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Synthesis and antileishmanial activities of 4,5-di-substituted acridines as compared to their 4-mono-substituted homologues

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Abstract—Newly synthesized 4,5-di-substituted acridines were assessed for in vitro antileishmanial activities as compared to those of their 4-mono-substituted homologues. Mono-substituted acridines exhibited a weak specificity for *Leishmania* parasites. Di-substituted acridines, on the contrary, displayed interesting amastigote-specific activities through a mechanism of action that might not involve intercalation to DNA. This antileishmanial property, associated with a low antiproliferative activity towards human cells, led to the identification of a new class of promising acridine derivatives such as 4,5-bis(hydroxymethyl)acridine with a nonclassical mechanism of action based on the inhibition of *Leishmania* internalization within macrophages. In the meantime, the effects of experimental lighting on the biological properties of acridines were assessed: experimental lighting did not significantly improve the antileishmanial activity of the compounds since it produced a greater toxicity against human cells.

1. Introduction

For hundreds of years, leishmaniases have been the cause of death among millions of people throughout the world. Since the 1990s, as a consequence of agro-industrial development, a significant expanse of *Leishmania*-endemic areas has been observed, correlated with a sharp increase of cases.[1,2] AIDS and other immunosuppressive conditions have also enhanced the risk of *Leishmania*–HIV co-infected people and contributed to the appearance of new severe clinical forms of the disease.[3] Nowadays, leishmaniases are spread over five continents and are endemic in the tropical and subtropical regions of 88 countries.[1] Among all leishmaniases, the visceral form also known as kala-azar is the most severe, since it produces, if untreated, a mortality rate of almost 100% independently to the immunological status of the patient.[2] For decades, treatment of kala-azar with injected pentavalent antimony has been successfully used, but since the 1990s, *Leishmania* parasites have developed resistances.[4,5] Amphotericin B and the oral anticancer drug miltefosine are considered at the moment to be the best second-line therapeutic solutions.[6] Nevertheless, they do not represent a safe treatment in all clinical cases and necessitate the research of new antileishmanial molecules.[6]

It has been established for many years that the planar structure of tricyclic rings conferred to acridine derivatives the ability to intercalate in DNA and to interfere with various metabolic processes in both prokaryotic and eukaryotic cells.[7,8] As a consequence, various natural and synthetic compounds of the acridine family have been selected for antibacterial or anticancer chemotherapy.[9] Recent developments in the biology of protozoa have also demonstrated that acridines could exert a powerful toxicity toward *Plasmodium*,[10] *Trypanosoma*[11] and *Leishmania*[12] parasites. However, their mechanisms of action toward protozoa are still poorly understood, rendering necessary the synthesis of new acridine series and the study of their antiparasitic profile.

Keywords: Leishmaniasis; Acridines; Bi-functional acridines; Photoactivation.
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For this purpose, we investigated the antiparasitic properties of newly synthesized bifunctional acridines by comparing their antileishmanial activities to those of their mono-substituted homologues. In the meantime, according to the statement that acridine dyes were photosensitizers[13] and demonstrated photo-enhanced bactericidal and antitumoral activities,[14] we explored the effects of simulated sunlight on the biological properties of both the series of compounds.

2. Results and discussion

2.1. Chemistry

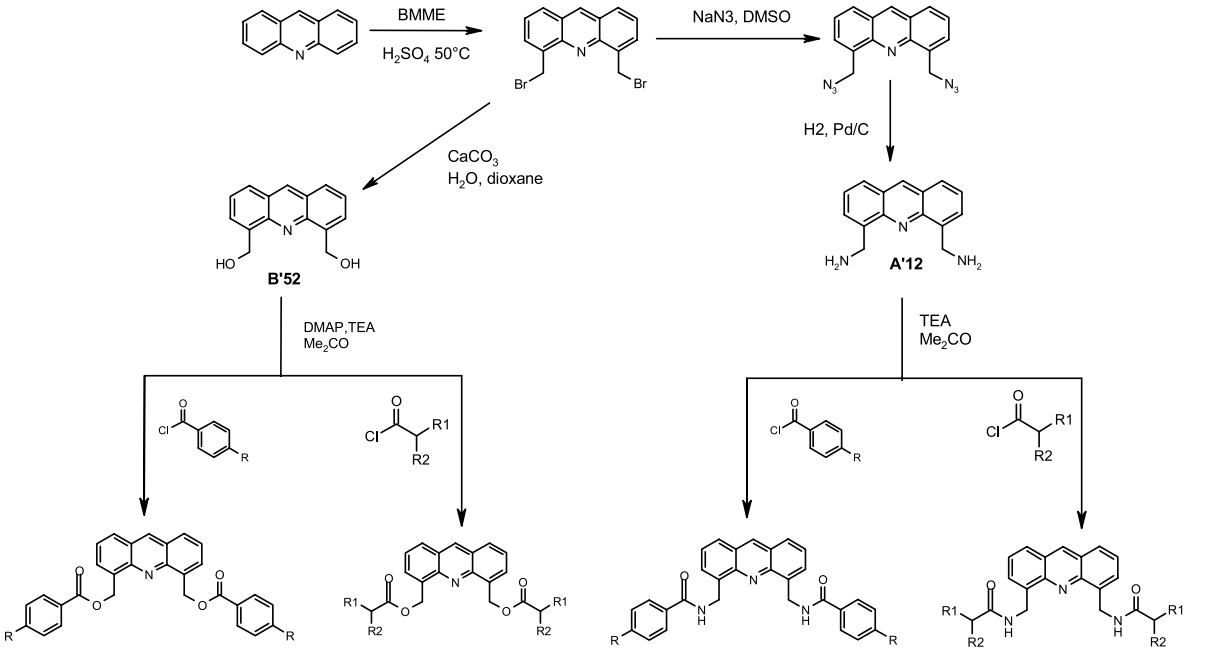
Mono-substituted acridines (**A** and **B** series) were prepared during a previous work.[15] 4,5-Bis(aminoethyl)-acridine **A′12** was prepared using acridine as a starting material according to a previously described method.[16] Compounds of the **A′** series were synthesized according to classical amide formation methods by reacting **A′12** hydrochloride with various carboxylic acid and acyl chloride derivatives as described in Figure 1. The parent molecule was first solubilized into acetone and the mixture was neutralized with concentrated NaOH. Under these experimental conditions, the generation of amide bindings was faster than the concomitant hydrolysis of acyl chloride functions and the excess of NaOH neutralized the HCl formed during the reaction.

Compounds of the **B′** series were prepared in a similar way by using 4,5-bis(hydroxymethyl)acridine **B′52** as starting material. The best solvent for these syntheses was CH_2Cl_2 that permitted an optimal solubility for all the components. However, with the addition of TEA alone, weak reaction yields were obtained. In order to resolve this problem, we added DMAP as an activator of acid function.

Each compound structure was characterized by 1D and 2D NMR sequences and their purity was confirmed by TLC, microanalysis, and HPLC.

2.2. Biological activity tests without irradiation

Antileishmanial activity was assessed on the referenced strain *L. infantum* (MHOM/FR/78/LEM75) in both its promastigote and its intracellular amastigote forms. Cytotoxicity was assessed on human monocytes (THP1 cell line). Among 4-substituted acridines (**A** and **B** series), compounds **A66**, **A27**, and **A60** appeared highly active toward human cells (IC_{50} = 3.2, 10.1–13.4 μM); only compound **A27** exerted a strong inhibitory effect on the promastigote form of the parasite (IC_{50} = 1.7 μM), while two compounds, **A33** and **A75**, produced an interesting selective antiamastigote activity (SI > 30). Concerning di-substituted acridines, none of the tested derivatives showed strong antiproliferative properties on human monocytes and promastigotes. Nevertheless, some of these compounds (**B′52**, **A′12**, **A′21**, **B′57**, **A′2**, and **A′11**) expressed interesting amastigote-specific activities, and more particularly compound **B′52**, 4,5-bis(hydroxymethyl)acridine, that displayed a strong inhibitory activity on the intracellular amastigote



Compounds	R	Yields
B'57	H	79%
B'63	Cl	75%
B'78	F	76%
B'62	OMe	81%
B'76	NMe_2	61%

Compounds	R1	R2	Yields
B'84	CH_2CH_2Cl	H	90%
B'81	$=CH_2$	-	68%

Compounds	R	Yields
A'1	H	92%
A'3	Cl	92%
A'2	F	96%
A'4	OMe	95%
A'11	NMe_2	64%

Compounds	R1	R2	Yields
A'21	CH_2CH_2Cl	H	95%
A'22	$=CH_2$	-	57%

Figure 1. Synthesis of 4,5-di-substituted acridines.

form of the parasite (IC_{50} = 0.6 μM), leading to a selective index over than 200.

2.3. Biological activity tests after irradiation

Irradiation procedure was performed with a solar simulator equipped with a xenon arc lamp. The results presented in Tables 1 and 2 revealed that almost all the acridine compounds exerted photo-inducible antiproliferative properties; however, experimental illumination neither enhanced antipromastigote abilities nor improved selective antiparasitic activity against *Leishmania* amastigotes.

2.4. Statistical analysis

Multifactor ANOVA was used for evaluating the effects of the light and the chemical structure of the compounds on their biological properties (Statgraphics 5.0 plus software, Statistical Graphics Corporation, USA). Cytotoxicity towards human cells poorly depended on the chemical nature of the substituted chains ($P > 0.05$); however, it was significantly enhanced by experimental lighting ($P < 0.001$). On the contrary, antileishmanial activity greatly depended on the nature of the substituted chains ($P < 0.001$), while it was poorly affected by lighting ($P > 0.05$): mono-substituted compounds were significantly more efficient on both the promastigote and the amastigote forms of the parasite than di-substituted acridines ($P < 0.01$), and nitrogen-bearing derivatives were significantly more active than their oxygen-bearing homologues ($P < 0.01$).

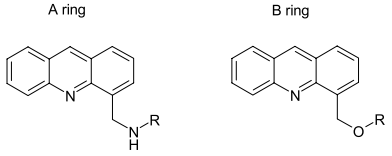
2.5. Additional experiments on B′52

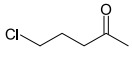
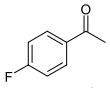
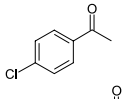
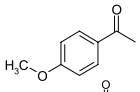
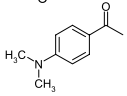
Complementary experiments were performed for studying the mechanism of **B′52** antileishmanial action. NO production was analyzed in adherent macrophages and inhibition of parasite internalization within macrophages was assessed by infecting adherent macrophages with promastigotes previously treated with various concentrations of **B′52**. The results showed that **B′52** did not increase NO production by macrophages (data not shown). On the contrary, the compound prevented macrophage infection by inhibiting promastigote internalization within human macrophages (Fig. 2).

3. Conclusion

Due to their capacity to interact with macromolecules,[8,9] acridine chromophores have been widely synthesized and assessed for their pharmacological activities including antibacterial, anticancer, and antiparasitic properties.[10–12] The results observed in the present study

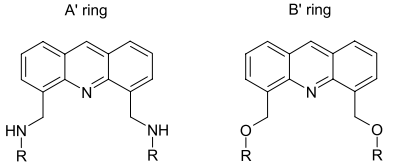
Table 1. Structures and biological properties of mono-substituted acridines

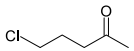
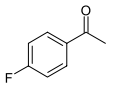
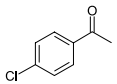
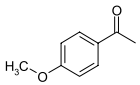
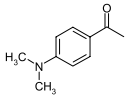


R	Ring	No.	Antiproliferative activity on human monocytes IC_{50} ± SD (μM)		Antileishmanial activity IC_{50} ± SD (μM)					
			Without photo-activation	With photo-activation	Without photo-activation			With photo-activation		
					PRO	AMA	SI	PRO	AMA	SI
H	A	**A27**	10.1 ± 1.2	2.4 ± 0.8	1.7 ± 0.4	3.4 ± 0.5	2.9	6.3 ± 1.1	4.8 ± 0.7	—
	B	**B48**	18.4 ± 2.4	5.3 ± 0.9	>200	32.7 ± 3.4	—	43.3 ± 4.6	Tox	—
	A	**A66**	3.2 ± 0.5	0.8 ± 0.1	45.2 ± 6.4	26.7 ± 3.8	—	13.1 ± 2.1	Tox	—
	B	**B68**	28.6 ± 3.1	13.2 ± 1.5	158.4 ± 8.9	153.1 ± 9.7	—	0.7 ± 0.2	Tox	—
	A	**A60**	13.4 ± 2.1	2.4 ± 0.6	175.8 ± 10.2	6.8 ± 1.9	1.9	42.7 ± 4.6	Tox	—
	B	**B69**	16.5 ± 1.3	18.2 ± 1.9	29.1 ± 2.6	2.6 ± 0.7	6.3	22.1 ± 1.4	4.1 ± 0.4	4.4
	A	**A37**	20.8 ± 5.4	69.6 ± 2.1	45.1 ± 8.1	13.2 ± 6.1	1.5	29.4 ± 3.5	9.4 ± 2.1	7.4
	B	**B50**	>200	6.1 ± 1.6	76.7 ± 12.3	>200	—	2.8 ± 0.3	Tox	—
	A	**A32**	>200	9.4 ± 2.4	65.3 ± 6.7	78.7 ± 8.9	4.6	29.5 ± 3.4	Tox	—
	B	**B61**	124.7 ± 11.4	1.3 ± 0.1	55.1 ± 6.6	>200	—	2.7 ± 0.5	Tox	—
	A	**A31**	85.1 ± 9.4	42.8 ± 6.1	>200	10.1 ± 2.1	8.4	22.9 ± 4.5	3.1 ± 0.2	13.8
	B	**B67**	>200	8.1 ± 1.9	61.6 ± 9.2	27.1 ± 3.8	14.5	2.42 ± 0.4	3.3 ± 0.1	2.4
	A	**A33**	>200	32.8 ± 5.1	32.6 ± 8.5	9.4 ± 2.3	42.8	26.4 ± 3.6	Tox	—
	B	**B59**	136.1 ± 15.4	8.7 ± 0.9	29.6 ± 5.4	7.4 ± 2.2	18.4	6.3 ± 1.4	8.1 ± 3.2	1.1
	A	**A75**	143.2 ± 20.1	4.8 ± 1.1	34.6 ± 3.1	4.7 ± 0.6	30.4	75.1 ± 5.6	9.4 ± 1.4	
	B	**B71**	153.2 ± 22.4	10.4 ± 1.7	65.9 ± 3.5	>200	—	9.6 ± 1.4	Tox	—

PRO: promastigotes; AMA: amastigotes; Tox: toxicity higher than antileishmanial activity; SI: selectivity index; SD: Standard deviations.

Table 2. Structures, physicochemical, and biological properties of di-substituted acridines



R	Ring	No.	Antiproliferative activity on human monocytes $IC_{50} \pm SD$ (μM)		Antileishmanial activity $IC_{50} \pm SD$ (μM)					
			Without photo-activation	With photo-activation	Without photo-activation			With photo-activation		
					PRO	AMA	SI	PRO	AMA	SI
H	A′	**A′12**	35.5 ± 2.1	5.2 ± 0.9	6.6 ± 1.9	3.4 ± 0.4	10.4	1.4 ± 0.2	0.6 ± 0.03	8.6
	B′	**B′52**	>200	24.3 ± 3.1	>200	0.6 ± 0.05	>200	>200	23.6 ± 4.1	1.0
	A′	**A′21**	48.2 ± 5.1	4.5 ± 0.7	4.6 ± 0.9	6.5 ± 0.9	7.4	7.3 ± 0.9	Tox	—
	B′	**B′84**	156.4 ± 12.4	12.6 ± 2.7	>200	>200	—	8.6 ± 1.1	2.3 ± 0.4	5.4
	A′	**A′22**	23.5 ± 3.7	15.4 ± 1.8	11.5 ± 2.1	>200	—	30.4 ± 5.4	>200	—
	B′	**B′81**	34.1 ± 2.9	18.6 ± 0.7	11.6 ± 0.4	>200	—	11.2 ± 0.7	Tox	—
	A′	**A′1**	98.1 ± 10.4	65.3 ± 4.1	3.6 ± 0.4	17.2 ± 4.1	5.6	41.2 ± 4.8	46.5 ± 7.1	1.4
	B′	**B′57**	145.1 ± 9.9	10.2 ± 2.5	>200	5.2 ± 1.4	27.9	>200	Tox	—
	A′	**A′2**	18.5 ± 9.9	5.3 ± 1.2	5.7 ± 0.5	1.8 ± 0.1	10.2	2.7 ± 0.1	Tox	—
	B′	**B′78**	26.6 ± 3.9	25.6 ± 4.7	>200	>200	—	155.2 ± 10.4	>200	—
	A′	**A′3**	94.1 ± 8.4	75.3 ± 4.1	29.2 ± 4.4	36.5	2.5	136.4 ± 9.8	21.4 ± 1.5	3.5
	B′	**B′63**	14.7 ± 2.1	12.3 ± 0.4	>200	>200	—	>200	Tox	—
	A′	**A′4**	124.1 ± 0.7	86.5 ± 0.4	62.6 ± 1.1	>200	—	>200	>200	—
	B′	**B′62**	21.2 ± 4.1	26.3 ± 0.7	>200	66.8 ± 1.4	—	>200	Tox	—
	A′	**A′11**	52.2 ± 6.7	36.6 ± 2.4	8.7 ± 1.4	5.3 ± 0.1	1.14	>200	Tox	—
	B′	**B′76**	31.4 ± 2.8	18.2 ± 0.8	>200	46.5 ± 5.8	—	>200	Tox	—

PRO: promastigotes; AMA: amastigotes; Tox: toxicity higher than antileishmanial activity; SI: selectivity index; SD : Standard deviations.

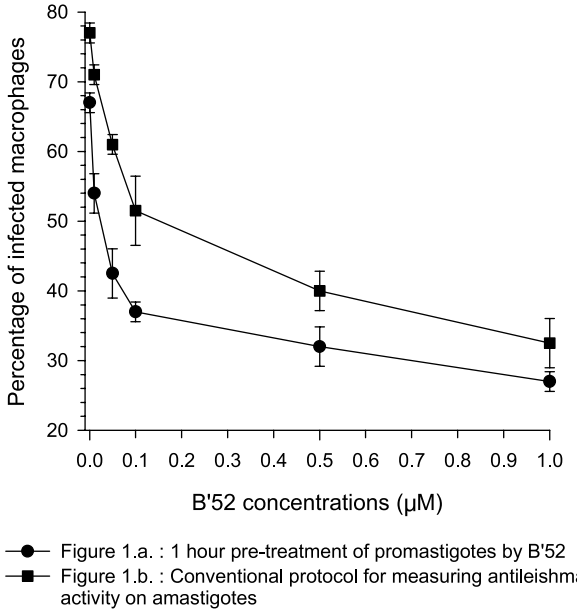


Figure 2. Effects of **B′52** on macrophage infection by *Leishmania* promastigotes (1.a.) and parasite proliferation within macrophages (1.b.).

clearly confirmed that various 4-substituted and 4,5-disubstituted acridines could exert interesting antileishmanial activities. Antiproliferative abilities towards human cells were shown to vary according to the length of the 4- or 4,5-substituted groups rather than the nature of the rings: mono-substituted acridines bearing a linear side chain exerted stronger activity than compounds bearing aryl chains, indicating that conformational mechanisms could play an important role in the interactions of the compounds with macromolecules.[17] Antileishmanial activities, on the contrary, depended on both the nature of the rings and the number of substituted groups. Mono-substituted acridines demonstrated stronger antipromastigote activities than their di-substituted homologues, and compounds bearing a nitrogen bond were more active than their oxygen bond bearing homologues. Di-substituted acridines were weakly active on the promastigote form of the parasite. These compounds appeared too cluttered on both sides to involve a strong intercalative binding with DNA. As a consequence, they did not demonstrate strong antiproliferative activity on tumoral cells. Nevertheless, some of these acridines, such as compounds **B′52**, **A′12**, **A′21**, **B′57**, **A′2**, and **A′11**, exhibited interesting amastigote-specific properties, indicating that they could exert another interaction mechanism with *Leishmania* parasite that does not imply direct DNA intercalation. Complementary experiments concerning **B′52** confirmed this statement, since they established that subtoxic concentrations of the compound prevented macrophages from *Leishmania* infection by a mechanism of action that significantly reduced parasite internalization. On this basis, additional in vitro and in vivo experiments should be performed in order to confirm the antileishmanial properties of this new class of compounds and to better elicit this mechanism of action.

4. Experimental

4.1. General procedures

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

4.2. Chemistry

4.2.1. 4,5-Bis(aminomethyl)acridine (A′12). 4,5-Bis(azidomethyl)acridine obtained from acridine according to the methodology previously described by Hess and Stewart[16] was dissolved into dioxane/ethanol (30 mL, 5:5, v:v) with Pd/C (100 mg, 10%). After a 4-h agitation period at room temperature with H_2, the mixture was filtered and washed with ethanol. The filtrate was evaporated, dissolved into $CHCl_3$, and filtered until final evaporation to give a brown oil (805 mg, 98%). ^{1}H NMR (300 MHz,$CDCl_3$, 25 °C): δ = 2.03 (s, 4H, 2′H), 4.50 (s, 4H, H-1′), 7.42 (dd, J = 8.5, 6.7 Hz, 2H, H-2), 7.64 (dd, J = 6.7, 0.7 Hz, 2H, H-3) 7.82 (dd, J = 8.5, 1.2 Hz, 2H, H-1), 8.65 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 44.42 (C-1), 125.56 (C-2), 126.45 (C-9a), 127.05 (C-1), 127.60 (C-3), 136.31 (C-9), 141.14 (C-4), 146.50 (C-4a) ppm. Anal. Calcd ($C_{15}H_{15}N_3$): C, 75.92; H, 6.37; N, 17.71. Found: C, 75.71; H, 6.35; N, 17.65.

A′12 hydrochloride was obtained by dissolving **A′12** into 5 mL MeOH and incorporating the mixture into 100 mL HCl saturated Et_2O. After an overnight incubation at room temperature under $CaCl_2$ atmosphere, the precipitate was filtered and washed with Et_2O to obtain yellow powder (1.053 g).

4.2.2. 4,5-Bis[(4-chlorobutylamido)-*N*-methyl]acridine (A′21). A′12 (500 mg, 1.60 mmol) was dissolved into 20 mL acetone at 0 °C and neutralized with 5 mL NaOH 1 N. A solution of 4-chlorobutyl chloride (500 mg, 3.55 mmol) in Me_2CO (10 mL) was added at 0 °C and the mixture was incubated at room temperature for 3 h. Then, the solvent was evaporated and the residual was dissolved into water/MeOH (20 mL, 8:2, v:v) under ultrasounds. The precipitate was filtered and washed with water to obtain white powder (681 mg, yield 95%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 2.01 (quint, J = 6.10 Hz, 4H, H-4′), 2.40 (t, 4H, J = 6.9 Hz, 4H, H-3′), 3.67 (t, J = 6.11 Hz, 4H, H-5′), 5.06 (d, J = 5.6 Hz, 4H, H-1′) 7.59 (t, J = 7.4 Hz, 2H, H-2), 7.69 (d, J = 6.3 Hz, 2H, H-3), 8.06 (d, J = 8.4 Hz, 2H, H-1), 8.48 (t, J = 5.6 Hz, 2H, H-2′), 9.09 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 28.53 (C-4′), 32.62 (C-3′), 39.08 (C-1′), 45.27 (C-5′), 125.78 (C-2), 126.05 (C-9a), 127.41 (C-1), 127.73 (C-3), 136.75 (C-9), 137.00 (C-4), 145.73 (C-4a), 171.58 (C-2a) ppm. Anal. Calcd ($C_{23}H_{25}Cl_2N_3O_2$): C, 61.89; H, 5.65; N, 9.41. Found: C, 62.11; H, 5.63; N, 9.37.

4.2.3. 4,5-Bis[(acrylamido)-*N*-methyl]acridine (A′22). **A′12** hydrochloride (500 mg, 1.60 mmol) was dissolved

into 5 mL water, neutralized with NaOH 5 N and extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried with $MgSO_4$ and evaporated. The residue was dissolved into 20 mL CH_2Cl_2 and TEA (0.7 mL, 498 mmol) at 0 °C. Then, a solution of acryloyl chloride (579 mg, 6.40 mmol) was incorporated into 5 mL CH_2Cl_2 under N_2 atmosphere and the mixture was incubated for 24 h at room temperature. The solvent was evaporated, and the residue was dissolved into 5 mL Me_2CO and placed into 30 mL water. The precipitate was filtered and washed with H_2O/MeOH (9:1, v:v) to obtain white powder (300 mg, yield 57%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.15 (d, J = 5.70 Hz, 4H, H-1′), 5.64 (dd, J = 10.1, 2.2 Hz, 2H, H-4′), 6.16 (dd, J = 17.2, 2.2 Hz, 2H, H-5′), 6.40 (dd, J = 17.2, 10.1 Hz, 2H, H-3′), 7.59 (t, J = 7.4 Hz, 2H, H-2), 7.70 (d, J = 6.3 Hz, 2H, H-3), 8.08 (d, J = 8.4 Hz, 2H, H-1), 8.67 (t, J = 5.7 Hz, 2H, H-2′), 9.11 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 39.22 (C-1′), 125.55 (C-4′), 125.83 (C-2), 126.14 (C-9a), 127.62 (C-1), 128.25 (C-3), 131.98 (C-3′), 136.72 (C-9), 136.90 (C-4), 145.81 (C-4a), 165.08 (C-2a′) ppm. Anal. Calcd ($C_{21}H_{19}N_3O_2$): C, 73.03; H, 5.54; N, 12.17. Found: C, 72.75; H, 5.56; N, 12.22.

4.2.4. 4,5-Bis[(benzamido)-*N*-methyl]acridine (A′1). A′12 hydrochloride (500 mg, 1.60 mmol) was dissolved into 50 mL acetone at 0 °C and neutralized with 7 mL NaOH 1 N. Benzoyl chloride (492 mg, 3.35 mmol) in 5 mL acetone was added at 0 °C and the mixture was incubated at room temperature for 3 h. The solvent was evaporated and the residue was mixed with H_2O/MeOH (20 mL, 5:5, v:v) under ultrasounds. The precipitate was filtered and washed successively with water and MeOH to obtain white powder (652 mg, yield 92%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.31 (d, J = 5.70 Hz, 4H, H-1′), 7.49 (m, 4H, H-4′), 7.52 (m, 2H, H-5′), 7.61 (dd, J = 8.1, 6.8 Hz, 2H, H-2), 7.73 (d, J = 6.8 Hz, 2H, H-3), 8.00 (d, J = 6.8 Hz, 4H, H-3′), 8.10 (d, J = 8.1 Hz, 2H, H-1), 9.12 (t, J = 5.7 Hz, 2H, H-2′), 9.15 (s, 1H, H-9) ppm. ^{13}C NMR (50 MHz, DMSO-d_6, 25 °C): δ = 39.92 (C-1′), 125.78 (C-2), 126.09 (C-9a), 127.39 (C-3*), 127.51 (C-3′), 127.65 (C-1*), 128.54 (C-4′), 131.41 (C-5′), 134.75 (C-3a′), 136.86 (C-4), 136.86 (C-9) 145.79 (C-4a), 166.81 (C-2a′) ppm. Anal. Calcd ($C_{29}H_{23}N_3O_2$): C, 78.18; H, 5.20; N, 9.43. Found: C, 78.25; H, 5.15; N, 9.51.

4.2.5. 4,5-Bis[(4-fluorobenzamido)-*N*-methyl]acridine (A′2). A′12 hydrochloride (500 mg, 1.60 mmol) was dissolved into 50 mL acetone at 0 °C and neutralized with 7 mL NaOH 1 N. 4-Fluorobenzoyl chloride (533 mg, 3.36 mmol) in 5 mL acetone was added at 0 °C and the mixture was incubated at room temperature for 3 h. The solvent was evaporated and the residue was mixed with H_2O/MeOH (20 mL, 5:5, v:v) under ultrasounds. The precipitate was filtered and washed successively with water and MeOH to obtain white powder (740 mg, yield 96%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.31(d, J = 5.60 Hz, 4H, H-1′), 7.32 (m, 4H, H-4′), 7.61 (t, J = 7.60 Hz, 2H, H-2), 7.73 (d, J = 6.8 Hz, 2H, H-3), 8.04 (m, 4H, H-3′), 8.09 (d, J = 8.3 Hz, 2H, H-1), 9.13 (t, J = 5.6 Hz, 2H, H-2′), 9.14 (s, 1H, H-9) ppm. ^{13}C NMR (50 MHz, DMSO-d_6, 25 °C): δ = 39.69 (C-1′), 115.29 (d, J = 22.4 Hz, C-4′) 125.28 (C-2), 125.99 (C-9a), 126.84 (C-3), 127.10 (C-1), 130.09 (d, J = 9.7 Hz, C-3′), 131.06 (d, J = 4.2 Hz, C-3′a), 136.74 (C-4), 136.74 (C-9), 145.68 (C-4a), 163.97 (d, J = 246.5 Hz, C-4′a), 165.59 (C-2a′) ppm. Anal. Calcd ($C_{29}H_{21}F_2N_3O_2$): C, 72.34; H, 4.40; N, 8.73. Found: C, 72.45; H, 4.28; N, 8.72.

4.2.6. 4,5-Bis[(4-chlorobenzamido)-*N*-methyl]acridine (A′3). A′12 hydrochloride (200 mg, 0.65 mmol) was dissolved into 25 mL acetone at 0 °C and neutralized with 5 mL NaOH 1 N. 4-Chlorobenzoyl chloride (237 mg, 1.36 mmol) in 5 mL acetone was added at 0 °C and the mixture was incubated at room temperature for 3 h. The solvent was evaporated and the residue was mixed with H_2O/MeOH (20 mL, 5:5, v:v) under ultrasounds. The precipitate was filtered and washed successively with water and MeOH to obtain white powder (652 mg, yield 92%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.29 (d, J = 5.5 Hz, 4H, H-1′), 7.56 (m, 4H, H-4′), 7.60 (dd, J = 6.2, 8.3 Hz, 2H, H-2), 7.73 (d, J = 6.2 Hz, 2H, H-3), 7.97 (m, 4H, H-3′), 8.10 (d, J = 8.3 Hz, 2H, H-1), 9.15 (s, 1H, H-9), 9.18 (t, J = 5.7 Hz, 2H, H-2′) ppm. ^{13}C NMR (50 MHz, DMSO-d_6, 25 °C): δ = 39.97 (C-1′), 125.82 (C-2), 126.12 (C-9a), 127.49 (C-3), 127.78 (C-1), 128.61 (C-3′), 129.45 (C-4′), 133.45 (C-3a′), 136.25 (C-4), 136.25 (C-4′a), 136.68 (C-9), 145.80 (C-4a), 165.75 (C-2a′) ppm. Anal. Calcd ($C_{29}H_{21}Cl_2N_3O_2$): C, 67.71; H, 4.11; N, 8.17. Found: C, 67.82; H, 4.03; N, 8.05.

4.2.7. 4,5-Bis[(4-methoxybenzamido)-*N*-methyl]acridine (A′4). A′12 hydrochloride (200 mg, 0.65 mmol) was dissolved into 25 mL acetone at 0 °C and neutralized with 5 mL NaOH 1 N. 4-Methoxybenzoyl chloride (232 mg, 1.36 mmol) in 5 mL acetone was added at 0 °C and the mixture was incubated at room temperature for 3 h. The solvent was evaporated and the residue was mixed with H_2O/MeOH (20 mL, 5:5, v:v) under ultrasounds. The precipitate was filtered and washed successively with water and MeOH to obtain white powder (300 mg, yield 95%). ^{1}H NMR (200 MHz, DMSO-d_6, 25 °C): δ = 3.81 (s, 6H, H-5′), 5.29 (d, J = 5.5 Hz, 4H, H-1′), 7.02 (m, 4H, H-4′), 7.59 (dd, J = 6.2, 8.3 Hz, 2H, H-2), 7.72 (d, J = 6.2 Hz, 2H, H-3), 7.97 (m, 4H, H-3′), 8.08 (d, J = 8.3 Hz, 2H, H-1), 8.99 (t, J = 5.7 Hz, 2H, H-2′), 9.11 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 39.80 (C-1′), 55.52 (C-5′), 113.75 (C-4′), 125.82 (C-2), 126.12 (C-9a), 126.92 (C-3a′), 127.37 (C-3), 127.66 (C-1), 129.31 (C-3′), 137.11 (C-4), 137.11 (C-9), 145.84 (C-4a), 161.79 (C-4′a), 166.24 (C-2a′) ppm. Anal. Calcd ($C_{31}H_{27}N_3O_4$): C, 73.65; H, 5.38; N, 8.31. Found: C, 73.73; H, 5.28; N, 8.42.

4.2.8. 4,5-Bis[(4-dimethylaminobenzamido)-*N*-methyl]acridine (A′11). A′12 hydrochloride (500 mg, 1.63 mmol) was dissolved into 20 mL acetone at 0 °C and neutralized with 5 mL NaOH 1 N. 4-Dimethylaminobenzoyl chloride (618 mg, 3.37 mmol) in 10 mL acetone was added at 0 °C and the mixture was incubated at room temperature for 3 h. The solvent was evaporated and the

residue was mixed with H_2O/MeOH (20 mL, 5:5, v:v) under ultrasounds. The precipitate was filtered and washed successively with water and MeOH to obtain white powder (542 mg, yield 64%). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 2.96 (s, 12H, H-5′), 5.27 (d, J = 5.7 Hz, 4H, H-1′), 6.71 (m, 4H, H-4′), 7.59 (dd, J = 8.4, 6.3 Hz, 2H, H-2), 7.71 (d, J = 6.3 Hz, 2H, H-3), 7.84 (m, 4H, H-3′), 8.08 (d, J = 8.4 Hz, 2H, H-1), 8.76 (t, J = 5.7 Hz, 2H, H-2′), 9.13 (s, 1H, H-9) ppm. ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): δ = 39.58 (C-1′), 39.82 (C-5′), 110.97 (C-4′), 121.26 (C-3a′), 125.71 (C-2), 126.02 (C-9a), 127.18 (C-3*), 127.65 (C-1*), 128.71 (C-3′), 137.35 (C-4), 136.76 (C-9), 145.78 (C-4a), 152.24 (C-4a′), 166.55 (C-2a′) ppm. Anal. Calcd ($C_{33}H_{33}N_5O_2$): C, 74.55; H, 6.26; N, 13.17. Found: C, 74.26; H, 6.28; N, 13.12.

4.2.9. 4,5-Bis(bromomethyl)acridine. BMME (6.08 g, 44.64 mmol) was added to a solution of acridine (2 g, 11.16 mmol) in H_2SO_4 (25 mL) at 50 °C. The mixture was maintained under nitrogen for 12 h and refreshed in ice for an hour. The precipitate was filtered and dissolved into $CHCl_3$, and the organic phase was dried with $MgSO_4$. After evaporation of the solvent, the residue was recrystallized into anhydrous EtO_2 to obtain yellow powder (2.49 g, yield 64%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.39 (s, 4H, H-1′), 7.46 (dd, 2H, J = 8.5, 6.8 Hz, H-2), 7.89 (d, 2H, J = 6.8 Hz, H-3), 7.93 (d, 2H, J = 8.5 Hz, H-1), 8.70 (s, 1H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 27.01 (C-1′), 126.94 (C-2), 127.59 (C-9a), 128.81 (C-1), 131.55 (C-3), 137.83 (C-4), 139.45 (C-9), 151.22 (C-4a) ppm. Anal. Calcd ($C_{15}H_{11}N_7$): C, 49.35; H, 3.04; N, 3.84. Found: C, 49.49; H, 3.03; N, 3.83.

4.2.10. 4,5-Bis(hydroxymethyl)acridine (B′52). 4,5-Bis(bromomethyl)acridine (2 g, 5.48 mmol) was dissolved into 60 mL dioxane and $CaCO_3$ (10 g, 99.91 mmol) in 60 mL water was added. The mixture was filtered, the solvent was evaporated, the residue was dissolved into 200 mL CH_2Cl_2 and the organic phase was dried with $MgSO_4$. The solid residue was purified by silicagel chromatography (CH_2Cl_2/EtOAc, 5:5, v:v) to obtain a yellow powder (1.21 g, yield 92%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 4.10 (s, 2H, H-2′), 5.33 (s, 4H, H-1′), 7.50 (dd, 2H, J = 8.5, 6.8 Hz, H-2), 7.73 (d, 2H, J = 6.8 Hz, H-3), 7.92 (d, 2H, J = 8.5 Hz, H-1), 8.78 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 64.02 (C-1′), 125.71 (C-2), 126.50 (C-9a), 127.80 (C-1), 128.49 (C-3), 137.23 (C-4), 137.75 (C-9), 146.40 (C-4a) ppm. Anal. Calcd ($C_{15}H_{13}NO_2$): C, 75.30; H, 5.48; N, 5.85. Found: C, 75.19; H, 5.47; N, 5.86.

4.2.11. Acridin-4,5-yldimethyl-bis-4-chlorobutyrate (B′84). B′52 (300 mg, 1.25 mmol) was dissolved into 20 mL Me_2CO. TEA (0.5 mL, 3.60 mmol), DMAP (383 mg, 3.13 mmol) and 4-chlorobutyryl chloride (388 mg, 2.75 mmol) diluted into 20 mL Me_2CO were added at 0 °C under drying atmosphere ($CaCl_2$), and the mixture was incubated for 24 h at room temperature. Then, the mixture was transferred into 100 mL basic water (5 mL NaOH, 5 N) for 1 h and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with pickle and dried with $MgSO_4$. The solvent was evaporated and the residue was purified by silicagel chromatography (CH_2Cl_2/EtOAc, 10:0 and 9:1, v:v) to obtain brown oil (504 mg, yield 90%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.18 (quint, J = 6.8 Hz, 5H, H-3′), 2.65 (t, J = 7.2 Hz, 4H, H-2′), 3.64 (t, J = 6.3 Hz, 4H, H-4′), 5.97 (s, 4H, H-1′), 7.52 (dd, J = 8.5, 6.9 Hz, 2H, H-2), 7.76 (d, J = 6.9 Hz, 2H, H-3), 7.94 (d, J = 8.5 Hz, 2H, H-1), 8.72 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 27.69 (C-3′), 31.29 (C-2′), 44.14 (C-4′), 63.09 (C-1′), 125.44 (C-2), 126.24 (C-9a), 128.13 (C-3), 128.48 (C-1), 134.37 (C-4), 136.14 (C-9), 145.97 (C-4a), 172.53 (C-2a′) ppm. Anal. Calcd ($C_{23}H_{23}Cl_2NO_4$): C, 61.62; H, 5.17; N, 3.12. Found: C, 61.38; H, 5.19; N, 3.13.

4.2.12. Acridin-4,5-yldimethyl-bis-acrylate (B′81). B′52 (300 mg, 1.25 mmol) was dissolved into 20 mL Me_2CO. TEA (0.5 mL, 3.60 mmol), DMAP (383 mg, 3.13 mmol), and acryloyl chloride (249 mg, 2.75 mmol) diluted into 20 mL Me_2CO were added at 0 °C under drying atmosphere ($CaCl_2$), and the mixture was incubated for 24 h at room temperature. Then, the mixture was transferred into 100 mL basic water (5 mL NaOH, 5 N) for 1 h. The precipitate was filtered and washed with water. The solid layer was purified by silicagel chromatography (CH_2Cl_2/EtOAc, 10:0 and 9:1, v:v) to obtain solid yellow crystal (295 mg, yield 68%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.86 (dd, J = 10.3, 1.5 Hz, 2H, H-3′), 6.07 (s, 4H, H-1′), 6.24 (dd, J = 17.4, 10.3 Hz, 2H, H-2′), 6.50 (dd, J = 17.4, 1.5 Hz, 2H, H-4′), 7.52 (dd, J = 8.4, 6.9 Hz, 2H, H-2), 7.78 (dd, J = 6.9, 1.1 Hz, 2H, H-3), 7.96 (d, J = 8.4 Hz, 2H, H-1), 8.76 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ = 63.04 (C-1′), 125.48 (C-2), 126.27 (C-9a), 128.05 (C-2′*), 128.36 (C-3*), 128.54 (C-1*), 130.97 (C-3′), 134.52 (C-4), 136.11 (C-9), 146.02 (C-4a), 166.15 (C-2a′) ppm. Anal. Calcd ($C_{21}H_{17}NO_4$): C, 72.61; H, 4.93; N, 4.03. Found: C, 72.91; H, 4.89; N, 4.00.

4.2.13. Acridin-4,5-yldimethyl-bis-benzoate (B′57). B′52 (300 mg, 1.25 mmol) was dissolved into 20 mL CH_2Cl_2. TEA (0.5 mL, 3.60 mmol), DMAP (383 mg, 3.13 mmol), and benzoyl chloride (370 mg, 2.63 mmol) diluted into 10 mL CH_2Cl_2 were added at 0 °C under drying atmosphere ($CaCl_2$). Then, the mixture was incubated for 3 h at room temperature. The solvent was evaporated, the residual was dissolved into 10 mL Me_2CO, and transferred into 80 mL of water. After 1 h, the precipitate was filtered and washed with water. The resulting dried solid was dissolved into 10 mL $CHCl_3$, clarified by filtration and evaporated. The residue was recrystallized in MeOH to obtain white powder (441 mg, yield 79%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.26 (s, 2H, H-1′), 7.42 (m, 4H, H-3′), 7.51 (m, 2H, H-2), 7.54 (m, 4H, H-4′), 7.86 (dd, 2H, J = 6.8, 1.1 Hz, H-3), 7.95 (d, 2H, J = 8.5 Hz, H-1), 8.14 (m, 4H, H-2′), 8.76 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 63.44 (C-1′), 125.53 (C-2), 126.33 (C-9a), 128.01 (C-3), 128.27 (C-1), 128.37 (C-3′), 129.78 (C-2′), 130.47 (C-2a′), 132.87 (C-4′), 134.82 (C-4), 136.14 (C-9), 146.10 (C-4a), 166.52 (C-2b′) ppm. Anal. Calcd ($C_{29}H_{21}NO_4$): C, 77.84; H, 4.73; N, 3.13. Found: C, 78.12; H, 4.71; N, 3.11.

4.2.14. Acridin-4,5-yldimethyl-bis-4-fluorobenzoate (B′78). B′52 (400 mg, 1.67 mmol) was dissolved into 20 mL of anhydrous Me_2CO with 0.5 mL TEA (3.60 mmol) at 0 °C under drying atmosphere ($CaCl_2$). 4-Fluorobenzoyl chloride (557 mg, 3.51 mmol) diluted into 10 mL Me_2CO were added and the mixture was incubated for 5 h at room temperature. Then, the mixture was transferred into 100 mL of basic water (5 mL NaOH, 5 N). After 1 h, the precipitate was filtered and washed with water. The resulting solid was dissolved into 10 mL $CHCl_3$, clarified by filtration, and evaporated. The residue was recrystallized in MeOH to obtain white powder (614 mg, yield 76%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.22 (s, 4H, H-1′), 7.07 (m, 4H, H-3′), 7.54 (dd, 2H, J = 8.5, 6.8 Hz, H-2), 7.84 (dd, 2H, J = 6.8, 1.2 Hz, H-3), 7.98 (d, 2H, J = 8.5 Hz, H-1), 8.12 (m, 4H, H-2′), 8.79 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 63.57 (C-1′), 115.48 (d, J = 21.8 Hz, C-3′), 125.50 (C-2), 126.31 (C-9a), 126.63 (d, J = 2.3 Hz, C-2a′) 128.18 (C-3), 128.54 (C-1), 132.26 (d, J = 9.2 Hz, C-2′), 134.56 (C-4), 136.18 (C-9), 146.10 (C-4a), 165.53 (C-2b′), 165.72 (d, J = 253.6 Hz, C-3a′) ppm. Anal. Calcd ($C_{29}H_{19}F_2NO_4$): C, 72.04; H, 3.96; N, 2.90. Found: C, 72.33; H, 3.94; N, 2.89.

4.2.15. Acridin-4,5-yldimethyl-bis-4-chlorobenzoate (B′63). B′52 (300 mg, 1.25 mmol) was dissolved into 20 mL Me_2CO with 0.5 mL TEA (3.60 mmol) and 4-chlorobenzoyl chloride (614 mg, 3.51 mmol) diluted into 10 mL Me_2CO was added at 0 °C under drying atmosphere ($CaCl_2$). The mixture was incubated for 5 h at room temperature and transferred into 100 mL basic water (5 mL NaOH, 5 N) for 1 h. The precipitate was filtered and washed with water and the solid layer was dissolved into 10 mL $CHCl_3$, clarified and evaporated to obtain a yellow powder (640 mg, yield 75%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.21 (s, 4H, H-1′), 7.35 (m, 4H, H-3′), 7.53 (dd, J = 8.5, 6.9 Hz, 2H, H-2), 7.84 (dd, J = 6.9, 0.8 Hz, 2H, H-3), 7.97 (dd, J = 8.5, 0.8 Hz, 2H, H-1), 8.02 (m, 4H, H-2′), 8.77 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 63.67 (C-1′), 125.50 (C-2), 126.33 (C-9a), 128.26 (C-3), 128.67 (C-3′), 128.73 (C-1), 128.85 (C-2′a), 131.10 (C-2′), 134.46 (C-4), 136.18 (C-9), 139.31 (C-3′a), 146.13 (C-4a), 165.61 (C-2′b) ppm. Anal. Calcd ($C_{29}H_{19}Cl_2NO_4$): C, 67.45; H, 3.71; N, 2.71. Found: C, 67.70; H, 3.69; N, 2.70.

4.2.16. Acridin-4,5-yldimethyl-bis-4-methoxybenzoate (B′62). B′52 (300 mg, 1.25 mmol) was dissolved into 20 mL CH_2Cl_2. TEA (0.5 mL, 3.60 mmol), DMAP (383 mg, 3.13 mmol) and 4-methoxybenzoyl chloride (471 mg, 2.75 mmol) diluted into 10 mL CH_2Cl_2 were added at 0 °C under drying atmosphere ($CaCl_2$). Then, the mixture was incubated for 5 h at room temperature. The solvent was washed with 70 mL NaOH 1 N, the organic layer was dried with $MgSO_4$ and the solvent was evaporated. The residue was purified by silicagel chromatography (CH_2Cl_2/EtOAc, 99:1, v:v) to obtain yellow powder (515 mg, yield 81%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.84 (s, 6H, H-4′), 6.23 (s, 4H, H-1′), 6.89 (m, 4H, H-3′), 7.52 (dd, 2H, J = 8.5, 6.8 Hz, 2H, H-2), 7.84 (dd, J = 6.8, 1.0 Hz, 2H, H-3), 7.95 (d, J = 8.5 Hz, 2H, H-1), 8.08 (m, 4H, H-2′), 8.76 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 55.37 (C-4′), 63.15 (C-1′), 113.62 (C-3′), 122.93 (C-2a′), 125.51 (C-2), 126.30 (C-9a), 127.89 (C-3), 128.18 (C-1), 131.79 (C-2′), 135.07 (C-4), 136.05 (C-9), 146.09 (C-4a), 163.32 (C-3a′), 166.26 (C-2b′) ppm. Anal. Calcd ($C_{30}H_{25}NO_6$): C, 73.36; H, 4.96; N, 2.76. Found: C, 73.07; H, 5.00; N, 2.77.

4.2.17. Acridin-4,5-yldimethyl-dimethylaminobenzoate (B′76). B′52 (500 mg, 2.09 mmol) was dissolved into 20 mL anhydrous Me_2CO containing 1 mL TEA (7.20 mmol) at 0 °C under drying atmosphere ($CaCl_2$). 4-Dimethylaminobenzoyl chloride (806 mg, 4.39 mmol) diluted into 40 mL Me_2CO was added and the mixture was incubated for 5 h at room temperature. Then, the mixture was transferred into 100 mL basic water (5 mL NaOH, 5 N). After 1 h, the precipitate was filtered and washed with water. The solid layer was dissolved into 10 mL $CHCl_3$, clarified by filtration, and evaporated. The residue was recrystallized in MeOH to obtain brown powder (680 mg, yield 61%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.02 (s, 12H, H-4′), 6.25 (s, 4H, H-1′), 6.65 (m, 4H, H-3′), 7.50 (dd, J = 8.5, 7.0 Hz, 2H, H-2), 7.84 (dd, J = 7.0, 1.2 Hz, 2H, H-3), 7.93 (d, J = 8.5 Hz, 2H, H-1), 8.03 (m, 4H, H-2′), 8.75 (s, 1H, H-9) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 40.07 (C-4′), 62.69 (C-1′), 110.77 (C-3′), 117.34 (C-2a′), 125.53 (C-2), 126.26 (C-9a), 127.53 (C-3*), 127.62 (C-1*), 131.50 (C-2′), 135.64 (C-4), 135.93 (C-9), 146.04 (C-4a), 153.33 (C-3a′), 166.87 (C-2b′) ppm. Anal. Calcd ($C_{33}H_{31}N_3O_4$): C, 74.28; H, 5.86; N, 7.87. Found: C, 74.58; H, 5.82; N, 7.82.

4.3. Biology

4.3.1. Irradiation procedure. Irradiation was performed with a solar simulator Suntest CPS+ (Atlas Material Testing Technology BV, Moussy le Neuf, France) equipped with a xenon arc lamp, a treated quartz filter to block infrared light, and a glass filter to block UVC light and to reduce the UVB light (UVA/UVB: 0.5/0.001 mW/cm^2, visible light 800 lx). The average light incident dose was 2 mJ/cm^2 for a period of 1 min.

4.3.2. Antileishmanial activity against promastigotes. *Leishmania infantum* promastigotes in late log phase were incubated in RPMI medium supplemented with 12% fetal calf serum at an average of 10^5 cells/mL and a range of acridine concentrations was aseptically incorporated into duplicate cultures (final DMSO concentration was <5%). Following a 48-h incubation period at 25 °C, promastigote growth was estimated by counting parasites with a hemacytometer. IC_{50} was defined as the drug concentration necessary to inhibit 50% of parasite growth.

4.3.3. Antileishmanial activity against intracellular amastigotes. Intracellular amastigote cultures were performed in human monocyte-derived macrophages according to the methodology previously described by us.[17] 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× magnification. IC_{50} was defined as the concentration of drug necessary to produce a 50% decrease of infected macrophages.

4.3.4. Toxicity against human monocytes. In vitro toxicity of acridine derivatives was assessed on human monocytes maintained in RPMI medium (Eurobio, Paris, France) supplemented with 10% fetal calf serum (Eurobio, Paris, France) at 37 °C in 5% CO_2 and replicated every week. A range of concentrations was incorporated in late log-phase monocytes (10^5 cells/ml) and cultures were incubated at 37 °C with 5% CO_2. After a 72-h incubation period, cell growth was measured by counting monocytes in a hemacytometer. IC_{50} was defined as the concentration of drug required to induce a 50% decrease of cell growth. An in vitro selective index (SI), corresponding to the ratio between antiparasitic and cytotoxic activities, was calculated according to the following formula: $SI = IC_{50-monocytes}/IC_{50-amastigotes}$.

4.3.5. Effects of compound B′52 on nitric oxide production by macrophages. Maturation of human 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 various concentrations of compound **B′52** in the presence or absence of 10 U/mL human recombinant interferon gamma. After 48 h at 37 °C, NO production was measured by assessing the nitrite content of culture supernatants by the method described by Ding et al.[18]: 100μL of fresh Griess reagent was added to equal volumes of culture supernatants and the optical density at 540 nm was measured after 15 min of incubation at room temperature. Nitrite concentrations were determined by using $NaNO_2$ diluted in DMEM as the standard.

4.3.6. Effects of compound B′52 on the phagocytic capacities of human macrophages. Assays were performed on human monocyte-derived macrophages. 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 various concentrations of compound **B′52** were incorporated into duplicate cultures. After a 48-h incubation period at 37 °C, cells were rinsed with fresh medium and infected with RPMI medium containing stationary-phase promastigotes (cells/promastigotes ratio = 1:10). After a 4-h incubation period at 37 °C (5% CO_2), promastigotes were removed by four successive washes with fresh medium, fixed with methanol and stained with 10% Giemsa stain. The percentage of macrophages containing adherent or intracellular parasites was analyzed microscopically at 1000× magnification.

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