Antitumor acridines with diaminoalkylo pharmacophoric group*

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Abstract: The substitution of acridine molecule in positions 1 and/or 4 with diaminoalkylo residue may result in obtaining derivatives displaying antitumor activity. The diaminoalkylo residue can be attached to acridine either directly or indirectly as a carboxamido moiety. In the former case, the presence of appropriate substituent in position *para* to diaminoalkylo residue is crucial for antitumor activity. Also, heterocyclic aromatic rings condensed with the acridine core can be considered as such substituents. Additional substituents introduced into the acridine core, especially those that may be transformed into quinoid systems, significantly increase antitumor activity of modified analogs. It is, however, of utmost importance that the presence of diaminoalkylo residue is the indispensable prerequisite for biological activity of acridines. Among several groups of synthesized diaminoalkyloacridines, the most potent antineoplastic properties toward a wide spectrum of transplantable tumors are exhibited by acridine-4-carboxamides, imidazo-, triazolo-, and pyrazoloacridinones. Two derivatives belonging to the above groups, acridine-4-carboxamide DACA and imidazoacridinone C-1311, are currently in clinical trials. Other derivatives exhibiting potent antitumor activity, that could be considered as close analogs of diaminolkyloacridines, are pyrazoloacridines, one of which is currently under clinical evaluation.

Our previous research on the mode of action of mitoxantrone and ametantrone (Fig. 1) demonstrated that diaminoalkylo groups present in the side chains of these antitumor drugs are responsible for their ability to induce, after metabolic activation, interstrand covalent cross-links in DNA of tumor cells. Metabolically transformed diaminoalkylo residues are involved in the formation of DNA cross-linking bonds [1,2]. Other authors showed, based on structure–activity relationship studies carried out for mitoxantrone, ametantrone, and analogs, that diaminoalkylo side chain plays a key role in the biological activity of these drugs; hence, it can be considered a pharmacophoric group (for review, see ref. 3).
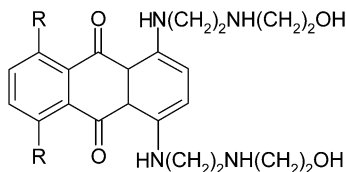

**Fig. 1** Anthracenediones: R = OH mitoxantrone, R = H ametantrone.

Most probably, the flat polycyclic anthracenedione moiety docks the drug within DNA double helix by intercalation and influences metabolic activation of diaminoalkylo side chains attached to it.

The demonstration that diaminoalkylo groups play a crucial role in both biological activity and DNA cross-linking ability of mitoxantrone and ametantrone suggested that the substitution of other planar polycyclic systems capable of DNA intercalation with such a pharmacophoric group may give rise to new antitumor compounds. We verified this hypothesis by designing new compounds consisting of diaminoalkylo groups attached to acridines belonging to classic DNA intercalating agents for which this phenomenon was described for the first time. The choice of acridines was dictated by our earlier interests in antitumor properties of this group of compounds, however, we used acridinones for further modifications since their structure closer resembled the structure of anthracenedione. Also, similarly as in the case of anthracenediones, and other antitumor drugs containing diaminoalkylo groups, the position 1 of acridinone core was chosen as the site of the diaminoalkylo side chain attachment. Mostly, the chains containing 2 or 3 methylene groups between amino groups were linked, because for such side chains, the highest biological activity was observed for anthracenediones.

As can be seen in Table 1, the substitution of acridinone in position 1 with diaminoalkylo group gave derivatives devoid of biological activity (compounds 1 and 2). The biologically active derivatives were obtained only when additionally the appropriate substituent was introduced in position 4, that is, in the position *para* to aromatic amine of diaminoalkylo moiety. Data presented in Table 1 show that the introduction into position 4 of such substituents as carboxy or amino group lead to inactive compounds, while the attachment of nitro or methyl group resulted in the compounds exhibiting significant biological activity. The role of a substituent in position 4 in the induction of antitumor activity of 1-diaminoalkyloacridinones is difficult to explain. Nitro and carboxy groups should incur similar elec-

Table 1 Structure of 1-diaminoalkyloacridinones and analogs and their antitumor activity against leukemia P388 in mice.
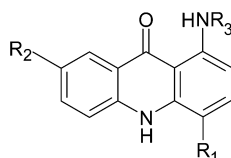
No.	R_1	R_2	R_3	Leukemia P388 O.D.** (mg/kg)	T/C*** (%)
1	H	H	$CH_2CH_2N(CH_3)_2$	6–200	N.A.****
2	H	H	$CH_2CH_2CH_2N(CH_3)_2$	6–200	N.A.
3	COOH	H	$CH_2CH_2N(CH_3)_2$	6–200	N.A.
4	COOH	H	$CH_2CH_2CH_2N(CH_3)_2$	6–200	N.A.
5*	NH_2	H	$CH_2CH_2N(CH_3)_2$	6–100	N.A.
6*	NH_2	H	$CH_2CH_2CH_2N(CH_3)_2$	6–100	N.A.
7*	NO_2	H	$CH_2CH_2N(CH_3)_2$	25	160
8*	NO_2	H	$CH_2CH_2CH_2N(CH_3)_2$	75	190
9	CH_3	H	$CH_2CH_2N(CH_3)_2$	100	205
10	CH_3	H	$CH_2CH_2CH_2N(CH_3)_2$	5	215
11	CH_3	OH	$CH_2CH_2N(CH_3)_2$	50	165
12	CH_3	OH	$CH_2CH_2CH_2N(CH_3)_2$	50	175
13	CH_3	H	CH_2CH_2OH	6–100	N.A.
14	CH_3	H	$CH_2CH_2CH_2OH$	6–100	N.A.

Data from publication [4]
**optimal dose
***survival time test/control
****nonactive

tronic effects toward amino group in position 1, thus, similar biological effects would be expected. This is not the case, since acridinones with nitro group are biologically active and carboxy-substituted analogs are not. Also, the role of substituents in position 4 does not seem to be associated with their susceptibility to metabolic activation leading to the formation of reactive metabolites capable of covalent binding to DNA. In the case of acridinones substituted in position 4 with either nitro or amino group, the final expected metabolites would be the same: as a result of enzymatic reduction of nitro group or enzymatic oxidation of amino group, the same reactive hydroxyamino derivative may be formed, which is able to bind covalently to DNA. One would thus expect that acridinone derivatives substituted with nitro or amino group should display comparable biological activity, in fact, only 4-nitro analogs are active.

In the group of 4-substituted derivatives of acridinone, over a dozen 1-diaminoalkylo-4-methyl-acridin-9-ones were obtained, several of which exhibited significant antitumor activity against leukemia P388 in mice [4]. Selected derivatives are presented in Table 1. The currently available data enable one to draw some conclusions concerning the relationship between the structure and biological activity of this group of compounds. The replacement of the terminal amino group in the side chain by hydroxyl or the attachment of a bulky moiety to this group, e.g., changing it into morpholine or piperidine moiety, abolishes biological activity. The necessity to retain the integrity of diaminoalkylo residue confirms its key role in the biological activity of compounds containing such a chain. The additional substituents in position 7 of acridinone core, like hydroxy or methoxy groups, do not show any substantial impact on biological activity that differs 1-diaminoalkylo-4-methylacridin-9-ones from compounds discussed below.

Once the crucial role of a substituent in position 4 for biological activity of diaminoalkyloacridinones was recognized, further modifications consisted in extending the acridinone system by the addition of another ring. This additional ring was presumed to have two functions: it should play a role of a substituent in the position *para* to the side chain and at the same time should improve the intercalative properties of a parent compound, as it is the case for other polycyclic compounds intercalating into DNA. The first group of compounds, designed based on the above assumptions, were diaminoalkyloimidazoacridinones, whose general structure is presented in Fig. 2A. Most of the derivatives obtained in this group exhibited significant antitumor activity toward leukemia P388 in mice [5]. Their antitumor activity was greatly increased when hydroxy group was introduced into position 8 [6]. We believe that the significance of 8-hydroxyl for biological activity of alkyloimidazoacridinones results from the possibility of formation of quinone-imine system involving this hydroxy group and acridine heterocyclic nitrogen in the neighboring ring. Such a quinonoimine system may be generated after enzymatic oxidation, and it may enhance the ability of imidazoacridinones to bind covalently to DNA by making positions 7 and 8 more susceptible to nucleophilic substitution. The performed studies on electrochemical
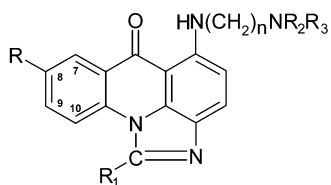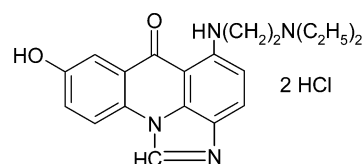
R = H, OH, OCH$_3$, t-butyl,
R$_1$-R$_3$ = H, CH$_3$, C$_2$H$_5$,
n = 2, 3

Fig. 2 A) Imidazoacridinones; B) Imidazoacridinone C-1311.

oxidation, as well as enzymatic oxidation by horseradish peroxidase, suggested that 8-OH group indeed easily undergoes oxidation and that this process is relevant for biological activity of imidazoacridinones [7,8]. Imidazoacridinones devoid of a substituent in position 8 or substituted in this position with methoxy group also display biological activity. Probably, in cells, both types of derivatives are converted into 8-OH derivatives as a result of, respectively, either hydroxylation or demethylation. This reasoning is supported by the fact that analogs with methyl or t-butyl substituent in this position lack biological activity [9]. On the other hand, the introduction of hydroxy or methoxy group in other positions in the same acridinone ring not only does not enhance the biological activity of imidazoacridinone but often decreases it [9]. The 8-OH imidazoacridinones represent, thus, an interesting example of antitumor compounds with two pharmacophoric groups: diaminoalkylo moiety and a latent quinonoimine system linked to and modulated by imidazo ring and its nitrogen atoms in particular. One of these nitrogen atoms takes part in the formation of the latent quinonoimine system, while the other occupies the position *para* to the side chain. 8-Hydroxy diaminoalkyloacridinones exhibit high antitumor activity not only against leukemias [1,6,9], but also toward other experimental tumors such as melanoma B16, colon adenocarcinoma Co26, Co38, MAC 15A, MAC 29 in mice, as well as toward xenografts of human colon cancer HT 29 in nude mice [10,11]. Imidazoacridinones display also potent cytotoxicity against a number of tumor cell lines of human and animal origin [4,6,11,12, and NCI protocols (unpublished results)]. The proliferation of cells growing in monolayer cultures and as multicellular spheroids is inhibited, to similar extent, which may at least in part explain the activity of imidazoacridinones toward solid tumors [13]. At the same time, these compounds display only weak mutagenicity [12]. The most active derivative, designated C-1311 (Fig. 2B), has been selected for clinical evaluation and is currently in the I stage of clinical studies.

Imidazoacridinones bind noncovalently to DNA, which was demonstrated by several techniques, e.g., by thermal DNA denaturation or spectroflourimetric titration [11,12,14]. However, no correlation between physicochemical DNA binding and biological activity could be found which suggested that noncovalent drug–DNA interactions did not play a decisive role in the activity of these compounds. Imidazoacridinones, after metabolic activation, bind covalently to DNA and induce interstrand covalent DNA cross-links in tumor cells [15]. They also inhibit topoisomerase II activity by stabilizing its cleavable complexes with DNA, and this effect is correlated with biological activity of imidazoacridinones [13]. It follows that their mode of action involves several routes, in particular topoisomerase II inhibition and covalent DNA cross-linking, preceded by metabolic activation. At the cellular level, the first observable effect of imidazoacridinones is the irreversible block of the cell cycle progression at the G2 stage [16]. This block appears to be sufficient to trigger the apoptosis of tumor cells [17] before DNA synthesis becomes inhibited. The description of other published results concerning imidazoacridinones can be found in a review [18], as well as in the references included in the hitherto-cited literature.

The next group of diaminoalkyloacridinones with additional ring includes triazoloacridinones (Fig. 3). Significant biological activity displays triazoloacridinones without additional substituents in acridinone ring system, but even higher antitumor potency toward several transplantable tumors in animals is exhibited by derivatives substituted in position 8 with hydroxy group. On the other hand, the substitution of the same position with chlorine, methyl, or nitro group or, in contrast to imidazoacridi-
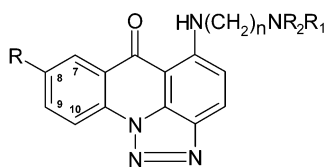

Fig. 3 Triazoloacridinones.

nones, also with methoxy group leads to inactive compounds [19]. All these findings point to the great importance of position 8 for biological activity of this group of acridines and strongly supports the hypothesis that it is involved in the formation of the pharmacophoric quinonoimine system, similarly as it was the case for imidazoacridinones.

Another group of acridine derivatives with additional ring is represented by pyrazoloacridones [20]. The structure of the compound designated KW-2170 belonging to this group, whose biological properties were most widely studied, is given in Fig. 4A. It exhibits potent antitumor activity toward several tumors in mice, as well as toward 17 xenografts of human tumors in nude mice [21]. Pyrazoloacridone KW-2170 displays also significant cytotoxicity in the case of tumor cell lines with MDR1-type resistance, as well as those resistant to adriamycin.

The potent antitumor properties toward leukemia P388 in mice, as well as significant cytotoxic activity toward cell lines resistant to adriamycin, are also displayed by some pyrimidoacridones (Fig. 4B) [22].
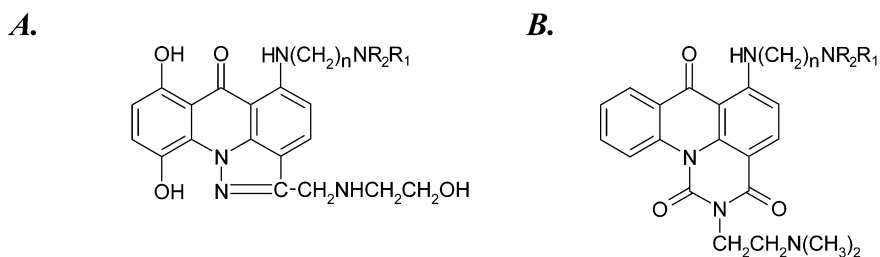
A.   **B.**

Fig. 4 A) Pyrazoloacridone KW-2170; B) Pyrimidoacridones.

Because of structural similarity, along with the group of diaminoalkylacridines, other acridine derivatives can be discussed, that is, 9-aminoacridine-4-carboxamides (Fig. 5A), in which one of the amino groups in the diaminoalkylo moiety is replaced by amido group. The resemblance of both mentioned groups of derivatives is supported by similar relationships between structure and biological activity. Most importantly, the presence of the side chain is an absolute prerequisite for biological activity. Its more substantial modifications, like methylation of amido nitrogen, leads to the loss of activity. The optimal biological activity is achieved when two nitrogens in the side chain are separated by 2 or 3 methylene groups [23]. The authors confirmed experimentally the importance of the position of the side chain attachment: derivatives with the side chain in position 1, 2, or 3 were biologically inactive (Table 2) [23,26]. This indicates that, as far as the position of the side chain is concerned, there exist very strict stereochemical requirements, most probably necessary for specific interactions with DNA. It is a very important observation, because in all so far obtained antitumor polycyclic compounds with diaminoalkylo residue, the side chain was always attached to the chromophore in the sites corresponding to positions 1 and/or 4 in acridine molecule. However, for any of these compounds, the influence of other positions of the side chain on biological activity has not been determined.
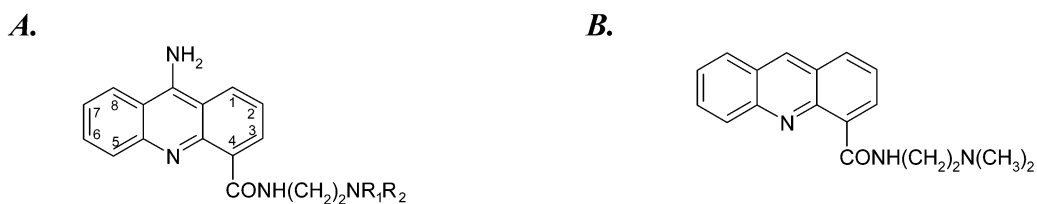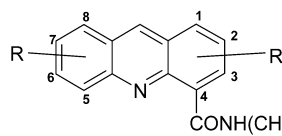
A.   **B.**

Fig. 5 A) 9-aminoacridine-4-carboxamides; B) DACA.

Table 2 Structures of acridine-4-carboxamides and their antitumor activity against leukemia P388 and Lewis lung carcinoma (based on data from [26]).

R—[acridine ring system, positions 1,2,3,4,5,6,7,8, N]—R
CONH(CH$_2$)$_n$N(CH$_3$)$_2$

No.	Side chain position	n	R	Leukemia P388		Lewis lung carcinoma	
				O.D.[*] (mg/kg)	T/C[**] (%)	O.D. (mg/kg)	T/C (%)
1	1	2	H	150	N.A.[***]		
2	2	2	H	225	N.A.		
3	3	2	H	150	N.A.		
4	4	2	H	66	191	100	>>300
5	4	3	H	150	218	150	184
6	4	4	H	100	243	100	200
7	4	5	H	225	134	150	183
8	4	6	H	100	132	150	N.A.
9	4	2	1-OCH$_3$	30	205	65	>>300
10	4	2	2-OCH$_3$	100	199	100	205
11	4	2	5-OCH$_3$	65	170	45	154
12	4	2	6-OCH$_3$	100	173	100	185
13	4	2	7-OCH$_3$	150	191	150	146
14	4	2	1-Cl	100	210	100	220
15	4	2	1-CH$_3$	100	171	100	167

*optimal dose

**survival time, test/control

***nonactive

Acridine-4-carboxamides devoid of amino group in position 9, in contrast to 9-aminoacridine-4-carboxamides, exhibit strong antitumor activity toward solid tumors, e.g., Lewis lung carcinoma (Table 2) or colon Co38 carcinoma in mice [26,27]. The most active representative of this group, that is *N*-[(dimethylamino)ethyl]acridine-4-carboxamide known also as DACA (Fig. 5B), is currently in the II phase of clinical studies [29 and references within].

An interesting observation was made as a result of structure–activity relationship studies carried out for DACA and its analogs. It was found that the substitution of position 7, *para* to heterocyclic nitrogen, with hydroxy group had no influence on biological activity. Thus, acridine–4-carboxamides behave in a similar fashion as 4-substituted diaminoalkyloacridones and differ in this regard from other groups discussed earlier. Other substituents in positions from 5 to 8 also did not influence significantly the biological properties of analogs of DACA [28].

9-Aminoacridine-4-carboxamides are able to intercalate into DNA and the dissociation kinetics of DNA binding is correlated with their biological activity [30]. Biologically active 9-aminoacridine-4-carboxamides induce, after metabolic activation, covalent interstrand cross-links in DNA of tumor cells. Such ability is not displayed by inactive derivatives [31]. DACA belongs to the inhibitors of topoisomerases I and II. It stabilizes the cleavable complexes of both these enzymes with DNA, and this property is regarded as the one underlying its biological activity [29]. The references to numerous publications, not mentioned here, concerning this very interesting group of acridine-4-carboxamides can be found in the hitherto-cited literature [23–31].

As it was the case with acridine-4-carboxamides, because of structural resemblance as well as similar relationship between structure and activity, also pyrazoloacridines whose general structure is given in Fig. 6A can by treated as analogs of diaminoalkyloacridines. Most of these compounds exhibit significant activity toward leukemia P388 in mice [32]. On the assumption that one of amino groups of the side chain is incorporated into pyrazolo ring, within the chain defined in such a way, similar relations between the structure and activity are observed as those typical for antitumor compounds so far discussed, containing diaminoalkylo moiety. The optimal for biological activity linker between two nitrogens consists of 2 or 3 methylene groups. The replacement of terminal amino group with chlorine or hydroxyl results in the loss of activity. Similarly, as in the case of imidazo- and triazoloacridinones, the presence in position 9 (*para* to the heterocyclic nitrogen) of free or slightly substituted hydroxy group is essential for biological activity [32]. Also, the substituent in the position *para* to the side chain plays an important role, as it was seen for diaminoalkyloacridines (Table 1). In the case of pyrazoloacridines, this position must be substituted with nitro group; its reduction leads to the loss of biological activity [33]. 9-Methoxypyrazoloacridine, whose structure is presented in Fig. 6B, is currently under the II phase of clinical evaluation (for review, see ref. 34).
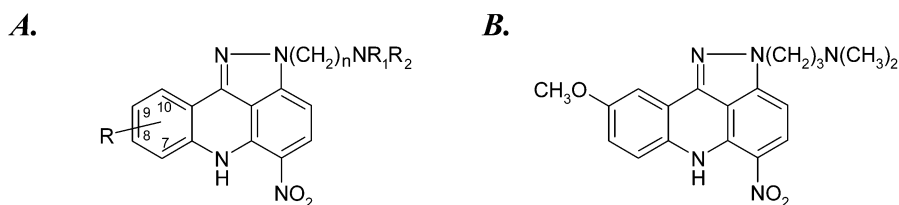

Fig. 6 A) Pyrazoloacridines; B) 9-Methoxypyrazoloacridine.

9-Methoxypyrazoloacridine blocks topoisomerases I and II, however, the mechanism of this inhibition is different from the one determined for other inhibitors, that is, it does not consist in stabilization of cleavable complexes between DNA and these enzymes [35]. After metabolic activation, pyrazoloacridines become capable of covalent interstrand DNA cross-linking. Biologically inactive amino derivative, obtained by reduction of nitro group in 9-methoxypyrazoloacridine, does not cross-link DNA, which points to the crucial role of DNA cross-linking in the mechanism of action of this compound ([36] and unpublished results by R. Majcher and J. Konopa).

In conclusion, numerous common structural elements, similarities as well as differences in the mode of action, and differentiated biological activity, suggest that in the group of the discussed acridine derivatives from among which three compounds were selected for clinical evaluation, further interesting discoveries can be expected.

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