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Original article

Synthesis and biological evaluation of novel 4,5-bis(dialkylaminoalkyl)-substituted acridines as potent telomeric G-quadruplex ligands

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ABSTRACT

Several 4,5-bis(dialkylaminoalkyl)-substituted acridines have been prepared starting from acridine and their telomeric G-quadruplex stabilizing properties were evaluated using FRET melting and TRAP (Telomerase Repeat Amplification Protocol Assay) experiments.

1. Introduction

Human telomeric DNA consists of tandem repeats of the sequence d(TTAGGG)$_n$ with a 3′ single-stranded extension (G-overhang) [1]. To sustain continuous cell proliferation, telomere length is maintained in cancer cells either by the activity of telomerase or by recombinations between telomeres (Alternative Lengthening of Telomeres) [2]. Almost two decades ago, it was shown that the telomeric G-overhang is proned to fold into G-quadruplex structures, leading to the inhibition of telomerase activity [3]. This finding was the initial paradigm to search for small molecules that have the ability to stabilize telomeric G-quadruplex in order to inhibit telomerase activity and reverse tumor cell immortalization. Prolonged treatment of tumor cells with G-quadruplex ligands was indeed shown to provoke a telomerase-like inhibition phenotype (including telomere shortening, delayed growth inhibition and senescence induction), as well as telomere uncapping (including apoptosis, telomere fusion, anaphase bridges, G-overhang degradation and TIFs) [4,5].

Synthetic small molecules such as anthraquinone derivatives were the first ligands identified to fold G-quadruplex and indirectly inhibit telomerase activity [6] (Fig. 1). Following this initial work, a number of quadruplex binding molecules have been reported to block telomere access to telomerase but correspond to weak inhibitors of telomerase extension [5,7]. Structure–activity relationship studies and physico-chemical measurements evidenced large aromatic ring-systems bearing positively charged side chains as main structural features to be efficient G-quadruplex ligands [8–10]. Secondary structure stabilization and thus telomerase inhibition implies π–π stacking between planar aromatic molecules and the external guanine-rich face of G-quadruplex and electrostatic interactions of positively charged functions with the phosphate backbone of DNA.

Among the numerous heterocyclic systems some substituted acridines excelled by their efficiency. Thus, a 3,6-bis(aminoalkyl) side-chain substituted acridine, BRACO 19 displayed potent G-quadruplex stabilization ability ($\Delta T_m = 27.5$ °C at 1 μM) and TRAP inhibitory potential (IC_{50}(TRAP) = 0.09 μM) [11–13]. In addition, BRACO 19 was reported as a cancer cell proliferation inhibitor [14]. Replacement of the C-9 dimethylaminoaniline moiety by a difluorobenzyl group allowed pharmacological profile improvements [15]. Albeit simple acridine appendage seemed to be very attractive for G-quadruplex stabilization it has been rapidly evidenced the influence of the substitution pattern. Thus, 2,7-bis(aminoalkyl)

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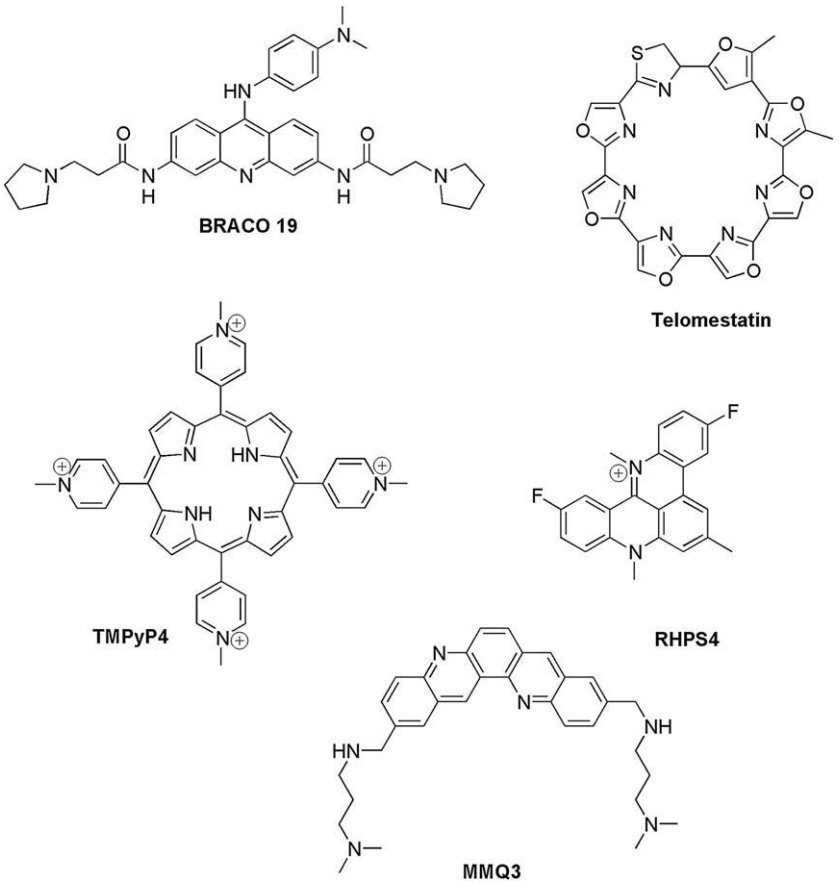


Fig. 1. Some selected G-quadruplex ligands.

substituted acridine derivatives were found to be modest G-quadruplex ligands (IC_{50}(TRAP) ≫ 10 μM) [16]. Other functionalized annulated acridines have also been reported as potential telomere maintenance inhibitors [17,18]. Although the chemistry of diverse 4,5-disubstituted acridines has been well documented due to Galy et al. [19–21], their biological applications, except for antileishmanial activities [20], remained unexploited. Having a long-standing experience in nitrogen containing heterocyclic chemistry we were interested in a systematic pharmacomodulation of 4,5-disusbtituted acridines aiming at the development of new G-quadruplex ligands.

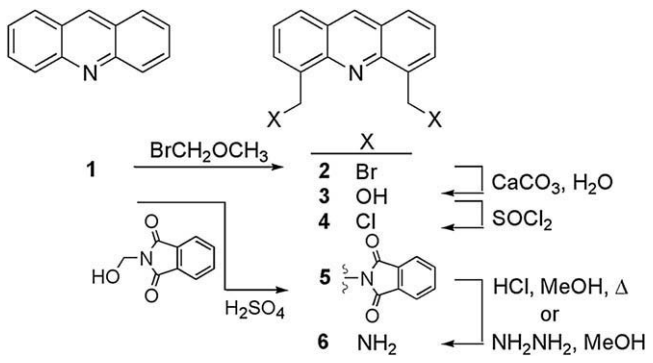
Herein we describe in full details the chemistry and preliminary pharmacological evaluation of some new 4,5-bis(dialkylaminoalkyl)-substituted acridines enabling the study of the impact of the side-chain length and the nature of protonable groups on G-quadruplex stabilization.

2. Results and discussion

2.1. Chemistry

4,5-Disubstituted acridine derivatives were prepared from acridine (**1**) by a linear synthesis based on simple functional group transformations (Scheme 1). Thus, 4,5-bis(halogenomethyl)acridines (**2**, **4**) were obtained from **1** *via* the corresponding hydroxymethyl derivative (**3**) following a well documented pathway. Similarly, aminomethyl functions on C-4 and C-5 carbons were introduced by simple electrophilic substitution using *N*-hydroxymethylphthalimide in sulfuric acid. Slight modification of Galy's method allowed the isolation of **5** in 84% yield. Deprotection of **5** was carried out in refluxing hydrochloric acid or by hydrazinolysis in 61 or 77% yield, respectively. We found that dihydrochloride salt of **6** was more stable for long-term storage.

The first series of pharmacomodulations concerned the synthesis of 4,5-bis(aminoalkylamidomethyl)-substituted acridines (Scheme 2). To this end, 4,5-bis(aminomethyl)acridine **6** was acylated with the homolog ω-chloroacylchlorides (n = 1,2,3) affording the corresponding 4,5-bis(chloroacylamide)-substituted acridines **7a** (n = 1), **7b** (n = 2) and **7c** (n = 3) in 77, 86 and 72% yield, respectively. Dialkylamino groups were smoothly incorporated by using the appropriate secondary amines in pure form or with solvent using in some cases NaI as activator. By these nucleophilic substitutions the corresponding dialkylaminoalkylamides **8**–**12** were obtained in varying chemical yields (23–87%). In parallel,



Scheme 1. Synthesis of some 4,5-difunctionalized acridine intermediates.

dimethylaminoacyl moiety was introduced with peptide coupling reagents (HOBt, DCC) starting from bisamine **6** to afford the corresponding 4,5-bis(dimethylaminoalkylamide) derivatives **8a**,**b** in 43 and 65% yield, respectively.

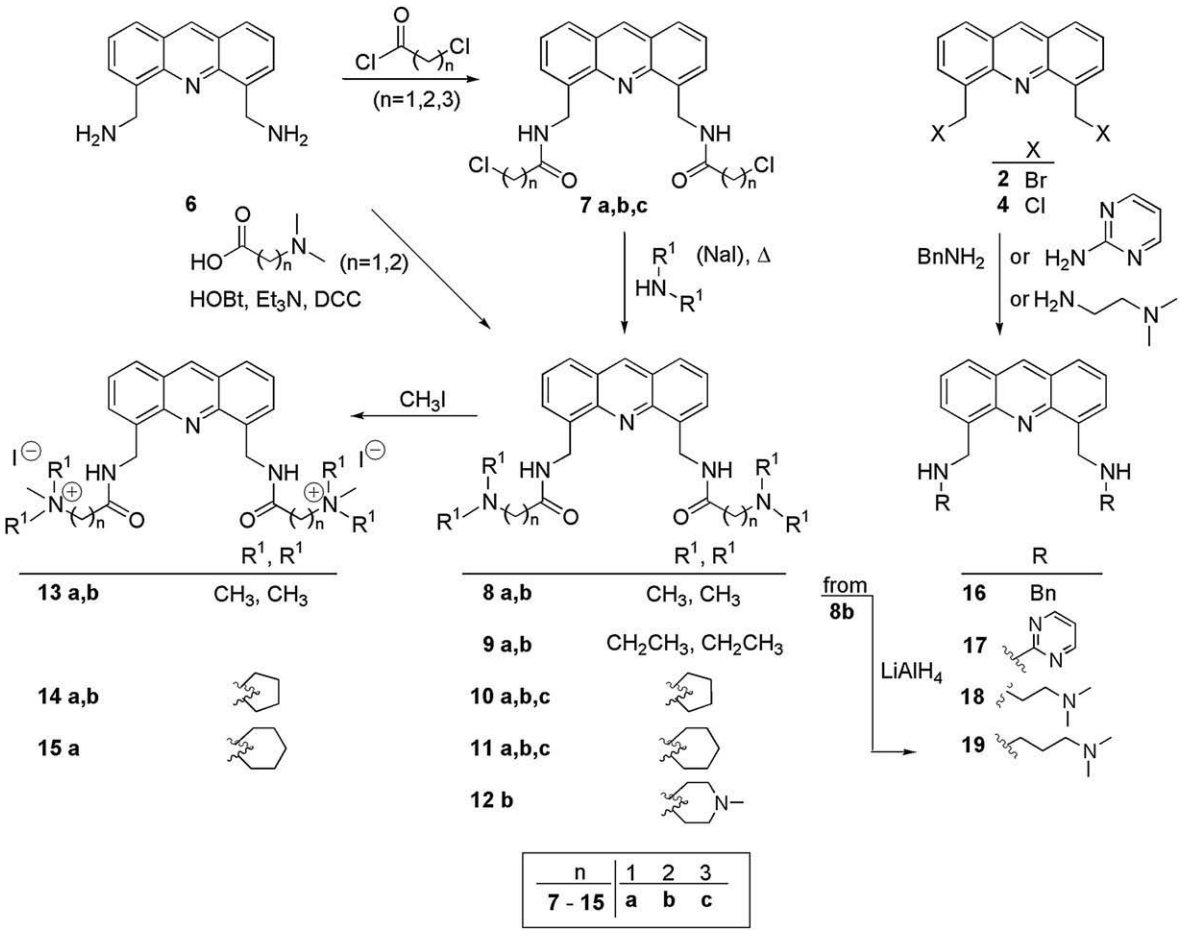
As some quaternary ammonium salts in the acridine series have been reported [22] to inhibit telomerase activity (TRAP assay) we prepared some side-chain methylated quaternary ammonium salts (**13a**,**b**; **14a**,**b**; **15a**) by simple treatment of the corresponding tertiary amines (**8a**,**b**; **10a**,**b**; **11a**) with methyliodide.

With 4,5-bis(halogenomethyl)acridines (**2**,**4**) in hand we have extended our study to more flexible, protonable dialkylaminoalkylamine-substituted acridines. To this end nucleophilic substitutions were carried out with benzylamine, 2-aminopyrimidine or 2-(dimethylamino)ethylamine leading to the corresponding substituted 4,5-bis(aminomethyl) derivatives **16**, **17** and **18**. The observed low yields are probably due to purification difficulties. In order to complete the collection of dimethylaminoalkylamine-substituted acridines the propyl side-chain substituted one (**19**) has also been prepared by $LiAlH_4$ reduction of **8b**. Newly synthesized acridine derivatives were fully characterized by classical spectroscopic methods (UV, IR, MS, ^1H and ^{13}C NMR). NMR spectra of some acridine derivatives were also recorded in DMSO-d_6 due to their low solubility.

2.2. Pharmacological evaluation

Biochemical evaluation of the synthesized compounds (**6**–**19**) was carried out in two successive steps. First, their G-quadruplex stabilization potential was determined by FRET (fluorescence resonance energy transfer) melting experiments onto telomeric sequence (F21D) using two ligand concentrations (20 μM or/and 10 μM). On the basis of these results the selected derivatives ($\Delta T_m > 5$ °C) were submitted to a second series of measurements at 5 and/or 1 μM ligand concentrations. Acridine derivatives displaying a ΔT_m value higher than 0.5 °C at 5 μM concentration were submitted to TRAP (telomerase repeat amplification protocol), an assay that measures the ability of a ligand to block polymerase extension of telomeric sequences [7]. The results of these experiments are summarized in Table 1 according to the structural feature of acridine side chain. Thus, the first series of derivatives concerns 4,5-dialkylaminoalkylamide substituted-ones (**8**–**15**). Amide function bearing derivatives are divided in two subclasses, the tertiary amine substituted ones (**8**–**12**) and the quaternary ammonium salts (**13**–**15**). Finally, some 4,5-bis(aminomethyl)-type acridine derivatives **16**–**19** completed our investigation. In all experiments, the 3,6,9-trisubstituted acridine derivative BRACO 19 was included to serve as a reference.

Global analysis of biological data confirmed that there is no simple correlation between G-quadruplex stabilization data (ΔT_m values) and TRAP inhibitory activity. Aminoalkylamide type side chain containing 4,5-disubstituted acridine derivatives (**8**–**12**) showed varying and relatively modest quadruplex complexing potential. This lower affinity may be explained by the formation of a hydrogen bond interaction between acridine nitrogen and NH of one of the amido groups contributing to the partial loss of conformational mobility and consequently to diminish interactions between chain-end protonated amine and DNA phosphate



Scheme 2. Synthesis of 4,5-bis(dialkylamino)alkyl substituted acridine derivatives.

Table 1
Telomeric G-quadruplex stabilization measured by FRET and TRAP inhibition.

No	FRET ΔT_m [°C]				IC_{50} (μM)
	20 μM	10 μM	5 μM	1 μM	TRAP
8a	3.6	–	–	–	>30
9a	2.4	–	–	–	–
10a	2.6	–	1.4	–	>30
11a	14.6	–	0.6	0	–
8b	8.1	–	5.0	–	>30
9b	10.7	9.1	7.2	2.6	–
10b	7.6	4.3	2.8	0	6
11b	10.4	7.3	6.6	2.2	>30
12b	4.6	2.6	0.5	0	>30
10c	16.4	11.9	8.8	3.0	13
11c	10.2	8.1	3.7	1.3	12
13a	16.7	–	5.9	–	7.3
14a	9.8	7.4	4.3	0.3	27
15a	12.6	8.8	6.8	2.6	30
13b	11.0	8.2	5.7	2.1	3
14b	12.6	8.7	6.8	2.3	4
6·(2HCl)	7.3	–	–	0	>30
16	4.8	–	–	–	–
17	0	–	–	–	–
18	10.2	–	4.0	–	5.6
19	17.7	–	6.7	–	0.15
BRACO 19	–	41.1	34.7	29.1	0.09

–: Not tested.

residues. Within the dialkylaminoalkylamide-substituted series (**8**–**12**) choosing a given amine, chain length proved to be important for both G-quadruplex stabilization and TRAP inhibition.

This chain length dependence observed in both dimethyl-(**8**) and diethylamino-(**9**) series became particularly evident when ΔT_m data of pyrrolidine derivatives (**10a**–**c**) were compared. Quadruplex stabilizing potency of pyrrolidine type homologs increased with the length of alkyl linkers. Acetamido derivative (**10a**) at 5 μM concentration afforded a small increase in melting temperature ($\Delta T_m = 1.4$ °C) relative to the control, while its butyramido counterpart (**10c**) gave a $\Delta T_m = 8.8$ °C under the same conditions. Comparison of TRAP inhibitory data showed a slightly better activity for propionamide (**10b**) type derivative ($IC_{50} = 6$ μM *vs.* 13 μM for **10b** and **10c**, respectively). In accordance with Neidle's previous findings [15] pyrrolidine bearing derivatives displayed the highest activity comparing to other amines. It is interesting to note that incorporation of a second basic function by replacing the piperidine ring with an *N*-methylpiperazine moiety didn't improve the TRAP inhibition (**11b** *vs.* **12b**).

Even if tertiary amine functions are considered to be protonated under physiological conditions we hoped that introduction of positively charged quaternary ammonium salts on side-chain termini would increase electrostatic interactions with the negatively charged DNA phosphate backbone. Indeed, comparison of pharmacological data of **14a** or **14b** to that of the tertiary amines (**10a** or **10b**, respectively) confirmed that quaternary ammonium function containing derivatives were more active species in both quadruplex stabilization and TRAP inhibition assays. Similar tendency was observed in dimethylamino- (**13** *vs.* **8**) and piperidine-substituted (**15a** *vs.* **11a**) series. Among the quaternary ammonium salts propionamido ($n = 2$) derivatives **13b** and **14b** proved to be the most active TRAP inhibitors with IC_{50} values of 3 and 4 μM, respectively.

Since 4,5-disubstituted acridines with amide function displayed micromolecular TRAP inhibitory potency in a third phase of our study we were interested in the corresponding 'in-chain' amino group containing acridine derivatives. Such structural modification would prevent acridine nitrogen from hydrogen bonding with amide function and render side chains more flexible and more hydrophile ready to participate in water-relayed hydrogen bonds in the grooves. The first amino group bearing derivatives showed that simple aminomethyl substituted acridine alone (**6**) or with arylaminomethyl chain (**16**,**17**) had very limited influence on G-quadruplex stabilization. Higher affinities were measured for dimethylaminoalkyl substituted counterparts (**18**,**19**) indicating the importance of electrostatic interactions in the recognition. Comparing 4,5-disubstituted alkylamino derivatives (**18**,**19**) to their acylamino counterparts (**8a**,**b**) we found that alkylamino-type molecules were at least ten-fold more potent in TRAP inhibition experiments (**18** *vs.* **8a**). Between **19** and **8b** this difference was even more significant. Both G-quadruplex stabilization and TRAP inhibitory activity seemed to be influenced by the chain length linking the two protonable amine functions. Thus, the highest inhibitory activity ($IC_{50} = 0.15$ μM) was obtained for the dimethylaminopropyl substituted derivative **19** (Fig. 2).

Generally, our 4,5-disubstituted acridine derivatives are less active than the reference BRACO 19, however the best one, **19** displayed an $IC_{50} = 0.15$ μM value, comparable to that of the reference product ($IC_{50} = 0.09$ μM).

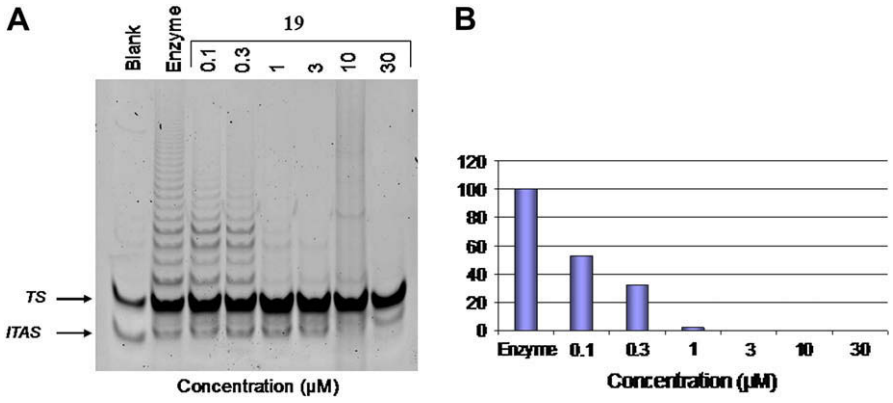


Fig. 2. Inhibition of telomerase activity by compound **19** in the TRAP assay. Compound **19**, at the indicated concentrations (0.1–30 μM), was added to 100 ng telomerase extract in the conditions of the TRAP assay (see, Section 4.2.2, TRAP assay). Enzyme: telomerase extract without compound. Blank: TRAP assay without telomerase extract. Arrows indicate the positions of TS and ITAS PCR products. Compound **19** inhibits telomere ladder formation at lower concentrations than ITAS formation. IC_{50}'s for TS and ITAS bands were calculated after scanning of the SYBR green I fluorescence in a Typhoon Phosphorimager and are reported in Table 1.

3. Conclusion

With the aim of completing the structure–activity relationship studies a series of 4,5-bis(dialkylaminoalkyl)-substituted acridines have been synthesized and evaluated as telomeric G-quadruplex binding agents. G-quadruplex stabilization and TRAP inhibition experiments evidenced the importance of the length and the nature of protonable side-chain residues. Among the 4,5-disubstituted acridine derivatives the dimethylaminopropyl substituted one (**19**) excelled by its sub-micromolar TRAP inhibitory activity ($IC_{50} = 0.15$ μM). Comparing to BRACO 19 the lower activity of **19** may be explained by its lower π-stacking surface and non-optimized side-chain length. Neidle's recent report [25] on the G-quadruplex recognition by BRACO 19 provides further insight into the fine structure of the complex. On the basis of thesis findings further pharmacomodulations and molecular modelling are envisaged toward the development of more potent G-quadruplex stabilizing agents.

4. Experimental

4.1. Chemistry

All solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Reactions and products were routinely monitored by thin-layer chromatography (TLC) on silica gel (Kieselgel 60 F_{254}, Merck). Column chromatography purifications were performed on CHROMAGEL® Silice 60 ACC 70–200 μm silica gel. Melting points were determined on a Reichert Thermovar hot-stage apparatus and are uncorrected. UV spectra were recorded in methanol solution on a Unicam 8700 UV/VIS apparatus. IR spectra were measured on a Perkin–Elmer Spectrum BX FTIR instrument. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AC 300 spectrometer using TMS as internal standard, chemical shifts were given in ppm (δ). Couplings expressed as s, br s, d, t, m correspond to singlet, broad-singlet, doublet, triplet and multiplet, respectively. For compounds **6**–**19**, exact chemical shifts assignments are given on the basis of their two-dimensional spectra (HMQC, HMBC) study. Mass spectra were recorded on an MSQ ThermoFinnigan apparatus using electronspray (ESI) or on a GCT Waters apparatus using electronimpact (EI) ionisation method.

4.1.1. Synthesis of 4,5-bis(bromomethyl)acridine (**2**)

Bromomethyl derivative **2** was prepared as described in the literature [19]. For some complementary spectral data see, Supplementary information.

4.1.2. Synthesis of 4,5-bis(hydroxymethyl)acridine (**3**)

Compound **3** was prepared by a slightly modified protocol of Galy et al. [20]. For details see, Supplementary information.

4.1.3. Synthesis of 4,5-bis(chloromethyl)acridine (**4**)

To a solution of **3** (500 mg, 2.09 mmol) in distilled CH_2Cl_2 (10 mL) was added $SOCl_2$ (381 μL, 5.22 mmol) under N_2 atmosphere. The solution was stirred at room temperature for 1 h. After concentration under reduced pressure, the mixture was extracted with EtOAc (3 × 50 mL) and washed with brine (20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **4** as a yellow powder (555 mg, 96 %); mp 158–160 °C. UV λ 252, 343, 358, 388 nm. IR (KBr) ν 3453, 2916, 1639, 1427, 1264, 1163, 754 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.79 (s, 1H, H-9), 7.99 (d, $J = 8.5$ Hz, 2H, H-1, H-8), 7.95 (d, $J = 6.8$ Hz, 2H, H-3, H-6) 7.55 (dd, $J = 8.5$, 6.8 Hz, 2H, H-2, H-7), 5.52 (s, 4H, 2 × H-1′, 2 × H-1″). ^{1}H NMR (DMSO-d_6) δ 9.20 (s, 1H, H-9), 8.22 (d, $J = 8.5$ Hz, 2H, H-1, H-8), 8.07 (d, $J = 6.7$ Hz, 2H, H-3, H-6) 7.67 (dd, $J = 8.5$, 6.7 Hz, 2H, H-2, H-7), 5.54 (s, 4H, 2 × H-1′, 2 × H-1″). ^{13}C NMR ($CDCl_3$) δ 146.0 (C-4a, C-10a), 136.7 (C-9), 135.9 (C-4, C-5), 130.6 (C-3, C-6), 128.9 (C-1, C-8), 126.6 (C-8a, C-9a), 125.8 (C-2, C-7), 43.1 (C-1′, C-1″). ^{13}C NMR (DMSO-d_6) δ 145.4 (C-4a, C-10a), 137.5 (C-9), 135.4 (C-4, C-5), 131.7 (C-3, C-6), 129.6 (C-1, C-8), 126.3 (C-8a, C-9a), 126.1 (C-2, C-7), 42.8 (C-1′, C-1″). MS (ESI+) $m/z = 276.1$ $[M + H]^+$. EI-MS m/z (%) = 277 (39, $[M]^+$), 276 (9), 275 (55, $[M]^+$), 241 (46), 240 (40), 239 (100). HRMS calcd. for $C_{15}H_{11}N^{35}Cl_2$ 275.0269; found 275.0263. HRMS calcd. for $C_{15}H_{11}N\ ^{35}Cl^{37}Cl$ 277.0239; found 277.0245. HRMS calcd. for $C_{15}H_{11}N\ ^{37}Cl_2$: 279.0210; found: 279.0219.

4.1.4. Synthesis of **6**

*4.1.4.1. 4,5-Bis(phthalimidomethyl)acridine (**5**).* Title compound **5** was prepared by a slightly modified protocol of Galy et al. [21]. For details see, Supplementary information.

*4.1.4.2. 4,5-Bis(aminomethyl)acridine dihydrochloride salt of **6**.* Dihydrochloride salt of **6** was prepared from **5** according to the experimental protocol of Galy et al. [21]. For details see, Supplementary information.

*4.1.4.3. 4,5-Bis(aminomethyl)acridine (**6**).* Prepared from dihydrochloride salt of **6** with a 1 N aqueous NaOH solution. For details see, Supplementary information.

4.1.5. Synthesis of compounds **8**–**12** from **6**

*4.1.5.1. Synthesis of compounds **8**–**12** from **6*** via *bis(chloroalkylamido) acridine derivatives **7a**–**c***

*4.1.5.1.1. Synthesis of compounds **7a**–**c**. General procedure*: A suspension of **6** in ω-chloroacyl chloride was heated under N_2 atmosphere (TLC monitoring). After cooling, the mixture was concentrated under reduced pressure and the crude product was purified by column chromatography or by crystallization from (Et_2O/EtOH).

*4.1.5.1.1.1. N,N′-[4,5-Acridindiylbis(methylene)]bis(chloroacetamide) (**7a**).* Acridine **6**: 170 mg (0.70 mmol); 2-chloroacetylchloride: 1.14 mL (14.4 mmol); Temperature: 80 °C; Reaction time: 8 h. Purification by column chromatography (eluent: CH_2Cl_2/MeOH, 95/5) gave **7a** as a pale beige powder (301 mg, 77%); mp 215–216 °C. UV λ 244, 341, 349, 358, 367, 387 nm. IR (KBr) ν 3436, 3277, 3074, 2925, 1643 (CO), 1546, 1423 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 9.17 (s, 1H, H-9), "8.83 (t, $J = 6.7$ Hz,....391.0707". 2H, 2 × NHCO), 8.13 (d, $J = 8.4$ Hz, 2H, H-1, H-8), 7.76 (d, $J = 6.7$ Hz, 2H, H-3, H-6), 7.64 (dd, $J = 8.4$, 6.7 Hz, 2H, H-2, H-7), 5.13 (d, $J = 5.7$ Hz, 4H, 2 × H-1′, 2 × H-1″), 4.24 (s, 4H, 2 × H-4′, 2 × H-4″). ^{13}C NMR (DMSO-d_6) δ 166.2 (2 × NHCO), 145.6 (C-4a, C-10a), 136.9 (C-9), 136.0 (C-4, C-5), 128.1 (C-3, C-6), 127.6 (C-1, C-8), 126.0 (C-9a, C-8a), 125.7 (C-2, C-7), 42.8 (C-4′, C-4″), 39.6 (C-1′, C-1″). MS (ESI+) $m/z = 390.1$ $[M + H]^+$. EI-MS m/z (%) = 391 (15, $[M]^+$), 390 (4), 389 (24, $[M]^+$), 314 (12), 312 (36), 284 (54). HRMS calcd. for $C_{19}H_{17}N_3O_2^{35}Cl_2$: 389.0698; found: 389.0727. HRMS calcd. for $C_{19}H_{17}N_3O_2^{37}Cl^{35}Cl$: 391.0668; found: 391.0707.

*4.1.5.1.1.2. N,N′-[4,5-Acridindiylbis(methylene)]bis(3-chloropropanamide) (**7b**).* Prepared in 86% yield from acridine derivative **6** and 3-chloropropionylchloride according to the *General procedure*. For details see, Supplementary information.

*4.1.5.1.1.3. N,N′-[4,5-Acridindiylbis(methylene)]bis(4-chlorobutanamide) (**7c**).* Title compound **7c** has already been described [20]. For a different protocol see, Supplementary information.

*4.1.5.1.2. Synthesis of compounds **8**–**12** from **7**. General procedure*: Chloroacyl substituted acridines **7** were treated with distilled pure amines using as solvent or in EtOH solution in the presence of NaI as activator. The mixture was heated under stirring under nitrogen atmosphere. After evaporation the reaction mixture was

diluted with CH_2Cl_2 and extracted with a saturated $NaHCO_3$ solution. The combined organic layers were dried with $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude residue by column chromatography, sometimes followed by recrystallization (Et_2O/EtOH, 9/1) afforded the corresponding amides **8**–**12**.

4.1.5.1.2.1. N,N′-*[4,5-Acridindiylbis(methylene)]bis(2-diethylaminoacetamide) (**9a**).* Chloroacetylamide: **7a**: 594 mg (1.52 mmol); NaI: 119 mg (0.76 mmol); EtOH: 6 mL. Dropwise addition of distilled diethylamine: 8.8 g (12.46 mL, 121.6 mmol); Temperature: 80 °C; Reaction time: 4 h. Purification by column chromatography (eluent: CH_2Cl_2/MeOH, 95/5) afforded **9a** as a brown amorphous solid (162 mg, 23%); mp 164–166 °C. UV λ 254, 336, 345, 356, 366, 375 nm. IR (KBr) ν 3436 (NH), 3348 (NH), 2969, 2916, 2811, 1674 (CO), 1498, 1252 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.80 (s, 1 H, H-9), 8.67 (t, $J = 5.5$ Hz, 2H, 2 × NHCO), 7.95 (d, $J = 7.8$ Hz, 2H, H-1, H-8), 7.78 (d, $J = 6.7$ Hz, 2H, H-3, H-6), 7.51 (dd, $J = 8.4$, 6.8 Hz, 2H, H-2, H-7), 5.25 (d, $J = 6.5$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.07 (s, 4H, 2 × H-4′, 2 × H-4″), 2,46 (q, $J = 7.1$ Hz, 8H, 4 × H-6′, 4 × H-6″), 0.80 (t, $J = 7.1$ Hz, 12H, 4 × CH_3). ^{13}C NMR ($CDCl_3$) δ 171.9 (2 × NHCO), 146.7 (C-4a, C-10a), 136.9 (C-9), 136.2 (C-4, C-5), 129.1 (C-3, C-6), 127.8 (C-1, C-8), 126.5 (C-9a, C-8a), 125.8 (C-2, C-7), 57.6 (C-4′, C-4″), 48.7 (2 × C-6′, 2 × C-6″), 40.5 (C-1′, C-1″), 12.0 (4 × CH_3). MS (ESI+) $m/z = 464.5$ $[M + H]^+$. HRMS calcd. for $C_{27}H_{37}N_5O_2$: 463.2947; found: 463.3079.

4.1.5.1.2.2. N,N′-*[4,5-Acridindiylbis(methylene)]bis[2-(1-pyrrolidinyl)acetamide] (**10a**).* Prepared in 31% yield from chloroacetylamide derivative **7a** and pyrrolidine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.3. N,N′-*[4,5-Acridindiylbis(methylene)]bis[2-(1-piperidinyl)acetamide] (**11a**).* Prepared in 49% yield from chloroacetylamide derivative **7a** and piperidine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.4. N,N′-*[4,5-Acridindiylbis(methylene)]bis(3-dimethylaminopropanamide) (**8b**).* Prepared in 48% yield from chloroacetylamide derivative **7b** and dimethylamine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.5. N,N′-*[4,5-Acridindiylbis(methylene)]bis(3-diethylaminopropanamide) (**9b**).* Prepared in 52% yield from chloroacetylamide derivative **7b** and diethylamine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.6. N,N′-*[4,5-Acridindiylbis(methylene)]bis[3-(1-pyrrolidinyl)propanamide] (**10b**).* Chloropropionylamide **7b**: 100 mg (0.24 mmol); NaI: 18 mg (0.12 mmol); EtOH: 4 mL; Dropwise addition of distilled pyrrolidine: 171 mg (200 μL, 2.40 mmol); Temperature: 120 °C; Reaction time: 2 h. Purification by column chromatography (eluent: MeOH/NH_4OH, 95/5) followed by recrystallization afforded **10b** as a yellow powder (102 mg, 87%); mp 169–171 °C. UV λ 253, 339, 349, 357, 389 nm. IR (KBr) ν 3418 (NH), 3277, 3066, 2951, 2792, 1643 (CO), 1550, 1454, 1427, 1348, 1229, 1128 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.92 (t, $J = 5.5$ Hz, 2H, 2 × NHCO), 8.77 (s, 1H, H-9), 7.94 (dd, $J = 8.4$, 1.0 Hz, 2H, H-1, H-8), 7.77 (dd, $J = 6.7$, 0.7 Hz, 2H, H-3, H-6), 7.51 (dd, $J = 8.4$, 6.7 Hz, 2H, H-2, H-7), 5.22 (d, $J = 5.9$ Hz, 4H, 2 × H-1′, 2 × H-1″), 2.75 (t, $J = 6.3$ Hz, 4H, 2 × H-5′, 2 × H-5″), 2.48 (t, $J = 6.3$ Hz, 4H, 2 × H-4′, 2 × H-4″), 2.41 (br s, 8H, 4 × H-7′, 4 × H-7″), 1.47 (m, 8H, 4 × H-8′, 4 × H-8″). ^{13}C NMR ($CDCl_3$) δ 172.5 (2 × NHCO), 146.7 (C-4a, C-10a), 137.1 (C-4, C-5), 136.5 (C-9), 129.1 (C-3, C-6), 127.7 (C-1, C-8), 126.7 (C-9a, C-8a), 125.9 (C-2, C-7), 53.5 (2 × C-7′, 2 × C-7″), 51.8 (C-5′, C-5″), 40.3 (C-1′, C-1″), 34.6 (C-4′, C-4″), 23.4 (2 × C-8′, 2 × C-8″). MS (ESI+) $m/z = 488.4$ $[M + H]^+$. EI-MS m/z (%) = 487 (3, $[M]^+$), 284 (56), 267 (110). HRMS calcd. for $C_{29}H_{37}N_5O_2$: 487.2947; found: 487.2980.

4.1.5.1.2.7. N,N′-*[4,5-Acridindiylbis(methylene)]bis[3-(1-piperidinyl)propanamide] (**11b**).* Prepared in 63% yield from chloropropionylamide derivative **7b** and piperidine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.8. N,N′-*[4,5-Acridindiylbis(methylene)]bis[3-(4-methylpiperazin-1-yl)propanamide] (**12b**).* Chloropropionylamide **7b**: 91.0 mg (0.22 mmol); distilled *N*-methylpiperazine: 792 mg (877 μL, 8.80 mmol); Temperature: 90 °C; Reaction time: 3 h. Purification by column chromatography (eluent: MeOH/NH_4OH, 99/1) followed by recrystallization afforded **12b** as a yellow powder (85 mg, 71%); mp 168–169 °C. UV λ 250, 340, 349, 357, 387 nm. IR (KBr) ν 3409 (NH), 3286, 3074, 2933, 2810, 1634 (CO), 1546, 1454, 1370, 1282, 1163, 1137, 1009 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.80 (s, 1H, H-9), 8.77 (t, $J = 5.7$ Hz, 2H, 2 × NHCO), 7.96 (d, $J = 8.6$ Hz, 2H, H-1, H-8), 7.80 (d, $J = 6.5$ Hz, 2H, H-3, H-6), 7.53 (dd, $J = 8.6$, 6.5 Hz, 2H, H-2, H-7), 5.20 (d, $J = 5.7$ Hz, 4H, 2 × H-1′, 2 × H-1″), 2.62 (t, $J = 6.3$ Hz, 4H, 2 × H-5′, 2 × H-5″), 2.45 (t, $J = 6.3$ Hz, 4H, 2 × H-4′, 2 × H-4″), 2.34 (br s, 8H, 4 × H-7′, 4 × H-7″), 1.94 (s, 14H, 4 × H-8′, 4 × H-8″, 2 × CH_3). ^{13}C NMR ($CDCl_3$) δ 172.2 (2 × NHCO), 146.7 (C-4a, C-10a), 136.9 (C-4, C-5), 136.4 (C-9), 129.3 (C-3, C-6), 127.7 (C-1, C-8), 126.6 (C-9a, C-8a), 125.9 (C-2, C-7), 54.51 (2 × C-7′, 2 × C-7″), 53.7 (C-5′, C-5″), 52.1 (2 × C-8′, 2 × C-8″), 45.5 (2 × CH_3), 40.3 (C-1′, C-1″), 32.4 (C-4′, C-4″). MS (ESI+) $m/z = 546.4$ $[M + H]^+$. EI-MS m/z (%) = 545 (18, $[M]^+$), 219 (100). HRMS calcd. for $C_{31}H_{43}N_7O_2$: 545.3478; found: 545.3477.

4.1.5.1.2.9. N,N′-*[4,5-Acridindiylbis(methylene)]bis[4-(1-pyrrolidinyl)-butanamide] (**10c**).* Prepared in 67% yield from chlorobutyramide derivative **7c** and pyrrolidine according to the *General procedure*. For details see, Supplementary information.

4.1.5.1.2.10. N,N′-*[4,5-Acridindiylbis(methylene)]bis[4-(1-piperidinyl)butanamide] (**11c**).* Chlorobutyramide **7c**: 250 mg (0.56 mmol); NaI: 168 mg (1.12 mmol); EtOH (10 mL); Dropwise addition of distilled piperidine: 476 mg (552 μL, 5.60 mmol); Temperature: 100 °C; Reaction time: 1 h. Purification by column chromatography (eluent: MeOH/NH_4OH, 99/1) afforded **11c** as a yellow powder (89 mg, 31%); mp 154–155 °C. UV λ 250, 340, 349, 357, 368, 386 nm. IR (KBr) ν 3423 (NH), 3254, 2923, 1642 (CO), 1514, 1453, 1411, 1216, 1210 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.72 (s, 1H, H-9), 8.39 (t, $J = 6.0$ Hz, 2H, 2 × NHCO), 7.90 (d, $J = 8.2$ Hz, 2H, H-1, H-8), 7.85 (d, $J = 6.7$ Hz, 2H, H-3, H-6), 7.49 (dd, $J = 8.2$, 6.7 Hz, 2H, H-2, H-7), 5.17 (d, $J = 6.0$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.06 (t, $J = 6.3$ Hz, 4H, 2 × H-4′, 2 × H-4″), 2.43 (t, $J = 6.3$ Hz, 4H, 2 × H-6′, 2 × H-6″), 2.02 (m, 8H, 2 × H-8′, 2 × H-8″, 2 × H-12′, 2 × H-12″), 1.85 (m, 8H, 2 × H-9′, 2 × H-9″, 2 × H-11′, 2 × H-11″), 1.25 (m, 4H, 2 × H-10′, 2 × H-10″). ^{13}C NMR ($CDCl_3$) δ 171.9 (2 × NHCO), 146.5 (C-4a, C-10a), 136.5 (C-4, C-5), 136.4 (C-9), 130.4 (C-3, C-6), 127.8 (C-1, C-8), 126.5 (C-9a, C-8a), 125.8 (C-2, C-7), 57.0 (C-6′, C-6″), 53.4 (C-8′, C-8″, C-12′, C-12″), 40.0 (C-1′, C-1″), 33.0 (C-4′, C-4″), 22.8 (C-9′, C-9″, C-11′, C-11″), 21.8 (C-10′, C-10″), 20.2 (C-5′, C-5″). MS (ESI+) $m/z = 544.4$ $[M + H]^+$. HRMS calcd. for $C_{33}H_{45}N_5O_2$: 515.3260; found: 515.3215.

*4.1.5.2. Synthesis of compounds **8a**,**b** from **6**. General procedure* for *N*-acylation of 4,5-bis(aminomethyl)acridine **6** using DCC/HOBt: To a solution of 1-hydroxybenzotriazole (HOBt), triethylamine (Et_3N) and *N*,*N*-dimethylaminoalkyl carboxylic acid hydrochloride salt in distilled CH_2Cl_2 was added the crude **6** dissolved in distilled CH_2Cl_2. The reaction mixture was stirred under N_2 atmosphere. After 0.5 h, a solution of *N*,*N*-dicyclohexylcarbodiimide (DCC) and Et_3N in distilled CH_2Cl_2 was added to the mixture. After stirring at room temperature, the mixture was diluted and filtered under reduced pressure. The filtrate was washed with 1 N aqueous NaOH solution and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography sometimes followed by recrystallization to afford title compounds.

4.1.5.2.1. N,N′-*[4,5-Acridindiylbis(methylene)]bis(2-dimethylaminoacetamide) (**8a**).* 4,5-Bis(aminomethyl)acridine **6**: 162 mg (0.68 mmol); CH_2Cl_2: 4 mL; HOBt: 202 mg (1.50 mmol); Et_3N: 96 mg (132 μL (0.95 mmol)); *N*,*N*-dimethylaminoglycine hydrochloride: 211 mg (1.50 mmol) dissolved in CH_2Cl_2 (4 mL); DCC: 310 mg

(1.50 mmol) and Et_3N (240 mg, 330 μL, 2.38 mmol) dissolved in CH_2Cl_2 (2 mL); Stirring: 72 h. Dilution with EtOAc (100 mL); Extraction and purification by column chromatography (eluent: CH_2Cl_2/MeOH, 90/10 → 80/20) gave **8a** as a yellow powder (119 mg, 43%); mp 152–155 °C. UV λ 245, 356, 386 nm. IR (KBr) ν 3356 (NH), 2942, 2828, 2775, 1678 (CO), 1661, 1528, 1414, 1264, 1145 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.82 (s, 1H, H-9), 8.60 (t, $J = 6.3$ Hz, 2H, 2 × NHCO), 7.97 (dd, $J = 8.5$, 1.1 Hz, 2H, H-1, H-8), 7.81 (dd, $J = 6.7$, 1.1 Hz, 2H, H-3, H-6), 7.52 (dd, $J = 8.5$, 6.7 Hz, 2H, H-2, H-7), 5.23 (d, $J = 6.3$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.03 (s, 4H, 2 × H-4′, 2 × H-4″), 2.22 (s, 12H, 4 × CH_3). ^1H NMR (DMSO-d_6) δ 9.19 (s, 1H, H-9), 8.63 (t, $J = 6.0$ Hz, 2H, 2 × NHCO), 8.13 (d, $J = 8.4$ Hz, 2H, H-1, H-8), 7.73 (d, $J = 6.0$ Hz, 2H, H-3, H-6), 7.64 (t, $J = 8.2$ Hz, 2H, H-2, H-7), 5.12 (d, $J = 6.0$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.06 (s, 4H, 2 × H-4′, 2 × H-4″), 2.26 (s, 12H, 4 × CH_3). ^{13}C NMR ($CDCl_3$) δ 170.2 (2 × NHCO), 146.8 (C-4a, C-10a), 136.9 (C-9), 136.0 (C-4, C-5), 129.5 (C-3, C-6), 127.9 (C-1, C-7), 126.6 (C-9a, C-8a), 125.8 (C-2, C-7), 63.1 (C-4′, C-4″), 45.8 (4 × CH_3), 40.5 (C-1′, C-1″). MS (ESI+) $m/z = 408.2$ $[M + H]^+$. EI-MS m/z (%) = 407 (100, $[M]^+$), 349 (52). HRMS calcd. for $C_{23}H_{29}N_5O_2$: 407.2321; found: 407.2308.

4.1.5.2.2. N,N′-*[4,5-Acridindiylbis(methylene)]bis(3-dimethylaminopropanamide)* (***8b***). Prepared in 65% yield from **6** and 3-(*N*,*N*-dimethylamino)propanoic acid hydrochloride according to the *General procedure*. For details see, Supplementary information.

*4.1.6. General procedure for the quaternization of **8a**,**b**, **10a**,**b** and **11a** with MeI*

To a solution of tertiary amines (**8**, **10**, **11**) in distilled CH_2Cl_2 was added at 0 °C, iodomethane dissolved in CH_2Cl_2. The suspension was stirred at room temperature under N_2 atmosphere, filtered, washed with diethylether and dried to give the corresponding quaternary ammonium salts.

*4.1.6.1. 2,2′-[4,5-Acridindiylbis(methyleneimino)]bis(*N,N,N-*trimethyl-2-oxoethylammonium) diiodide (**13a**).* Prepared in 91% yield from **8a** and iodomethane according to the *General procedure*. For details see, Supplementary information.

*4.1.6.2. 2,2′-[4,5-Acridindiylbis(methyleneimino)]bis[*N-*methyl*-N-*(2-oxoethyl)pyrrolidinium] diiodide (**14a**).* Prepared in 61% yield from **10a** and iodomethane according to the *General procedure*. For details see, Supplementary information.

*4.1.6.3. 2,2′-[4,5-Acridindiylbis(methyleneimino)]bis[*N-*methyl*-N-*(2-oxoethyl)piperidinium] diiodide (**15a**).* Tertiary amine **11a**: 58.0 mg (0.12 mmol); CH_2Cl_2: 10 mL; MeI: 851 mg (375 μL, 6.0 mmol); Stirring: 15 h. Yield: 65 mg (70%). Yellow powder; mp 231–233 °C. UV λ 252, 338, 347, 356, 367, 386 nm. IR (KBr) ν 3427 (NH), 3216, 3048, 2934, 1674 (CO), 1529, 1445, 1252, 758 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.22 (s, 1H, H-9), 9.06 (br s, 2H, 2 × NHCO), 8.18 (d, $J = 8.2$ Hz, 2H, H-1, H-8), 7.82 (d, $J = 6.4$ Hz, 2H, H-3, H-6), 7.67 (t, $J = 7.5$ Hz, 2H, H-2, H-7), 5.19 (d, $J = 5.4$ Hz, 4H, 2 × H-1′, 2 × H-1″), 4.31 (s, 4H, 2 × H-4′, 2 × H-4″), 3.66–3.50 (m, 8H, 4 × H-6′, 4 × H-6″), 3.31 (s, 6H, 2 × CH_3), 1.86 (br s, 8H, 4 × H-7′, 4 × H-7″), 1.58 (br s, 4H, 2 × H-8′, 2 × H-8″). ^{13}C NMR (DMSO-d_6) δ 163.6 (2 × NHCO), 145.7 (C-4a, C-10a), 137.2 (C-9), 135.6 (C-4, C-5), 128.5 (C-3, C-6), 128.1 (C-1, C-8), 126.2 (C-9a, C-8a), 125.9 (C-2, C-7), 61.5 (2 × C-6′ 2 × C-6″), 60.8 (C-4′, C-4″), 49.4 (2 × CH_3), in DMSO peaks (C-1′, C-1″), 20.8 (C-8′, C-8″), 19.6 (2 × C-7′, 2 × C-7″). MS (ESI+) $m/z = 644.4$ $[M–I]^+$. Anal. calcd. for $C_{31}H_{43}I_2N_5O_2$: 48.26 C%, 5.61 H%, 9.08 N%; found: 48.39 C%, 5.91 H%, 9.37 N%.

*4.1.6.4. 2,2′-[4,5-Acridindiylbis(methyleneimino)]bis(*N,N,N-*trimethyl-3-oxopropylammonium) diiodide (**13b**).* Tertiary amine **8b**: 100 mg (0.23 mmol); CH_2Cl_2: 8 mL; MeI: 1.63 g (715 μL, 11.5 mmol); Stirring: 24 h. Yield: 139 mg (84%). Yellow powder; mp 158–159 °C. UV λ 220, 254, 355, 386 nm. IR (KBr) ν 3436 (NH), 3066, 1652 (CO), 1533, 1476, 1251 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.19 (s, 1H, H-9), 8.70 (t, $J = 5.7$ Hz, 2H, 2 × NHCO), 8.15 (d, $J = 8.1$ Hz, 2H, H-1, H-3), 7.77 (d, $J = 6.2$ Hz, 2H, H-3, H-6), 7.64 (t, $J = 7.1$ Hz, 2H, H-2, H-7), 5.14 (d, $J = 5.7$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.66 (t, $J = 7.7$ Hz, 4H, 2 × H-5′, 2 × H-5′), 3.11 (s, 18H, 6 × CH_3), 2.86 (t, $J = 7.7$ Hz, 4H, 2 × H-4′, 2 × H-4″). ^{13}C NMR (DMSO-d_6) δ 168.7 (2 × NHCO), 145.8 (C-4a, C-10a), 137.1 (C-9), 136.5 (C-4, C-5), 127.9 (C-3, C-6), 127.7 (C-1, C-8), 126.2 (C-9a, C-8a), 125.9 (C-2, C-7), 62.0 (C-5′, C-5″), 52.5 (6 × CH_3), 39.2 (C-1′, C-1″), 29.3 (C-4′, C-4″). MS (ESI+) $m/z = 592.2$ $[M–I]^+$. Anal. calcd. for $C_{27}H_{39}I_2N_5O_2$: 45.07 C%, 5.46 H%, 9.74 N%; found: 45.38 C%, 5.69 H%, 9.48 N%.

*4.1.6.5. 2,2′-[4,5-Acridindiylbis(methyleneimino)]bis[*N-*methyl*-N-*(3-oxopropyl)pyrrolidinium] diiodide (**14b**).* Tertiary amine **10b**: 50.0 mg (0.10 mmol); CH_2Cl_2: 10 mL; MeI: 710 mg (310 μL, 5.0 mmol); Stirring: 2 h. Yield: 55 mg (72%). Brown amorphous solid. UV λ 220, 253, 356, 386 nm. IR (KBr) ν 3436 (NH), 3268, 3066, 2951, 1652 (CO), 1537, 1458, 1423, 1238 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.19 (s, 1H, H-9), 8.71 (t, $J = 5.4$ Hz, 2H, 2 × NHCO), 8.14 (d, $J = 8.4$ Hz, 2H, H-1, H-8), 7.78 (d, $J = 6.6$ Hz, 2H, H-3, H-6), 7.65 (t, $J = 7.6$ Hz, 2H, H-2, H-7), 5.14 (d, $J = 5.4$ Hz, 4H, 2 × H-1′, 2 × H-1″), 3.69 (t, $J = 7.6$ Hz, 4H, 2 × H-5′, 2 × H-5″), 3.50 (d, $J = 5.0$ Hz, 8H, 4 × H-7′, 4 × H-7″), 3.03 (s, 6H, 2 × CH_3), 2.88 (t, $J = 7.6$ Hz, 4H, 2 × H-4′, 2 × H-4″), 2.11 (sl, 8H, 4 × H-8′, 4 × H-8″). ^{13}C NMR (DMSO-d_6) δ 168.8 (2 × NHCO), 145.8 (C-4a, C-10a), 137.0 (C-9), 136.5 (C-4, C-5), 128.0 (C-3, C-6), 127.7 (C-1, C-8), 126.2 (C-9a, C-8a), 125.9 (C-2, C-7), 63.8 (2 × C-7′, 2 × C-7″), 59.7 (C-5′, C-5″), 47.8 (2 × CH_3), 39.1 (C-1′, C-1″), 30.0 (C-4′, C-4″), 21.3 (2 × C-8′, 2 × C-8″). MS (ESI+) $m/z = 644.3$ $[M–I]^+$. Anal. calcd. for $C_{31}H_{43}I_2N_5O_2$: 48.26 C%, 5.61 H%, 9.08 N%; found: 48.58 C%, 5.99 H%, 9.41 N%.

*4.1.7. Synthesis of compounds **16**–**19***

Procedure A: To a suspension of primary amine, K_2CO_3, and tetrabutylammonium hydrogen sulfate (TBAHS) in distilled CH_2Cl_2 was added 4,5-bis(bromomethyl)acridine **2** and the mixture was stirred at 50 °C for 3–24 h under N_2 atmosphere. The precipitate was filtered, the filtrate was extracted with CH_2Cl_2 (×2) and washed with 1 N aqueous NaOH solution.

Procedure B: To a solution of **4** in distilled DMSO was added the primary amine and the reaction mixture was stirred at room temperature. The mixture was extracted with EtOAc (×2) and washed with brine.

Purification (*Procedures A and B*): the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was crystallized or purified by column chromatography.

*4.1.7.1. 4,5-Bis(benzylaminomethyl)acridine (**16**). Procedure B*: 4,5-bis(chloromethyl)acridine **4**: 50 mg (0.18 mmol); DMSO: 15 mL; benzylamine: 235 mg (240 μL, 0.40 mmol); Stirring: 6 h. Extraction: EtOAc (2 × 25 mL) and brine (10 mL). Purification by column chromatography (CH_2Cl_2/MeOH, 90/10) afforded **16** as a beige powder (33 mg; 44%); mp 350 °C. UV λ 252, 358 nm. IR (KBr) ν 3445 (NH), 2916, 2704, 1635, 1427, 754 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.77 (s, 1H, H-9), 7.94 (d, $J = 8.5$ Hz, 2H, H-1, H-8), 7.68 (d, $J = 6.7$ Hz, 2H, H-3, H-6), 7.49 (t, $J = 7.6$ Hz, 2H, H-2, H-7), 7.37–7.23 (m, 10H, 2 × H-5′, 2 × H-5″, 2 × H-6′, 2 × H-6″, H-7′, H-7″), 4.51 (s, 4H, 2 × H-1′, 2 × H-1″), 3.79 (s, 4H, 2 × H-3′, 2 × H-3″). ^1H NMR (DMSO-d_6) δ 9.90 (br s, 2H, H-2′, H-2″), 9.30 (s, 1H, H-9), 8.31 (d, $J = 8.3$ Hz, 2H, H-1, H-8), 8.08 (d, $J = 6.5$ Hz, 2H, H-3, H-6), 7.74–7.69 (m, 6H, H-2, H-7, 2 × H-5′, 2 × H-5″), 7.42 (m, 6H, 2 × H-6′, 2 × H-6″, H-7′, H-7″), 4.93 (s, 4H, 2 × H-1′, 2 × H-1″), 4.41 (s, 4H, 2 × H-3′, 2 × H-3″). ^{13}C NMR ($CDCl_3$) δ 146.6 (C-4a, C-10a), 138.8 (C-4′, C-4″), 136.8 (C-9), 136.1 (C-4, C-5), 130.3 (C-3, C-6), 128.6 (2 × C-6′, 2 × C-6″), 128.4 (2 × C-5′, 2 × C-5″),

127.9 (C-1, C-8), 127.2 (C-7′, C-7″), 126.5 (C-9a, C-8a), 125.6 (C-2, C-7), 52.4 (C-3′, C-3″), 50.3 (C-1′, C-1″). ^{13}C NMR (DMSO-d_6) δ 145.7 (C-4a, C-10a), 138.0 (C-9), 133.9 (C-3, C-6), 132.0 (C-4, C-5), 130.4 (2 × C-6′, 2 × C-6″), 130.2 (C-1, C-8), 129.6 (C-4′, C-4″), 128.9 (C-7′, C-7″), 128.6 (2 × C-5′, 2 × C-5″), 126.2 (C-9a, C-8a), 125.7 (C-2, C-7), 50.4 (C-3′, C-3″), 45.9 (C-1′, C-1″). MS (ESI+) m/z = 418.30 $[M+H]^+$. EI-MS m/z (%) = 417 (9, $[M]^+$), 310 (100), 219 (45). HRMS calcd. for $C_{29}H_{27}N_3$: 417.2205; found: 417.2216.

*4.1.7.2. 4,5-Bis(2-pyrimidinylaminomethyl)acridine (**17**). Procedure A*: 4,5-bis(bromomethyl)acridine **2**: 200 mg (0.55 mmol); pyrimidine: 88 mg (86 μL, 1.10 mmol); K_2CO_3: 304 mg (2.20 mmol); TBAHS: 20 mg (0.06 mmol); CH_2Cl_2: 20 mL; Stirring: 24 h. Extraction of the filtrate: CH_2Cl_2 (2 × 15 mL) and 1 N aqueous NaOH (5 mL). Purification by column chromatography (CH_2Cl_2/MeOH, 95/5) afforded **17** as a yellow powder (50 mg; 23%); mp 215–216 °C. UV λ 256, 356, 387 nm. IR (KBr) ν 3251 (NH), 3031, 1590, 1528, 1454, 1418, 1392, 1282 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H, H-9), 8.29 (m, 6H, 2 × H-4′, 2 × H-4″, H-2′, H-2″), 7.92 (d, J = 8.4 Hz, 2H, H-1, H-8), 7.84 (d, J = 6.7 Hz, 2H, H-3, H-6), 7.48 (dd, J = 8.4, 6.7 Hz, 2H, H-2, H-7), 6.55 (br s, 2H, H-2′, H-2″), 6.52 (t, J = 4.8 Hz, 2H, H-5′, H-5″), 5.42 (d, J = 6.0 Hz, 4H, 2 × H-1′, 2 × H-1″). ^{13}C NMR ($CDCl_3$) δ 161.6 (C-3′, C-3″), 157.8 (2 × C-4′, 2 × C-4″), 146.4 (C-4a, C-10a), 136.5 (C-9), 136.0 (C-4, C-5), 129.3 (C-3, C-6), 127.4 (C-1, C-8), 126.3 (C-9a, C-8a), 125.3 (C-2, C-7), 110.1 (C-5′, C-5″), 42.2 (C-1′, C-1″). MS (ESI+) m/z = 394.2 $[M+H]^+$. HRMS calcd. for $C_{23}H_{19}N_7$: 393.1754; found: 393.1702.

*4.1.7.3. 4,5-Bis[2-(N,N-dimethylamino)ethylaminomethyl]acridine (**18**). Procedure B*: 4,5-bis(chloromethyl)acridine **4**: 700 mg (0.25 mmol); DMSO: 7 mL; 2-(dimethylamino)ethylamine: 48.5 mg (60 μL, 0.55 mmol); Stirring: 3 h. Extraction with EtOAc (2 × 20 mL) and brine (10 mL) afforded **18** as a brown oil (13 mg, 14%). UV λ 213, 253, 358 nm. IR (KBr) ν 3441 (NH), 2912, 1616, 1389, 1132, 754 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H, H-9), 7.93 (d, J = 8.6 Hz, 2H, H-1, H-8), 7.78 (d, J = 6.6 Hz, 2H, H-3, H-6), 7.50 (dd, J = 8.6, 6.6 Hz, 2H, H-2, H-7), 4.60 (s, 4H, 2 × H-1′, 2 × H-1″), 2.84 (t, J = 6.2 Hz, 4H, 2 × H-3′, 2 × H-3″), 2.50 (t, J = 6.2 Hz, 4H, 2 × H-4′, 2 × H-4″), 2.10 (s, 12H, 4 × CH_3). ^{13}C NMR ($CDCl_3$) δ 146,6 (C-4a, C-10a), 136.8 (C-9), 136.5 (C-4, C-5), 129.9 (C-3, C-6), 127.7 (C-1, C-8), 126.5 (C-8a, C-9a), 125.6 (C-2, C-7), 58.6 (C-4′, C-4″), 50.9 (C-1′, C-1″), 46.4 (C-3′, C-3″), 45.4 (4 × CH_3). MS (ESI+) m/z = 380.29 $[M+H]^+$. HRMS calcd. for $C_{23}H_{33}N_5$: 379.2736; found: 379.2707.

*4.1.7.4. 4,5-Bis[3-(*N,N*-dimethylamino)propylaminomethyl]acridine (**19**).* To a suspension of **8b** (200 mg, 0.46 mmol) in 1,2-dimethoxyethane (DME) (4 mL) was carefully added at 0 °C lithium aluminum hydride ($LiAlH_4$) (174 mg, 4.60 mmol). The suspension was stirred at room temperature under N_2 atmosphere for 24 h and heated at 60 °C for 3 h. The reaction mixture was first quenched under stirring with water, the precipitate was filtered and washed successively with CH_2Cl_2 (6 × 10 mL), DME (40 mL) and EtOAc (20 mL). The filtrate was evaporated under reduced pressure and the residue was extracted with CH_2Cl_2 (2 × 80 mL) and washed with 1 N aqueous NaOH solution (60 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product (102 mg) was purified by column chromatography (eluent: CH_2Cl_2/MeOH/NH_4OH, 20/80/10) to give **19** (27 mg; 14 %) as a brown amorphous solid; mp 152–155 °C. UV λ 254, 356, 389 nm. IR (KBr) ν 3421 (NH), 2910, 1632, 1533, 1410, 1385, 1112, 758 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H, H-9), 7.91 (d, J = 8.5 Hz, 2H, H-1, H-8), 7.73 (d, J = 6.6 Hz, 2H, H-3, H-6), 7.48 (dd, J = 8.5, 6.6 Hz, 2H, H-2, H-7), 4.50 (s, 4H, 2 × H-1′, 2 × H-1″), 2.78 (t, J = 7.1 Hz, 4H, 2 × H-3′, 2 × H-3″), 2.29 (t, J = 7.1 Hz, 4H, 2 × H-5′, 2 × H-5″), 2.13 (s, 12H, 4 × CH_3), 1.76 (m, 4H, 2 × H-4′, 2 × H-4″). ^{13}C NMR ($CDCl_3$) δ 146.7 (C-4a, C-10a), 136.9 (C-4, C-5), 136.7 (C-9), 129.8 (C-3, C-6), 127.6 (C-1, C-8), 126.5 (C-9a, C-8a), 125.6 (C-2, C-7), 58.0 (C-5′, C-5″), 51.4 (C-1′, C-1″), 48.0 (C-3′, C-3″), 45.4 (4 × CH_3), 27.8 (C-4′, C-4″). MS (ESI+) m/z = 408.3 $[M+H]^+$. HRMS calcd. for $C_{25}H_{37}N_5$: 407.3049; found: 407.3163.

4.2. Biochemical evaluations

4.2.1. FRET melting experiments

Initial screening experiments were performed on a LightCycler real-time PCR instrument (Roche, Basel, Switzerland) as described previously [23] using a fluorescent oligonucleotide F21D (5′-FAM-GGGTTAGGGTTAGGGTTAGGG-DabCyl-3′), alone or in the presence of tested compound. Assays were performed in a buffer containing 0.2 μM F21D, 10 mM cacodylate, (pH = 8.0), 0.1 M LiCl, and 5 mM KCl. Excitation wavelength was 470 nm, and emission of fluorescein was recorded at 530 nm.

4.2.2. TRAP assay

The TRAP reaction was performed as previously described [24]. PCR was performed in a final 50 μL reaction volume composed of a 45 μL reaction mixture containing 20 mM Tris–HCl (pH 8.0), 50 μM dNTPs, 1.5 mM $MgCl_2$, 63 mM KCl, 1 mM EGTA, 0.005% Tween 20, 20 μg/mL Bovine Serum Albumin, 0.1 μg primer TS (5′-AATCCGTCG AGCAGAGTT-3′), 0.1 μg primer CXext (5′-GTGCCCTTACCCTTACCCTT ACCCTAA-3′), 0.1 μg picoM primer NT (5′-ATCGCTTCTCGGCCTTTT-3′), 0.01 attoM TSNT internal control (5′-ATTCCGTCGAGCAGAGTTAA AAGGCCGAGAAGCGAT-3′), 2.5 U Taq DNA polymerase (DyNAzyme II DNA polymerase, Ozyme) and 200 ng HT1080 cells CHAPS extract. Compounds and distilled water were added under a volume of 5 μL. PCR was performed in an Eppendorf Mastercycler equipped with a hot lid and incubated for 15 min at 30 °C, 1 min at 92 °C followed by 30 cycles, as follows: 30 s at 92 °C, 30 s at 52 °C, 30 s at 72 °C. After amplification, 8 μL of loading buffer containing 20% sucrose, 5× TBE, 0.2% bromophenol blue and 0.2% xylene-cyanol were added to the reaction. Amplified products were resolved on a 12% nondenaturing polyacrylamide gel in 1× TBE and stained with SYBR Green I (Roche). Fluorescence was analyzed with a Typhoon 9210 PhosphorImager (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK).

HT1080 human lung carcinoma was obtained from the American Type Culture Collection (Manassas, VA). The cells were grown in Dulbecco's modified Eagle's medium with GlutaMAX (Invitrogen), supplemented with 10% fetal calf serum and antibiotics.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.04.021.

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