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Short communication

Synthesis and antileishmanial activity of 6-mono-substituted and 3,6-di-substituted acridines obtained by acylation of proflavine

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Abstract

Two new series of diaminoacridinic derivatives obtained from proflavine and *N*-(6-amino-3-acridinyl)acetamide were synthesised and assessed for their cytotoxic and antileishmanial activities. Two compounds, *N*-[6-(acetylamino)-3-acridinyl]acetamide and *N*-[6-(benzoylamino)-3-acridinyl]benzamide demonstrated highly specific antileishmanial properties against the intracellular amastigote form of the parasite. Structure–activity relationships established that the antiproliferative activity against human cells was greatly enhanced by the presence of a benzoylamino group in 6-mono-substituted acridines, while the presence of two acetylamino or benzoylamino groups in 3,6-di-substituted acridines strongly increased the specificity of the molecules for *Leishmania* parasite, suggesting that symmetric conformations could preferentially interfere with *Leishmania* metabolism.

Keywords: Acridines; Cytotoxicity; Antileishmanial activity; DNA-binding activity

1. Introduction

For hundreds of years, leishmaniases have been the cause of death among millions of people throughout the world [1]. Transmitted by the bite of a sand fly, they are due to an intracellular protozoa belonging to the genus *Leishmania* which colonizes human macrophages and produces a large spectrum of clinical manifestations [1,2]. Among all leishmaniases, the visceral form also known as kala-azar is the most severe [1–3]. Characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and anaemia, this disease can produce, if untreated, a mortality rate of almost 100%, independently on the immunological status of the patient [3]. Pentavalent antimony, Amphotericin B and the oral anticancer drug miltefosine are considered at the moment as the best therapeutic solutions [4,5] for the treatment of visceral leishmaniasis, however, they do not represent a safety treatment in all clinical cases, rendering necessary the research of new antileishmanial molecules.

Due to their rapid replication, Trypanosomatidae protozoa have shown many similarities with tumour cells and various anticancer drugs have been successfully tested for their antileishmanial activity. Since the 1990s, various acridine derivatives which have shown strong antiproliferative activities on human transformed cells, have been considered as promising agents for anticancer and/or antiparasitic therapy [6]. Among these compounds, proflavine, widely used as a biochemical tool for studying the properties of DNA and RNA *in vivo* and *in vitro* [7], showed some remarkable antiproliferative effects on hamster melanoma [8], indicating that synthesis of new derivatives of the 3,6-diaminoacridine series would present new promising anticancer and/or antiparasitic agents.

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In order to open a new way in antitumor drug research, an efficient synthetic route to monofunctional 6-substitued acridine and bifunctional 3,6-di-substituted acridine derivatives has been developed on the basis of direct electrophilic substitution of acridinic moiety. This synthetic route led to a wide range of acridinic derivatives which could be linked to various side chains. On this basis we synthesised two new series of diaminoacridinic derivatives obtained from proflavine and *N*-(6-amino-3-acridinyl)acetamide and studied their antiproliferative and antileishmanial activities as compared to their capacity to bind with DNA.

2. Chemistry

Our synthetic pathway was based on the preparation of symmetric and non-symmetric diaminoacridines by mono- or di-acylation: proflavine **1**, used as hemisulfate, was allowed to stand with different anhydrides to yield the corresponding amides **2a–c** as reported in Fig. 1.

The *in situ* neutralisation of **1** with NEt_3 in pyridine was not competitive to the acylation reaction because of the higher kinetic of amide toward acid formation. The formed hydrosulfate was then neutralized with excess base. No significant yield augmentation was observed using neutral proflavine. Compound **1** was also allowed to react with different benzoylchlorides to yield the corresponding amides shown in Fig. 2 with medium to good yields. Moreover, a useful synthetic route has been developed to yield selectively the mono-*N*-(6-amino-3-acridinyl)acetamide **4** from proflavine **1**: we used a modified procedure from Albert and Linnell [9] using acetic anhydride to give only one monomer with 84% yield. The reaction was fully regioselective and did not require protection of the second amino substituent. The reaction allowed the desymetrization of the molecule as the electrophilic substitution only proceeded on one side of the molecule, because of the strong withdrawing electronic effect of the acetylamino group. Then, mono-acetyl **4** was reacted with different benzoylchlorides to yield good yields of the corresponding mono-amides **4a–e**, as reported in Fig. 3.

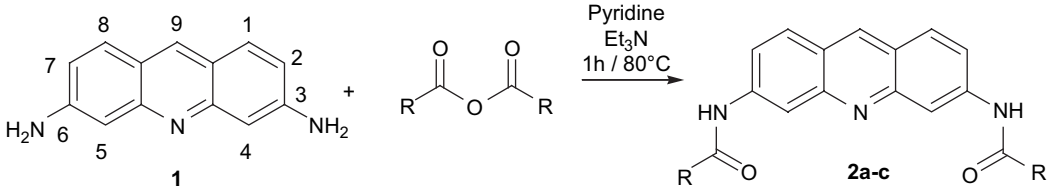
3. Pharmacology

The antiproliferative properties of acridine derivatives were evaluated against transformed human monocytes (THP1 cells), while antileishmanial activities were assessed against parasites of the species *Leishmania infantum* responsible for kala-azar, the most severe visceral form of leishmaniasis, on both its extracellular promastigote and intracellular amastigote forms. In parallel, the capacity of the compounds to non-covalently interact with DNA by intercalating into the DNA major groove was evaluated by the DNA–methyl green bioassay.

4. Results and discussion

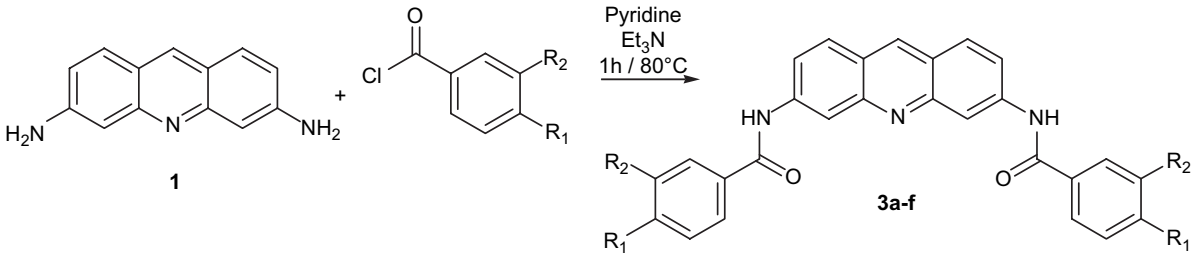
Results are summarized in Table 1 and schematised in Fig. 4. They showed that the pharmacological properties of the compounds greatly depended on the number and the chemical nature of the substituents. Compound **4** (*N*-(6-amino-3-acridinyl)acetamide), the parent molecule of the **4a–e** series, exerted an interesting antiproliferative activity on human monocytes ($IC_{50\text{-monocytes}} = 4.7$ μM) while it was deprived from toxicity toward *Leishmania* parasites on both their extracellular and intracellular forms. Comparison with results observed for proflavine indicated that the replacement of an amino group by an acetylamino group slightly reduced the cytotoxicity of the compounds but deprived the derivatives from antileishmanial activity.

Its bifunctional homologue **2a** (*N*-[6-(acetylamino)-3-acridinyl]acetamide) on the contrary, showed a weak toxicity against human cells and *Leishmania* promastigotes ($IC_{50\text{-monocytes}} > 500$ μM and $IC_{50\text{-promastigotes}} > 300$ μM) associated with a strong anti-amastigote property ($IC_{50\text{-amastigotes}} = 4.5$ μM). These values indicated that the replacement of the amino group by a second acetylamino group on position 6 greatly modified the biological properties of the molecule, rendering it less toxic for mammalian cells and highly specific for the intracellular form of the parasite. Compounds **2b** (*N*-[6-(propionylamino)-3-acridinyl]propanamide) and **2c** (*N*-[6-(butyrilamino)-3-acridinyl]butanamide) were more efficient than compound **2a** against human cells and promastigotes,



Comp	R	Yields	MW	Molecular formula	Mp °C
2a	CH_3	43 %	293	$C_{17}H_{15}N_3O_2$	260
2b	C_2H_5	65 %	321	$C_{19}H_{19}N_3O_2$	265
2c	C_3H_7	32 %	321	$C_{19}H_{17}N_3O_2$	216

Fig. 1. Synthesis of compounds **2a** to **2c**.

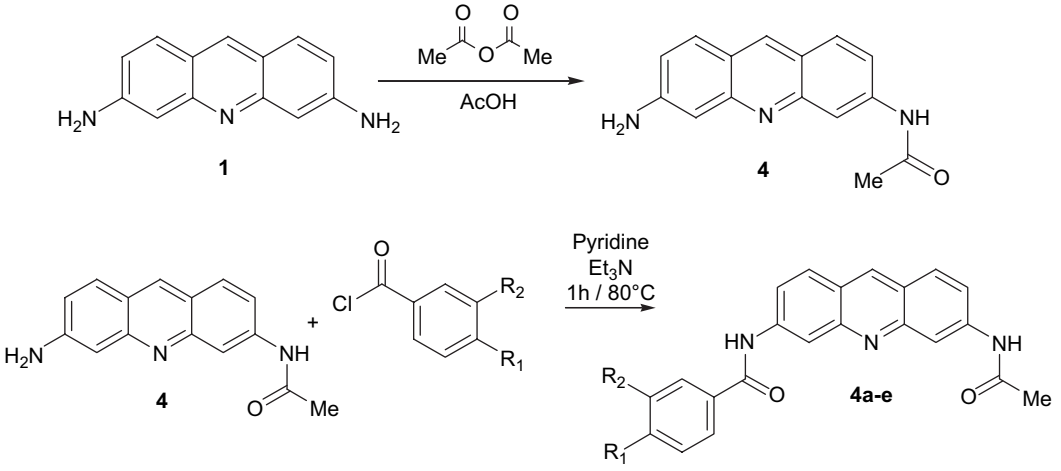


Comp	R_2	R_1	Yields	MW	Molecular formula	Mp °C
3a	H	H	66 %	417	$C_{27}H_{19}N_3O_2$	248
3b	H	Cl	90 %	486	$C_{27}H_{17}N_3O_2Cl_2$	370
3c	H	F	96 %	453	$C_{27}H_{17}N_3O_2F_2$	290
3d	H	OMe	51 %	477	$C_{29}H_{23}N_3O_4$	252
3e	OMe	OMe	39 %	537	$C_{31}H_{27}N_3O_6$	207

Fig. 2. Synthesis of compounds **3a** to **3e**.

suggesting that their toxicity increased with the length of the 3,6-di-substituted linear chains. Inversely, their anti-amastigote activity proportionally decreased. These results implied that such molecules could react with biological structures by two different mechanisms. The first one, weakly specific for the cell type, was highly dependent on the length of the 3,6-di-substituted linear chains. The second one, strongly specific of the amastigote–macrophage system, was essentially due to the combined action of the two 3,6-di-substituted acetylamino groups. Compound **3a** (*N*-[6-(benzoylamino)-3-acridinyl]benzamide), bearing benzoylamino groups on positions 3 and 6, demonstrated a strong affinity for both parasite forms with $IC_{50\text{-promastigotes}}$ and $IC_{50\text{-amastigotes}}$ averaging 1.1 and 4.3 μM, respectively, while it exhibited a lower antiproliferative activity against human monocytes. This suggested that replacement of linear chains by aryl rings greatly increased the specificity of the molecule for *Leishmania* metabolism. However, addition of a fluoro-, chloro- or methoxy-group on the 3,6-di-substituted aryl chains considerably reduced the specificity of the compounds for *Leishmania* parasite, rendering them toxic against human cells.

Compounds **4a–4e** obtained from *N*-(6-amino-3-acridinyl) acetamide **4**, exerted a strong antiproliferative activity against human monocytes but were far less active on *Leishmania*



Comp	R_2	R_1	Yields	MW	Molecular formula	Mp °C
4a	H	H	63 %	355	$C_{22}H_{17}N_3O_2$	152
4b	H	Cl	83 %	390	$C_{27}H_{16}ClN_3O_2$	282
4c	H	F	84 %	373	$C_{27}H_{16}FN_3O_2$	292
4d	H	OMe	74 %	385	$C_{23}H_{19}N_3O_3$	170
4e	OMe	OMe	72 %	415	$C_{24}H_{21}N_3O_4$	165

Fig. 3. Synthesis of compounds **4a** to **4e**.

Table 1
Cytotoxicity and antileishmanial activity of proflavine derivatives

Ref.[a]	Cytotoxicity against monocytes $IC_{50\text{-monocytes}}$ (μM)	Antileishmanial activity			DNA-binding activity $IC_{50\text{-DNA}}$ (μM)
		$IC_{50\text{-promastigotes}}$ (μM)	$IC_{50\text{-amastigotes}}$ (μM)	Specificity index (SI)	
4	4.7 ± 0.6	90.8 ± 5.5	Toxic[b]	–	59.1 ± 5.2
2a	737.5 ± 12.4	341.5 ± 15.4	4.5 ± 1.2	163.8	>250
2b	23.2 ± 3.2	159.8 ± 12.4	7.7 ± 1.4	3.0	170.9 ± 8.1
2c	18.9 ± 6.4	46.7 ± 3.2	12.8 ± 5.1	1.47	102.5 ± 6.6
3a	110.3 ± 15.2	4.31 ± 1.2	1.1 ± 0.2	100.2	>250
3b	2.49 ± 0.3	0.11 ± 0.03	0.2 ± 0.01	11.3	>250
3c	0.77 ± 0.1	1.7 ± .7	0.03 ± 0.01	23.3	>250
3d	13.4 ± 2.1	4.8 ± 1.2	1.1 ± 0.2	12.1	>250
3e	26.8 ± 4.2	16.1 ± 3.3	0.8 ± 0.03	32.7	>250
4a	5.4 ± 0.8	186.2 ± 10.4	Toxic[b]	–	>250
4b	1.9 ± 0.1	11.5 ± 2.7	2.9 ± 0.4	–	>250
4c	1.1 ± 0.1	132.7 ± 14.1	0.67 ± 0.04	–	>250
4d	1.76 ± 0.1	86.9 ± 5.4	Toxic[b]	–	>250
4e	2.1 ± 0.3	202.9 ± 14.1	1.3 ± 0.2	1.6	>250
Amphotericin B	154.5 ± 9.2	0.38 ± 0.1	0.14 ± 0.03	1103 ± 26.4	–
Doxorubicin	–	–	–	–	1.38 ± 0.8
Proflavine	2.21 ± 3.4	1.3 ± 0.5	0.12 ± 0.06	18.1 ± 7.3	>250

[a] Ref.: compound references.
[b] Toxic: toxicity observed on human macrophages at concentrations that did not display antileishmanial activity.

parasites than their di-substituted homologues of the **3a–e** series. They indicated that a symmetric conformation was essential for a specific antileishmanial activity.

Due to their tricyclic structure, acridines have been largely studied for their capacity to bind with DNA. Concerning our series of proflavine derivatives, only four compounds demonstrated this ability (Table 1): compound **4** (*N*-(6-amino-3-acridinyl)acetamide) exhibited the highest affinity for DNA, while compounds **2b** (*N*-[6-(propionylamino)-3-acridinyl]propanamide) and **2c** (*N*-[6-(butyrilamino)-3-acridinyl]butanamide) were less active. Results established that the capacity of derivatives to bind with DNA greatly depended on the presence of the 6-amino group (compound **4**), while linear chains in 3,6-di-substituted derivatives were far less active. No correlation could be observed by the rank correlation test of Spearman between cytotoxicity or antileishmanial activity and DNA-binding activity ($P > 0.05$), suggesting that binding to DNA could not be considered as the main mechanism responsible for antiproliferative effects in human cells and *Leishmania* parasites.

5. Conclusion

Acridine family includes a wide range of tricyclic molecules with various biological properties. Considered as potential antiparasitic agents since the 1990s, numerous acridine derivatives have been synthesised and successfully assessed for their antimalarial, trypanocidal or antileishmanial properties [10–12]. On this basis, authors recently demonstrated that diverse compounds of the 9-substituted [13], 4-substituted and 4,5-di-substituted aminoacridine [14] series exerted potent antileishmanial activities, however, the mechanisms by which such molecules could specifically interact with parasite metabolism remain unclear and extensive additional studies are necessary to complete current data.

Results observed in the present study clearly demonstrated that some derivatives of the 6-substituted and 3,6-di-substituted aminoacridine family could exert interesting anticancer and/or antileishmanial activities. Among the 14 compounds tested, compound **3c** (4-fluoro-*N*-[6-(4-fluorobenzoyl)amino)-3-acridinyl]benzamide exhibited the highest anticancer properties against human monocytes, while compounds **2a** *N*-[6-(acetylamino)-3-acridinyl] acetamide and **3a** *N*-[6-(benzoylamino)-3-acridinyl]benzamide exerted the most specific antileishmanial

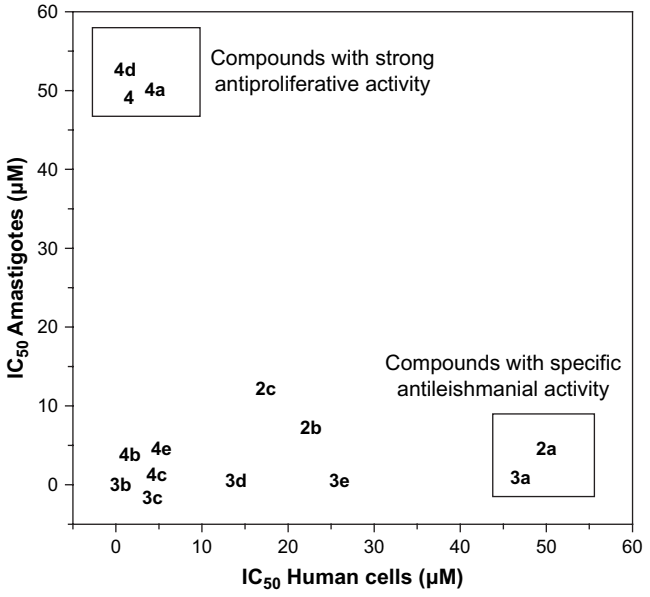


Fig. 4. Schematic representation of antileishmanial and antiproliferative activities.

activities, suggesting that acridine compounds could interact with mammalian and protozoan cells by different mechanisms.

Structure–activity relationships led to the identification of some molecular structures responsible for the specificity of the compounds for *Leishmania* parasites or for human cells. Anticancer properties were shown to vary according to the number and the quality of the substituents: they were greatly enhanced by the presence of a single benzoylamino group in 6-mono-substituted acridines as compared to their 3,6-di-substituted homologues, or by the presence of a fluoro-, a chloro- or a methoxy-substituent in both mono-substituted or di-substituted derivatives.

Antiproliferative activities against *Leishmania* promastigotes were preferentially related to the presence of two benzoylamino groups in di-substituted derivatives. Moreover, they appeared to depend on the presence of a chloro-, a fluoro- or a methoxy-group in both mono-substituted and di-substituted compounds.

Strong anti-amastigote activities could be observed with almost all the acridine compounds, nevertheless the most specific properties were obtained for compounds bearing 3,6-acetylamino groups or 3,6-benzoylamino groups, indicating that a symmetric conformation with acetylamino or benzoylamino substituents was essential for such specific antileishmanial activity.

Cytotoxicity and antileishmanial activity were not correlated with the properties of the derivatives to bind with DNA, indicating that other mechanisms of action should be investigated. Nevertheless the interaction with DNA observed with compound **4** confirmed previous studies concerning the capacities of acridines to bind with DNA [15,16] and illustrated the important role of the amino group in acridine–DNA interactions. However, methyl green is a major groove binding molecule which does not detect the entire DNA-binding agents and more particularly acridines capable of forming minor groove adducts [17]. Consequently, complementary experiments should be performed in order to better analyse the interactions of 6-substituted and 3,6-di-substituted acridines with macromolecules.

On the other hand, results observed on human macrophages indicated that some of the acridine derivatives could have an indirect antileishmanial activity by interfering with *Leishmania*–macrophage interactions. They also suggested that acridines could react with cellular structures by a wide range of mechanisms and justified the constant need of extended biological studies.

6. Experimental

6.1. General procedures

All reagents were of analytical grade, dried and purified when necessary. Proflavine derivatives were dissolved in sterile dimethyl sulfoxide (analytical grade, Sigma) and stored frozen at −70 °C until used.

6.2. Chemistry

Melting points were performed on a Electrothermal 9200 melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were measured on a BRUKER AC 300 spectrometer (300.13 MHz for ^{1}H).

6.2.1. N-[6-(Acetylamino)-3-acridinyl]acetamide (**2a**)

Hemisulfate of 3,6-diaminoacridine **1** (1 g, 3.25 mmol) was dissolved in acetic anhydride (1.9 ml, 20 mmol) and 0.25 g of anhydrous sodium acetate. The mixture was warmed at 100 °C for 2 h. The obtained precipitate was filtered and the grey solid was thoroughly washed with a solution of ammonium hydroxide 10%. Recrystallization from ethanol yielded pure **2a** as orange crystalline powder. Yield 43%. M.p. 260 °C. NMR ^{1}H (DMSO-d_6): 10.35 (NH), 8.86 (9-H), 8.49 (4-H and 5-H), 7.59 (2-H and 7-H), 8.03 (1-H and 8-H), 2.15 (CH_3-1′). Anal. Calc. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.59; H, 5.10; N, 14.36.

6.2.2. N-[6-(Propionylamino)-3-acridinyl]propanamide (**2b**)

Hemisulfate of 3,6-diaminoacridine (300 mg, 1 mmol) was dissolved in pyridine (6 ml) at 25 °C and NEt_3 (0.5 ml) was added. Then, propionic anhydride (2.1 equiv, 0.27 ml) was added dropwise at 50 °C and stirring was continued for 50 min more at 80 °C. The resulting mixture was poured into water (80 ml). The obtained precipitate was filtered and washed with water. The resulting solid was recrystallized from ethanol to give an orange powder **2b**. Yield 65%. M.p. 265 °C. NMR ^{1}H (DMSO-d_6): 10.28 (NH), 8.81 (9-H), 8.48 (4-H and 5-H), 8.01 (1-H and 8-H), 7.60 (2-H and 7-H), 2.43 (CH_2-1′), 1.13 (CH_3-2′). Anal. Calc. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 71.04; H, 5.92; N, 13.03.

6.2.3. N-[6-(Butyrilamino)-3-acridinyl]butanamide (**2c**)

As reported for **2b** but with (0.35 ml) of butyric anhydride. After work up the recovered solid was recrystallized from ethanol to give an orange powder **2c**. Yield 32%. M.p. 216 °C. NMR ^{1}H (DMSO-d_6): 10.27 (NH), 8.80 (9-H), 8.50 (4-H and 5-H), 7.98 (1-H and 8-H), 7.58 (2-H and 7-H), 2.38 (CH_2-1′), 1.67 (CH_2-2′), 1.09 (CH_3-3′). Anal. Calc. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.21; H, 6.65; N, 12.05.

6.2.4. N-[6-(Benzoylamino)-3-acridinyl]benzamide (**3a**)

Hemisulfate of 3,6-diaminoacridine **1** (300 mg, 1 mmol) was dissolved in pyridine (6 ml) at 25 °C and NEt_3 (0.5 ml) was added. Then, benzoylchloride (2.1 equiv., 0.25 ml) was added dropwise at 40 °C and the stirring was left for 50 min more at 80 °C. The mixture was poured into water (80 ml). The precipitate was filtered and washed with water. The resulting solid was recrystallized from ethanol to give an orange powder **3a**. Yield 66%. M.p. 248 °C. NMR ^{1}H (DMSO-d_6): 10.69 (NH), 8.95 (9-H), 8.73 (4-H and 5-H), 8.13 (1-H and 8-H), 8.05 (CH-2′), 7.92 (2-H and 7-H), 7.65 (CH-4′), 7.58

(CH-3′). Anal. Calc. for $C_{27}H_{19}N_3O_2$: C, 77.68; H, 4.59; N, 10.07. Found: C, 77.71; H, 4.55; N, 10.03.

*6.2.5. 4-Chloro-N-[6-(4-chlorobenzoyl)amino)-3-acridinyl]benzamide (**3b**)*

As reported for **3a** but with 4-chlorobenzoylchloride, (0.24 ml). After work up the resulting solid was recrystallized from ethanol to give an orange powder **3b**. Yield 90%. M.p. 370 °C. NMR ^{1}H (DMSO-d_6): 10.71 (NH), 8.92 (9-H), 8.68 (4-H and 5-H), 8.11 (1-H and 8-H), 8.06 (CH-2′), 7.89 (2-H and 7-H), 7.66 (CH-3′). Anal. Calc. for $C_{27}H_{17}N_3O_2Cl_2$: C, 66.68; H, 3.52; N, 8.64. Found: C, 66.65; H, 3.49; N, 8.61.

*6.2.6. 4-Fluoro-N-[6-(4-fluorobenzoyl)amino)-3-acridinyl]benzamide (**3c**)*

As reported for **3a** but with 4-fluorobenzoylchloride (0.25 ml). After recrystallization from ethanol **3c** was obtained as an orange powder. Yield 96%. M.p. 290 °C. NMR ^{1}H (DMSO-d_6): 10.77 (NH), 9.07 (9-H), 8.76 (4-H and 5-H), 8.16 (1-H and 8-H), 8.13 (CH-2′), 7.91 (2-H and 7-H), 7.41 (CH-3′). Anal. Calc. for $C_{27}H_{17}N_3O_2F_2$: C, 71.52; H, 3.78; N, 9.27. Found: C, 71.49; H, 3.80; N, 9.30.

*6.2.7. 4-Methoxy-N-[6-(4-methoxybenzoyl)amino)-3-acridinyl]benzamide (**3d**)*

As described for **3a**, but with 4-methoxybenzoylchloride (0.29 ml). After recrystallization from ethanol **3d** was obtained as a yellow powder. Yield 71%. M.p. 252 °C. NMR ^{1}H (DMSO-d_6): 10.50 (NH), 8.91 (9-H), 8.65 (4-H and 5-H), 8.10 (1-H and 8-H), 8.03 (CH-2′), 7.89 (2-H and 7-H), 7.09 (CH-3′), 3.85 (OCH_3). Anal. Calc. for $C_{29}H_{23}N_3O_4$.: C, 72.94; H, 4.85; N, 8.80. Found: C, 72.91; H, 4.84; N, 8.83.

*6.2.8. 3,4-Dimethoxy-N-[6-(3,4-dimethoxybenzoyl)amino)-3-acridinyl]benzamide (**3e**)*

As described for **3a**, but with 3,4-dimethoxybenzoyl chloride (0.42 g). After recrystallization from ethanol **3e** was obtained as a yellow powder. Yield 61%. M.p. 207 °C. NMR ^{1}H (DMSO-d_6): 10.51 (NH), 8.92 (9-H), 8.67 (4-H and 5-H), 8.12 (1-H and 8-H), 7.91 (2-H and 7-H), 7.93 (CH-6′), 7.65 (CH-2′), 7.08 (CH-5′), 3.86 (OCH_3), 3.85 (OCH_3). Anal. Calc. for $C_{31}H_{27}N_3O_6$: C, 69.26; H, 5.06; N, 7.82. Found: C, 69.28; H, 5.09; N, 7.79.

*6.2.9. N-(6-Amino-3-acridinyl)acetamide (**4**)*

3,6-Diaminoacridine **1** (2.5 g, 12 mmol) was dissolved in a mixture of glacial acetic acid (10 ml) and acetic anhydride (2.5 ml). The mixture was warmed until solidification in a water bath, heating was continued 15 min more and the resulting solid turned yellow, then acetic acid (40 ml) was added. The mixture was warmed for 15 min under stirring at 120 °C. The resulting precipitate was filtered and thoroughly washed with 10% ammonium hydroxide. The grey compound was recrystallized from ethanol to yield pure **4** as a yellow powder. Yield 80%. M.p. 260 °C. NMR ^{1}H (DMSO-d_6): 10.97 (NH), 9.05 (9-H), 8.62 (4-H), 8.10 (1-H), 7.97 (8-H), 7.82 (NH_2), 7.58 (2-H), 7.17 (7-H), 6.89 (5-H), 2.19 (CH_3-1′). Anal. Calc. for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.68; H, 5.19; N, 16.70.

*6.2.10. N-[6-(Acetylamino)-3-acridinyl]benzamide (**4a**)*

N-(6-Amino-3-acridinyl)acetamide (0.2 g, 0.8 mmol)) was dissolved in pyridine (4 ml) at room temperature, then NEt_3 (0.3 ml) was added. Benzoylchloride (2.1 equiv, 0.2 ml, 1.68 mmol) was added dropwise at 40 °C under stirring; the stirring was maintained for 45 min at 80 °C. The mixture was poured into water (80 ml). The obtained precipitate was filtered and washed with water. The solid was finally recrystallized from ethanol to yield a yellow powder **4a**. Yield 63%. M.p. 152 °C. NMR ^{1}H, recorded as hydrochloride, (DMSO-d_6): 11.41 (NH_2), 11.29 (NH), 9.46 (9-H), 8.96 (5-H), 8.78 (4-H), 8.26 (8-H), 8.24 (1-H), 8.10 (7-H), 8.08 (CH-2′), 7.83 (2-H), 7.65 (CH-4′), 7.56 (CH-3′), 2.22 (CH_3-1′). Anal. Calc. for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.32; H, 4.84; N, 11.79.

*6.2.11. N-[6-(Acetylamino)-3-acridinyl]-4-chlorobenzamide (**4b**)*

As described for **4a**, but with 4-chlorobenzoylchloride (0.2 ml). After recrystallization from ethanol **4b** was obtained as a yellow powder. Yield 83%. M.p. 282 °C. NMR ^{1}H (DMSO-d_6): 10.69 (NH), 10.36 (NH), 8.86 (9-H), 8.64 (5-H), 8.48 (4-H), 8.08 (8-H), 8.07 (CH-2′), 8.02 (1-H), 7.86 (7-H), 7.65 (CH-3′), 7.60 (2-H), 2.13 (CH_3-1′). Anal. Calc. for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.76; H, 4.17; N, 10.76.

*6.2.12. N-[6-(Acetylamino)-3-acridinyl]-4-fluorobenzamide (**4c**)*

As described for **4a**, but with 4-fluorobenzoylchloride (0.2 ml). The mixture was poured into water (80 ml). The precipitate was filtered and washed with water. After recrystallization from ethanol **4c** was obtained as a yellow powder. Yield 84%. M.p. 292 °C. NMR ^{1}H (DMSO-d_6): 10.64 (NH), 10.35 (NH), 8.87 (9-H), 8.63 (5-H), 8.51 (4-H), 8.08 (8-H), 8.11 (CH-2′), 8.05 (1-H), 7.87 (7-H), 7.60 (2-H), 7.41 (CH-3′), 2.17 (CH_3-1′). Anal. Calc. for $C_{22}H_{16}FN_3O_2$: C, 70.77; H, 4.32; N, 11.25. Found: C, 70.79; H, 4.35; N, 11.23.

*6.2.13. N-[6-(Acetylamino)-3-acridinyl]-4-methoxybenzamide (**4d**)*

As described for **4a**, but with 4-methoxybenzoylchloride (0.23 ml). After recrystallization from ethanol **4d** was obtained as a yellow powder. Yield 74%. M.p. 170 °C. NMR ^{1}H (DMSO-d_6): 10.46 (NH), 10.35 (NH), 8.86 (9-H), 8.64 (5-H), 8.51 (4-H), 8.08 (8-H), 8.05 (CH-2′), 8.03 (1-H), 7.89 (7-H), 7.61 (2-H), 7.10 (CH-3′), 2.42 (OCH_3), 2.15 (CH-1′). Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90. Found: C, 71.65; H, 4.99; N, 10.88.

*6.2.14. N-[6-(Acetylamino)-3-acridinyl]-3,4-dimethoxybenzamide (**4e**)*

As described for **4a**, but with 3,4-dimethoxybenzoyl chloride (0.34 g). **4e** was obtained as a yellow powder. Yield

72%. M.p. 165 °C. NMR ^{1}H (DMSO-d_6): 10.48 (NH), 10.37 (NH), 8.86 (9-H), 8.65 (5-H), 8.52 (4-H), 8.08 (8-H), 8.05 (CH-2′), 8.05 (1-H), 7.91 (CH-6′), 7.89 (7-H), 7.66 (CH_2-2′), 7.61 (2-H), 7.10 (CH_2-3′), 6.98 (CH_2-5′), 2.15 (CH-1′), 3.86 (OCH_3), 3.85 (OCH_3). Anal. Calc. for $C_{24}H_{21}N_3O_4$: C, 69.39; H, 5.10; N, 10.11. Found: C, 69.41; H, 5.13; N, 10.09.

6.3. Biology

6.3.1. Antiproliferative activity toward human transformed monocytes

The effects of acridines on the growth of human monocytes were assessed by colorimetric determination of cell viability using the oxidation–reduction indicator Alamar Blue®. Cytotoxicity was assessed on the THP1 human monocyte cell line (ATCC, Manassas VA, USA) by colorimetric determination of cell viability using the oxidation–reduction indicator Alamar Blue® [18]. Late log-phase human monocytes were incubated in RPMI medium containing 10% Alamar Blue® supplemented with 10% foetal calf serum (Eurobio, Paris, France) and a range of acridine concentrations was incorporated in duplicate cultures (final DMSO concentration less than 5%). After a 72 h incubation period at 37 °C with 5% CO_2, reduction of Alamar Blue® from blue to red was measured by absorbance monitoring at 570 and 630 nm. $IC_{50\text{-monocytes}}$ was defined as the concentration of acridine required to induce a 50% decrease of cell growth, corresponding to a 50% reduction of alamar Blue® as compared to the control culture.

6.3.2. Antileishmanial activity against promastigotes

The effects of acridines on the growth of *Leishmania* promastigotes were assessed by colorimetric determination of parasite viability using the oxidation–reduction indicator Alamar Blue® [19]. Antileishmanial activity was assessed on the referenced strain *L. infantum* (MHOM/FR/78/LEM75). Promastigotes in late log-phase were incubated in RPMI medium (without phenol red) containing 10% Alamar Blue® supplemented with 12% foetal calf serum and a range of acridine concentrations was aseptically incorporated into duplicate cultures (final DMSO concentration less than 5%). Following a 48 h incubation period at 25 °C, Alamar Blue® reduction from blue to red was assessed by absorbance monitoring at 570 and 630 nm. $IC_{50\text{-promastigotes}}$ was determined as the concentration of acridine necessary to inhibit 50% of parasite growth, corresponding to a 50% reduction of alamar Blue® as compared to the control culture.

6.3.3. Antileishmanial activity against intracellular amastigotes

Intracellular amastigote cultures were performed in human monocyte-derived macrophages according to the methodology previously described by Ogunkolade et al. [20]. Maturation of monocytes into adherent macrophages was performed by treating exponentially-growing monocytes (10^5 cells/ml) with 1 μM phorbol myristate acetate (Sigma). After a 48 h incubation period at 37 °C (5% CO_2) in chamber-slides (Fisher, Paris, France), cells were rinsed with fresh medium and suspended in RPMI medium containing stationary-phase promastigotes (cells/promastigotes ratio = 1/10). After a 24 h incubation period at 37 °C (5% CO_2), promastigotes were removed by four successive washes with fresh medium. Adapted dilutions of chemical compounds were added in duplicate chambers and cultures were incubated for 96 h at 37 °C (5% CO_2). Negative controls treated by solvent (DMSO) and positive controls containing a range of amphotericin B (Sigma, St Louis, Mo, USA) concentrations were added to each set of experiments. At the end of the incubation period, cells were harvested with analytical grade methanol (Sigma) and stained with 10% Giemsa stain (Eurobio, Paris, France). The percentage of infected macrophages in each assay was determined microscopically at 1000 times magnification. The antileishmanial activity of acridines derivatives against the intracellular amastigote form of the parasite was expressed by $IC_{50\text{-amastigotes}}$ corresponding to the concentration of derivatives required to induce a 50% decrease of infected macrophages. The specificity index (SI) corresponding to the ratio between toxicity and antileishmanial activity was expressed as follows: $SI = IC_{50\text{-monocytes}}/IC_{50\text{-amastigotes}}$.

6.3.4. Intercalation to DNA

The capacities of proflavine derivatives to bind with DNA were assessed by the DNA–methyl green bioassay [21], using triphenyl methane methyl green dye that may reversibly interact with DNA according to a reaction that can be followed spectrophotometrically. Twenty milligrams of DNA–methyl green (Sigma, St. Louis, MO, USA) were suspended in 100 mL of 0.05 M Tris–HCl buffer, pH 7.5, containing 7.5 mM of magnesium sulfate and stirred at 37 °C for 24 h. Two hundred microlitres of the above solution were added to each well and various concentrations of acridine derivatives were added. The initial absorbance of each sample was measured at 630 nm. After a 24-hour incubation period in the dark at room temperature, the final absorbance of the samples was measured as above. The readings were corrected for the initial absorbance and normalized as percentages of the untreated DNA–methyl green absorbance value. The $IC_{50\text{-DNA}}$ corresponded to the concentration of chemical compounds that induced a 50% decrease of DNA-related absorbance.

6.3.5. Statistical analysis

Correlations between cytotoxicity observed on human monocytes or antileishmanial activity and DNA-binding were performed by the rank correlation test of Spearman by using the Statgraphic plus (V5) software (Manugistics Inc., USA).

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