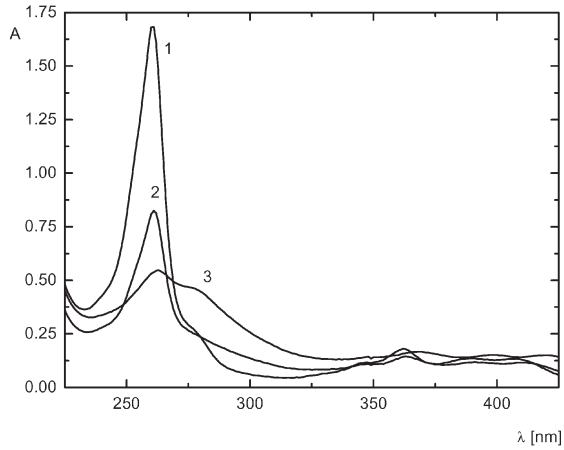


FIG. 2
The absorption spectra of compounds BG 138 (*1*), BG 980 (*2*), and BG 979 (*3*) ($c = 2 \times 10^{-5}$ mol l^{-1}, pH 8)

the absorption maxima of "head-to-tail" aggregates are shifted bathochromically relative to the monomer (*J*-bands are formed); the absorption maxima of "head-to-head" aggregates are shifted hypsochromically (*H*-bands). According to our results, the degree of aggregation of the studied compounds depends on the type of substituent on the sulfur atom. As an example, the absorption spectra of compounds with increasing alkyl length (BG 138 and BG 980) and with the phenyl group in the side chain (BG 979) are given in Fig. 2. The absorbance of the absorption maxima of monomers ($\lambda \cong 260$ nm) decrease and the new absorption maxima of aggregates ($\lambda \cong 280$ nm) are formed. We have found that the presence of ethanol affects the aggregation, too. However, we have determined that this effect begins at the concentration of ethanol higher than 10%; the concentration of ethanol in the measured solutions was lover than 1%. The protonized forms of derivatives do not aggregate because of the repulsion of their positive charges.

In the presence of (2-hydroxypropyl)cyclodextrins (Fig. 3), the absorbance of absorption maximum of aggregate ($\lambda \cong 280$ nm for all aggregates) decreases and the absorbance of absorption maximum of monomer of base ($\lambda \cong 260$ nm) increases. Thus, (2-hydroxypropyl)cyclodextrins suppress the aggregation, as only monomers can be included in the cavity and can form the inclusion complexes. Mainly monomeric forms are pharmaceutically

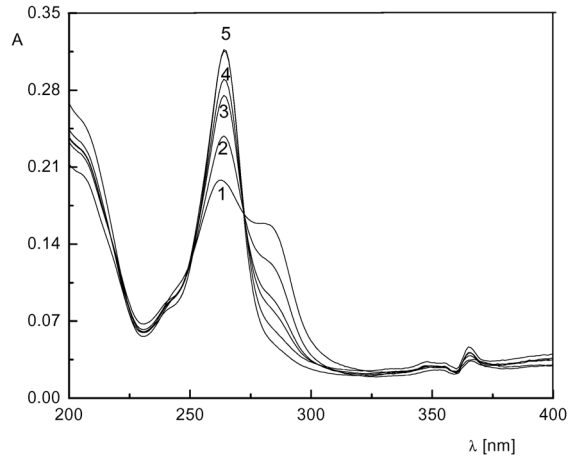


FIG. 3
The absorption spectra of compound BG 375 ($c = 1 \times 10^{-5}$ mol l^{-1}, pH 8) in presence of growing concentration of (2-hydroxypropyl)-α-cyclodextrin (in mol l^{-1}): 0 (*1*), 2×10^{-3} (*2*), 4×10^{-3} (*3*), 6×10^{-3} (*4*), 8×10^{-3} (*5*)

active and, therefore, (2-hydroxypropyl)cyclodextrins could have a positive effect in medical formulations.

Association Constants of Inclusion Complexes

The formation of the inclusion complexes in the presence of increasing concentrations of (2-hydroxypropyl)cyclodextrins results in increase in the absorbance of the main absorption band of the base ($\lambda_{max} \cong 260$ nm for all the compounds). The protonized forms of the compounds do not interact with (2-hydroxypropyl)cyclodextrins. The stoichiometry and association constants K_{as} of inclusion complexes of the selected compounds were evaluated from their absorption spectra using the Benesi–Hildebrand method[26], i.e. the linear dependence of $1/\Delta A$ vs $1/c$ for the complexes with the stoichiometry 1:1. As an example, the absorption spectra of the negligibly aggregating compound BG 138 in the presence of increasing concentration of (2-hydroxypropyl)-α-cyclodextrin are given in Fig. 4.

The binding isotherms of BG 138 and of the aggregating compounds BG 980 are given in Fig. 5. It follows from this figure that the degree of interaction depends on the compound structure and on the cavity size of (2-hydroxypropyl)cyclodextrins. The constant absorbance value is attained for the interaction with (2-hydroxypropyl)-β-cyclodextrin in both cases, in-

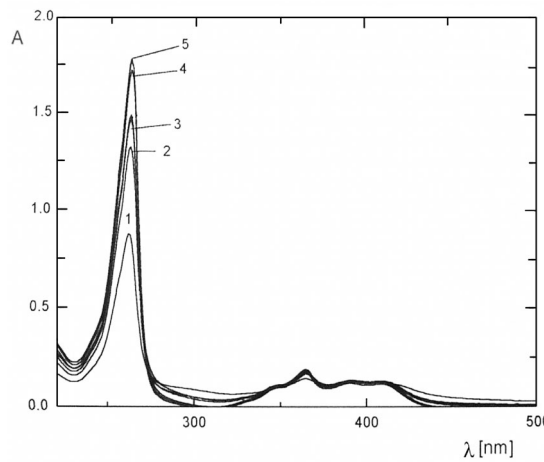


FIG. 4
The absorption spectra of compound BG 138 ($c = 1 \times 10^{-5}$ mol l^{-1}, pH 8) in presence of growing concentration of (2-hydroxypropyl)-α-cyclodextrin (in mol l^{-1}): 0 (*1*), 6×10^{-3} (*2*), 8×10^{-3} (*3*), 1×10^{-2} (*4*), 2.5×10^{-2} (*5*)

dicating that the complexes are formed at the used (2-hydroxypropyl)-β-cyclodextrin concentrations. The continually increasing absorbance in the interaction with (2-hydroxypropyl)-α-cyclodextrin and (2-hydroxypropyl)-γ-cyclodextrin indicates that the complexes are weaker and a higher excess of cyclodextrins is necessary for their formation. However, this could not be realized because of the insufficient solubility of (2-hydroxypropyl)-cyclodextrins.

The stoichiometry sulfanylacridine:cyclodextrin 1:1 was found in all cases. The obtained values of K_{as} are given in Table III. Thus, the K_{as} values decrease with increasing alkyl length in the interaction with (2-hydroxypropyl)-α-cyclodextrin. The highest values of K_{as} of the inclusion complexes of the compound with alkylsulfanyl group were obtained for the interaction with (2-hydroxypropyl)-β-cyclodextrin, the derivative with phenylsulfanyl group forms the most stable complex with (2-hydroxypropyl)-γ-cyclodextrin.

NMR Study of Aggregation and Association with (2-Hydroxypropyl)cyclodextrins

The aggregation of 9-sulfanylacridines and their association with (2-hydroxypropyl)cyclodextrins were studied by ^{1}H NMR, 2D-NOESY, and ROESY in detail[27]. The concentration and temperature dependences of ^{1}H NMR spectra confirmed aggregation of the studied compounds; the aggregation is suppressed in the presence of (2-hydroxypropyl)cyclodextrins. In the

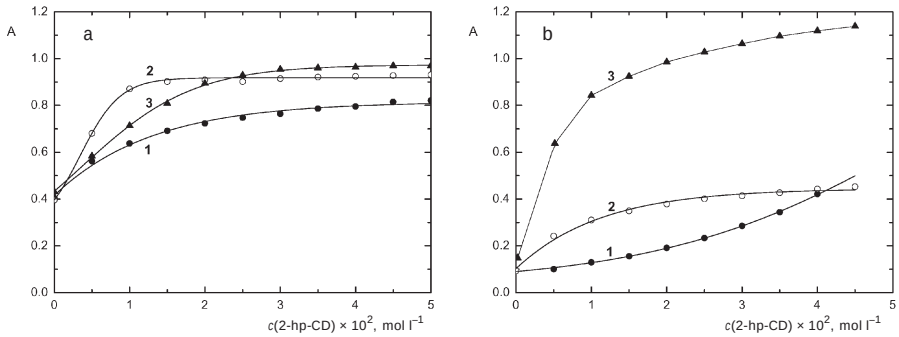


FIG. 5

The binding isoterms of compounds BG 138 (a) and BG 980 (b) in the presence of (2-hydroxypropyl)-α-cyclodextrin (*1*), (2-hydroxypropyl)-β-cyclodextrin (*2*), and (2-hydroxypropyl)-γ-cyclodextrin (*3*). Measurements were performed against blank solutions with the same concentrations of the cyclodextrins; (c(compound) = 2.5 × 10^{-6} mol l^{-1}, pH 8)

^{1}H NMR spectra of BG 180, the considerable proton shifts in the position of 3 and 5 of glucose units of cyclodextrins give evidence on the occupation of cyclodextrin cavity. The protons in the position 6 and 6′ of glucose units are affected, too. The space contacts of cyclodextrin protons in the position 6 with the acridine protons in the positions 1, 2, 7, and 8 are significant in 2D NOESY. The ethyl group on the sulfur is in contacts with cyclodextrin protons in the position 3 and 5 in such a way that the $–CH_3$ is nearer to H-3, and $–CH_2–$ is nearer to H-5. It can be concluded that this ethyl group is inserted into narrow rim of cyclodextrin cavity. Similarly, compound BG 979 interacts with (2-hydroxypropyl)-β-cyclodextrin and (2-hydroxypropyl)-γ-cyclodextrin by insertion its phenyl ring into cyclodextrin cavity. No signs of the insertion of acridine rings were found in any of the cases. The interaction with the protonized forms is very weak; the compounds are near to the narrow rim of cyclodextrin cavity.

The Solubility of 9-Sulfanylacridines

The effect of cyclodextrins on the solubility of drugs is one of the often studied effects[28]. Generally, the inclusion complexes with cyclodextrins are more water soluble than the original drugs[29]. We have studied the effect of (2-hydroxypropyl)cyclodextrins on the solubility of compounds BG 375

TABLE III
The association constants of studied 9-sulfanylacridines with (2-hydroxypropyl)cyclodextrins

Compound	K_{as}, mol^{-1}l[a]		
	α-CD	β-CD	γ-CD
BG 138	946	1720	587
BG 180	462	149	42
BG 980	52	459	373
BG 375	73	666	145
BG 376	36	220	74
BG 979	–[b]	288	496
BG 463	–[b]	312	262

[a] Relative standard deviations are lower than 15%. [b] Negligible interaction.

and BG 980. Their solubility increases about six times (Fig. 6). Thus, the use of cyclodextrins as carriers of 9-sulfanylacridines would increase their biological availability.

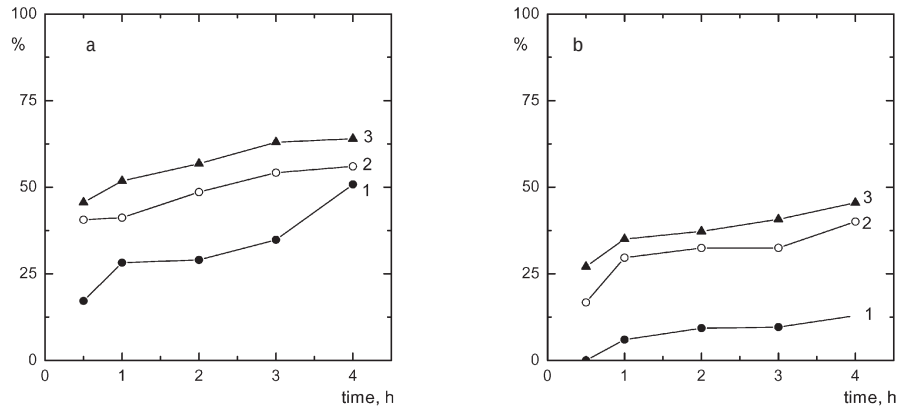


FIG. 6
The solubility of compounds BG 375 (a) and BG 980 (b) in the presence of (2-hydroxypropyl)-α-cyclodextrin (*1*), (2-hydroxypropyl)-β-cyclodextrin (*2*), and (2-hydroxypropyl)-γ-cyclodextrin (*3*). The solubility is related to the batch amount of compound

CONCLUSION

The dissociation constants of the studied 9-sulfanylacridines, differing in the length of alkylsulfanyl groups and with phenylsulfanyl groups, substituted in the acridine ring by methoxy or amino groups, range between 5 and 7. The effect of (2-hydroxypropyl)cyclodextrins on these values is very small (decrease in pK_a values of tenths of units). The free bases of 9-sulfanylacridines aggregate in aqueous solutions. (2-Hydroxypropyl)cyclodextrins suppress the aggregation by the formation of inclusion complexes with monomeric form of 9-sulfanylacridines. The association constants of these complexes were determined; their values depend on both the structure of the compound and on the cyclodextrin cavity size. The formation of inclusion complexes improves the solubility of studied compounds.

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