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Short communication

Some new 1-nitro acridine derivatives as antimicrobial agents

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Summary – Some new 1-nitro acridine derivatives were prepared and characterized. These compounds were screened for evaluating their antimicrobial properties against Gram+ bacteria, Gram– bacteria, and mycobacteria as well as fungi. With reference to the results obtained, 1-nitro-4-aminoethylamine-9-alkylthioacridines must be considered as promising lead molecules.

Résumé – **Quelques nouveaux dérivés acridiniques nitrés en position 1, agents antimicrobiens.** *De nouveaux acridiniques nitrés en position 1 ont été préparés et étudiés pour leurs activités antimicrobiennes. Les meilleurs résultats ont été obtenus avec ceux portant un groupement thioether greffé en position 9 et une substitution aminoéthylamine en 4.*

nitro acridines / antimicrobial agents

Introduction

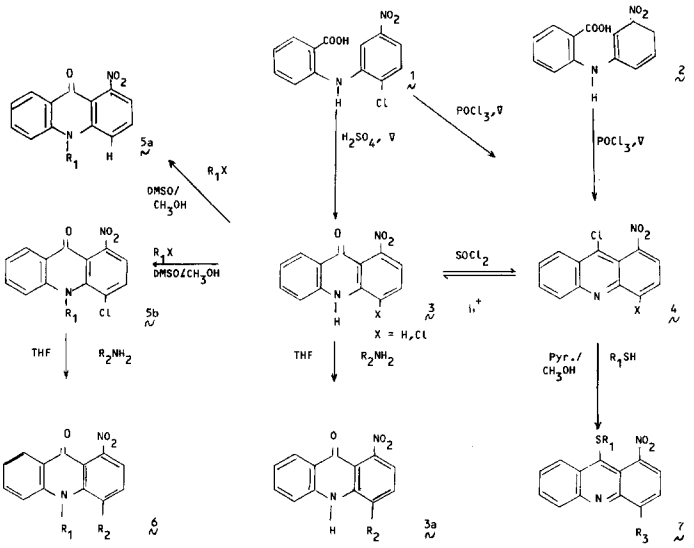
Acridine derivatives are well-known antibacterial agents. Moreover, a low antifungal activity has been reported for some 9-amino acridines [1, 2]. As regards nitro substituted acridines, despite the fact that these compounds were tested clinically with a view to cure pleural empyema [3], the major uses mentionned refer either to streptococcal infection of cattle or to experimental diseases in laboratories [4, 5, 6]. For example, 1-nitro acridines are of interest with regard to *Salmonella typhimurium, Bacillus subtilis* [7, 8], *Micrococcus lysodeiticus* [9] and *Staphylococcus aureus* whilst 1-nitro-9-acridinones are mildly effective against *Escherichia coli, Streptococcus pneumoniae,* and *Branhamella catarrhalis, Bacillus cereus* and Coryne bacteria [10]. In addition, the mechanism of 1-nitro-9-amino-acridine derivatives which show anticancer properties, has been recently detailed [11, 12, 13].

With this in mind, some new 1-nitro acridines were prepared and evaluated as antimicrobial agents.

Chemistry

Anthranilic acids **1** and **2** lead to the acridine derivatives [14]. These acids are prepared by condensing *ortho*-chloro benzoic acid with a suitably substituted aniline [14, 15]. Thus, the 10H-9-acridinones **3** are obtained from **1**, by using sulfuric acid as cyclizing agent. On the other hand, the 9-chloro acridines **4** are obtained from **1** and **2**, by using phosphorus oxychloride as cyclizing agent. Hydrolysis of **4** in acidic medium gives **3** [14], whilst the reaction is reversed by using thionyl chloride [15]. Finally, 9-thioalkyl-acridines **7** are obtained when a mercaptan reacts with **4** in basic medium [16] while alkylation of **3** under various conditions, leads to the alkyl-substituted 1-nitro-9-acridinones **5** or **6**.

These synthetic pathways are schematized in Scheme 1. In addition, the compounds prepared are listed in Table I.



Scheme 1. Synthetic pathways.

Biological results and discussion

Minimal inhibitory concentrations (MIC), minimal bactericidal concentrations (MBC), and minimal fungicidal concentrations (MFC) are gathered in Table II, while results concerning intercalation of drugs into DNA are presented in Table III.

With respect to these results, some comments can be given.

Table I. List of compounds prepared.

Compounds	R_1	R_2	R_3
3a	–	$(CH_2)_2NH_2$	–
5a	$(CH_2)_2N(C_2H_5)_2$	–	–
5b	CH_3	–	–
6a	CH_3	$(CH_2)_2NH_2$	–
7a	CH_2CH_3	–	–
7b	$(CH_2)_2-N(C_2H_5)_2$	–	–
7c	$(CH_2)_3-N(CH_3)_2$	–	–
7d	$C(=NH)NH_2$	–	–
7e	CH_2CH_3	–	$S-CH_2CH_3$

Antibacterial activity

On the one hand, comparison between the MIC of compounds **5a** and **7b** shows that acridine thioethers are more potent than the corresponding *N*-substituted-9-acridinones. With respect to this, one must remember that a similar result occurred when the antiparasitic activity of related compounds was screened [17]. However, as growth inhibitory effect on *E. coli* and *Mycobacterium smegmatis* is only enhanced, whilst there are no changes in growth inhibitory effect on *S. aureus,* activity could be correlated to cell penetration rather than to a change in affinity towards the admitted target.

On the other hand, comparison between **7a, 7b, 7c** and 9-ethylthio-acridine **8a,** 9-(3′-dimethyl aminopropylthio)-acridine **8b,** 9-(2′-diethylaminoethylthio)-acridine **8c** [18] leads to the conclusion that the nitro group slightly enhances growth inhibition, except in the case of *M. smegmatis*. Moreover, there is some evidence that a protonatable nitrogen branched on the tricyclic moiety favours interaction with DNA, but there is a little evidence that this group favours antibacterial activity.

Finally, attention must be drawn to compounds **3a** and **6a**. Indeed, data indicate that these compounds are the most effective compounds as growth inhibitors. In particular, bacteridical activity of **6a** against *E. coli* and *S. aureus* must be emphasized. In addition, these compounds show a strong affinity for DNA. However, at the present time we are still unable to correlate these results with a peculiar molecular feature, although the

Table II. *In vitro* antimicrobial activities.

Compounds	*E. coli*		*S. aureus*		*M. smegmatis*		*C. albicans*	
	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC
3a	500*	50	500	50	> 500**	50	> 500	100
5a	> 1000	500	> 1000	500	> 1000	500	> 1000	1000
6a	500	50	500	100	> 500	50	500	50
7a	500	100	> 500	500	> 500	100	> 500	500
7b	> 500	100	> 500	500	> 500	100	> 500	500
7c	> 500	100	> 500	500	> 500	100	> 500	500
7d	> 500	100	> 500	500	> 500	500	> 500	500
7e	> 500	> 500	> 500	500	> 500	500	> 500	500
8a	10000	1000	10000	1000	10000	100	***	***
8b	10000	500	> 10000	1000	10000	100	***	***
8c	1000	1000	10000	1000	10000	100	***	***
8d	5000	500	5000	500	5000	100	5000	500

*Results are given in $\mu g/ml$.
**> Means no activity at the concentration mentioned. Moreover, higher concentrations have not been tested due to the low solubility of the compound.
***Tests have not been performed.

Table III. DNA binding parameters and effect on denaturation temperature of the nucleic acid.

Compounds	Isobestic point (nm)	Bathochromic shift (nm)	Binding affinity $K \times 10^5$	No. of binding sites	ΔTm (°C)*
3a	480	5	14.30	0.387	+3
5a	442	2	3.89	0.2	+2.6
6a	495	5	11.02	0.28	+3
7a	**	0	0	0	0
7d	500	90	6.9	0.24	+2.6
7e	**	0	0	0	0
8a	**	0	0	0	0
8b	***	2	7.65	0.2	+3.3
8c	410	2	8.75	0.2	+2

*ΔTm = Tm(DNA + ligand)−Tm(DNA).
**There are no isobestic points.
***Isobestic point has not been identified.

pharmacophoric groups $N-(CH_2)-N$ could be considered as the responsible one, as this was previously shown in the case of tumors growth inhibitors [19].

Antifungal activity

The fungistatic activity of the most of compounds prepared does not differ significantly from the activity of some other acridine derivatives such as 9-octylthio-acridine **8d** [18], which was selected as a model. Yet, compounds **3a** and **6a** are still remarkable with the reference to the latter activity so that the aminoethylamino substituted acridinic moiety behaves as a lead structure for new antimicrobial derivatives.

Experimental protocols

Chemistry

Melting points were determined in open capillary tubes on a Buchi-Tottoli apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker AM200 spectrometer at ambient temperature with tetramethylsilane as internal standard. Data are expressed in p.p.m. Analytical results for C, H and N were within ± 0.4% of the theoretical values.

1-Nitro-4-(2'-aminoethylamino)-10H-9-acridinone ***3a***

A mixture of 1-nitro-4-chloro-10H-9-acridinone [20] (2.75 g, 10 mmol), ethylene-diamine (1.20 g, 20 mmol), and tetrahydrofuran (75 ml) was heated at 80°C with stirring for 24 h. Solvent was evaporated and the residue dissolved in absolute ethanol. The solution was then concentrated before the compound was precipitated by the addition of aqueous ammonia (yield: 50%); mp: 168−170°C; ^1H NMR ($CDCl_3$) δ: 10.2 (s, H); 8.3 (d, 2H); 7.65 (t, H); 7.4 (d, H); 7.25 (m, H); 6.2 (d, H); 3.35 (m, 2H); 3.05 (t, 2H).

1-Nitro-10-(2'-diethylaminoethyl)-9-acridinone ***5a***

A mixture of 1-nitro-10H-9-acridinone [21] (2.40 g, 10 mmol), 1-chloro-2-(diethylamino)-ethane (1.36 g, 10 mmol), and triethylbenzylammonium chloride (1.14 g, 5 mmol), 50% aqueous potassium hydroxide (50 ml) and butanone (100 ml) was refluxed with stirring for 3 h. The organic layer was separated, dried with sodium sulfate and evaporated. The dried residue was recrystallized from absolute ethanol (yield: 53%); mp: 183°C; ^1H NMR (TFAA−d) δ: 8.45 (d, H); 8.1 (m, 3H); 7.6 (m, 2H); 5.35 (t, 2H); 3.8 (m, 6H); 1.6 (t, 6H).

1-Nitro-4-chloro-10-methyl-9-acridinone ***5b***

A mixture of 1-nitro-4-chloro-10H-9-acridinone [20] (2.75 g, 10 mmol), potassium hydroxide (1.12 g), and dimethylsulfoxide (20 ml), methanol (25 ml) was stirred for 10 min before methyliodide (2.84 g, 20 mmol) was added. The solution was heated at 50°C under stirring for 15 h. The crude product precipitated when water was added. The precipitate was washed with water and dried, then recrystallized from chloroform-ethanol (v/v) (yield: 80%); mp: 247−250°C; ^1H NMR ($CDCl_3$) δ: 8.3 (d, H); 7.8 (t, 2H); 7.5 (d, H); 7.35 (d, H); 7.2 (d, H); 4.0 (s, 3H); ^{13}C NMR ($DMSO-d_6$) δ: 147.46 (C−1); 116.74 (C−2); 136.09 (C−3); 124.62 (C−4); 117.87 (C−5); 134.80 (C−6); 122.96 (C−7); 125.44 (C−8); 174.13 (C−9); 116.03 (C−11); 145.43 (C−12); 142.81 (C−13); 122.54 (C−14); 43.59 (CH_3).

1-Nitro-4-(2'-aminoethylamino)-10-methyl-9-acridinone dihydrochloride ***6a***

A mixture of 1-nitro-4-chloro-10-methyl-9-acridinone (2.89 g, 10 mmol) **5b**, ethylenediamine (1.20 g, 20 mmol), and tetrahydrofuran (75 ml) was heated at 80°C with stirring for 24 h. Solvent was evaporated and the residue dissolved in absolute ethanol. The solution was then concentrated before the compound was precipitated by the addition of hydrochloric ether. Hydrochloride was recrystallized from methanol (yield: 67%); mp: 196−200°C; ^1H NMR ($CDCl_3$) δ: 10.4 (s, H); 8.2 (d, H); 7.55 (t, H); 7.3 (t, 2H); 7.15 (t, H); 3.85 (s, 3H); 3.25 (q, 2H); 3.0 (m, 2H).

1-Nitro-9-thioethyl-acridine ***7a***

A mixture of 1-nitro-9-chloro-acridine [22] (2.89 g, 10 mmol), ethanethiol (0.75 g, 12 mmol), potassium carbonate (2 g), and anhydrous acetone (40 ml) was refluxed with stirring for 25 min. After filtration, the solvent was evaporated *in vacuo*. The crude product was recrystallized from ether-petroleum ether (yield: 55%); mp: 93°C; ^1H NMR ($CDCl_3$) δ: 8.75 (d, H); 8.4 (d, H); 8.2 (d, H); 7.9 (m, 4H); 2.75 (q, 2H); 1.0 (t, 3H).

1-Nitro-9-(2'-diethylaminoethylthio)-acridine ***7b***

A mixture of 1-nitro-9-chloro-acridine [22] (1.03 g, 4 mmol), 2-diethylaminoethanethiol hydrochloride (0.76 g, 4.5 mmol), freshly distilled pyridine (20 ml), and methanol (10 ml) was refluxed with stirring for 15 min before being heated at 100°C for 40 min. After cooling, the solution was diluted with ether and left to stand overnight. The reddish crystals so obtained were washed with ether (yield: 40%); mp: 195°C; ^1H NMR (TFAA−d) δ: 9.0 (d, H); 8.7 (t, H); 8.5 (d, 2H); 8.35 (m, 2H); 8.25 (m, H); 3.65 (m, 2H); 3.55 (m, 2H); 3.4 (m, 4H); 1.4 (t, 6H).

1-Nitro-9-(3'-dimethylaminopropylthio)-acridine ***7c***

A mixture of 1-nitro-9-chloro acridine [22] (1.17 g, 4.5 mmol), freshly distilled pyridine (20 ml), and methanol (10 ml) was refluxed for 15 min. 3-Dimethylaminopropanethiol (5 mmol) was then added. The mixture was heated at 100°C with stirring for 40 min. After cooling, the solution was diluted with ether. Crude product was collected by filtration before being dissolved in methanol. Petroleum ether was added until the solution was turbid. The latter was then allowed to stand overnight. Brown crystals were obtained (yield: 35%); mp: 135−137°C; ^1H NMR (TFAA−d) δ: 8.95 (d, 2H); 8.75 (t, H); 8.55 (t, H); 8.15 (m, 3H); 3.45 (q, 2H); 3.15 (s, 6H); 2.8 (m, 2H); 2.35 (m, 2H).

1-Nitro-9-ureidylthio-acridine ***7d***

A mixture of 1-nitro-9-chloro-acridine [22] (1.55 g, 6 mmol), thiourea (0.91 g, 12 mmol), and 1,2-propanediol (30 ml) was heated at 120°C with stirring for 30 min. After cooling, 10% aqueous potassium hydroxide was added till alkaline reaction. Crude product was collected by filtration and washed with water. The expected compound was recrystallized from acetone (yield: 45%); mp: >260°C; ^1H NMR (TFAA−d) δ: 9.15 (m, 4H); 9.1 (m, 2H); 8.7 (d, H); ^{13}C NMR ($DMSO-d_6$) δ: 146.84 (C−1); 115.33 (C−2); 132.26 (C−3); 125.05 (C−4); 119.52 (C−5); 130.73 (C−6); 122.15 (C−7); 129.14 (C−8); 149.27 (C−9); 111.66 (C−11); 143.26 (C−12); 140.34 (C−13); 121.59 (C−14); 147.51 (S−C−N).

1-Nitro-4,9-diethylthio-acridine ***7e***
A mixture of 1-nitro-4,9-dichloro-acridine [23] (2.93 g, 10 mmol), ethanethiol (1.49 g, 24 mmol), potassium hydroxide (2 g), and methanol (75 ml) was refluxed with stirring for 15 min. The solvent was evaporated and the crude product was dissolved in ether. The ether was evaporated. The residue was dissolved in a small quantity of acetone. The compound was precipitated by addition of a small amount of petroleum ether (yield: 44%); mp: 90°C; ^{1}H NMR ($CDCl_3$) δ: 8.7 (d, H); 8.5 (d, H); 7.85 (t, 2H); 7.75 (t, H); 7.3 (t, H); 3.15 (q, 2H); 2.75 (q, 2H); 1.55 (t, 3H); 0.9 (t, 3H).

Biology

Microbiology
Activity of compounds was evaluated in a microplate assay on the following strains: *E. coli* ATCC 10536, *S. aureus* ATCC 9144, *M. smegmatis* CNCM 7326 and *C. albicans* CNCM 1180-79. Stock solutions of drugs and dilutions of the latter were prepared in acetone / water (1:1) due to the fact that compounds tested are poorly soluble in pure water. Concentrations tested were the following: 10 000, 5 000, 1 000, 500, 100, 50, 10, 5, 1 μg / ml.

Growth inhibition was evaluated by mixing equal volumes (100 μl) of drug dilutions and of a double-strength both inoculated with (2 ± 1) 10^5 viable cells per ml: Nutrient Broth Difco supplemented with 0.1% dextrose and 0.15% yeast extract, for bacteria and Sabouraud Broth for yeast. Positive growth control and negative growth control as well as controls for absorbance at each concentration tested, were simultaneously performed. The optical density was determined at 620 nm on a Titertek Multiskan photometer after 48 h *(E. coli, S. aureus)*, or 72 h *(M. smegmatis, C. albicans)*. MIC is defined as the lowest concentration allowing less than 20% of growth.

Lethal activity was determined from a mixture of 75 μl of cell suspension [(2 ± 1) 10^7 viable cells per ml] and 75 μl of the drug dilution, incubated for 15 min at 21°C. Survivors were counted on plate count agar (Difco) for bacteria and on yeast agar (yeast extract, 5 g dextrose, 20 g; M / V) for the yeast, by plating 1 μl × 10 of the 1 / 10 dilution in a suitable neutralizer (5% fresh egg yolk supplemented with 3% polysorbate 80; v / v), after incubation at 37°C for 48 h or 72 h in the case of *M. smegmatis*. MBC and MFC are defined as the lowest concentrations able to reduce by at least 10^5 the number of viable cells.

DNA binding
Interaction with DNA of the compounds selected, was investigated by UV spectral shift determination, UV spectrophotometric titration, and determination of the effect on the denaturation temperature (Tm) of DNA, all of which are well-documented methods for investigating intercalation of drugs into DNA [24, 25].

Assays were carried out at 25°C on a Philipps PU 8800 spectrophotometer. Solutions of dyes were prepared in pH 7.0 buffer (0.018 mol / l NaCl; 0.003 mol / l Tris), so that the final concentrations was exactly $2.5 \cdot 10^{-5}$ M. Aliquots of DNA solution (about 1 mg / ml) were sequentially added with stirring until there were no significant changes in the observed extinction of the dyes solutions. DNA affinity and number of binding sites were estimated by using the Scatchard plot method [26].

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