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Synthesis, cytotoxicity evaluation, and molecular modeling studies of 2,N^{10}-substituted acridones as DNA-intercalating agents

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Abstract

Acridine-based compounds possess anticancer activities by intercalating to DNA. Although they have chemotherapeutic potential, acridine-based compounds are not used to treat cancer. In this study, 2,N^{10}-acridone derivatives are designed and synthesized based on acridone, a ketone derivative of acridine. Herein, acridone is functionalized with alkyl side chains containing terminal nitrogen-based moieties at the N^{10}-position and substituted at the C2-position. The products are evaluated for in vitro cytotoxicity against four cancer cell lines: Molt-3, HepG2, A549, and HuCCA-1. The derivative bearing two butyl piperidine side chains at the C2- and N^{10}-positions is the most active, with IC_{50} values ranging from 2.96 to 9.46 μM. Molecular modeling studies supported the binding of the derivatives to DNA by intercalation, thereby confirming the observed cytotoxic effects.

Keywords

acridone, cytotoxicity, intercalating agent, synthesis

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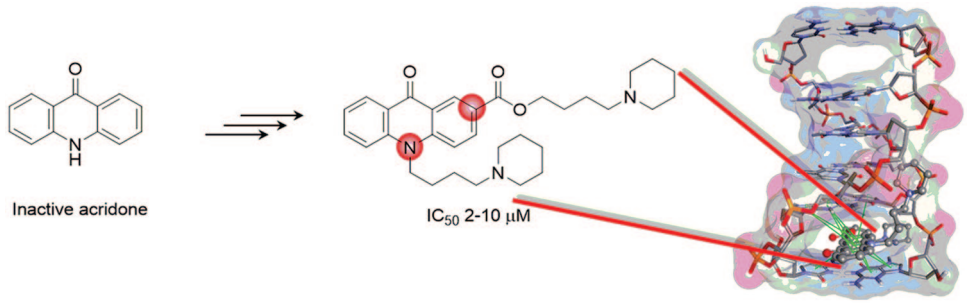

Introduction

Cancer is a class of disease characterized by an abnormal growth of tissues that can metastasize. In 2018, the World Health Organization (WHO)[1] reported approximately 9.6 million deaths from cancers worldwide. Undeniably, cancer is a serious health concern globally. The development of chemotherapeutics faces challenging obstacles including the development of potent small molecule drugs against mutant forms of protein targets with high selectivity and tolerable adverse effects. Thus, research to develop new anticancer drugs with the above-mentioned properties is highly desirable. Existing anticancer chemotherapeutic

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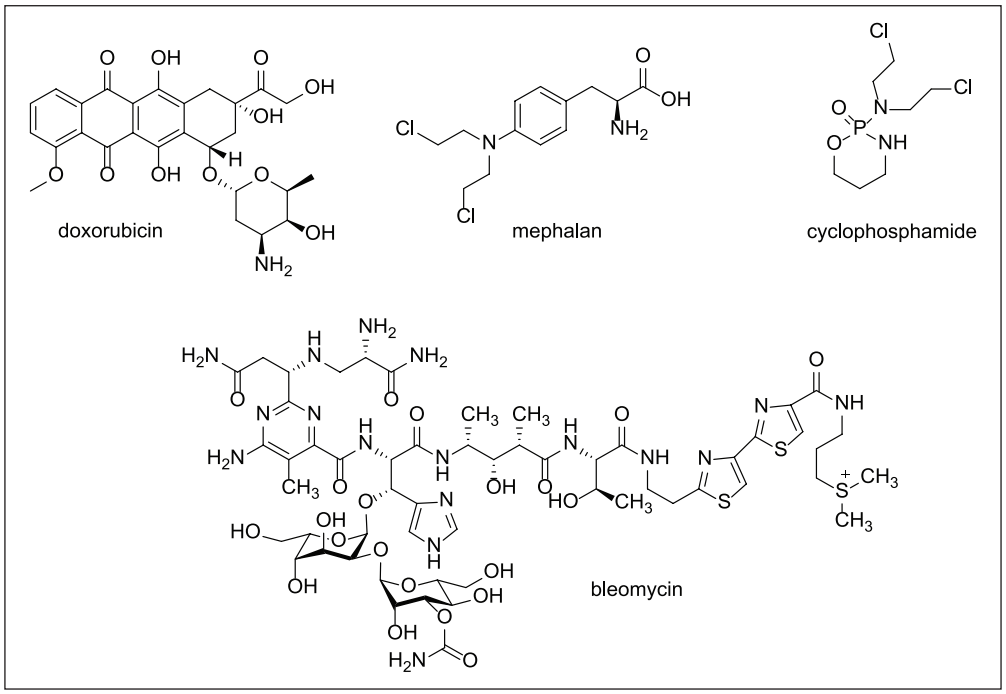


Figure 1. Structures of some DNA-targeting anticancer drugs.

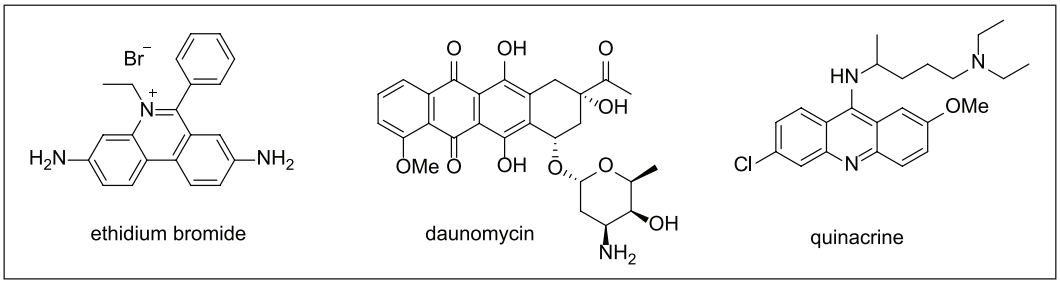


Figure 2. Chemical structures of known DNA-intercalating agents: ethidium bromide, daunomycin, and quinacrine.

agents exert different mechanisms of action, for example, kinase inhibition, inhibition of DNA transcription activity, manipulation of tubulin dynamics, and DNA-targeting compounds. DNA is one of the most viable and successful targets for anticancer chemotherapy as seen by the approval of many DNA-targeting anticancer drugs,[2] for example, bleomycin, doxorubicin, mephalan, and cyclophosphamide (Figure 1).

Herein, we have developed DNA-targeting anticancer agents that can interfere with the DNA base-pairing system through intermolecular interactions. This mechanism causes conformational changes in the DNA structure and often disrupts the recognition of DNA-binding proteins such as DNA polymerases, transcription factors, and topoisomerases.[3] Furthermore, intercalation is one of the most important mechanisms of action exhibited by DNA-targeting drugs, which are classified as DNA-intercalating agents. The mechanism of action relies on the flat planar structure of the drug, which can be inserted between DNA base pairs. The stability of the complex is considerably influenced by van der Waal's forces, hydrophobic interactions, π–π stacking interactions, and electrostatic forces.[4] Various well-known compounds, such as ethidium bromide, daunomycin, and quinacrine, interact with DNA through intercalation (Figure 2).

Acridone (**1**) is a flat planar heterocyclic scaffold consisting of three *ortho*-fused aromatic rings with a keto group and a nitrogen atom at the 9- and 10-positions, respectively. The flat planar shape allows acridone to intercalate between DNA bases disrupting the intermolecular bonding networks of the DNA molecule. Hence, acridone-based compounds are regarded to have chemotherapeutic potential. Numerous naturally occurring acridone alkaloids have been isolated and studied for their biological activities, including antiproliferative,[5–7] antiviral,[8,9] and antiparasitic[10] activities. Some well-recognized naturally occurring cytotoxic acridone-based compounds include acronycine and glyfoline (Figure 3). These compounds have been studied and structurally modified to improve their potency and solubility for clinical applications as anticancer drug candidates.[11–13]

Recognizing the potential of acridone-based compounds as DNA-intercalating chemotherapeutic agents, we focused our investigations on developing qualitative structure-activity relationships (SARs) by means of synthetic structural modifications and experimentally testing for the cytotoxic activities of the derivatives in vitro against four cancer cell lines: acute lymphoblastic leukemia (Molt-3),

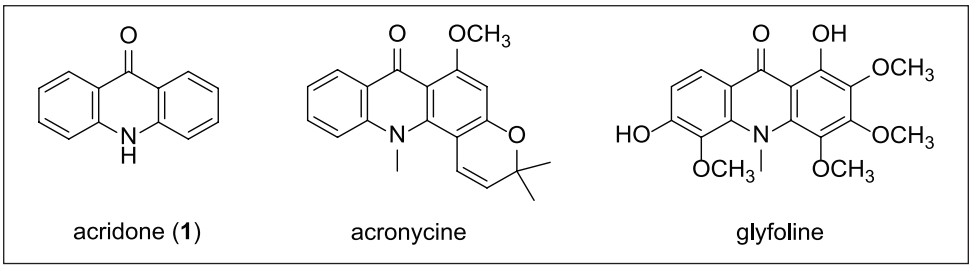
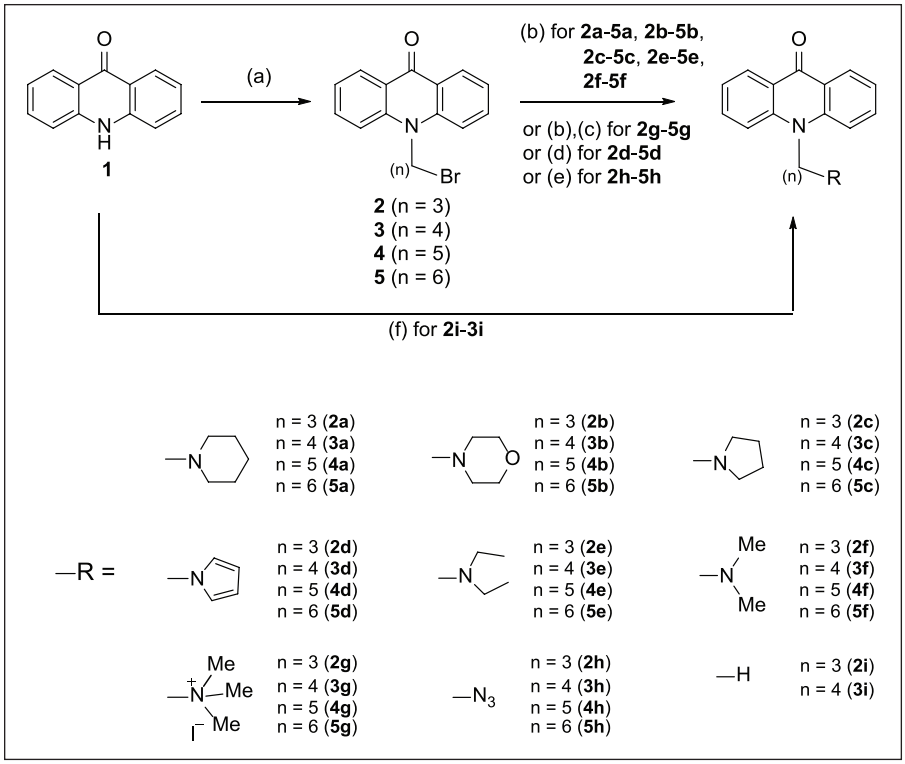


Figure 3. Chemical structures of acridone (**1**) and the naturally occurring acridone-based cytotoxic compounds acronycine and glyfoline.



Scheme 1. (a) NaH, DMF, dibromoalkane, 80 °C, 0.5 h; (b) amine, CH_3CN, rt, 24 h; (c) MeI, CH_2Cl_2, rt, 18 h; (d) NaOH, DMSO, pyrrole, rt, 2 h; (e) NaN_3 (0.5 M) in DMSO, rt, 1 h; (f) NaH, DMF, PrI (for **2i**), or BuBr (for **3i**), 80 °C, 0.5 h.

hepatocarcinoma (HepG2), lung carcinoma (A549), and cholangiocarcinoma (HuCCA-1). Inspired by the structure of quinacrine, we introduced various basic side chains to the core structure to enhance the electrostatic binding of acridone to the DNA target. The structures of the derivatives comprise an acridone core scaffold, which can form π–π interactions with the DNA base pair and the basic side chain at the C2-terminus, creating an electrostatic interaction with the negative phosphate backbone of DNA. Furthermore, molecular modeling was employed to understand the basic binding conformations of the compounds and interactions with the DNA target.

Results and discussion

Synthesis and cytotoxicity of N^{10}-substituted acridones

Acridone (**1**) was shown to be almost inactive against all the four tested cell lines, despite its planar structure (data from our preliminary cytotoxicity screening). This finding may partly be due to its structural simplicity and lack of functionalities that can allow extensive interactions with DNA. We modified the SARs between acridone and the target molecules by adding alkyl amines with varying chain lengths and different terminal nitrogen-based moieties to the N^{10}-position of the acridone tricyclic core via a two-step reaction, as shown in Scheme 1.

Commercially available acridone (**1**) was used as the starting material for the *N*-alkylations (Scheme 1). A strong base was required for deprotonation to achieve *N*-alkylation due to the weak basic nature of the nitrogen in acridone. Using sodium hydride as the base, acridone could be alkylated with different dibromoalkanes in dimethylformamide at 80 °C to afford the corresponding N^{10}-bromoalkyl acridones (**2**–**5**) in acceptable yields. An *O*-alkylated compound was formed as a by-product accompanied with the *N*-alkylated product but was unstable and readily transformed into acridone (**1**) under mild acidic conditions. The number of methylenes in the alkyl

chain affected the alkylation reaction. Under the same conditions, alkylation reactions with butyl to hexyl chain spacers were accomplished with acceptable yields. Other undesired side products were obtained from the alkylation of acridone with dibromopropane, whereas alkylation with dibromoethane did not occur. The resulting bromoalkyl acridones **2**–**5** underwent nucleophilic substitution with various nitrogen-containing moieties to yield N^{10}-substituted acridones bearing an alkyl side chain with a nitrogen atom at the terminal position. The first set of acridone derivatives was synthesized with varying alkyl chain lengths and terminal nitrogen-based moieties as side chains (Scheme 1). The synthesized compounds were evaluated for their cytotoxic activities and the results are summarized in Table 1.

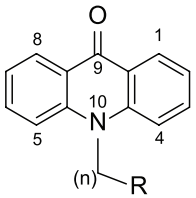
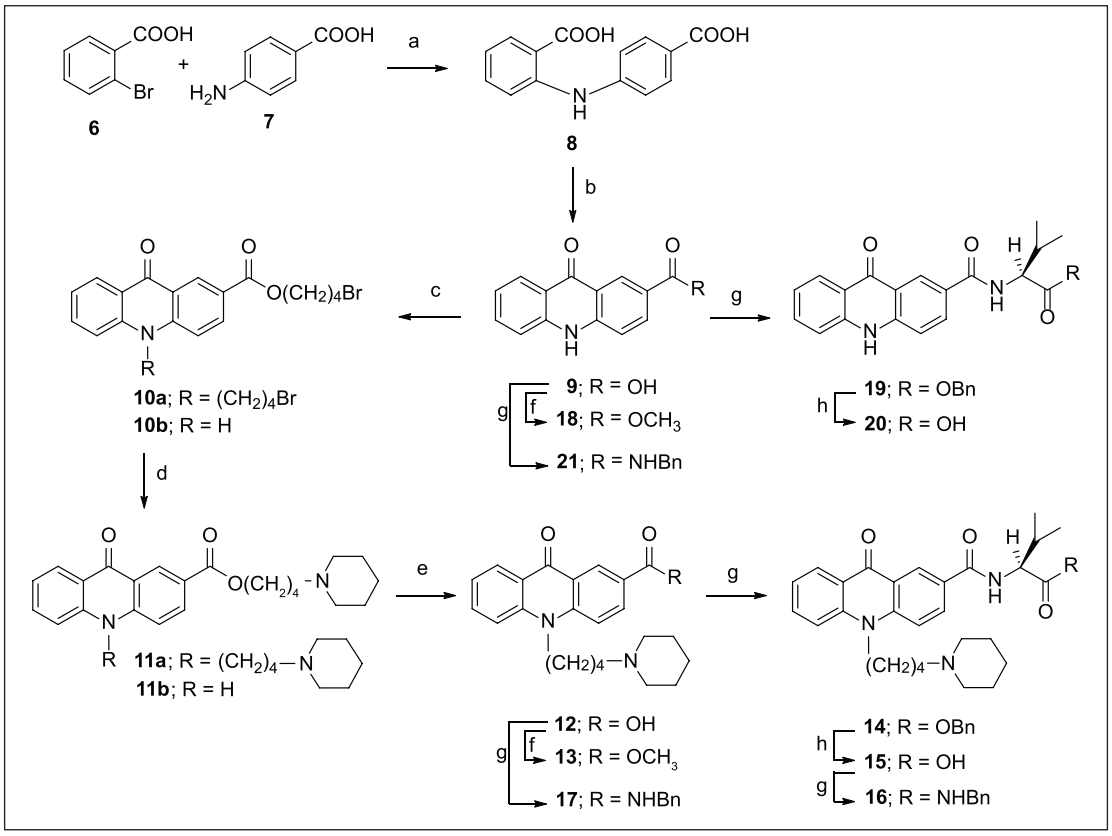


Table 1. Cytotoxic activities and the Moriguchi octanol-water partition coefficient (MlogP) values of N^{10}-substituted acridone derivatives.

Compound	R	n	IC_{50} (μM)[a]				ΔG (kcal mol^{-1})	MlogP
			Molt-3	HepG2	A549	HuCCA-1		
Acridone		0	Inactive[b]	15.5 ± 6.7	Inactive	Inactive	−6.6	2.44
2a		3	11.1 ± 3.9	17.2 ± 4.6	43.3 ± 3.9	61.6 ± 12.2	−7.3	3.14
3a		4	14.8 ± 2.5	14.2 ± 3.3	38.9 ± 5.1	56.9 ± 2.8	−7.4	3.36
4a		5	14.6 ± 2.5	12.0 ± 3.6	41.1 ± 10.6	58.5 ± 0.6	−7.5	3.58
5a		6	19.9 ± 1.2	14.9 ± 5.7	44.2 ± 7.0	64.0 ± 1.4	−7.0	3.79
2b		3	43.9 ± 4.7	Inactive	Inactive	Inactive	−7.3	2.11
3b		4	22.8 ± 0.2	38.7 ± 5.2	67.0 ± 6.3	103.1 ± 2.7	−7.5	2.34
4b		5	16.6 ± 2.6	42.9 ± 0.0	77.1 ± 8.1	97.1 ± 1.4	−7.2	2.55
5b		6	18.1 ± 0.6	27.5 ± 5.9	57.0 ± 0.6	93.1 ± 3.1	−7.2	2.77
2c		3	14.9 ± 3.6	16.3 ± 3.5	40.9 ± 5.3	63.1 ± 5.5	−7.0	2.92
3c		4	16.9 ± 3.0	16.7 ± 3.6	38.1 ± 6.6	55.9 ± 6.6	−6.9	3.14
4c		5	14.8 ± 3.6	19.8 ± 4.5	52.1 ± 6.8	68.3 ± 5.7	−6.8	3.36
5c		6	16.9 ± 3.1	18.2 ± 4.4	49.3 ± 1.8	60.1 ± 7.7	−6.8	3.58
2d		3	19.2 ± 2.4	Inactive	Inactive	Inactive	−7.0	2.77
3d		4	26.5 ± 5.2	69.6 ± 0.0	Inactive	Inactive	−6.7	2.99
4d		5	25.7 ± 5.1	53.0 ± 6.4	Inactive	Inactive	−6.8	3.21
5d		6	23.8 ± 0.3	65.9 ± 11.7	Inactive	Inactive	−6.9	3.42
2e		3	17.2 ± 6.3	11.6 ± 1.3	33.1 ± 3.4	69.5 ± 2.1	−6.3	3.31
3e		4	21.0 ± 0.9	14.0 ± 2.7	42.7 ± 5.1	74.2 ± 16.2	−6.3	3.54
4e		5	21.8 ± 0.6	17.5 ± 5.2	49.9 ± 3.0	68.4 ± 8.4	−6.5	3.75
5e		6	23.5 ± 3.7	17.5 ± 7.0	54.0 ± 1.2	80.0 ± 1.8	−6.1	3.97
2f		3	14.1 ± 0.2	15.6 ± 2.7	35.7 ± 4.7	50.0 ± 6.8	−6.2	2.85
3f		4	15.6 ± 3.7	21.0 ± 5.2	64.0 ± 1.9	87.8 ± 13.2	−6.8	3.09
4f		5	13.6 ± 1.2	20.6 ± 5.0	61.7 ± 1.2	73.7 ± 7.8	−6.6	3.31
5f		6	12.2 ± 3.1	18.6 ± 6.2	51.7 ± 2.8	62.9 ± 3.3	−6.4	3.54
2g		3	100.8 ± 6.6	Inactive	Inactive	Inactive	−6.7	−0.60
3g		4	38.7 ± 9.6	Inactive	Inactive	Inactive	−6.8	−0.37
4g		5	80.2 ± 29.9	Inactive	Inactive	Inactive	−6.7	−0.15
5g		6	inactive	Inactive	Inactive	Inactive	−6.7	0.07
2h	N_3	3	27.2 ± 4.5	98.9 ± 10.4	Inactive	Inactive	−6.9	2.25
3h		4	34.1 ± 11.1	79.6 ± 9.4	157.5 ± 9.7	Inactive	−6.6	2.49
4h		5	33.5 ± 11.8	83.9 ± 13.2	150.3 ± 9.2	150.3 ± 18.5	−6.8	2.73
5h		6	24.9 ± 5.4	63.3 ± 12.6	135.9 ± 11.0	Inactive	−6.7	2.96
2i	H	3	31.8 ± 0.5	80.2 ± 14.2	192.8 ± 5.4	173.0 ± 1.5	−6.2	3.21
3i		4	44.2 ± 2.6	95.6 ± 6.9	Inactive	Inactive	−6.1	3.45
Doxorubicin	–	–	ND[c]	0.4 ± 0.2	0.2 ± 0.0	0.9 ± 0.7	−9.2	−0.82

[a]Results are expressed as mean ± standard error; average of three independent experiments.
[b]$IC_{50} \geqslant 50\,\mu g\,mL^{-1}$.
[c]Not determined.



Scheme 2. (a) Cu, K_2CO_3, DMF, reflux, 4 h; (b) Conc. H_2SO_4, 100 °C, 4 h; (c) NaH, DMF, dibromobutane, 80 °C, 1 h; (d) piperidine, CH_3CN, rt, 24 h; (e) 5% NaOH, EtOH, reflux, 1 h; (f) $SOCl_2$, reflux, 3 h then cold MeOH; (g) amino acid or amine, HOBt, EDCI, DIPEA, DMF, rt, 24 h; (h) H_2, 10% Pd/C, MeOH, rt, 24 h.

Table 1 shows that the introduction of the side chains, particularly tertiary amine, to the N^{10}-position of the parent acridone (**1**) led to cytotoxicity against the tested cell lines. Acridones with alkyl piperidine **2a**–**5a**, alkyl morpholine **2b**–**5b**, alkyl pyrrolidine **2c**–**5c**, alkyl diethylamine **2e**–**5e**, and alkyl dimethylamine **2f**–**5f** demonstrated improved cytotoxicity, whereas acridones with alkyl pyrrole **2d**–**5d**, alkyl azide **2h**–**5h**, and alkyl ammonium salts **2g**–**5g** were inactive or displayed modest activity. These results are presumably due to their poor ability to interact with DNA. Varying the chain length from three to six carbons resulted in slight differences in the cytotoxicities. Among the tested cell lines, Molt-3 was the most sensitive against alkyl piperidines **2a**–**5a**, alkyl pyrrolidines **2c**–**5c**, alkyl diethylamines **2e**–**5e**, and alkyl dimethylamines **2f**–**5f**, with comparable IC_{50} values ranging from 11 to 23 μM. For the HepG2 cell line, acridones bearing alkyl piperidine **2a**–**5a**, alkyl pyrrolidine **2c**–**5c**, and alkyl diethylamine **2e**–**5e** units displayed cytotoxicities within 12−20 μM, which were comparable with that of unsubstituted acridone (**1**). For the A549 cell line, acridones containing alkyl piperidine **2a**–**5a** exhibited the highest improvement in cytotoxicity, with IC_{50} values ranging from 39 to 44 μM. For the HuCCA-1 cell line, acridones with alkyl piperidine **2a**–**5a** and alkyl pyrrolidine **2c**–**5c** substituents showed the highest improvement in cytotoxicity, with comparable IC_{50} values ranging from 56 to 68 μM. Hence, introducing an alkyl piperidine at N^{10} of acridone significantly improved its cytotoxicity compared with alkyl morpholine. Alkyl piperidines ($pK_a = 11.22$) had a higher level of positive-charged forms at physiological pH compared to alkyl morpholines ($pK_a = 8.36$) because of the higher basicity of the former. The high extent of electrostatic interactions imposed by alkyl piperidine derivatives contributed to the lower IC_{50} values compared with those of alkyl morpholines. The slightly higher pK_a values of alkyl pyrrolidines (e.g. pK_a 11.27) compared with those of alkyl piperidines (e.g. pK_a 11.22) resulted in slightly lower cytotoxicities. This phenomenon could be due to the higher level of positive-charged forms of alkyl pyrrolidine acridones, as reflected in the lower Moriguchi partition coefficients (MlogP), thereby preventing them from entering into the cell. It is noted that MlogP was calculated with nitrogen atoms negatively contributing to the parameter.[14] Despite the low value of MlogP calculated from doxorubicin caused by the negative contribution of the nitrogen and an oxygen atoms, this drug is actually carried into cells by organic anion transporting peptide 1A/1B (OATP1A/1B).[15] Among alkyl piperidine derivatives **2a**–**5a**, butyl and pentyl piperidines (**3a** and **4a**, respectively) were equally effective against the tested cell lines. However, the cytotoxicity was significantly compromised after lengthening the alkyl chain by adding six carbons. This finding could be due to the higher degree of freedom of the acridone moiety to escape from base-pair stacking.

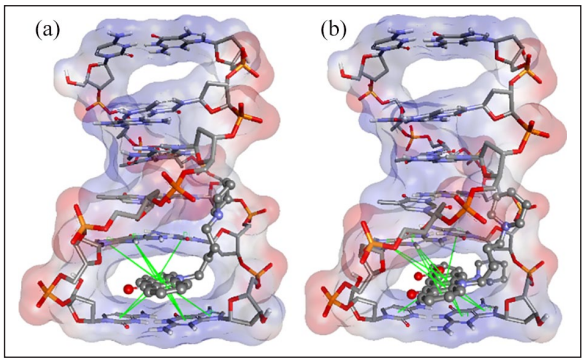


Figure 4. Binding conformation of N^{10}-substituted acridone derivative **3a** (a) and C2,N^{10}-substituted acridone derivative **11a** (b). The DNA fragment is represented as sticks, and ligands are represented as balls and sticks. The DNA surface has been rendered. Blue, red, and gray surfaces represent partial positive, negative, and neutral charges, respectively. Green solid lines are π–π stacking interactions and red dotted lines depict hydrogen bonding.

Introducing a butyl piperidine to the N^{10} of acridone led to the highest cytotoxicity for compound **3a**. This finding encouraged us to improve the cytotoxicity of **3a**. The addition of a butyl piperidine side chain to the aromatic ring of **3a** was aimed toward enhancing the cytotoxicity by increasing the binding interaction to the negative groove of DNA. Evidence suggests that conjugating an amino acid to compounds of interest can improve their bioavailability in tissues.[16] Therefore, acridone **3a** was conjugated to an amino acid in an effort to enhance the cytotoxicity. A carboxyl group was used to connect the butyl piperidine side chain or amino acid to the aromatic ring of acridone **3a**. Hence, acridone-2-carboxylic acid was prepared and used as a precursor for the synthesis of 2,N^{10}-substituted acridones.

Synthesis and cytotoxicity of 2,N^{10}-substituted acridones

The precursor, acridone-2-carboxylic acid (**9**), was prepared via two steps (Scheme 2) to obtain key intermediates for synthesis of 2,N^{10}-substituted compounds. The Ullmann condensation of 2-bromobenzoic acid (**6**) with 4-aminobenzoic acid (**7**) in the presence of copper and potassium carbonate in refluxing *N,N*-dimethylformamide yielded diphenylamine-2,4′-dicarboxylic acid (**8**).[17] Treatment of **8** with concentrated sulfuric acid formed the desired acridone-2-carboxylic acid (**9**). A butyl piperidine side chain was incorporated into **9** through the previously described method. The use of NaH and 1,4-dibromobutane led to the generation of *N,O*- and *O*-alkylated products (**10a** and **10b**, respectively). Stirring the resulting bromobutyl acridone **10a** with piperidine in acetonitrile at room temperature yielded acridone **11a** containing two butyl piperidine side chains. Reaction under the same conditions with **10b** provided **11b**. The basic hydrolysis of **11a** afforded acridone **12** with a free carboxylic acid. Simple methyl ester **13** was prepared to investigate the cytotoxicity. The conjugation of **12** with an amino acid was aimed toward enhancing the cytotoxicity. The amino acid, L-valine benzyl ester, was conjugated to acridone **12** by using 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) to obtain acridone **14** in good yield. The benzyl ester group of **14** was removed by hydrogenation to form acridone **15**. A benzyl amide protecting group was incorporated to yield acridone **16**. The simple benzyl amide of **12** (compound **17**) was also prepared to investigate its cytotoxicity. Compounds **11a** and **12**–**16** were subjected to cytotoxic testing. The corresponding molecules of these compounds without the N^{10}-butyl piperidine side chain were also prepared by following similar procedures and were also subjected to cytotoxic testing (compounds **18**, **19**, **20**, and **21**). The IC_{50} values of the substituted acridone derivatives and those of **3a** are summarized in Table 2.

Considering C2-derivatized **3a**, the presence of a polar carboxyl moiety at C2 in compound **12** led to it being inactive in cytotoxicity studies. The inactivity of **12** could be due to the high hydrophilicity, as shown by the low partition coefficient (MlogP), thereby prohibiting cellular uptake. Decreasing the polarity of the compounds might increase their cytotoxicity. Hence, the carboxyl group of **12** was converted into a butyl piperidine ester (compound **11a**), a methyl ester (compound **13**), and a benzyl amide (compound **17**), which greatly improved their activities. The conjugation of **12** with L-valine amino acid led to compound **15**, in which the presence of the carboxyl group resulted in inactivity. Therefore, the uptake of acridone derivatives was not facilitated by amino acid transporting proteins but by passive diffusion. To confirm the above argument, the free acid was protected as a benzyl ester (compound **14**) and a benzyl amide (compound **17**), resulting in the recovery of the activity. When the R group was the same, for example, compound **11a**, with the N^{10}-butyl piperidine side chain, exhibited higher cytotoxic activity compared to **11b** without the N^{10}-attached group. This finding indicates the crucial role of the N^{10}-butyl piperidine side chain in the cytotoxicity of acridone. Introducing a butyl piperidine ester, a methyl ester, a benzyl amide, a L-valine benzyl ester, and a L-valine benzyl amide at the C2-position of **3a** increased the cytotoxicity. Compound **11a**, which was obtained from the addition of butyl piperidine ester at the C2-position of **3a**, exhibited the highest improvement in cytotoxicity against the tested cell lines. The cytotoxicity of **11a** was improved by 4.5-, 4.8-, 7.5- and 6-fold against Molt-3, HepG2, A549, and HuCCA-1, respectively, compared with **3a**.

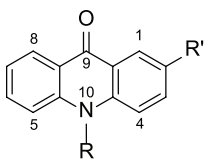
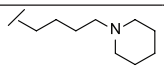
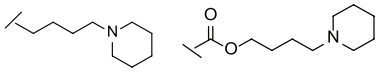
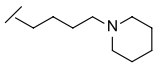
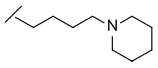
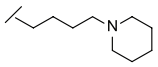
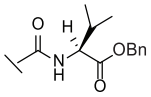
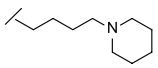
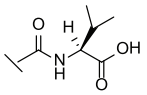
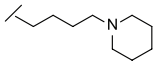
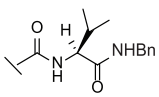
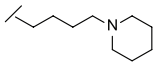
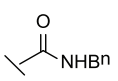
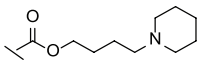
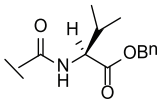
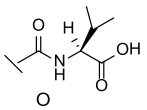
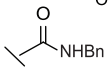


Table 2. Cytotoxic activities and MlogP values of the acridone derivatives substituted at C-2 and/or the N^{10}-position.

Compound	R	R′	IC_{50} (μM)[a]				ΔG (kcal mol^{-1})	MlogP
			Molt-3	HepG2	A549	HuCCA-1		
3a		H	14.8 ± 2.5	14.2 ± 3.3	38.9 ± 5.1	56.9 ± 2.8	−7.4	3.36
11a			3.3 ± 0.5	3.0 ± 0.5	5.2 ± 0.5	9.5 ± 0.3	−7.8	4.00
12		COOH	Inactive[b]	Inactive	Inactive	Inactive	−7.7	2.98
13		COOMe	3.6 ± 0.4	4.3 ± 1.5	10.8 ± 1.9	25.6 ± 2.5	−7.8	3.20
14			6.1 ± 0.2	7.0 ± 0.5	8.9 ± 0.9	9.4 ± 0.3	−7.9	3.99
15			Inactive	Inactive	Inactive	Inactive	−6.7	2.81
16			9.6 ± 1.9	35.3 ± 3.5	19.3 ± 3.2	20.9 ± 3.3	−7.8	3.59
17			8.2 ± 0.6	2.0 ± 0.2	7.5 ± 1.1	9.9 ± 3.3	−8.7	3.84
9	H	COOH	Inactive	Inactive	Inactive	Inactive	−7.0	2.06
11b	H		12.4 ± 0.7	18.9 ± 7.5	20.2 ± 2.0	25.9 ± 1.8	−7.5	2.98
18	H	COOMe	43.9 ± 12.2	5.9 ± 3.4	11.1 ± 0.4	12.1 ± 2.8	−6.9	2.32
19	H		6.9 ± 1.0	13.8 ± 3.5	16.7 ± 3.1	22.5 ± 1.3	−8.3	3.40
20	H		Inactive	Inactive	Inactive	Inactive	−7.6	2.05
21	H		Inactive	Inactive	105.7 ± 11.5	Inactive	−8.3	3.15
Doxorubicin	–	–	ND[c]	0.7 ± 0.1	0.1 ± 0.0	0.5 ± 0.4	−9.2	−0.82
3′-desamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin			ND	ND	ND	ND	−9.4	ND

[a]Results are expressed as mean ± standard error; average of three independent experiments.
[b]$IC_{50} \geqslant 50\ \mu g\ mL^{-1}$.
[c]Not determined.

Molecular modeling of 2,N^{10}-substituted acridones

Molecular modeling was conducted using the AutoDock Vina 1.1.2 software suite[18] to elucidate the binding modes and binding energies of the acridone derivatives. A double-stranded DNA crystal structure was obtained from the Protein Data Bank (PDB code: 2DES)[19,20] and was used as the docking scaffold. The native co-crystallized ligand, 3′-desamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin, was removed and then docked again to the DNA crystal structure. The docked conformation and the co-crystallized conformation were superimposed (Figure S1 in the Supplementary Information) and showed similar orientations, which means that our method can be used further for all the derivatives.

All the derivatives were docked to the DNA crystal structure and the binding energies measured as ΔG values are shown in Tables 1 and 2. The docking results revealed that the

positive control, doxorubicin, had the lowest $\Delta G = -9.2\,kcal\,mol^{-1}$, suggesting that this compound binds very tightly, as reflected in the very low value of IC_{50} (<1 μM). The ΔG values of the N^{10}-substituted acridone derivatives tended to be more positive with ΔG values ranging between −6.1 and $-7.5\,kcal\,mol^{-1}$ compared to the more cytotoxic C2,N^{10}-substituted acridone derivatives with ΔG values ranging between −6.7 and $-9.1\,kcal\,mol^{-1}$, in congruence with the cytotoxicity results. Depicted in Figure 4(a), the tricyclic acridone core is inserted and stabilized by π–π stacking with the base pairs in DNA, thereby perturbing DNA base-pairing interactions. The N^{10}-alkyl side chains (Figures 4(a) and (b)) interact with the DNA minor groove, most probably through van der Waal's interactions, and the nitrogen-based moieties could potentially form electrostatic attractions with the phosphate backbones. For the C2,N^{10}-substituted acridone derivatives (Figure 4(b)), the C2-substituted ester is predicted to interact with the DNA major groove.

Conclusion

The introduction of two butyl piperidine side chains at the C2- and N^{10}-positions of acridone (compound **11a**) could enhance the cytotoxicity of acridine-based compounds. The optimal pK_a (piperidinyl moiety) improved the ability of the derivative to bind to the DNA phosphate backbone and enter the cell compared with the other derivatives, that is, morpholine-, pyrrolidine-, pyrrole-, 3° and 4° amines and azide-substituted compounds. The substitution of the butyl piperidine side chain at the C2-position could provide tight binding of the compound to DNA, as supported by the increased binding energy from the computational calculations. The incorporation of two butyl piperidines at the C2- and N^{10}-positions of acridone did not affect the cellular uptake of the compound. In addition, the positive-charged piperidinium moieties could electrostatically bind to the DNA phosphate backbone, facilitating the tight binding DNA. As a result, compound **11a** showed the greatest enhanced DNA intercalation.

Experimental

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Bruker AVANCE 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers. Chemical shifts are reported as δ values in ppm relative to tetramethylsilane. Mass spectra were obtained on a Finnigan Polaris GCQ mass spectrometer, while accurate masses (HRMS) were obtained using a Bruker Micro TOF in APCI or ESI positive mode. Infrared (IR) spectra were recorded in terms of cm^{-1} on a Perkin Elmer Spectrum One FTIR spectrometer. Melting points were determined on an SMP3 melting point apparatus. Column chromatography analysis was performed on Merck silica gel 60 (70–230 mesh).

General procedure for the preparation of bromoalkyl acridones 2–5

A solution of acridone (1 mmol) in *N,N*-dimethylformamide (10 mL) was added to a stirred solution of sodium hydride (3 mmol) in *N,N*-dimethylformamide (10 mL). The mixture was heated at 80 °C for 15 min, treated with the appropriate dibromoalkane (6 mmol) and heated at 80 °C for an additional 0.5 h. The reaction mixture was cooled, water was added, and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography.

10-(3′-N-Bromopropyl)acridone (***2***):[21] (32%) yellow solid, m.p. 113.5 °C–114.5 °C; FTIR, ν_{max} (cm^{-1}): 1631, 1596, 1489, 1461, 1377, 1177, 752, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.68–7.63 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.22 (t, *J* = 7.5 Hz, 2H), 4.48 (t, *J* = 7.7 Hz, 2H), 3.56 (t, *J* = 6.0 Hz, 2H), 2.35–2.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 134.1 (2C), 128.1 (2C), 122.5 (2C), 121.4 (2C), 114.2 (2C), 44.5, 30.2, 29.4; MS (EI): *m/z* (relative intensity): 315 (M^+, 20), 208 (100), 180 (26), 152 (13); HRMS (APCI): *m/z* calcd for $C_{16}H_{15}NOBr$ $[M + H]^+$: 316.0332; found: 316.0339.

10-(4′-N-Bromobutyl)acridone (***3***):[22] (57%) yellow solid, m.p. 145.5 °C–146.5 °C; FTIR, ν_{max} (cm^{-1}): 1631, 1601, 1492, 1461, 1264, 1176, 732, 704; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.75–7.68 (m, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.28 (t, *J* = 7.7 Hz, 2H), 4.34 (t, *J* = 7.7 Hz, 2H), 3.52 (t, *J* = 6.5 Hz, 2H), 2.05–2.15 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 134.0 (2C), 128.0 (2C), 122.4 (2C), 121.3 (2C), 114.4 (2C), 45.1, 32.7, 29.7, 25.7; MS (EI): *m/z* (relative intensity): 329 (M^+, 23), 208 (100), 180 (21); HRMS (APCI): *m/z* calcd for $C_{17}H_{17}NOBr$ $[M + H]^+$: 330.0488; found: 330.0491.

10-(5′-N-Bromopentyl)acridone (***4***): (75%) yellow solid, m.p. 114.5 °C–115.0 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1597, 1490, 1460, 1377, 1290, 1264, 1176, 753, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.78–7.68 (m, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 4.35 (t, *J* = 8.2 Hz, 2H), 3.47 (t, *J* = 6.5 Hz, 2H), 2.05–1.91 (m, 4H), 1.78–1.68 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 133.9 (2C), 128.0 (2C), 122.5 (2C), 121.3 (2C), 114.4 (2C), 45.8, 33.3, 32.2, 26.3, 25.4; MS (EI): *m/z* (relative intensity): 343 (M^+, 27), 208 (100), 180 (17); HRMS (APCI): *m/z* calcd for $C_{18}H_{19}NOBr$ $[M + H]^+$: 344.0644; found: 344.0647.

2.2.4 *10-(6′-N-Bromohexyl)acridone* (***5***): (70%) yellow solid, m.p. 103.0 °C–104.0 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1597, 1490, 1460, 1377, 1290, 1264, 1176, 753, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.74–7.67 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 4.36 (t, *J* = 8.3 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.05–1.90 (m, 4H), 1.75–1.55 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.9 (2C), 128.0 (2C), 122.4 (2C), 121.2 (2C), 114.4 (2C), 45.9, 33.5, 32.5, 27.8, 27.0,

26.1; MS (EI): *m/z* (relative intensity): 357 (M^+, 33), 208 (100), 195 (19), 180 (13); HRMS (APCI): *m/z* calcd for $C_{19}H_{21}NOBr$ $[M + H]^+$: 358.0801; found: 358.0795.

General procedure for the preparation of N^{10}-substituted acridones

Preparation of compounds 2a–5a, 2b–5b, 2c–5c, 2e–5e, and 2f–5f. Amine (5 mmol) was added to a stirred solution of bromoalkyl acridone (1 mmol) in acetonitrile (25 mL). The reaction mixture was stirred at room temperature for 24 h and concentrated under reduced pressure. The residue was treated with water and extracted with dichloromethane. The combined organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography.

Preparation of compounds 2d–5d. Pyrrole (3 mmol) was added to a mixture of sodium hydroxide (9 mmol) in dimethyl sulfoxide (5 mL) and the mixture was stirred at room temperature for 30 min. After cooling in an ice bath, the mixture was treated with a solution of bromoalkyl acridone (1 mmol) in dimethyl sulfoxide (15 mL) and then stirred at room temperature for 2 h. Water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography.

Preparation of compounds 2g–5g. A solution of **2f**–**5f** (1 mmol) in dichloromethane (10 mL) was treated with iodomethane (5 mmol) and the mixture was stirred under argon at room temperature for 12 h. The precipitate was collected, washed with dichloromethane, and dried to yield trimethylammonium salts **2g**–**5g**.

Preparation of compounds 2h–5h. A solution of bromoalkyl acridone (1 mmol) in dimethyl sulfoxide (3 mL) was treated with sodium azide (0.5 M) in dimethyl sulfoxide (3 mL, 1.5 mmol) and the mixture was stirred at room temperature for 3 h. Water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography.

Preparation of compounds 2i and 3i. A solution of acridone (1 mmol) in *N,N*-dimethylformamide (10 mL) was added to a stirred suspension of sodium hydride (3 mmol) in *N,N*-dimethylformamide (10 mL). The mixture was heated at 80 °C for 15 min, treated with propyl iodide (for **2i**) or 1-bromobutane (for **3i**) (5 mmol), and heated at 80 °C for an additional 0.5 h. The reaction mixture was cooled and then water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography.

*10-(3′-N-Piperidinopropyl)acridone (**2a**)*:[23] (67%) yellow solid, m.p. 135.0 °C−136.0 °C; FTIR, ν_{max} (cm^{-1}): 1634, 1598, 1489, 1462, 1377, 1262, 1177, 753, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.80−7.60 (m, 4H), 7.29 (t, *J* = 7.6 Hz, 2H), 4.47 (t, *J* = 8.4 Hz, 2H), 2.55−2.40 (m, 6H), 2.10−2.00 (m, 2H), 1.75−1.60 (m, 4H), 1.60−1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.0, 141.9 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.8 (2C), 55.7, 54.8 (2C), 44.1, 26.1 (2C), 24.8, 24.4; MS (EI): *m/z* (relative intensity): 320 (M^+, 8), 208 (4), 180 (4), 124 (10), 98 (100); HRMS (APCI): *m/z* calcd for $C_{21}H_{25}N_2O$ $[M + H]^+$: 321.1961; found: 321.1954.

*10-(4′-N-Piperidinobutyl)acridone (**3a**)*:[23] (96%) yellow solid, m.p. 115.5 °C−116.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1598, 1490, 1461, 1377, 1264, 1177, 752, 732, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.74−7.60 (m, 4H), 7.31−7.25 (m, 2H), 4.37 (t, *J* = 7.6 Hz, 2H), 2.50−2.40 (m, 6H), 2.00−1.91 (m, 2H), 1.80−1.70 (m, 2H), 1.70−1.60 (m, 4H), 1.60−1.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 141.7 (2C), 133.8 (2C), 127.8 (2C), 122.4 (2C), 121.1 (2C), 114.8 (2C), 57.9, 54.6 (2C), 45.9, 26.1 (2C), 24.6, 24.5, 23.6; MS (EI): *m/z* (relative intensity): 334 (M^+, 44), 208 (10), 138 (21), 98 (100); HRMS (APCI): *m/z* calcd for $C_{22}H_{27}N_2O$ $[M + H]^+$: 335.2118; found: 335.2125.

*10-(5′-N-Piperidinopentyl)acridone (**4a**)*: (77%) yellow solid, m.p. 121.5 °C−122.5 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1597, 1490, 1460, 1376, 1290, 1262, 1176, 753, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.74−7.68 (m, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 2H), 4.32 (t, *J* = 8.4 Hz, 2H), 2.38−2.31 (m, 6H), 2.00−1.89 (m, 2H), 1.70−1.44 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.5 (2C), 59.2, 54.7 (2C), 46.1, 27.0, 26.7, 26.0 (2C), 25.0, 24.4; MS (EI): *m/z* (relative intensity): 348 (M^+, 30), 208 (13), 154 (14), 98 (100); HRMS (APCI): *m/z* calcd for $C_{23}H_{29}N_2O$ $[M + H]^+$: 349.2274; found: 349.2281.

*10-(6′-N-Piperidinohexyl)acridone (**5a**)*: (77.5%) yellow solid, m.p. 81.0 °C−82.0 °C; FTIR, ν_{max} (cm^{-1}): 1631, 1598, 1492, 1460, 1377, 1289, 1263, 1178, 756, 674; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.78−7.73 (m, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 4.36 (t, *J* = 8.0 Hz, 2H), 3.00−2.77 (m, 6H), 2.00−1.75 (m, 8H), 1.66−1.40 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 134.0 (2C), 128.0 (2C), 122.5 (2C), 121.2 (2C), 114.5 (2C), 57.9, 53.7 (2C), 45.8, 27.1, 26.8, 26.4, 24.5, 23.5 (2C), 22.7; MS (EI): *m/z* (relative intensity): 362 (M^+, 5), 208 (12), 149 (25), 98 (98), 86 (100); HRMS (APCI): *m/z* calcd for $C_{24}H_{31}N_2O$ $[M + H]^+$: 363.2431; found: 363.2438.

*10-(3′-N-Morpholinopropyl)acridone (**2b**)*:[23] (78.5%) yellow solid, m.p. 114.0 °C−114.5 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1598, 1491, 1462, 1378, 1290, 1263, 1178, 1115, 754, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.75−7.62 (m, 4H), 7.29 (t, *J* = 7.4 Hz, 2H), 4.49 (t, *J* = 7.5 Hz, 2H), 3.80 (t, *J* = 4.5 Hz, 4H), 2.55−2.47 (m, 6H),

2.12−2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.0, 141.8 (2C), 133.8 (2C), 128.0 (2C), 122.4 (2C), 121.2 (2C), 114.6 (2C), 67.0 (2C), 55.4, 53.8 (2C), 43.7, 24.3; MS (EI): *m*/*z* (relative intensity): 322 (M^+, 6), 279 (8), 222 (12), 208 (10), 180 (9), 100 (100); HRMS (APCI): *m*/*z* calcd for $C_{20}H_{23}N_2O_2$ $[M + H]^+$: 323.1754; found: 323.1750.

10-(4′-N-Morpholinobutyl)acridone (***3b***):[23] (73%) yellow solid, m.p. 143.5 °C−144.5 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1600, 1493, 1462, 1378, 1265, 1116, 731, 703; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.74−7.69 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.30−7.26 (m, 2H), 4.37 (t, *J* = 8.3 Hz, 2H), 3.78 (t, *J* = 4.8 Hz, 4H), 2.55−2.47 (m, 6H), 2.02−1.93 (m, 2H), 1.82−1.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.2 (2C), 114.6 (2C), 67.0 (2C), 57.6, 53.7 (2C), 45.8, 24.4, 23.2; MS (EI): *m*/*z* (relative intensity): 336 (M^+, 10), 293 (14), 222 (9), 208 (23), 180 (15), 142 (14), 100 (100); HRMS (APCI): *m*/*z* calcd for $C_{21}H_{25}N_2O_2$ $[M + H]^+$: 337.1911; found: 337.1908.

10-(5′-N-Morpholinopentyl)acridone (***4b***): (75%) yellow solid, m.p. 116.5 °C−117.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1597, 1490, 1460, 1376, 1290, 1261, 1176, 1116, 753, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.75−7.70 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 4.34 (t, *J* = 8.2 Hz, 2H), 3.74 (t, *J* = 4.6 Hz, 4H), 2.50−2.37 (m, 6H), 2.02−1.91 (m, 2H), 1.70−1.54 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 128.0 (2C), 122.5 (2C), 121.2 (2C), 114.4 (2C), 66.9 (2C), 58.7, 53.8 (2C), 46.0, 27.0, 26.2, 24.7; MS (EI): *m*/*z* (relative intensity): 350 (M^+, 4), 332 (9), 319 (15), 307 (19), 264 (20), 222 (18), 208 (41), 180 (20), 100 (100); HRMS (APCI): *m*/*z* calcd for $C_{22}H_{27}N_2O_2$ $[M + H]^+$: 351.2067; found: 351.2065.

10-(6′-N-Morpholinohexyl)acridone (***5b***): (77%) yellow solid, m.p. 95.5 °C−96.0 °C; FTIR, ν_{max} (cm^{-1}): 1634, 1598, 1490, 1460, 1376, 1290, 1262, 1176, 1116, 753, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.74−7.69 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.31−7.26 (m, 2H), 4.32 (t, *J* = 8.2 Hz, 2H), 3.73 (t, *J* = 4.6 Hz, 4H), 2.47–2.42 (m, 4H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.98−1.89 (m, 2H), 1.62−1.52 (m, 4H), 1.50−1.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.5 (2C), 66.9 (2C), 58.9, 53.8 (2C), 46.0, 27.2, 27.1, 26.8, 26.5; MS (EI): *m*/*z* (relative intensity): 364 (M^+, 4) 346 (23), 333 (37), 321 (32), 277 (16), 222 (18), 208 (43), 100 (100); HRMS (APCI): *m*/*z* calcd for $C_{23}H_{29}N_2O_2$ $[M + H]^+$: 365.2224; found: 365.2225.

10-(3′-N-Pyrrolidinopropyl)acridone (***2c***): (69%) yellow solid, m.p. 70.5 °C−71.5 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1598, 1490, 1461, 1377, 1290, 1263, 1177, 753, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.77−7.68 (m, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 4.50 (t, *J* = 7.7 Hz, 2H), 2.75−2.62 (m, 6H), 2.22−2.11 (m, 2H), 1.92−1.85 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.0, 141.8 (2C), 133.9 (2C), 127.9 (2C), 122.4 (2C), 121.2 (2C), 114.7 (2C), 54.2 (2C), 52.9, 43.9, 26.4, 23.5 (2C); MS (EI): *m*/*z* (relative intensity): 306 (M^+, 13), 208 (6), 180 (6), 110 (7), 84 (100); HRMS (APCI): *m*/*z* calcd for $C_{20}H_{23}N_2O$ $[M + H]^+$: 307.1805; found: 307.1819.

10-(4′-N-Pyrrolidinobutyl)acridone (***3c***): (93%) yellow solid, m.p. 108.5 °C−109.5 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1597, 1490, 1461, 1378, 1264, 1177, 752, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.73−7.68 (m, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.30−7.25 (m, 2H), 4.37 (t, *J* = 8.3 Hz, 2H), 2.62−2.53 (m, 6H), 2.03−1.94 (m, 2H), 1.86−1.73 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.7 (2C), 55.3, 54.1 (2C), 45.9, 25.7, 24.9, 23.5 (2C) ppm; MS (EI): *m*/*z* (relative intensity): 320 (M^+, 25), 208 (9), 180 (11), 124 (21), 84 (100); HRMS (APCI): *m*/*z* calcd for $C_{21}H_{25}N_2O$ $[M + H]^+$: 321.1961; found: 321.1962.

10-(5′-N-Pyrrolidinopentyl)acridone (***4c***): (73%) yellow solid, m.p. 113.0 °C−114.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1597, 1490, 1460, 1377, 1290, 1263, 1176, 753, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.75−7.70 (m, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 4.34 (t, *J* = 8.2 Hz, 2H), 2.58−2.50 (m, 6H), 2.01−1.91 (m, 2H), 1.85−1.78 (m, 4H), 1.74−1.56 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.8 (2C), 133.8 (2C), 128.0 (2C), 122.5 (2C), 121.2 (2C), 114.5 (2C), 56.3, 54.3 (2C), 46.1, 28.6, 27.1, 25.0, 23.4 (2C); MS (EI): *m*/*z* (relative intensity): 334 (M^+, 13), 208 (11), 180 (8), 140 (17), 84 (100); HRMS (APCI): *m*/*z* calcd for $C_{22}H_{27}N_2O$ $[M + H]^+$: 335.2118; found: 335.2106.

10-(6′-N-Pyrrolidinohexyl)acridone (***5c***): (71%) yellow solid, m.p. 76.0 °C−77.0 °C; FTIR, ν_{max} (cm^{-1}): 1630, 1596, 1491, 1460, 1377, 1263, 1177, 755, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.77−7.72 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 4.34 (t, *J* = 8.0 Hz, 2H), 3.08 (br s, 4H), 2.88 (t, *J* = 7.9 Hz, 2H), 2.10−1.81 (m, 8H) 1.63−1.45 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 134.0 (2C), 127.9 (2C), 122.4 (2C), 121.2 (2C), 114.5 (2C), 55.6, 53.7 (2C), 45.8, 27.0, 26.8, 26.5, 26.4, 23.3 (2C); MS (EI): *m*/*z* (relative intensity): 348 (M^+, 13), 208 (8), 180 (9), 110 (11), 84 (100); HRMS (APCI): *m*/*z* calcd for $C_{23}H_{29}N_2O$ $[M + H]^+$: 394.2274; found: 349.2283.

10-(3′-(N-Pyrrole)propyl)acridone (***2d***): (78%) yellow solid, m.p. 154.5 °C−155.5 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1598, 1491, 1461, 1291, 1264, 1177, 732, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.68−7.60 (m, 2H), 7.26 (t, *J* = 7.0 Hz, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 6.78 (t, *J* = 2.0 Hz, 2H), 6.30 (t, *J* = 2.1 Hz, 2H), 4.25 (t, *J* = 8.1 Hz, 2H), 4.15 (t, *J* = 6.2 Hz, 2H), 2.40−2.26 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.4 (2C), 134.0 (2C), 128.0 (2C), 122.4 (2C), 121.3 (2C), 120.8 (2C), 114.0 (2C), 109.1 (2C), 46.8, 43.1, 28.9; MS (EI): *m*/*z* (relative intensity): 302 (M^+, 41), 222 (62), 208 (35), 180 (20), 152 (13), 81 (100); HRMS (APCI): *m*/*z* calcd for $C_{20}H_{19}N_2O$ $[M + H]^+$: 303.1492; found: 303.1494.

10-(4′-(N-Pyrrole)butyl)acridone (***3d***): (66%) yellow solid, m.p. 145.0 °C−146.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1603, 1594, 1491, 1458, 1377, 1172, 752, 726, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, *J* = 8.0, 1.6 Hz, 2H), 7.72−7.65 (m, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.30−7.22 (m, 2H), 6.69 (t, *J* = 2.1 Hz, 2H), 6.19 (t, *J* = 2.1 Hz, 2H), 4.25 (t, *J* = 8.2 Hz, 2H), 4.00 (t, *J* = 6.8 Hz, 2H), 2.08−1.84 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 133.9

(2C), 128.0 (2C), 122.4 (2C), 121.3 (2C), 120.4 (2C), 114.4 (2C), 108.5 (2C), 49.0, 45.6, 28.6, 24.4; MS (EI): *m/z* (relative intensity): 316 (M^+, 40), 222 (23), 208 (100), 180 (31), 152 (16), 122 (49), 80 (46); HRMS (APCI): *m/z* calcd for $C_{21}H_{21}N_2O$ $[M + H]^+$: 317.1648; found: 317.1640.

10-(5′-(N-Pyrrole)pentyl)acridone (***4d***): (63%) yellow solid, m.p. 109.0 °C−110.0 °C; FTIR, ν_{max} (cm^{-1}): 1630, 1597, 1492, 1458, 1375, 1279, 1174, 749, 720, 699; ^1H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.74−7.68 (m, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.2 Hz, 2H), 6.67 (t, *J* = 2.0 Hz, 2H), 6.18 (t, *J* = 2.0 Hz, 2H), 4.29 (t, *J* = 8.2 Hz, 2H), 3.94 (t, *J* = 7.0 Hz, 2H), 1.96−1.87 (m, 4H), 1.57−1.47 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 128.0 (2C), 122.5 (2C), 121.2 (2C), 120.4 (2C), 114.4 (2C), 108.1 (2C), 49.2, 45.8, 31.2, 26.8, 24.1; MS (EI): *m/z* (relative intensity); 330 (M^+, 35), 236 (26), 222 (46), 208 (100), 195 (17), 180 (40), 149 (27), 80 (43); HRMS (APCI): *m/z* calcd for $C_{22}H_{23}N_2O$ $[M + H]^+$: 331.1805; found: 331.1804.

10-(6′-(N-Pyrrole)hexyl)acridone (***5d***): (70%) yellow solid, m.p. 82.0 °C−83.0 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1597, 1490, 1460, 1376, 1289, 1262, 1176, 753, 723, 673; ^1H NMR (300 MHz, $CDCl_3$): δ 8.56 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.73−7.76 (m, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.27 (t, *J* = 7.7 Hz, 2H), 6.65 (t, *J* = 2.1 Hz, 2H), 6.15 (t, *J* = 2.1 Hz, 2H), 4.27 (t, *J* = 8.1 Hz, 2H), 3.90 (t, *J* = 7.0 Hz, 2H), 1.94−1.77 (m, 4H), 1.58−1.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 133.9 (2C), 128.0 (2C), 122.4 (2C), 121.2 (2C), 120.4 (2C), 114.4 (2C), 108.0 (2C), 49.4, 45.9, 31.5, 27.1, 26.6, 26.5; MS (EI): *m/z* (relative intensity): 344 (M^+, 80), 264 (36), 222 (50), 208 (100), 196 (36), 180 (40), 80 (38); HRMS (APCI): *m/z* calcd for $C_{23}H_{25}N_2O$ $[M + H]^+$: 345.1961; found: 345.1952.

10-(3′-(N-Diethylamino)propyl)acridone (***2e***):[24] (69%) yellow solid, m.p. 100.5 °C−101.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1598, 1490, 1461, 1376, 1291, 1265, 1178, 752, 673; ^1H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.75−7.62 (m, 4H), 7.28 (t, *J* = 7.8 Hz, 2H), 4.45 (t, *J* = 8.0 Hz, 2H), 2.70−2.60 (m, 6H), 2.10−2.00 (m, 2H), 1.13 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.0, 141.8 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.7 (2C), 50.2, 46.9 (2C), 44.3, 25.4, 11.7 (2C); MS (EI): *m/z* (relative intensity): 308 (M^+, 9), 208 (7), 180 (6), 166 (9), 86 (100); HRMS (APCI): *m/z* calcd for $C_{20}H_{25}N_2O$ $[M + H]^+$: 309.1961; found: 309.1966.

10-(4′-(N-Diethylamino)butyl)acridone (***3e***):[24] (94%) yellow solid, m.p. 63.0 °C−64.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1598, 1490, 1461, 1378, 1290, 1264, 1177, 752, 674; ^1H NMR (400 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.74−7.69 (m, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 2H), 4.36 (t, *J* = 8.3 Hz, 2H), 2.67−2.57 (m, 6H), 2.00−1.90 (m, 2H), 1.82−1.72 (m, 2H), 1.10 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.7 (2C), 52.1, 46.6, 46.0 (2C), 24.8, 24.1, 11.3 (2C); MS (EI): *m/z* (relative intensity): 322 (M^+, 19), 208 (14), 180 (9), 112 (13), 86 (100); HRMS (APCI): *m/z* calcd for $C_{21}H_{27}N_2O$ $[M + H]^+$: 323.2118; found: 323.2107.

10-(5′-(N-Diethylamino)pentyl)acridone (***4e***):[24] (85%) yellow solid, m.p. 61.5 °C−62.5 °C; FTIR, ν_{max} (cm^{-1}): 1630, 1597, 1492, 1459, 1378, 1290, 1263, 1178, 751, 671; ^1H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, *J* = 8.1 Hz, 2H), 7.70 (t, *J* = 7.0 Hz, 2H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.26 (t, *J* = 7.5 Hz, 2H), 4.29 (t, *J* = 8.1 Hz, 2H), 2.60−2.45 (m, 6H), 2.00−1.85 (m, 2H), 1.70−1.50 (m, 4H), 1.05 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.9 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.6 (2C), 52.6, 47.0, 46.1 (2C), 27.0, 26.9, 24.9, 11.6 (2C); MS (EI): *m/z* (relative intensity): 336 (M^+, 3), 208 (5), 180 (4), 126 (6), 86 (100); HRMS (APCI): *m/z* calcd for $C_{22}H_{29}N_2O$ $[M + H]^+$: 337.2274; found: 337.2278.

10-(6′-(N-Diethylamino)hexyl)acridone (***5e***):[24] (79%) yellow solid, m.p. 150.0 °C−151.0 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1597, 1490, 1460, 1376, 1289, 1262, 1176, 754, 674; ^1H NMR (400 MHz, $CDCl_3$): δ 8.56 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.75−7.69 (m, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.28 (t, *J* = 7.7 Hz, 2H), 4.32 (t, *J* = 8.1 Hz, 2H), 2.81 (q, *J* = 7.2 Hz, 4H), 2.71−2.66 (m, 2H), 1.96−1.87 (m, 2H), 1.75−1.65 (m, 2H), 1.63−1.54 (m, 2H), 1.50−1.42 (m, 2H), 1.20 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.8, 141.6 (2C), 133.9 (2C), 127.8 (2C), 122.3 (2C), 121.1 (2C), 114.5 (2C), 52.0, 46.6 (2C), 45.9, 27.0 (2C), 26.5, 25.4, 10.1 (2C); MS (EI): *m/z* (relative intensity): 350 (M^+, 23), 335 (9), 321 (9), 208 (23), 180 (13), 86 (100); HRMS (APCI): *m/z* calcd for $C_{23}H_{31}N_2O$ $[M + H]^+$: 351.2431; found: 351.2430.

10-(3′-(N-Dimethylamino)propyl)acridone (***2f***):[25] (78%) yellow solid, m.p. 86.0 °C−86.5 °C; FTIR, ν_{max} (cm^{-1}): 1634, 1597, 1490, 1461, 1377, 1291, 1261, 1178, 753, 674; ^1H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.75−7.69 (m, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 4.46 (t, *J* = 7.7 Hz, 2H), 2.48 (t, *J* = 6.6 Hz, 2H), 2.33 (s, 6H), 2.11−2.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 141.8 (2C), 133.9 (2C), 127.9 (2C), 122.4 (2C), 121.2 (2C), 114.7 (2C), 56.4, 45.5 (2C), 43.9, 25.4; MS (EI): *m/z* (relative intensity): 280 (M^+, 100), 236 (46), 222 (51), 209 (43), 180 (31) 166 (40), 152 (28), 58 (74); HRMS (APCI): *m/z* calcd for $C_{18}H_{21}N_2O$ $[M + H]^+$: 281.1648; found: 281.1642.

10-(4′-(N-Dimethylamino)butyl)acridone (***3f***): (95%) yellow solid, m.p. 96.5 °C−97.5 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1596, 1490, 1460, 1378, 1290, 1264, 1177, 1043, 753, 674; ^1H NMR (400 MHz, $CDCl_3$): δ 8.57 (dd, *J* = 8.0, 1.5 Hz, 2H) 7.72 (m, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 2H), 4.37 (t, *J* = 8.3 Hz, 2H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.31 (s, 6H), 2.01−1.91 (m, 2H), 1.80−1.71 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.1 (2C), 114.6 (2C), 58.8, 45.9, 45.3 (2C), 24.9, 24.6; MS (EI): *m/z* (relative intensity): 294 (M^+, 5), 222 (3), 208 (3), 180 (3), 100 (4), 71 (4), 58 (100); HRMS (APCI): *m/z* calcd for $C_{19}H_{23}N_2O$ $[M + H]^+$: 295.1805; found: 295.1797.

10-(5′-(N-Dimethylamino)pentyl)acridone (***4f***): (71%) yellow solid, m.p. 65.0 °C−65.5 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1591, 1492, 1459, 1376, 1261, 1181, 1037, 754, 673; ^1H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, *J* = 8.0, 1.4 Hz,

2H), 7.73 (m, 2H), 7.49 (d, J=8.7 Hz, 2H), 7.29 (t, J=7.8 Hz, 2H), 4.34 (t, J=8.1 Hz, 2H), 2.36 (t, J=6.6 Hz, 2H), 2.28 (s, 6H), 2.01−1.91 (m, 2H), 1.68−1.55 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 128.0 (2C), 122.5 (2C), 121.1 (2C), 114.4 (2C), 59.4, 46.0, 45.4 (2C), 27.3, 27.0, 24.7; MS (EI): *m*/*z* (relative intensity): 308 (M^+, 4), 208 (3), 180 (3), 149 (4), 114 (11), 58 (100); HRMS (APCI): *m*/*z* calcd for $C_{20}H_{25}N_2O$ $[M+H]^+$: 309.1961; found: 309.1958.

10-(6′-(N-Dimethylamino)hexyl)acridone ***(5f)***: (95%) yellow solid, m.p. 102.5 °C−103.0 °C; FTIR, ν_{max} (cm^{-1}): 1629, 1594, 1494, 1461, 1380, 1291, 1265, 1179, 748, 670; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, J=8.0, 1.6 Hz, 2H), 7.72 (m, 2H), 7.48 (d, J=8.7 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 4.33 (t, J=8.0 Hz, 2H), 2.35−2.30 (m, 2H), 2.26 (s, 6H), 2.00−1.90 (m, 2H), 1.63−1.44 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.8 (2C), 128.0 (2C), 122.5 (2C), 121.1 (2C), 114.5 (2C), 59.5, 46.1, 45.3 (2C), 27.5, 27.1 (2C), 26.8; MS (EI): *m*/*z* (relative intensity): 322 (M^+, 5), 208 (4), 180 (4), 167 (6), 149 (19), 58 (100); HRMS (APCI): *m*/*z* calcd for $C_{21}H_{27}N_2O$ $[M+H]^+$: 323.2118; found: 323.2113.

10-(3′-(N-Trimethylammonium)propyl)acridone ***(2g)***: (99% from **2f**) yellow solid, m.p. 266.0 °C−267.0 °C; FTIR, ν_{max} (cm^{-1}): 1608, 1590, 1497, 1460, 1267, 1179, 1020, 759, 673; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.37 (d, J=7.9 Hz, 2H), 8.00−7.80 (m, 4H), 7.38 (t, J=7.4 Hz, 2H), 4.51 (t, J=7.7 Hz, 2H), 3.70 (t, J=7.8 Hz, 2H), 3.12 (s, 9H), 2.30−2.15 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.5, 141.3 (2C), 134.4 (2C), 126.9 (2C), 122.6 (2C), 121.5 (2C), 115.9 (2C), 62.3, 52.5 (3C), 42.2, 20.7; MS (EI): *m*/*z* (relative intensity): 280 ($[M-CH_3]^+$, 7), 208 (7), 142 (17), 127 (7), 58 (100); HRMS (ESI): *m*/*z* calcd for $C_{19}H_{23}N_2O$ $[M]^+$:295.1805; found: 295.1796.

10-(4′-(N-Trimethylammonium)butyl)acridone ***(3g)***: (87% from **3f**) yellow solid, m.p. 260.0 °C−261.0 °C; FTIR, ν_{max} (cm^{-1}): 1627, 1607, 1594, 1492, 1457, 1261, 1174, 754, 676; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (dd, J=8.1, 1.3 Hz, 2H), 7.89−7.81 (m, 4H), 7.35 (t, J=6.6 Hz, 2H), 4.51 (t, J=8.2 Hz, 2H), 3.48−3.38 (m, 2H), 3.43 (s, 9H), 2.07−1.97 (m, 2H), 1.84−1.75 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9, 141.8 (2C), 134.7 (2C), 127.3 (2C), 122.1 (2C), 121.8 (2C), 116.2 (2C), 65.5, 52.9 (3C), 45.2, 24.1, 19.9; MS (EI): *m*/*z* (relative intensity): 294 ($[M-CH_3]^+$, 4), 208 (6), 142 (13), 69 (36), 58 (100); HRMS (APCI): *m*/*z* calcd for $C_{20}H_{25}N_2O$ $[M]^+$: 309.1961; found: 309.1951.

10-(5′-(N-Trimethylammonium)pentyl)acridone ***(4g)***: (61% from **4f**) yellow solid, m.p. 263.0 °C−263.5 °C; FTIR, ν_{max} (cm^{-1}): 1627, 1607, 1596, 1494, 1459, 1378, 1265, 1178, 756, 674; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (d, J=7.8 Hz, 2H), 7.85−7.81 (m, 4H), 7.36−7.30 (m, 2H), 4.50 (f, J=7.8 Hz, 2H), 3.36−3.34 (m, 11H), 1.85−1.46 (m, 2H), 1.72−1.63 (m, 2H), 1.60−1.51 (m, 2H), 1.41−1.32 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9, 141.9 (2C), 134.7 (2C), 127.2 (2C), 122.1 (2C), 121.7 (2C), 116.4 (2C), 65.6, 52.7 (3C), 45.3, 26.9, 23.2, 22.5; MS (EI): *m*/*z* (relative intensity): 296 ($[M-27]^+$, 12), 268 (9), 185 (9), 69 (63), 55 (100); HRMS (APCI): *m*/*z* calcd for $C_{21}H_{27}N_2O$ $[M]^+$: 323.2118; found: 323.2109.

10-(6′-(N-Trimethylammonium)hexyl)acridone ***(5g)***: (99% from **5f**) yellow solid, m.p. 103.0 °C−104.0 °C; FTIR, ν_{max} (cm^{-1}): 1627, 1607, 1596, 1494, 1459, 1378, 1265, 1178, 756, 674; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (d, J=7.8 Hz, 2H), 7.85−7.81 (m, 4H), 7.36−7.30 (m, 2H), 4.48 (t, J=7.8 Hz, 2H), 3.36−3.34 (m, 11H), 1.85−1.46 (m, 2H), 1.72−1.63 (m, 2H), 1.60−1.51 (m, 2H), 1.41−1.32 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9, 141.9 (2C), 134.7 (2C), 127.2 (2C), 122.1 (2C), 121.7 (2C), 116.3 (2C), 65.8, 52.7 (3C), 45.5, 27.1, 26.1, 25.9, 22.4; MS (EI): *m*/*z* (relative intensity): 322 ($[M-CH_3]^+$, 4), 208 (9), 180 (4), 142 (13), 58 (100); HRMS (APCI): *m*/*z* calcd for $C_{22}H_{29}N_2O$ $[M]^+$: 337.2274; found: 337.2265.

10-(3′-N-Azidopropyl)acridone ***(2h)***: (89%) yellow solid, m.p. 78.5 °C−79.5 °C; FTIR, ν_{max} (cm^{-1}): 2094, 1632, 1595, 1490, 1461, 1377, 1290, 1261, 1177, 751, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, J=7.8, 2H), 7.75−7.69 (m, 2H), 7.50 (d, J=8.7 Hz, 2H), 7.31−7.24 (m, 2H), 4.44 (t, J=7.8 Hz, 2H), 3.58 (t, J=6.0 Hz, 2H), 2.19−2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.8, 141.6 (2C), 134.0 (2C), 128.1 (2C), 122.5 (2C), 121.4 (2C), 114.2 (2C), 48.9, 43.0, 26.6; MS (EI): *m*/*z* (relative intensity): 278 ($[M]^+$, 59), 250 (13), 208 (100), 195 (34), 180 (53), 166 (28); HRMS (APCI): *m*/*z* calcd for $C_{16}H_{15}N_4O$ $[M+H]^+$: 279.1240; found: 279.1238.

10-(4′-N-Azidobutyl)acridone ***(3h)***: (95%) yellow solid, m.p. 112.5 °C−113.5 °C; FTIR, ν_{max} (cm^{-1}): 2094, 1632, 1596, 1491, 1460, 1379, 1290, 1263, 1175, 753, 673; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (dd, J=8.0, 1.6 Hz, 2H), 7.76−7.71 (m, 2H), 7.48 (d, J=8.7 Hz, 2H), 7.32−7.27 (m, 2H), 4.38 (t, J=8.1 Hz, 2H), 3.45 (t, J=6.6 Hz, 2H), 2.08−1.90 (m, 2H), 1.88−1.80 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 141.6 (2C), 134.0 (2C) 128.0 (2C), 122.4 (2C), 121.3 (2C), 114.3 (2C), 51.0, 45.4, 26.2, 24.5; MS (EI): *m*/*z* (relative intensity): 292 ($[M]^+$, 66), 220 (59), 208 (100), 195 (20), 180 (43), 152 (23); HRMS (APCI): *m*/*z* calcd for $C_{17}H_{17}N_4O$ $[M+H]^+$: 293.1397; found: 293.1387.

10-(5′-N-Azidopentyl)acridone ***(4h)***: (72%) yellow solid, m.p. 79.0 °C−80.0 °C; FTIR, ν_{max} (cm^{-1}): 2085, 1634, 1594, 1489, 1458, 1377, 1263, 1171, 755, 672; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (t, J=7.9 Hz, 2H), 7.73 (t, J=8.2 Hz, 2H), 7.47 (d, J=8.7 Hz, 2H), 7.29 (t, J=7.4 Hz, 2H), 4.34 (t, J=7.6 Hz, 2H), 3.36 (t, J=6.3 Hz, 2H), 2.00−1.90 (m, 2H), 1.78−1.60 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.8, 141.7 (2C), 133.9 (2C), 128.0 (2C), 122.5 (2C), 121.2 (2C), 114.3 (2C), 51.2, 45.8, 28.7, 26.8, 24.1; MS (EI): *m*/*z* (relative intensity): 306 ($[M]^+$, 27), 208 (100), 196 (44), 180 (44), 166 (19), 152 (22); HRMS (APCI): *m*/*z* calcd for $C_{18}H_{19}N_4O$ $[M+H]^+$: 307.1553; found: 307.1545.

10-(6′-N-Azidohexyl)acridone ***(5h)***: (96%) yellow solid, m.p. 77.0 °C−78.0 °C; FTIR, ν_{max} (cm^{-1}): 2093, 1634, 1598, 1491, 1460, 1376, 1290, 1262, 1176, 754, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (dd, J=8.0, 1.5 Hz, 2H), 7.75−7.70 (m, 2H), 7.47 (d, J=8.7 Hz, 2H), 7.29 (t, J=7.8 Hz, 2H), 4.33 (t, J=8.1 Hz, 2H), 3.32 (t, J=6.5 Hz, 2H), 2.00−1.90 (m, 2H), 1.70−1.50 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 141.7 (2C), 133.9 (2C), 128.0

(2C), 122.4 (2C), 121.2 (2C), 114.4 (2C), 51.3, 45.9, 28.8, 27.1, 26.5, 26.4; MS (EI): *m/z* (relative intensity): 320 ($[M]^+$, 78), 292 (15), 208 (98), 180 (100), 166 (54), 152 (60), 140 (47); HRMS (APCI): *m/z* calcd for $C_{19}H_{21}N_4O$ $[M + H]^+$: 321.1710; found: 321.1699.

*10-(N-propyl)acridone (**2i**)*:[26] (74%) yellow solid, m.p. 130.0 °C−131.0 °C; FTIR, ν_{max} (cm^{-1}): 1634, 1597, 1490, 1458, 1377, 1290, 1263, 1174, 747, 670; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.75−7.70 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.31−7.26 (m, 2H), 4.31 (t, *J* = 8.3 Hz, 2H), 2.03−1.93 (m, 2H), 1.17 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 141.8 (2C), 133.8 (2C), 127.9 (2C), 122.4 (2C), 121.2 (2C), 114.5 (2C), 47.7, 20.5, 11.1; MS (EI): *m/z* (relative intensity): 237 ($[M]^+$, 54), 208 (100), 180 (13), 166 (8), 152 (8), 140 (5); HRMS (APCI): *m/z* calcd for $C_{16}H_{16}NO$ $[M + H]^+$: 238.1226; found: 238.1231.

*10-(N-butyl)acridone (**3i**)*:[26] (70%) yellow solid, m.p. 98.5 °C−99.0 °C; FTIR, ν_{max} (cm^{-1}): 1633, 1596, 1489, 1459, 1375, 1290, 1259, 1175, 751, 671; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.75−7.70 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.31−7.26 (m, 2H), 4.34 (t, *J* = 8.3 Hz, 2H), 1.97−1.88 (m, 2H), 1.65−1.55 (m, 2H), 1.09 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 141.7 (2C), 133.8 (2C), 128.0 (2C), 122.4 (2C), 121.1 (2C), 114.5 (2C), 45.9, 29.2, 20.2, 13.8; MS (EI): *m/z* (relative intensity): 251 ($[M]^+$, 41), 208 (100), 180 (21), 166 (9), 152 (8), 140 (6); HRMS (APCI): *m/z* calcd for $C_{17}H_{18}NO$ $[M + H]^+$: 252.1383; found: 252.1377.

*Diphenylamine-2,4′-dicarboxylic acid (**8**)*:[27] A mixture of 2-bromobenzoic acid (**6**) (2.0 g, 9.95 mmol), 4-aminobenzoic acid (**7**) (1.37 g, 9.95 mmol), potassium carbonate (1.38 g, 9.95 mmol) and copper powder (60 mg, 3% w/w) in N,N-dimethylformamide (10 mL) was refluxed for 4 h. The mixture was cooled and slowly added to aqueous HCl (1:1) (50 mL). The precipitate was collected, washed with hot water, and dried to provide the title compound.

*9(10H)-Acridone-2-carboxylic acid (**9**)*:[27] Diphenylamine-2,4′-dicarboxylic acid (**8**) (2.57 g, 10.0 mmol) in concentrated H_2SO_4 (5 mL) was heated at 100 °C for 4 h. The reaction mixture was cooled and poured into ice water. The precipitate was filtered and washed with hot water. The solid was redissolved in 5% aqueous sodium hydroxide and filtered. The filtrate was diluted with an equal volume of ethanol and acidified with acetic acid. The precipitate was filtered off, washed with water, and dried to yield **9** (1.70 g, 71% from 2-bromobenzoic acid). m.p. > 300 °C; FTIR, ν_{max} (cm^{-1}): 3387, 1634, 1595, 1576, 1399, 1136; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.16 (s, 1H), 8.85 (s, 1H), 8.22 (t, *J* = 8.3 Hz, 2H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.9, 167.2, 143.3, 140.9, 133.9, 133.4, 128.6, 126.1, 124.0, 121.8, 120.9, 119.7, 117.7, 117.6; MS (EI): *m/z* (relative intensity): 239 ($[M]^+$, 100), 222 (32), 195 (24), 139 (28), 97 (45), 83 (44); HRMS (ESI): *m/z* calcd for $C_{14}H_{10}NO_3$ $[M + H]^+$: 240.0655; found: 240.0652.

*10-(4′-N-Bromobutyl)acridone-2-(4-bromobutyl)carboxylate (**10a**) and 9(10H)-Acridone-2-(4-bromobutyl) carboxylate (**10b**)*: A solution of **9** (500 mg, 2.09 mmol) in *N,N*-dimethylformamide (25 mL) was added to a stirred suspension of sodium hydride (250 mg, 6.25 mmol) in *N,N*-dimethylformamide (10 mL). The mixture was heated at 80 °C for 15 min, treated with 1,4-dibromobutane (1.5 mL, 12.71 mmol) and heated at 80 °C for an additional 1 h. The reaction mixture was cooled and water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to provide **10a** (245 mg, 23%) and **10b** (226 mg, 29%).

10a: Yellow solid, m.p. 107.5 °C−108.0 °C; FTIR, ν_{max} (cm^{-1}): 1716, 1639, 1610, 1596, 1482, 1297, 1246, 1139, 754; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.16 (s, 1H), 8.84 (d, *J* = 7.9 Hz, 1H), 8.31 (d, *J* = 8.9 Hz, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 9.0 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 1H), 4.45−4.35 (m, 4H), 3.60−3.50 (m, 4H), 2.20−1.90 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.5, 165.8, 144.2, 141.5, 134.4, 134.3, 130.5, 128.1, 122.9, 122.8, 122.3, 121.6, 114.7, 114.6, 64.1, 45.5, 33.2, 32.5, 29.5, 29.3, 27.4, 25.6; MS (EI): *m/z* (relative intensity): 507 ($[M]^+$, 36), 386 (98), 356 (12), 306 (11), 252 (100), 224 (15), 165 (16); HRMS (ESI): *m/z* calcd for $C_{22}H_{24}Br_2NO_3$ $(M + H)^+$: 508.0117; found: 508.0125.

10b: Yellow solid, m.p. 257.5 °C−258.5 °C; FTIR, ν_{max} (cm^{-1}): 1713, 1634, 1593, 1562, 1526, 1478, 1293, 1243, 1130, 756, 676; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.84 (d, *J* = 2.0 Hz, 1H), 8.25−8.20 (m, 2H), 7.81−7.76 (m, 1H), 7.63−7.56 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 4.35 (t, *J* = 6.4 Hz, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.00−1.86 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.7, 165.3, 143.7, 140.8, 134.1, 133.1, 128.6, 126.1, 122.1, 122.0, 120.9, 119.7, 117.9, 117.7, 63.9, 34.7, 29.1, 27.1; MS (EI): *m/z* (relative intensity): 373 ($[M]^+$, 44), 239 (96), 222 (100), 194 (42), 166 (23), 139 (29); HRMS (ESI): *m/z* calcd for $C_{18}H_{17}BrNO_3$ $[M + H]^+$: 374.0386; found: 374.0389.

*10-(4′-N-Piperidinobutyl)acridone-20[(N-piperidino) butyl]carboxylate (**11a**)*: A solution of **10a** (1 mmol) and piperidine (5 mmol) in acetonitrile was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure, the residue was treated with water and then extracted with dichloromethane. The combined organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield **11a** in 97% yield as a yellow solid: m.p. 104.5 °C−105.5 °C; FTIR, ν_{max} (cm^{-1}): 1703, 1639, 1602, 1479, 1251, 1137, 758; ^{1}H NMR (300 MHz, acetone-d_6): δ 9.04 (s, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 7.2 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 1H), 4.52 (t, *J* = 8.2 Hz, 2H), 4.37 (t, *J* = 6.6 Hz, 2H), 2.50−2.30 (m, 12H), 2.10−1.35 (m, 20H); ^{13}C NMR (75 MHz, acetone-d_6): δ 177.5, 166.2, 145.4, 142.7, 135.1, 134.4, 130.2, 128.0, 123.6 (2C), 122.8, 122.4, 117.0 (2C), 65.6, 59.4, 58.1, 55.3 (2C), 55.2 (2C), 46.8, 27.6, 27.0 (2C), 26.9 (2C), 25.4, 25.3, 24.8, 24.2, 23.6; MS (EI): *m/z* (relative intensity): 368 (6), 284 (6), 185

(14), 129 (47), 98 (93), 83 (91), 55 (100); HRMS (APCI): *m/z* calcd for $C_{32}H_{44}N_3O_3$ $[M+H]^+$: 518.3377; found: 518.3379.

*9(10H)-Acridone-2-[(N-piperidino)butyl]carboxylate (**11b**)*: A solution of **10b** (1 mmol) and piperidine (5 mmol) in *N,N*-dimethylformamide was stirred at room temperature for 24 h. Water was added to the mixture and the solvent was removed under reduced pressure. The precipitate was collected, washed with water, and then dichloromethane to give **11b** in 92% yield as a yellow solid: m.p. 143.5 °C−144.5 °C; FTIR, ν_{max} (cm^{-1}): 1711, 1627, 1594, 1575, 1525, 1475, 1275, 1241, 763; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.17 (s, 1H), 8.84 (d, *J*=2.1 Hz, 1H), 8.25−8.19 (m, 2H), 7.81−7.75 (m, 1H), 7.65−7.58 (m, 2H), 7.33 (t, *J*=7.2 Hz, 1H), 4.35−4.31 (m, 2H), 2.65−2.55 (m, 6H), 1.80−1.42 (m, 10H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.7, 165.3, 143.7, 140.8, 134.1, 133.0, 128.6, 126.1, 122.1, 121.9, 120.9, 119.6, 117.9, 117.7, 64.4, 57.1, 53.3 (2C), 26.1 (2C), 24.4, 23.1, 21.9; MS (EI): *m/z* (relative intensity): 378 ($[M]^+$, 23), 284 (2), 252 (10), 138 (24), 98 (100); HRMS (APCI): *m/z* calcd for $C_{23}H_{27}N_2O_3$ $[M+H]^+$:379.2016; found: 379.2024.

*10-(4′-N-Piperidinobutyl)acridone-2-carboxylic acid (**12**)*: A stirred solution of **11a** (248 mg, 0.48 mmol) in ethanol (5 mL) was treated with 2% aqueous NaOH (5 mL) and refluxed for 1 h. The reaction mixture was cooled and concentrated under reduced pressure to remove the solvent. Water was added to the residue and the mixture was neutralized with 5 M HCl. The yellow precipitate was filtered and washed with water to give **12** (180 mg, 99%) as a yellow solid: m.p. 136.0 °C−137.0 °C; FTIR, ν_{max} (cm^{-1}): 3369, 1614, 1593, 1557, 1484, 1376, 1268, 781, 756; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H), 8.34 (d, *J*=7.6 Hz, 1H), 8.24 (d, *J*=8.6 Hz, 1H), 8.00–7.80 (m, 3H), 7.40 (t, *J*=6.0 Hz, 1H), 4.55–4.45 (m, 2H), 3.20−2.90 (m, 6H), 2.10−1.90 (m, 2H), 1.90−1.70 (m, 6H), 1.60−1.45 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.4, 166.7, 143.9, 141.3, 134.7, 134.0, 129.0, 126.8, 123.3, 122.2, 122.0, 120.8, 116.4, 116.3, 55.5, 52.0 (2C), 45.2, 24.2, 22.4 (2C), 21.6, 20.4; MS (EI): *m/z* (relative intensity): 378 ($[M]^+$, 23), 252 (10), 138 (24), 98 (100); HRMS (APCI): *m/z* calcd for $C_{23}H_{27}N_2O_3$ $[M+H]^+$: 379.2016; found: 379.2015.

*10-(4′-N-Piperidinobutyl)acridone-2-methylcarboxylate (**13**)*: Compound **12** (74 mg, 0.20 mmol) in thionyl chloride (1 mL) was refluxed for 3 h. The reaction mixture was cooled and added dropwise to cold methanol. The solution was then treated with water and extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield **13** (63 mg, 82%) as a yellow solid: m.p. 126.5 °C−127.5 °C; FTIR, ν_{max} (cm^{-1}): 1717, 1643, 1608, 1481, 1297, 1250, 766; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.18 (s, 1H), 8.54 (d, *J*=8.1 Hz, 1H), 8.29 (d, *J*=8.7 Hz, 1H), 7.74 (t, *J*=7.2 Hz, 1H), 7.64 (d, *J*=8.7 Hz, 2H), 7.32 (t, *J*=7.2 Hz, 1H), 4.41 (t, *J*=8.0 Hz, 2H), 3.96 (s, 3H), 2.60−2.40 (m, 6H), 2.05−1.60 (m, 8H), 1.60−1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.7, 166.5, 144.4, 141.7, 134.3, 134.1, 130.5, 128.0, 122.8, 122.7, 122.1, 121.6, 115.1, 115.0, 57.7, 54.5 (2C), 52.1, 46.2, 25.7 (2C), 24.6, 24.2, 23.2; MS (EI): *m/z* (relative intensity): 392 ($[M]^+$, 21), 167 (3), 149 (9), 138 (19), 98 (100); HRMS (ESI): *m/z* calcd for $C_{24}H_{29}N_2O_3$ $[M+H]^+$: 393.2173; found: 393.2177.

*N-(10-(4′-N-Piperidinobutyl)acridone-2-carbonyl)-L-valine benzyl ester (**14**)*: A solution of **12** (100 mg, 0.26 mmol), L-valine benzyl ester hydrochloride (64.5 mg, 0.26 mmol), HOBt (53.6 mg, 0.40 mmol), and EDCI (76 mg, 0.40 mmol) in *N,N*-dimethylformamide (10 mL) was stirred at −10 °C for 10 min. The solution was then treated with DIPEA (0.14 mL, 0.78 mmol) and stirred at −10 °C to room temperature for 24 h. Water was added and the mixture was extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous ammonium chloride, brine, saturated aqueous sodium hydrogen carbonate, and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield **14** (70%) as a yellow solid: m.p. 89.0 °C−90.0 °C; FTIR, ν_{max} (cm^{-1}): 2934, 1739, 1637, 1608, 1482, 1466, 1264, 1181, 1155, 757; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (d, *J*=2.4 Hz, 1H), 8.60 (dd, *J*=8.0, 1.2 Hz, 1H), 8.34 (dd, *J*=9.0, 2.0 Hz, 1H), 7.81−7.70 (m, 3H), 7.40−7.32 (m, 5H), 6.93 (d, *J*=8.4 Hz, 1H), 5.26−5.18 (m, 2H), 4.86 (t, *J*=5.2 Hz, 1H), 4.48−4.43 (m, 2H), 2.50−2.45 (m, 4H), 2.35−2.30 (m, 1H), 2.02−1.96 (m, 2H), 1.82−1.75 (m, 2H), 1.70−1.63 (m, 6H), 1.55−1.45 (m, 2H), 1.02 (t, *J*=7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7, 171.9, 166.3, 143.6, 141.6, 135.3, 134.3, 133.4, 128.6(2C), 128.4 (3C), 127.9, 126.3, 125.8, 122.6, 122.0, 121.2, 115.5, 115.1, 67.0, 57.7, 57.6, 54.4 (2C), 46.1, 31.6, 25.5 (2C), 24.6, 24.0, 23.0, 19.1, 18.0; MS (EI): *m/z* (relative intensity): 567 ($[M]^+$, 13), 415 (3), 376 (3), 207 (9), 138 (41), 98 (100); HRMS (ESI): *m/z* calcd for $C_{35}H_{42}N_3O_4$ $[M+H]^+$: 568.3170; found: 568.3161.

*N-(10-(4′-N-Piperidinobutyl)acridone-2-carbonyl)-L-valine (**15**)*: To a solution of **14** (30 mg, 0.05 mmol) in methanol (3 mL) was added 10% Pd/C (4 mg, 10% w/w) and the suspension was stirred under a H_2 atmosphere at room temperature for 24 h. The reaction was filtered through Celite and eluting with methanol. The filtrate was concentrated under reduced pressure to yield **15** (80%); FTIR, ν_{max} (cm^{-1}): 1632, 1603, 1593, 1481, 1463, 1369, 1260, 761; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.65−8.55 (m, 1H), 8.33 (d, *J*=7.2 Hz, 1H), 8.24 (d, *J*=8.3 Hz, 1H), 7.92 (d, *J*=8.7 Hz, 2H), 7.90−7.70 (m, 1H), 7.40−7.30 (m, 1H), 4.55−4.40 (m, 2H), 4.28 (t, *J*=7.1 Hz, 1H), 2.55−2.35 (m, 6H), 2.30−2.15 (m, 1H), 1.85−1.40 (m, 10H), 1.00−0.90 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.5, 173.4, 165.6, 143.0, 141.4, 134.4, 132.9, 126.8, 126.7, 126.5, 121.8 (2C), 120.7, 116.2, 116.0, 58.9, 56.8, 53.6 (2C), 45.2, 29.8, 25.2 (2C), 24.0, 23.8, 22.2, 19.5, 18.9; MS (EI): *m/z* (relative intensity): 477 ($[M]^+$, 1), 415 (16), 373 (61), 282 (39), 250 (28), 138 (27), 98 (100), 91 (76); HRMS (ESI): *m/z* calcd for $C_{28}H_{36}N_3O_4$ $[M+H]^+$: 478.2700; found: 478.2693.

*N-(10-(4′-N-Piperidinobutyl)acridone-2-carbonyl)-L-valine benzyl amide (**16**)*: A procedure analogous to the preparation of **14** and starting from **15** and benzyl amine was conducted to obtain compound **16** in 60% yield as a yellow solid: FTIR, ν_{max} (cm^{-1}): 3284, 2931, 1633, 1607,

1481, 1257, 757; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (d, *J*=2.4 Hz, 1H), 8.55 (d, *J*=8.0 Hz, 1H), 8.23 (dd, *J*=9.0, 2.4 Hz, 1H), 7.78−7.67 (m, 3H), 7.35−7.23 (m, 5H), 7.11 (d, *J*=8.4 Hz, 1H), 6.59 (br s, 1H), 4.52−4.39 (m, 5H), 2.49−2.40 (m, 6H), 2.37−2.30 (m, 1H), 2.00−1.92 (m, 2H), 1.85−1.75 (m, 2H), 1.70−1.64 (m, 4H), 1.55−1.47 (m, 2H), 1.10−1.05 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7, 171.1, 166.6, 143.7, 141.7, 137.9, 134.3, 133.2, 128.7 (2C), 128.0, 127.8 (2C), 127.5, 126.2, 126.1, 122.7, 122.0, 121.3, 115.6, 115.2, 59.5, 57.8, 54.6 (2C), 46.2, 43.6, 31.0, 26.0 (2C), 24.6, 24.4, 23.3, 19.5, 18.5; MS (EI): *m/z* (relative intensity): 566 ($[M]^+$, 12), 368 (10), 207 (17), 138 (40), 98 (100); HRMS (ESI): *m/z* calcd for $C_{35}H_{43}N_4O_3$ $[M+H]^+$: 567.3330; found: 567.3333.

*10-(4′-N-Piperidinobutyl)acridone-2-carboxylic acid benzylamide (**17**)*: A procedure analogous to the preparation of **14** and starting from **12** and benzyl amine was used to obtain compound **17** in 72% yield as a yellow solid: m.p. 77.0 °C−78.0 °C; FTIR, ν_{max} (cm^{-1}): 1632, 1607, 1480, 1299, 1260, 756; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.47 (d, *J*=7.8 Hz, 1H), 8.36 (d, *J*=9.0 Hz, 1H), 7.80−7.60 (m, 3H), 7.40−7.20 (m, 5H), 4.66 (d, *J*=5.7 Hz, 2H), 4.38 (t, *J*=7.2 Hz, 2H), 2.60−2.40 (m, 6H), 2.00−1.60 (m, 8H), 1.55−1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.8, 166.2, 143.4, 141.6, 138.2, 133.3, 133.6, 128.6 (2C), 127.8 (2C), 127.4 (2C), 126.7, 125.5, 122.4, 121.9, 121.1, 115.6, 115.1, 57.7, 54.5 (2C), 46.1, 44.1, 25.8 (2C), 24.5, 24.2, 23.2; MS (EI): *m/z* (relative intensity): 467 ($[M]^+$, 20), 376 (3), 341 (3), 207 (9), 138 (39), 98 (100); HRMS (ESI): *m/z* calcd for $C_{30}H_{34}N_3O_2$ $[M+H]^+$: 468.2645; found: 468.2640.

*9(10H)-Acridone-2-methylcarboxylate (**18**)*:[28] A procedure analogous to the preparation of **13** and starting from **9** was used to obtain compound **18** in 87% yield as a yellow solid: m.p. >300 °C; FTIR, ν_{max} (cm^{-1}): 1717, 1633, 1595, 1575, 1293, 764; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.16 (s, 1H), 8.82 (d, *J*=2.1 Hz, 1H), 8.23 (dd, *J*=8.1, 1.5 Hz, 1H), 8.18 (dd, *J*=8.7, 2.1 Hz, 1H), 7.80-7.74 (m, 1H), 7.61 (d, *J*=8.4 Hz, 1H), 7.58 (d, *J*=7.5 Hz, 1H), 7.34-7.28 (m, 1H), 3.89 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.7, 165.7, 143.6, 140.7, 134.0, 132.9, 128.6, 126.0, 122.0, 121.7, 120.9, 119.6, 117.9, 117.7, 52.1; MS (EI): *m/z* (relative intensity): 253 ($[M]^+$, 100), 222 (84), 195 (20), 167 (29), 149 (76), 97 (37), 57 (50); HRMS (ESI): *m/z* calcd for $C_{15}H_{12}NO_3$ $[M+H]^+$: 254.0812; found: 254.0807.

*N-(9(10H)-Acridone-2-carbonyl)-L-valine benzyl ester (**19**)*: A procedure analogous to the preparation of **14** and starting from **9** was used to obtain **19** in 73% yield as a yellow solid: m.p. 211.0 °C−212.0 °C; FTIR, ν_{max} (cm^{-1}): 1716, 1642, 1556, 1521, 1479, 1267, 751, 737; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.98 (s, 1H), 8.93 (d, *J*=7.5 Hz, 1H), 8.87 (s, 1H), 8.27 (d, *J*=8.1 Hz, 1H), 8.20 (d, *J*=8.7 Hz, 1H), 7.77 (t, *J*=7.5 Hz, 1H), 7.58 (d, *J*=6.6 Hz, 2H), 7.39−7.28 (m, 5H), 5.10 (d, *J*=4.2 Hz, 2H), 4.37 (t, *J*=7.5 Hz, 1H), 2.29−2.22 (m, 1H), 1.00 (d, *J*=6.0 Hz, 3H), 0.95 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.9, 171.7, 166.5, 142.7, 140.8, 136.0, 133.9, 132.6, 128.4 (2C), 128.1, 128.0 (2C), 126.5, 126.3, 126.1, 121.7, 120.8, 119.5, 117.6, 117.3, 65.8, 59.0, 29.5, 19.3, 19.2; MS (EI): *m/z* (relative intensity): 428 ($[M]^+$, 2), 293 (5), 238 (30), 222 (100), 194 (18), 166 (8), 139 (7); HRMS (APCI): *m/z* calcd for $C_{26}H_{25}N_2O_4$ $[M+H]^+$: 429.1809; found: 429.1803.

*N-(9(10H)-Acridone-2-carbonyl)-L-valine (**20**)*: A procedure analogous to the preparation of **15** and starting from **19** was used to obtain compound **20** in 82% yield as a yellow solid: m.p. 200.0 °C (dec.); FTIR, ν_{max} (cm^{-1}): 3273, 1628, 1517, 1474, 1311, 1154, 757; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H), 8.82 (s, 1H), 8.57 (d, *J*=7.5 Hz, 1H), 8.25 (d, *J*=8.1 Hz, 1H), 8.20 (d, *J*=8.7 Hz, 1H), 7.76 (t, *J*=7.2 Hz, 1H), 7.60 (d, *J*=7.2 Hz, 2H), 7.29 (t, *J*=7.2 Hz, 1H), 4.30 (t, *J*=6.6 Hz, 1H), 2.30−2.20 (m, 1H), 1.05−0.90 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.9, 173.4, 166.0, 142.6, 140.8, 133.8, 132.4, 126.7, 126.2, 126.1, 121.6, 120.8, 119.5, 117.6, 117.3, 58.8, 29.7, 19.4, 18.9; MS (EI): *m/z* (relative intensity): 293 ($[M-45]^+$, 7), 279 (12), 227 (5), 167 (30), 149 (100), 71 (26); HRMS (APCI): *m/z* calcd for $C_{19}H_{19}N_2O_4$ $[M+H]^+$: 339.1339; found: 339.1345.

*9(10H)-Acridone-2-carboxylic acid benzylamide (**21**)*:[29] A procedure analogous to the preparation of **14** and starting from **9** and benzyl amine was used to obtain compound **21** in 67% yield as a yellow solid: m.p. >300 °C; FTIR, ν_{max} (cm^{-1}): 1622, 1579, 1549, 1523, 1308, 756, 736; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.08 (s, 1H), 9.28 (br t, *J*=5.2 Hz, 1H), 8.85 (s, 1H), 8.26 (d, *J*=8.0 Hz, 1H), 8.23 (d, *J*=8.8 Hz, 1H), 7.76 (t, *J*=7.6 Hz, 1H), 7.61 (d, *J*=8.0 Hz, 1H), 7.59 (d, *J*=7.5 Hz, 1H), 7.40−7.20 (m, 5H), 4.52 (d, *J*=5.4 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.9, 165.6, 142.6, 140.8, 139.9, 133.8, 132.2, 128.3 (2C), 127.3 (2C), 126.7 (2C), 126.1, 125.8, 121.7, 120.8, 119.6, 117.6, 117.4, 42.7; MS (EI): *m/z* (relative intensity): 328 ($[M]^+$, 47), 222 (100), 195 (64), 166 (19), 139 (19), 106 (12); HRMS (ESI): *m/z* calcd for $C_{21}H_{17}N_2O_2$ $[M+H]^+$: 329.1284; found: 329.1287.

In vitro cytotoxicity

The cytotoxic activities of compounds **1**, **5**, **8**, and **11**–**15** were evaluated against several cancer cell lines, namely, Molt-3, HepG2, HuCCA-1, and A549. Cells in the logarithmic growth phase were seeded in 96-well plates (Costar, 3599, USA) at a density of 10,000–15,000 cells/well. The plates were incubated at 37 °C in a humidified atmosphere with 95% air and 5% CO_2 for 24 h. The culture was then treated with a medium containing either the test compound or the vehicle to the desired final concentration and incubated for another 48 h. The cell monolayer was washed with phosphate-buffered saline (pH 7.2), fixed with 95% ethanol, stained with crystal violet solution, and lysed with 0.1 N HCl in methanol. The absorbance of the lysate was recorded at 540 nm on an automatic microtiter plate reader (Multiskan Ascent, Labsystem). All tests were carried out in triplicate, and the mean value was calculated. Activity is expressed as IC_{50} (concentration of substance that inhibits 50% of cell growth) by using doxorubicin (Sigma Aldrich) as a standard drug.

Molecular modeling

The chemical structures of the selected compounds were built using the Scigress software suite 2.8.1 and optimized using

the MM2 force field. The X-ray crystal structure of DNA was obtained from the Protein Data Bank database, code 2DES. AutoDockTools version 1.5.6 graphic user interface was used to prepare the crystal structure. Hydrogen atoms were added, and crystallographic water and co-crystallized 3′-desamino-3′-(2-methoxy-4-morpholinyl)-doxorubicin ligand were deleted. AutoDock Vina 1.1.2 was used as the docking engine. The grid box was set to 40, 40, and 15 Å for each of the x, y, and z dimensions. The center search space coordinates were set at x, y, z=–0.093, 0.946, and −8.586, respectively. The exhaustiveness that influences the thoroughness of the search pose was set to 15. DNA was kept rigid, and the ligands were free for pose search. The number of docking runs was set to 20 for each ligand, and the docked conformers were ranked according to the ΔG values (kcal mol^{-1}).

MlogP calculation

The Dragon 7.0 software suite was used to calculate the Moriguchi log P (MlogP) values to determine the lipophilicity of the compounds. Cheminformatics software was used to calculate for molecular descriptors.

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Supplemental material

Figure S1 and ^{1}H NMR and ^{13}C NMR spectra are provided in the Supplementary Information.

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