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Biological active acridine derivatives. Part 4: Synthesis and antiviral activity of some bis-acridinylated diamides

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Nine bis-acridine derivatives have been synthesized in search of structures with antiviral properties. Synthesis of target compounds was provided by a standard peptide-like coupling procedure using aliphatic diamines and protected amino acids following protective group removing and acridinylation by means of 9-methoxyacridine. Two out of nine compounds tested demonstrate high protective activity of Vero cells against HSV infection. Antiviral activity was observed only for compounds containing a pentamethylene fragment as a linker. The alanyl-containing derivative was less active than those containing valyl- and phenylalanyl. Most of synthesized compounds were less toxic than *N,N'*-bis-(acridinyl) hexamethylenediamine.

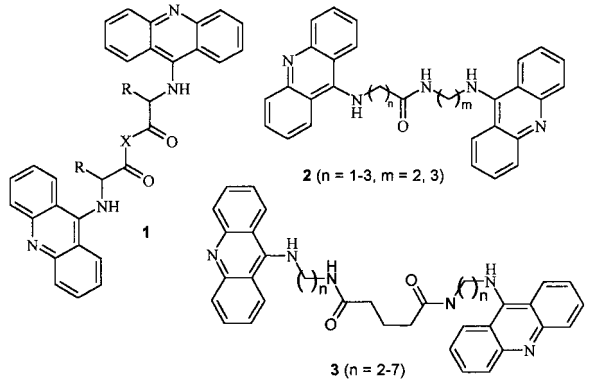
1. Introduction

A large number of compounds including fluoren-9-ones and 9-aminoacridines demonstrate both intercalative and antiviral properties [1–3]. Intercalation may cause different changes in the nucleic acids, so it may be one of the numerous modes of antiviral action of such compounds.
Affinity constants of bis-intercalators are several orders higher than those of the parent mono-derivatives [4], so, reinforcement of the inhibition of the DNA-depending processes and their antiviral activity may be expected for bis-intercalators. On the other hand, cytotoxicity can be increased [5]. A solution of this problem may be the including of an amide or ester group into the linking chain structure [6, 7] because of the cleavage ability of such compounds in physiological conditions [5]. Besides, the introduction of both amide and methylene fragments into the target molecules allows a regulation of linking chain flexibility [5]. Therefore we supposed high antiviral activity of bis-acridines such as **1** [6, 7].
Bis-intercalation mode of the drug-DNA interaction was shown for the four out of six similar compounds **2** [5] and for all similar compounds **3** [4]. The structural similarity of compounds **1**, **2** and **3** allows to suggest the bis-intercalation mode of the **1**-DNA interactions, so, as the first step of investigation we tested antiviral and cytostatic (as MNC) activity for synthesized compounds only.

2. Investigations, results and discussion

2.1. Chemistry

The target compounds **1a**–**1i** were synthesized (see Scheme) by aminoacylation of aliphatic diamines containing variable number of methylene groups by a routine procedure [8] following removing of the BOC-groups by means of hydrochloride acid and acridinylation of the correspondent diaminodiamides using excess 9-methoxyacridine. All steps were provided without isolation of the intermediates. Final compounds **1a**–**1i** were crystallized spontaneously after freezing from the reaction mixture generally in a pure state. In other cases a single recrystallization from methanol led to chromatographically homogeneous substances. Composition of the synthesized compounds was proved by microanalysis data (for all compounds the C, H, N – contents did not differ from the calculated values by more than 0.2–0.3%), the structure was confirmed by UV, IR and ^{1}H NMR spectroscopy and MS. The optical activity of the chiral objects was characterized polarimetrically in methanol solution at 15 °C at 0.25 % (m/v) concentration. In the UV spectra of compounds **1a**–**1i**, two intensive absorption bands were observed with maximum in a range of 250–270 nm (different π–π' absorption transitions of the acridine aromatic system) and 410–440 nm (π–π' transitions of the conjugated aminoacridine system). In the IR spectra the following characteristic absorption bands were observed: 3415, 3300 cm^{-1} (N–H non-associate and associate respectively); 3060–2850 cm^{-1} (C–H aliphatic, aromatic, and acridine); 1690–1650 cm^{-1} (C=O amide). Peaks of the $[M\text{-}2HCl]^+$ ions are present in MS of all sythesized compounds, but are not intensive. Fragmentation under electron strike is characteristic for the attached structures. In ^{1}H NMR spectra the quantity and shifts of proton's signals (Table 1) correspond to the quantity and type of the non-equivalent proton groups. The absence of additional signals from the corresponding protons of possible diastereomers allows to consider racemization at sythesis as insignificant.



2.2. Biology

Antiviral activity of the synthesized compounds **1a**–**1i** against herpes simplex virus (HSV) in Vero cells was investigated. 1,6-Bis-(acridin-9-yl-amino)hexane (**4**); tilorone (**5**); carboxymethylacridan-9-one sodium salt (CMA) (**6**) were used as comparison compounds. The antiviral activity was investigated following a routine method [12]. The results obtained (Table 2) correspond to the working hypothesis. Thus, two (**1f** and **1h**) out of the nine bis-acridines screened display high activity, practically depressing

Table 1: Physico-chemical properties of the synthesized compounds

Compd.	MW	Formula	Yield (%)	M.p. (°C)	$[M]^{+}$*	$[\alpha]_D$	^{1}H NMR: AM-250 "Bruker" (250.13 MHz, TMS, DMSO-d_6, ppm):
1a	671.7	$C_{37}H_{40}Cl_2N_6O_2$	49	195–198	598	−41.1	0.79 (s, 6 H, C(CH_3)$_2$); 1.70 (d, 6 H, 2 CH_3); 3.04 (m, 4 H, CH_2N); 5.19 (m, 2 H, CH); 7.50, 7.97, 8.48 (m, 16 H, CH-acridine); 8.72 (s, 2 H, CO−NH); 9.46 (s, 2 H, N<u>H</u>−CH); 14.17 (s, 2 H, HCl)
1b	657.7	$C_{36}H_{38}Cl_2N_6O_2$	45	205–207	584	−44.9	1.42 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$); 1.63 (d, 6 H, 2 CH_3); 3.09 (m, 4 H, CH_2N); 5.03 (m, 2 H, CH); 7.48, 7.92, 8.44 (m, 16 H, CH-acridine); 8.74 (s, 2 H, CO−NH); 9.45 (s, 2 H, N<u>H</u>−CH); 14.27 (s, 2 H, HCl)
1c	671.7	$C_{37}H_{40}Cl_2N_6O_2$	48	189–190	598	+5.93	1.22 (m, 2 H, $(CH_2)_2C\underline{H}_2(CH_2)_2$); 1.36 (m, 4 H, $CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$); 1.64 (d, 6 H, 2 CH_3); 3.07 (m, 4 H, CH_2N); 5.02 (m, 2 H, CH); 7.47, 7.92, 8.46 (m, 16 H, CH-acridine); 8.70 (s, 2 H, CO−NH); 9.45 (s, 2 H, N<u>H</u>−CH); 14.35 (s, 2 H, HCl)
1d	685.7	$C_{38}H_{42}Cl_2N_6O_2$	47	>270 (dec.)	612	−751	0.64 (d, 6 H, 2 CH_3); 0.78 (d, 6 H, 2 CH_3); 3.27 (s, 4 H, CH_2); 3.50 (m, 2 H, C<u>H</u>(CH_3)$_2$); 4.39 (d, 2 H, CH−N); 7.41, 7.79, 8.48 (m, 16 H, CH-acridine); 9.10 (s, 2 H, CO−NH); 9.24 (s, 2 H, N<u>H</u>−CH); 14.04 (s, 2 H, HCl)
1e	713.8	$C_{40}H_{46}Cl_2N_6O_2$	48	207–209	640	−393	0.85 (d, 6 H, 2 CH_3); 0.98 (d, 6 H, 2 CH_3); 1.75 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$); 2.88 (m, 4 H, CH_2N); 3.39 (m, 2 H, C<u>H</u>(CH_3)$_2$); 4.40 (d, 2 H, CH−N); 7.48, 7.82, 8.78 (m, 16 H, CH-acridine); 9.19 (s, 2 H, CO−NH); 9.68 (s, 2 H, N<u>H</u>−CH); 14.01 (s, 2 H, HCl)
1f	727.8	$C_{41}H_{48}Cl_2N_6O_2$	47	195–196	654	−244	0.85 (d, 6 H, 2 CH_3); 0.98 (d, 6 H, 2 CH_3); 1.29 (m, 2 H, $(CH_2)_2C\underline{H}_2(CH_2)_2$; 1.46 (m, 4 H, $CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$); 3.02 (m, 4 H, CH_2N); 3.26 (m, 2 H, C<u>H</u>(CH_3)$_2$); 4.41 (d, 2 H, CH−N); 7.47, 7.94, 8.76 (m, 16 H, CH-acridine); 9.17 (s, 2 H, CO−NH); 9.72 (s, 2 H, N<u>H</u>−CH); 14.15 (s, 2 H, HCl)
1g	741.8	$C_{42}H_{50}Cl_2N_6O_2$	45	190–192	668	−215	0.85 (d, 6 H, 2 CH_3); 0.99 (d, 6 H, 2 CH_3); 1.35 (m, 4 H, $(CH_2)_2C\underline{H}_2C\underline{H}_2(CH_2)_2$); 1.55 (m, 4 H, $CH_2C\underline{H}_2(CH_2)_2C\underline{H}_2CH_2$); 2.97 (m, 4 H, CH_2N); 3.28 (m, 2 H, C<u>H</u>(CH_3)$_2$); 4.42 (d, 2 H, CH−N); 7.51, 7.80, 8.77 (m, 16 H, CH-acridine); 9.21 (s, 2 H, CO−NH); 9.77 (s, 2 H, N<u>H</u>−CH); 14.22 (s, 2 H, HCl)
1h	823.9	$C_{49}H_{48}Cl_2N_6O_2$	47	179–181	750	−234	1.39 (m, 2 H, $(CH_2)_2C\underline{H}_2(CH_2)_2$); 1.48 (m, 4 H, $CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$); 3.18 (m, 4 H, CH_2N); 3.47 (m, 4 H, CH_2Ph); 5.17 (m, 2 H, CH−N); 6.95, 7.22 (m, 10 H, 2 C_6H_5); 7.40, 7.83, 8.44 (m, 16 H, CH-acridine); 9.01 (s, 2 H, CO−NH); 9.52 (s, 2 H, N<u>H</u>−CH); 14.18 (s, 2 H, HCl)
1i	739.8	$C_{42}H_{48}Cl_2N_6O_2$	49	198–200	666	−24.3	0.85 (d, 6 H, 2C<u>H</u>$_3$$CH_2$); 0.97 (t, 6 H, 2 CH_3); 1.23 (m, 4 H, C<u>H</u>$_2$$CH_3$); 3.60 (m, 2 H, C<u>H</u>(CH_3)); 3.77 (m, 8 H, CH_2N); 5.49 (d, 2 H, C<u>H</u>−N); 7.53, 7.93, 8.52 (m, 16 H, CH-acridine); 9.02 (s, 2 H, NH); 15.01 (s, 2 H, HCl)
4**	543.5	$C_{32}H_{32}Cl_2N_4$	75	178–180 (free base)	472	–	
5**	483.5	$C_{25}H_{36}Cl_2N_2O_3$	70	236 (dec.)	410	–	
6**	274.3	$C_{15}H_{11}NO_3Na$	60	220 (methylester)	–	–	

*Dihydrochlorides **1a–1i** have no molecular ion peak in their MS but they have $[M\text{-}2\ HCl]^{+}$ free basis peak, ** synthesized earlier [9, 10, 11].

HSV reproduction completely, besides one compound – **1c** – was less active; compounds **4, 5** and **6** were inactive. Among the bis-acridines, only the pentamethylene-containing ones demonstrate antiviral activity (**1f** and **1h** – high and **1c** – low). Compounds with a linker chain including the even quantity of methylene groups (**1b, 1d, 1e, 1g, 1i**) and/or a steric restricted linker chain (**1a** and **1i**) were inactive.

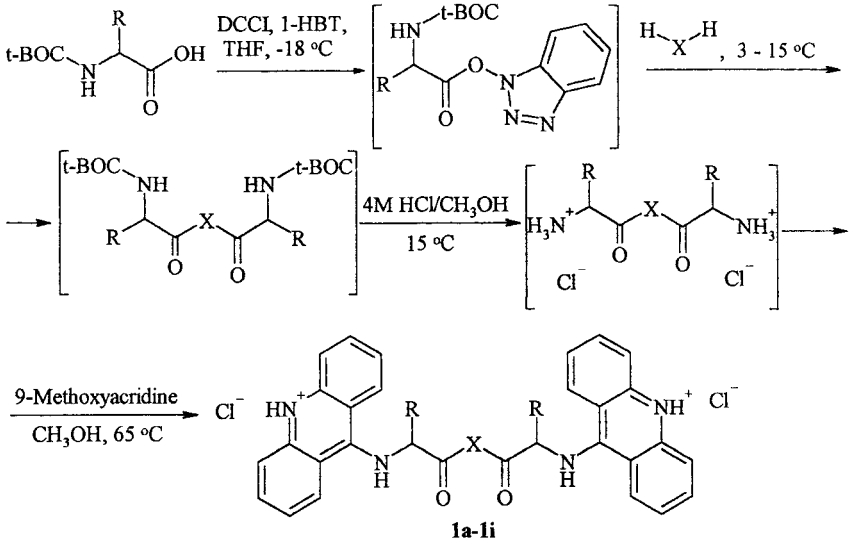
Therefore, active antivirals were found among the diacridines synthesized. Further search for analogues with an odd number of methylene groups in the linking chain seems reasonable to elucidate SAR. On the other hand, insertion of hydrophobic amino acids into the linker chain seems also promising. Comparative investigations of the affinity of the studied compounds to nucleosomes and purified DNA as well as AT/GC-specificity is in progress now to clarify the mode of antiviral action.

Table 2: Biological properties of the synthesized compounds

Compd.	MNC, μg/ml	MAC, μg/ml	CTI	IVTC, lg TCD_{50}	IVTD, lg TCD_{50}	II, lg TCD_{50}
1a	20	Inactive		6.0	5.0	1.0*
1b	10	Inactive		6.0	5.3	0.7*
1c	10	Inactive		6.0	4.0	2.0**
1d	20	Inactive		6.0	5.6	0.4*
1e	50	Inactive		6.0	5.0	1.0*
1f	20	5.0	4	6.0	1.0	5.0**
1g	20	Inactive		6.0	toxic	–
1h	50	5.0	10	6.0	1.0	5.0**
1i	20	Inactive		6.0	toxic	–
4	<10	Inactive		6.0	5.3	0.7*
5	50	Inactive		6.0	5.0	1.0*
6	50	Inactive		6.0	5.0	1.0*

MNC – maximal non-toxic concentration; MAC – minimal active concentration causing the 50% inhibition of the viral-specific cytopathic action; CTI- chemoterapeutical index (ratio of MNC/MAC); IVTC and IVTD – infectious viral titre (IVT) in the control and in the experiment respectively (trials were provided using 5 μg/ml concentration of the tested compounds); II – inhibition index (difference between IVTC and IVTD), IVT – the last dilution of the virus-containing media that still have possibility to produce the cytopathogenic effect; * $P > 0.1$; ** $P < 0.05$

Scheme



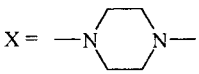
1a-1i

t-BOC - tert-Butyloxycarbonyl, DCCI - dicyclohexylcarbodiimide,
1-HBT - 1-hydroxybenzotriazol, THF - tetrahydrofuran

R = -CH_3 (L) : X = -$NHCH_2C(CH_3)_2CH_2NH$- (**1a**), -$NH(CH_2)_4NH$- (**1b**), -$NH(CH_2)_5NH$- (**1c**);

R = -$CH(CH_3)_2$ (L): X = -$NH(CH_2)_2NH$- (**1d**), -$NH(CH_2)_4NH$- (**1e**), -$NH(CH_2)_5NH$- (**1f**), -$NH(CH_2)_6NH$- (**1g**);

R = -$CH_2C_6H_5$ (L): X = -$NH(CH_2)_5NH$- (**1h**)

X =  : R = -$CH(CH_3)C_2H_5$ (L) (**1i**)

3. Experimental

3.1. Synthesis: N,N'-Bis-(L-acridin-9-valylamino)ethane dihydrochloride (**1d**)

To the solution of 3.131 g (14.41 mmol) t-BOC-Val-OH and 2.206 g (14.41 mmol) 1-hydroxybenzotriazole in 30 ml of cooled (−18 °C) anh. THF, 3.09 g (14.98 mmol) of DCCI dissolved in 10 ml THF was added and kept at this temperature for 2 h. Then the temperature was risen up to 15 °C, 0.45 ml (0.4 g, 6.67 mmol) ethylenediamine in 10 ml THF was added and left for a night at 3 °C. Dicyclohexylurea was filtered, the filtrate was evaporated, and the residue was dissolved in $CHCl_3$. The received solution was washed gradually with 1 M HCL (2×50 ml), H_2O (2×50 ml), 5% $NaHCO_3$ (3×50 ml), and the saturated solution of NaCl (2×50 ml), then dried by $MgSO_4$ and evaporated to dryness. To the residue 10 ml of the 4 M HCl solution in dry CH_3OH were added and left at room temperature for 3 h. The solvent was removed at lowered pressure. The prepared residue was washed with $(CH_3)_2CO$ and dried in vacuum at the room temperature for 24 h and was dissolved in 50 ml of dry CH_3OH. To the solution 2.791 g (13.34 mmol) of 9-methoxyacridine were added and refluxed with intensive stirring until the end of reaction (control by TLC): 30 ml of CH_3OH were evaporated under lowered pressure. The precipitate was filtered, washed with CH_3OH until no methoxyacridine was detectable (TLC) in the filtrate and dried in vacuum at 40–50 °C. Yield: 47% (2.15 g); m.p. > 270 °C (decomposed); MS: MX-1321 [70 ev, 220 °C, m/z (I, % max)]: 612 (M^+, 17), 569 (19), 363 (21), 306 (5), 249 (100), 235 (6), 219 (6), 207 (15). UV: Specord M-40 [CH_3OH, nm (lgε)]: λ_{max} = 262 (4.91), 418 (4.22), 440 (4.08).

The compounds **1a–1c**, **1e–1i** were similary prepared as dihydrochlorides. $C_{38}H_{42}Cl_2N_6O_2$

3.2. Biological test

HSV (L_2 stamm) cultivated in a culture of Vero cells was used for antiviral trials. HSV infectious titre in the cell culture was 6.0 lgTCD_{50}/0.1 ml (TCD – tissue citopathogenic dosage). Mixture of the equal volumes of the media 199 and Igle with addition of 10% of inactivated calf serum and antibiotics (penicillin and canamicine 100 units/ml of each) was used as

the growth medium. The Igle medium containing 2% of calf serum was used as supportive medium.

MNC determination was carried out in the experiments using 10, 20, 50, 100, 300 μg/ml of drug. Not less then 10 experiments were conducted for each concentration of drug. Test tubes with cells were incubated at 37 °C for 7 days and the cultures were reviewed daily for the cytopathogenic effects' exposure. The degree of the last one was determined by the change of the cells morphology according to the 4-grade system (from 1+ to 4+). The maximal drug concentration of not causing degeneration of the cells was determined as MNC, and not more than 1/2 of drug's MNC was used for the antiviral screening. For the MAC determination the test-virus was added in a dosage of 100 TCD_{50}/0.1 ml into the each test tube with cells culture, containing 1 ml of the cultural medium and incubated for 1 h at 37 °C. After virus adsorption on the cells, the medium was removed and the cells were washed thrice by medium 199. After that, different drug concentrations were added to the supportive medium. Cell cultures were reviewed daily for three days in order to detect cytopathic changes. The absence of a cytopathogenic activity in the experimental cultures while it was present in the control cultures allows to determine the drug's MAC. The drug was considered active at a decrease of the virus reproduction level for 1.8–2.0 lg and more. Virus-specific cytophatic action was evaluated daily according to a generally accepted method [13].

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