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Linker-modified triamine-linked acridine dimers: Synthesis and cytotoxicity properties in vitro and in vivo

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Abstract—The preparation and cytotoxicity properties of a series of N^{ε}-substituted triamine-linked acridine dimers are described. Most acridine dimer derivatives reveal highly potent in vitro cytotoxicity properties and DNA binding activity. Several acridine dimers were selected to study their action in vivo. These acridine dimers have demonstrated a narrow safe margin, as has adriamycin, but higher maximum tolerate dose (MTD) in comparison with that of adriamycin in ICR mice. The acridine dimers also demonstrated various anit-COLO 205 solid tumor activities in vivo. Compound **1** has shown the most potent solid tumor inhibition.

1. Introduction

DNA is an important target in cancer therapy. The planar structure of DNA intercalating drugs can strongly bind to DNA resulting in the death of cancer cells. Several representative antitumor agents of this class are traditionally used in clinics, such as actinomycin D, adriamycin and daunomycin. Recently, several intercalating antitumor agents were also developed as Amsarcine,[1,2] Mitoxantrone[3] or are progressed in clinical trials as 4-carboxamidoacridine DACA,[4,5] Anthrapyrazoles,[6,7] and pyrazolo[3–5] nitroacridine (NSC366140).[8,9]

Acridine is a potential anticancer chromophore (lead) for developing the anticancer drug because its planar structure can strongly bind to DNA. Many studies have used acridine as a lead to design more active compounds in studying DNA binding and killing cancer cells.[2,8,10–19] Therefore, increasing the binding affinity between agents and DNA and retaining the agent–DNA bound form are the two hypothesized rational designs to increase the sensitivity of killing cancer cells. To obtain a bis-derivative connecting two planar cores by a bridge results in increasing agents' DNA-binding affinity and effectiveness of killing cancer cells has been investigated in many studies.[10c,11,14,16,19–21] Some successful examples are the bis(naphthalimide) analogue,[22] DMP 840,[23,24] reported to be a topoisomerase II poison; LU 79553,[25] which has been highly effectively against tumor xenografts in vivo; and a series of bisimidazoacridones (WMC-26)[14,26] are in progress under preclinical and clinical trials.

In comparison with monomer, the two planar structures connecting appropriate linker length are considered to be two major factors for increasing cancer cell cytotoxicity. The optimal linker length connecting bisintercalators is required for DNA binding affinity and biological response in previous studies.[2a,10c,29–31] To look for improving the DNA binding affinity and cell killing effect on these issues, the various substituents on the nitrogen atom was introduced at the center of appropriate lengths of linkers connecting two acridines to evaluate the impacting influence of DNA binding affinity and cancer cells cytotoxicity by these substituents. Herein, the bridge lengths of 6 or 7 carbons on the triamine

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linker were adopted and a series of N^{ε}-substituted triamine-linked acridine dimers were synthesized to investigate their anticancer activity in vitro and in vivo. This series of N^{ε}-substituted triamine-linked acridine dimers have demonstrated potent DNA intercalating activity and anticancer activity in vitro in comparison with that of anticancer drug, adriamycin. However, the DNA intercalating affinity activity was not obviously correlated with cell cytotoxicity in this series of compounds. Several acridine dimer derivatives were selected to further perform in vivo studies. These acridine dimers have shown a narrow safe margin in testing ICR mice. The maximum tolerate dose (MTD) of ICR mice for compound **1**, compound **3**, compound **5**, compound **23**, compound **31** and adriamycin is 10, 22.5, 30, 10, 10 and 4 mg/kg, respectively. The compounds **1** and **23** demonstrated their anti-COLO 205 solid tumor activity in SCID mice. Compound **1** was found to have the most solid tumor inhibition in vivo.

2. Results and discussion

2.1. Synthesis

Series of bisacridines were connected by various linkers. Two separate routes for synthesizing the N^{ε}-substituted triamine-linked acridine dimers were employed. One method involves condensation of an N^{ε}-substituted-*N*,*N*-bis(3-aminopropyl)amine with 6,9-dichloro-2-methoxy-acridine using potassium carbonate in DMF, and followed by salt formation. The N^{ε}-substituted-*N*,*N*-bis(3-aminopropyl)amine was commercially obtained or prepared by reductive amination of 3,3-iminodipropionitrile with aldehyde and followed by nitrile reduction (Scheme 1). A second route involves reductive amination of N^{ε}-unsubstituted triamine-linked dimer with aldehyde and followed by salt formation (Scheme 2).

2.2. Biological evaluation

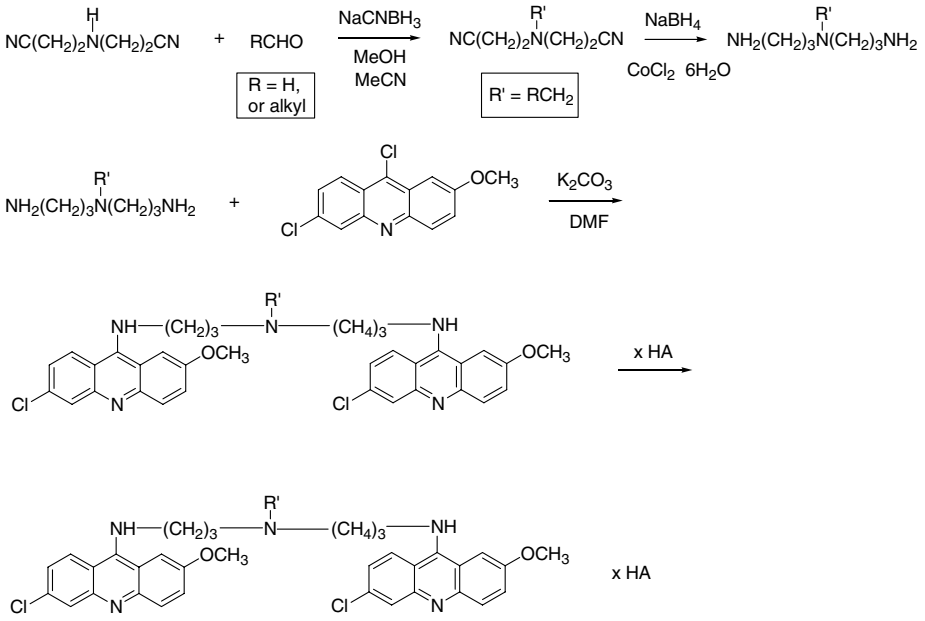
Various N^{ε}-substituents of the triamine linkers with six or seven carbon numbers length were synthesized to investigate for their DNA intercalating activity in vitro, and antitumor activity in vitro and in vivo.

2.2.1. In vitro cytotoxicity. Most acridine bisintercalators with **6** or **7** carbons of triamine-linked derivatives have demonstrated cancer cell killing activity with COLO205, HAT22T, SK-BR-3 and MOLT-4 human cancer cell lines by in vitro cytotoxicity assays. The various R-moieties attached to triamine linker do not have an increased or diminished effect on cancer killing activity in vitro in comparison to the R = H group (compound **1** or compound **2**). The IC_{50} (μM) values for triamine-linked derivatives were between 0.01 and 5.6 against COLO 205, between 0.06 and 6.95 against HA22T, between 0.05 and 7.31 against SK-BR-3 and between $\leqq$0.01 and 0.71 against MOLT-4. Most of them have shown comparable or more potent anticancer activity in vitro than that of adriamycin. Like the traditional cancer drug property such as adriamycin, most compounds have revealed more killing sensitivity (IC_{50}) in MOLT-4 cell in comparison with that of HA22T, COLO205 and SK-BR-3 cells. However, compound **9**, compound **12** and compound **14** have demonstrated similar cancer killing effect among COLO205, SK-BR-3 and MOLT-4 cells. Compound **12** and compound **14** have the least killing effect for HA22T cells among the four cancer cells.

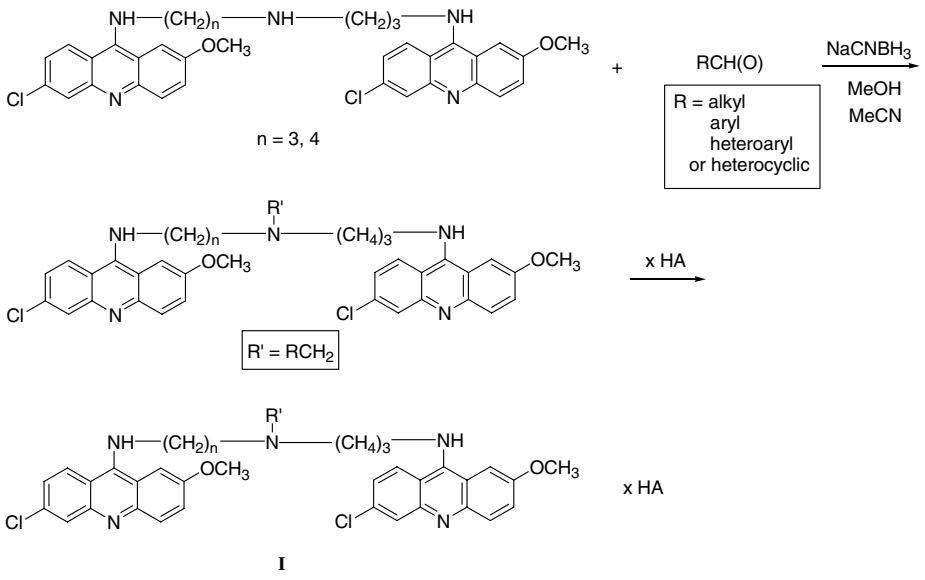
Among these derivatives, compound **5**, compound **7**, compound **8**, compound **20**, compound **30**, compound **31** or compound **34** are more selective against the COLO 205 cell line (IC_{50} is 0.09, 0.01, 0.89, 0.37, 0.01, 0.01, 0.13 μM) in comparison with HA22T (IC_{50} is 5.33, 0.51, 6.95, 4.73, 0.06, 3.97, 1.72 μM) or SK-BR-3 (IC_{50} is 7.31, 0.72, 6.14, 5.07, 0.07, 0.72, 1.29 μM). Except MOLT-4 cells from blood tumors, these acridine dimers seem to potentially have more selectivity against COLO 205 cells compared with HA22T and SK-BR-3 cells from solid tumors (Table 1). The reasons for the enhanced cytotoxicities of acridine dimers bearing N^{ε}-substituents on COLO 205 cells are not clear. It is possible that some proteins expressed in CLLO 205 cells interact with these substitutes of acridine dimers resulting in increasing cytotoxicity sensitivity. It is possible that the attractive force between DNA and bisintercalators was enhanced by hydrogen bonding between DNA bases (acceptor) and these substitutes (donor) or by interaction of negatively charged phosphodiester linkages of DNA and positively charged ammonium cations of these substituents.

2.2.2. DNA intercalating activity. 5-Fu, a negative control, did not show DNA intercalating activity (Fig. 1a). The SC_{50} (50% DNA shift concentration) of adriamycin, a well-known DNA intercalator, was 10–15 μM. Most of the polyamine-linked acridine dimers showed bisintercalating properties with 2–4 μM SC_{50} as compound **6** (Fig. 1b). Most bisacridine derivatives increase the DNA intercalating activity several folds compared to that of adriamycin monomer; except compound **21**, compound **28** or compound **30** (>20, >20, 2–20 μM) (Table 1). As a comparison between DNA intercalating affinity and anticancer activity in vitro for this series of bisintercalators and adriamycin, the increasing DNA intercalating activity of bisacridine derivatives was not accompanied with the enhancement of their anticancer activity in vitro. There is no apparent correlation between the DNA binding and killing activity against HeLa cells (**31**). Therefore, it is difficult to evaluate the rational design for structure–activity relationship among these compounds because the cytotoxicity did not correlate with DNA binding affinity.

2.2.3. Maximum tolerate dose (MTD). The bisacridine derivative acetate salts were prepared and dissolved in 2.5% cremophor/ddH_2O (v/v) for animal study. In order to observe the safety margin in vivo for acridine dimer-serial compounds, five acridine dimer derivatives (compounds **1**, **3**, **5**, **23**, **31**) were selected to determine their MTD in ICR mice. All testing compounds have shown a safe margin (the dose range interval between all surviving animals and all dead animals) that is too narrow.



Scheme 1.

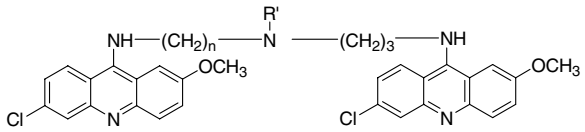


Scheme 2.

For example, compound **1** can kill all animals at 15 mg/kg, but all animals can survive at 10 mg/kg. The toxic effect observed over MTD is that the treated mice have increasing heart-beat, tremor, secrete mucus from nostril and mouth, and progress to death. It is usual with cytotoxic agents such as DNA intercalating agent as adriamycin that test animals lose body weight due to their toxicity effect during the experimental periods under MTD. However, the body weight loss compared to control group for the bisintercalator treatments was little or no loss through the experimental periods under MTD. Therefore, the bisintercalators have shown less toxicity when compared to cytotoxic agents as adriamycin in this study. (Data not shown.) The MTD of ICR mice for compound **1**, compound **3**, compound **5**, compound **23**, compound **31** and adriamycin is 10, 22.5, 30, 10, 10 and 4 mg/kg, respectively (Table 2).

2.2.4. In vivo antihuman COLO 205 solid tumor activity. Four compounds at MTD were introduced into nude mice carrying human COLO205 solid tumor by iv administration with a schedule of $(Q2D \times 3) \times 2$. Compound **1** has shown the most (80%) tumor inhibition at MTD in comparison with that of other testing compounds (compound **3**, 2%; compound **23**, 24%; compound **31**, 0%) (Table 3). The major difference among the set of four acridine dimers is the N^{ε}-substituent R in their linkers. They are H (compound **1**), CH_3 (compound **3**), CH_2(3′-furyl) (compound **23**) and CH_2(2′-pyridyl) (compound **31**), respectively. In vitro anticancer

Table 1. In vitro cytotoxicity[a] and DNA intercalating activity of acridine bis-intercalator derivatives



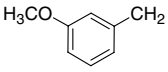
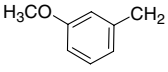
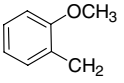
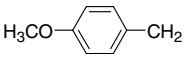
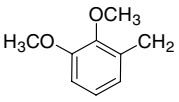
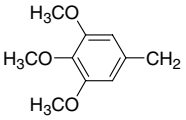
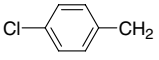
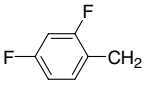
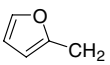
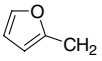
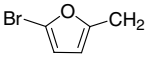
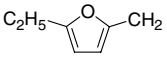
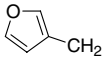
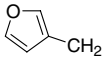
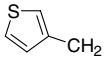
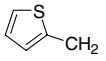
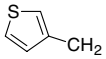
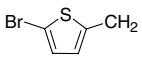
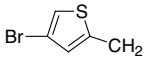
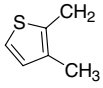
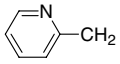
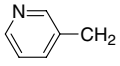
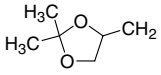
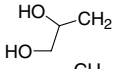
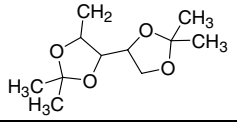
Compound[b]	*n*	R′	IC_{50}[a,c] (μM)				SC_{50}[d] (μM)
			COLO205	HA22T	SK-BR-3	MOLT-4	gel shifting
Adriamycin	—	—	0.26	0.31	0.29	0.02	10–15
1	3	H	0.41	0.33	0.77	<0.02	4–5
2	4	H	0.13	0.33	0.05	<0.02	4
3	3	CH_3	0.16	0.62	0.70	<0.02	NT
4	3	*n*-C_3H_7	1.46	5.98	5.78	<0.02	2–4
5	3	C_2H_5	0.09	5.33	7.31	<0.02	NT
6	3	CH_2—cyclopropyl	1.27	0.48	0.82	<0.01	2–4
7	4	CH_2—cyclopropyl	0.01	0.51	0.72	<0.01	2–4
8	3	CH_2(*n*-amyl)	0.89	6.95	6.14	0.09	NT
9	3	$C_6H_5CH_2$	0.75	0.70	0.70	0.70	2–4
10	3	$C_6H_5CH_2CH_2$	0.70	0.65	0.68	<0.01	NT
11	3		0.35	0.42	0.68	<0.01	2–4
12	4		0.25	5.66	1.30	0.71	NT
13	3		3.61	0.93	0.71	<0.01	2–4
14	4		0.39	5.45	0.86	0.71	NT
15	3		0.64	0.62	0.79	0.01	2–4
16	3		4.50	4.42	5.04	0.06	NT
17	3		0.20	0.58	0.53	<0.01	NT
18	3		0.77	0.23	0.78	<0.01	NT
19	3		0.24	0.61	0.84	<0.01	2–4
20	4		0.37	4.73	5.07	0.06	NT
21	3		0.41	1.11	4.24	<0.01	>20
22	3		2.56	3.32	5.25	0.10	NT

Table 1 (*continued*)

Compound[b]	*n*	R′	IC_{50}[a,c] (μM)				SC_{50}[d] (μM)
			COLO205	HA22T	SK-BR-3	MOLT-4	gel shifting
23	3		0.46	0.36	0.69	0.01	2–4
24	4		0.32	0.44	0.71	0.03	NT
25	3		0.56	1.41	1.17	0.07	NT
26	4		0.50	0.44	0.68	0.04	NT
27	4		0.25	3.59	0.99	<0.01	NT
28	3		0.85	1.15	5.80	<0.01	>20
29	3		0.72	0.84	0.70	0.06	NT
30	3		0.01	0.06	0.07	<0.01	2–20
31	3		0.01	3.97	0.72	<0.01	2–4
32	3		0.50	0.74	0.64	<0.01	2–4
33	3		5.60	3.43	6.61	<0.01	NT
34	3		0.13	1.72	1.29	<0.01	NT
35	3		2.19	4.68	4.73	0.06	NT

NT, not tested.
[a] 50% growth inhibition, determined by MTT assay; see in vitro cytotoxicity section for details.
[b] Compound was tested in triplicate.
[c] Cancer cell lines origin: COLO205 (colon), HA22T (liver), SK-BR-3 (ovary), MLOT-4 (peripheral blood).
[d] 50% DNA shift concentration, see DNA intercalating assay section.

activity assay and in DNA intercalating assays cannot find obvious differences among the four compounds. However, in vivo study shows that compound **3** has higher MTD, whereas the MTD for the other three compounds was slightly different in this study. Only compound **1** has demonstrated potent antiCOLO solid tumor activity at MTD in SCID mice. Whereas, compound **3**, which had higher MTD, did not show antisolid tumor activity. The molecular dimension for H and CH_3 (R-group) in triamine linker of acridine dimer does not occupy considerable volume in space or a dramatically attractive force in biochemistry reaction. Therefore, it is interesting to further explore the possible reasons for the dramatic difference of pharmacology effects between H and CH_3 (R-group) in triamine linker of the acridine dimers, as seen in MTD and antisolid tumor in vivo study.

3. Conclusion

Series of N^{ε}-substituted triamine-linked acridine dimers were synthesized and their biological evaluation was performed. We have found that most acridine dimers have demonstrated potent DNA binding affinity and anticancer activity in various cancer cell line studies. However, there is no apparent correlation between the DNA binding affinity and killing activity in this study.

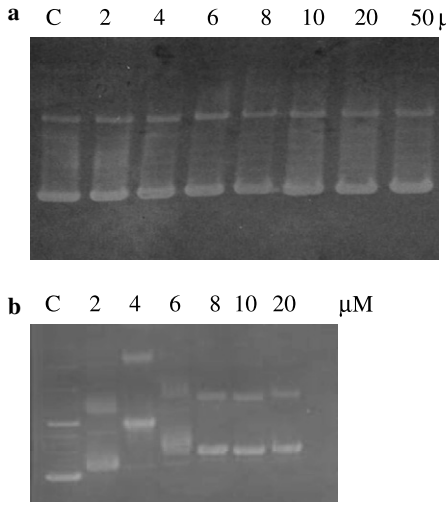


Figure 1. DNA intercalating activity of 5-Fu and compound **6**. The (a) 5-Fu, not unwind pUC 19 plasmid DNA, and (b) bisacridine derivative compound **6** unwind pUC 19 plasmid DNA. The negatively supercoiled pUC19 (1 μg) were incubated with increasing concentrations of 5-Fu or compound **6** for 30 min at 25 °C in reaction mixtures, pH 7.4, and then run on a 1.0% agarose gel. C refers to the negatively supercoiled form of pUC 19.

Table 2. MTD of Acridine Dimer derivatives in ICR mice

Compound	MTD (mg/kg)
1	10
3	22.5
5	30
23	10
31	10
Adriamycin	4

Table 3. Anticolon solid tumor efficacy study at MTD of acridine dimer derivatives in SCID mice

Compound	MTD (mg/kg)	TGI (%)[a]
1	10	80
3	22.5	2
23	10	24
31	10	0

[a] TGI (%): percentage of tumor growth inhibition. Tumor growth inhibition was selected at date after last dosing.

Most acridine dimers have also demonstrated more killing sensitivity to MOLT-4 cells from blood tumors in comparison with cancer cells (COLO205, SK-BR-3 or HA22T) from solid tumors that is a general property of cell killing agent of current market anticancer drug as adriamycin. However, compound **9**, compound **12** and compound **14** have demonstrated a similar cancer killing effect among COLO205, SK-BR-3 and MOLT-4 cells. Here, we also found that compound **5**, compound **7**, compound **8**, compound **20**, compound **30**, compound **31** and compound **34** are more selective against the COLO 205 cell line in comparison with HA22T or SK-BR-3. Several acridine dimers were selected to study their in vivo effect. They have demonstrated a narrow safe margin, like the traditional cancer killing drug adriamycin, but a higher maximum tolerate dose (MTD) in ICR mice. The acridine dimers also demonstrated various anit-COLO 205 solid tumor activities in vivo. Compound **1** has shown the most potent solid tumor inhibition.

4. Experimental

4.1. Materials

MOLT-4 and COLO 205 cell lines were obtained from American Tissue Culture Company (ATCC). SK-BR-3 was kindly provided by Dr. Ming-Chie Hung, Anderson Cancer Center, Houston, Texas, USA. HA22T/VGH was kindly provided by Veterans General Hospital, Taipei, Taiwan, ROC. JM 83 bacteria carrying a pUC19 plasmid was kindly provided by Dr. Ming-Ching Kao, National Defense Medical Center, Taipei, Taiwan, ROC. All the synthetic reagents are commercially available. Compounds **1**–**35** were prepared by the synthetic pathways illustrated in Schemes 1 and 2.

4.2. Biological evaluation

4.2.1. Cell culture. COLO 205 cells were grown as a monolayer and MOLT-4 cells were grown as a suspension in RPMI 1640 (Invitrogen Com., 31800-022) with 10% fetal bovine serum (FBS) (Hylcone, defined). HA22T/VGH cells were grown as a monolayer in DMEM/F12 (Dulbecco's modified Eagle medium: Nutrient Mixture F12 (Ham) (1:1) (Invitrogen Com., 21127-022)) powder medium supplemented with 10% FBS and 100 μM nonessential amino acid (Invitrogen Com., 11140-050). SK-BR-3 were grown as a monolayer in DMEM/F12 medium with 10% FBS. The cells were grown in culture medium and maintained in a humidified atmosphere of 5% CO_2–95% air incubator at 37 °C. The JM83 bacteria were grown in LB medium containing 1 μg/ml ampicillin at 37 °C. The bacterial culture was collected when the OD was between 0.8 and 1.0.

4.2.2. In vitro cytotoxicity assay. Single cell suspensions were obtained by mechanical disaggregation of the floating cell line (MOLT-4) or by trypsinization of the monolayer cultures (COLO 205, HA22T/VGH, SK-BR-3) and counted by trypan blue exclusion. All testing compounds were dissolved in DMSO (dimethyl sulfoxide) (Sigma, D8779) as stock solutions and serially diluted with DPBS buffer for use. The MTT (Sigma, M2128) cytotoxicity assay was used for acridine dimers-serial compounds and adriamycin to evaluate their growth inhibition effects on four human tumor cell lines. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) metabolic assay was performed as described by Monks et al., 1991.[27] In brief, the cells were seeded at a density of 6000–10,000 cells into microtiter plates (Nunc, 167008) and incubated in 180 μl culture medium overnight. Then the cells were treated with compounds, respectively, at various concentrations for 72 h. After treatment, viable cells were reacted with 20 μl MTT solution (5 mg/ml) at 37 °C for 4 h. Next,

the 170 μl medium was removed and the remainder, which contained the MTT–formazan crystals, was dissolved with 200 μl DMSO. Finally, the absorbance was measured at 545 nm and reference at 690 nm. (E_{max}, Molecular Device Inc.) Five concentrations of compounds at 100, 10, 1, 0.1, 0.01 μg/ml were used. Cell growth inhibition was calculated according to the formula $(1 - (\text{OD of drug treatment/OD of control})) \times 100\%$. The IC_{50} was obtained by 50% growth inhibition at a particular drug concentration by plotting the drug concentration against the growth inhibition percentage.

4.2.3. Plasmid isolation. The JM 83 bacteria containing a pUC19 plasmid were cultured and then harvested by centrifugation with 4000 rpm for 15 min at 4 °C. The pellets were lyzed by alkali method and the DNA plasmids were collected as the lower band (corresponding to the closed circular DNA) of a centrifuge tube after CsCl–ethidium bromide gradient centrifugation. This collection method is described in, for example, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press. (1989).[28] After removal of ethidium bromide (Sigma. E8751) from the DNA plasmids with 1-butanol saturated with water, the CsCl (Sigma, C3309) was removed by dialysis with TE (pH 8.0) buffer.

4.2.4. DNA intercalating assay. The pUC19 plasmid (1 μg in each reaction) was incubated with various concentrations of solutions containing various triamine-linked acridine dimers for 30 min at 25 °C in 20 μl reaction mixtures (10 mM Tris–HCl, pH 7.4, 0.1 mM EDTA (Sigma, E9884) and 5 mM NaCl). After reaction, the DNA mixture was run on a 1.0% agarose gel (Sigma, A5304) with running buffer (40 mM Tris–acetate, pH 8.0, and 2 mM EDTA) at 10 V/cm current. Then, the gel was stained with ethidium bromide and destained with distilled water. The shifts of the DNA bands were visualized using UV light and analyzed by photography. The DNA band-shifting pattern formed a sigmoidal curve. The 50% shift concentration (SC_{50}) corresponded to a 50% DNA band shift at the particular concentration according to the curve of the DNA band-shifting photograph.

4.2.5. Laboratory animals. The ICR or SCID mice (16–18 g, 4–5 weeks old) were obtained from the animal center at the medical center of National Taiwan University, Taipei, ROC and allowed to acclimate to their new environment for one week. The mice were given a sterilized pellet diet and sterilized water ad libitum, and housed under specific pathogen-free conditions in a temperature range between 23 and 25 °C, and a humidity of about 50 ± 10%. The lighting was automatically operated on a 12-h light/dark cycle.

4.2.6. In vivo maximum tolerate dose determination (MTD). The ICR mice were randomly divided into 6 groups. Each group contained 5 mice. The control group was given the 2.5% cremophor/ddH_2O only (Sigma, C5135). The treatment groups were given the compounds dissolved in 2.5% cremophor/ddH_2O at various concentrations with a $(Q2D \times 3) \times 2$ schedule. The physical activity, body weight and survival rate were observed for MTD determination. The MTD is described as the highest dose that all mice can tolerate and survive in this study.

4.2.7. In vivo anticolon solid tumor activity. The COLO205 cells were subcutaneously inoculated into the dorsal site of the SCID mice. The solid tumors were grown up to a size of about 500 mm^3, then isolated following the cultivations and named as COLO205F1 cells. The COLO205F1 cells were subcutaneously inoculated into the dorsal site of the SCID mice for compound testing. These tumors were grown to a size of approximately 100–200 mm^3. The mice were sorted according to body weight into several groups with five mice per group. The compounds, suspended in 2.5% cremophor/ddH_2O solvent, were injected into tail veins following MTD on $(Q2D \times 3) \times 2$ therapy schedule. Tumor weights at the beginning of chemotherapy and at weekly intervals thereafter were estimated using two dimensional caliper measurements and calculated with the formula for an ellipsoid. Tumor weight was $L \times W^2 \times 0.5$, where L is the major axis and W is the width of the tumor. The percentage of tumor growth inhibition (% TGI) was calculated according to the formula $(1 - (\text{mean tumor weight of treated group/mean tumor weight of control group})) \times 100\%$.

4.3. Chemistry

4.3.1. 3-[*N*-(2-Cyanoethyl)-*N*-ethylamino]-propionitrile. To a stirred mixture of 3,3′-iminodipropionitrile (14.4 g, 0.12 mol), acetaldehyde (25.7 g, 0.58 mol), methanol (50 ml) and acetonitrile (400 ml) was added sodium cyanoborohydride (9.6 g, 0.15 mol). After being stirred at room temperature overnight, the solvent was evaporated and the residue was diluted with dichloromethane and water with stirring. The separated organic layer was washed with water, dried and evaporated to give a yellow oil, which was distilled by a Kugelrohr apparatus to give the title compound (9.2 g, 52%). ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.86 (t, J = 7.0 Hz, 4H), 2.64 (q, J = 7.0 Hz, 4H), 2.48 (t, J = 7.0 Hz, 4H), 1.08 (t, J = 7.0 Hz, 3H); MS (70 eV) m/z (%) 152.3 (M^++1, 100), 126.2 (20); HRMS: calcd for $C_8H_{13}N_3$ (M^+) 151.1109, found 151.1111. The title compound was identical in all respects with the material prepared by the method described in Whitmore et al., 1944.[32]

4.3.2. 3-[*N*-(2-Cyanoethyl)-*N*-cyclopropylmethylamino]-propi 1onitrile. The title compound was prepared from 3,3′-iminodipropionitrile and cyclopropane carboxaldehyde by the method described in the former procedure in 54% yield. ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.86 (t, J = 7.0 Hz, 4H), 2.42 (t, J = 7.0 Hz, 4H), 2.39 (d, J = 7.2 Hz, 1H), 0.75 (m, 1H), 0.45 (m, 2H), 0.06 (m, 2H); MS (70 eV) m/z (%) 178.3 (M^++1, 15), 124.2 (100); HRMS: calcd for $C_{10}H_{15}N_3$ (M^+) 177.1266, found 177.1268.

4.3.3. *N*-[1-(3-Aminopropyl)-*N*-1-ethyl]-propane-1,3-diamine. The title compound was prepared by the method described in Satoh et al., 1969.[33] To a solution of

3-[*N*-2-cyanoethyl]-*N*-ethylamino]-propionitrile (15.1 g, 0.1 mol) and cobaltous chloride hexahydrate (47.6 g, 0.2 mol) in methanol (600 ml) was added sodium borohydride (38 g, 1.0 mol) in portions with stirring at 20 °C. To the mixture was added 200 ml of 3 N hydrochloric acid. After removal of methanol by distillation and unreacted nitrile by extraction with ether, the aqueous layer was made alkaline by addition of a concentrated ammonium hydroxide solution, which was then extracted with 100 ml of ethyl acetate three times. The combined organic extracts were washed with brine, dried over sodium sulfate, and the solvent evaporated to give the title compound (9.6 g, ~60%) as a crude oil, which was used for the preparation of compound **5** without purification. An analytical amount of the crude oil was characterized from its di-Cbz derivative: To a solution of the crude oil (0.2 g, ~1.0 mmol) in dichloromethane (4 mL) containing triethylamine (0.24 g, 2.4 mmol) was added at 0 °C, a solution of benzylchloroformate (0.34 g, 2.0 mmol) in dichloromethane (3 mL). After being stirred at 0 °C for 1 h and rt for 3 h, the reaction mixture was washed with water. The separated organic layer was dried ($MgSO_4$) and evaporated. Purification by silica gel column chromatography (eluent: ethyl acetate–hexane) provided the di-Cbz derivative as amber oil (0.28 g, 66%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.98 (t, J = 7.2 Hz, 3H), 1.60 (t, J = 6.6 Hz, 4H), 2.43 (t, J = 6.6 Hz, 4H), 2.47 (q, J = 7.2 Hz, 2H), 3.22 (m, 4H), 5.08 (s, 4H), 5.65 (br s, 2H), 7.33 (m, 10H); MS (70 eV) *m/z* (%) 428.2 (M^++1, 20), 335.2 (100), 291.5 (45); HRMS: calcd for $C_{24}H_{33}N_3O_4$ (M^+) 427.2471, found 427.2475. The title compound can also be prepared by catalytic hydrogenation of 3-[*N*-(2-cyanoethyl)-*N*-cyclopropylmethylamino]-propionitrile (**4**, **3**, **2**) under high pressure.[32]

4.3.4. *N*-[1-(3-Aminopropyl)]-*N*-[1-(cyclopropylmethyl)]-propane-1,3-diamine. The title compound was prepared from 3-[*N*-(2-cyanoethyl)-*N*-cyclopropylmethyl amino]-propionitrile by the method described in the former procedure as a crude oil in 60% yield, which was used for preparation of compound **6** without purification. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.50–3.00 (m, 8H), 2.30 (d, J = 6.4 Hz, 2H), 1.60 (m, 4H), 0.90 (m, 1H), 0.50 (m, 2H), 0.12 (m, 2H); MS (70 eV) *m/z* (%) 185.3 (M^+, 12), 132.2 (20), 100.2 (100), 86.1 (50); HRMS: calcd for $C_{10}H_{23}N_3$ (M^+) 185.1892, found 185.1896.

4.3.5. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (1). A mixture of 6,9-dichloro-2-methoxy-acridine (20.0 g, 71.9 mmol), 3,3′-diaminodipropylamine (5.5 g, 41.9 mmol) and potassium carbonate anhydrous (20.0 g, 144.9 mmol) in DMF (200 ml) was stirred at 118 °C for 16 h. The resulting solution was filtered and distilled on vacuum to remove DMF. The residue was triturated with dichloromethane–H_2O and filtered. The yellow powder was further triturated with acetone (250 ml) to give 8.6 g of the title compound. The mother liquid crystallized upon standing at 0 °C, which was filtered and triturated with acetone to give 3.0 g of the title compound. The mother liquid was evaporated and chromatographed using 1:10:0.1 (v/v) methanol/ethyl acetate/triethylamine to give the 1.6 g of the title compound. The combined yield is 13.2 g (60%). Mp 157–159 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.28 (d, J = 9.25 Hz, 2H), 7.81 (s, 2H), 7.77 (d, J = 9.3 Hz, 2H), 7.56 (s, 2H), 7.35 (d, J = 9.3 Hz, 2H), 7.17 (d, J = 9.3 Hz, 2H), 3.87 (s, 6H), 3.79 (t, J = 6.4 Hz, 4H), 2.69 (t, J = 6.4 Hz, 4H), 1.85 (m, 4H). The title compound was identical in all respects with the material prepared by the method described in Le Pecq et al., 1975.[29]

Mp 157–159 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) 8.28 (d, J = 9.25 Hz, 2H), 7.81 (s, 2H), 7.77 (d, J = 9.3 Hz, 2H), 7.56 (s, 2H), 7.35 (d, J = 9.3 Hz, 2H), 7.17 (d, J = 9.3 Hz, 2H), 3.87 (s, 6H), 3.79 (t, J = 6.4 Hz, 4H), 2.69 (t, J = 6.4 Hz, 4H), 1.85 (m, 4H).

4.3.6. Preparation of the acetic acid salt of compound 1. A mixture of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (0.25 g, 0.41 mmol), acetic acid (77.3 mg, 1.30 mmol) and methanol (20 ml) was stirred at refluxing temperature until the mixture became homogeneous (about 10 min). The mixture was filtered, evaporated and the residue was triturated with diethyl ether to afford the title compound (0.33 g, 95%). Mp 161–163 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.29 (d, J = 9.3 Hz, 2H), 7.82 (s, 2H), 7.78 (d, J = 9.3 Hz, 2H), 7.59 (s, 2H), 7.38 (d, J = 9.3 Hz, 2H), 7.25 (d, J = 9.3 Hz, 2H), 3.88 (s, 6H), 3.79 (m, 4H), 2.72 (m, 4H), 1.89 (s, 12 H), 1.88 (m, 4H).

4.3.7. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-butane-1,4-diamine (2). The title compound was prepared from spermidine by the procedure described in the preparation of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine in 62% yield. Mp 163–165 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.70–8.00 (m, 6H), 7.10–7.40 (m, 6H), 3.85–3.88 (m, 8H), 3.71 (m, 2H), 2.86 (m, 2H), 2.70 (m, 2H), 1.88 (m, 2H), 1.81 (m, 2H), 1.68 (m, 2H); Anal. ($C_{36}H_{39}Cl_2N_5O_2$) C, H, N. The title compound was identical in all respects with the material prepared by the method described in Le Pecq et al., 1975.[29]

4.3.8. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-methyl-propane-1,3-diamine (3). N-Alkylated triamine-linked acridine dimers were prepared according to the method shown in Scheme 1. As a typical example:

A mixture of 6,9-dichloro-2-methoxy-acridine (6.43 g, 23.1 mmol), *N*,*N*-bis(3-aminopropyl)methylamine (1.67 g, 11.5 mmol) and 4-dimethylaminopyridine (2.82 g, 23.1 mmol) in DMF (100 ml) was stirred at 85–90 °C for 4 h under N_2. The reaction mixture was filtered and the filtrate was distilled under vacuum to remove DMF. The residue was diluted with chloroform and water to give a solid suspension. The solid was collected and the chloroform solution was washed twice with water, dried and evaporated. The residue was combined with the solid and chromatographed on silica gel using ethyl acetate as eluent to give the title compound

(4.1 g, 56%) as a yellow powder. Mp 154–155 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.80–7.95 (m, 6H), 7.00–7.30 (m, 6H), 3.76 (s, 6H), 3.74 (m 4H), 2.54 (m, 4H), 2.32 (s, 3H), 1.85 (m, 4H); Anal. ($C_{36}H_{39}Cl_2N_5O_2$) C, H, N. The title compound was identical in all respects with the material prepared by the method described in Le Pecq et al., 1975.[29]

4.3.9. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-propyl-propane-1,3-diamine (4). *Typical example:* To a mixture of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (0.61 g, 1.0 mmol), propionaldehyde (1.47 g, 20.4 mmol), methanol (3 ml) and acetonitrile (3 ml) was added sodium cyanoborohydride (0.7 g) at 0 °C for 0.5 h and the ice bath was removed. Stirring was continued for 18 h. The solution was diluted with ethyl acetate and washed with water. The organic layer was evaporated and purified on silica using methanol/ethyl acetate/triethylamine (1:10:0.1, v/v) as eluent to give a reductive-amination product, which was diluted with small amount of methanol and 5 N HCl with stirring and the HCl salt was collected and triturated with acetone to give **4** (0.45 g, 54%) as the HCl salt. Mp 192–194 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.92 (m, 2H, NH^+), 9.70 (m, 1H, NH^+), 8.35 (m, 2H), 8.00 (m, 2H), 7.82 (s, 2H), 7.74 (d, J = 9.5 Hz, 2H), 7.49 (d, J = 9.5 Hz, 2H), 7.29 (d, J = 9.5 Hz, 2H), 4.08 (m, 2H), 3.83 (s, 6H), 3.20 (m, 4H), 2.94 (m, 2H), 2.35 (m, 4H), 1.60 (m, 2H), 0.78 (t, J = 7.0 Hz, 3H); Anal. ($C_{37}H_{44}Cl_7N_5O_2{\cdot}0.2H_2O{\cdot}0.1CH_3OH$) C, H, N. The free base **4** was liberated by neutralization with 10% potassium carbonate aqueous solution. ^{13}C NMR of the free base is as follows: ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 155.9, 151.0, 137.1, 135.8, 135.5, 129.1, 125.8, 124.8, 124.1, 123.6, 116.0, 114.7, 100.3, 56.1, 52.6, 49.7, 47.4, 28.5, 27.5, 12.2.

The following compounds were prepared in a manner similar to the above-described procedure except that a different aliphatic aldehyde or the acridine dimer homologue (compound **2**) was employed for the preparation of N-substituted triamine-linked acridine. The yields and analytical data are reported as follows:

4.3.10. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-ethyl-propane-1,3-diamine (5). Yield: 63%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.80–8.10 (m, 2H), 7.30 (m, 2H), 7.14 (m, 2H), 7.08 (m, 2H), 3.83 (s, 6H), 3.76 (m, 4H), 2.67 (m, 6H), 1.88 (m, 4H), 1.10 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.4, 150.8, 146.0, 135.7, 128.4, 128.0, 131.5, 124.6, 123.8, 123.5, 116.4, 113.8, 101.1, 55.5, 52.1, 49.7, 48.0, 27.8, 13.6; MS (20 eV) m/z (%) 641.4 ($M^+(2Cl^{35})$, 100), 467.3 (63); Anal. ($C_{36}H_{37}Cl_2N_5O_2{\cdot}0.1H_2O$) C, H, N.

4.3.11. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-cyclopropyl-methyl-propane-1,3-diamine (6). Yield: 65%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.99 (s, 2H), 7.92 (d, J = 9.3 Hz, 2H), 7.90 (s, 2H), 7.33 (m, 2H), 7.19 (s, 2H), 7.12 (m, 2H), 3.80 (s, 6H), 3.79 (t, J = 6.5 Hz, 4H), 2.75 (t, J = 6.5 Hz, 4H), 2.47 (d, J = 6.5 Hz, 2H), 1.89 (t, J = 6.5 Hz, 4H), 0.87 (m, 1H), 0.50 (m, 2H), 0.10 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.2, 152.3, 146.5, 146.2, 143.2, 139.1, 136.5, 129.3, 125.4, 124.5, 124.0, 116.6,101.9, 62.0, 56.2, 53.2, 50.1, 28.0, 8.3, 4.7; MS (70 eV) m/z (%) 667.5 ($M^+(2Cl^{35})$, 100), 611.6 (25), 602.3 (33), 499.7 (40), 467.3 (50), 423.2 (50); Anal. ($C_{38}H_{39}Cl_2N_5\ O_2{\cdot}0.25H_2O$) C, H, N.

4.3.12. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-cyclopropyl-methyl-butane-1,4-diamine (7). Yield: 70%, amorphous powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.44 (m, 2H), 7.95–7.99 (m, 4H), 7.76 (m, 2H), 7.55 (m, 2H), 7.37 (m, 2H), 4.06 (s, 6H), 3.91 (m, 2H), 3.83 (m, 2H), 2.58 (m, 2H), 2.48 (m, 2H), 2.25 (d, J = 6.2 Hz, 2H), 1.90 (m, 2H), 1.73 (m, 2H), 1.45 (m, 2H), 0.70 (m, 1H), 0.38 (m, 2H), 0.02 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 156.1, 155.5, 151.2, 150.6, 147.9, 145.9, 145.6, 135.5, 135.3, 130.6, 130.2, 127.5, 127.0, 125.4, 125.0, 124.7, 124.3, 124.0, 123.5, 117.9, 116.6, 115.5, 114.3, 101.9, 100.2, 59.7, 56.0, 54.4, 53.2,5 0.9, 50.4, 30.0, 27.5, 24.2, 8.4, 4.5; MS (70 eV) m/z (%) 681.1 ($M^+(2Cl^{35})$, 40), 636.9 (20), 423.2 (100); MS (70 eV) m/z (%) 682.1 ($M^+(2Cl^{35})$+1, 40), 612.1 (100); Anal. (C39H41Cl2N5 O2.0.2H2O) C, H, N.

4.3.13. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-hexyl-propane-1,3-diamine (8). Yield: 65%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.80–8.10 (m, 6H), 7.32 (m, 2H), 7.16 (s, 2H), 7.12 (m, 2H), 3.82 (s, 2H), 3.77 (t, J = 7.2 Hz, 4H), 2.63 (t, J = 7.2 Hz, 4H), 2.52 (m, 2H), 1.87 (t, J = 7.2 Hz, 4H), 1.45 (m, 2H), 1.15–1.35 (m, 6H), 1.15–1.35 (m, 6H), 0.86 (t, J = 7.2 Hz, 4H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 164.1, 155.7, 150.7, 145.8, 135.3, 130.4, 127.2, 125.3, 124.3, 123.8, 117.3, 114.9, 101.1, 56.0, 55.0, 52.9, 50.0, 32.0, 28.3, 27.6, 26.4, 22.9, 14.4; MS (70 eV) m/z (%) 698.1 ($M^+(2Cl^{35})$+1, 40), 612.1 (100); Anal. ($C_{40}H_{45}Cl_2N_5O_2{\cdot}0.15H_2O$) C, H, N.

N-(Aryl)-methyl-, *N*-(heteroaryl)methyl-, or *N*-(heterocyclyl)methyl-triamine-linked acridine dimers were prepared according to Scheme 2.

4.3.14. *N*-Benzyl-*N*′-(6-chloro-2-methoxy-acridin-9-yl)-*N*-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (9). *Typical example:* To a mixture of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (0.614 g, 1 mmol), benzaldehyde (1.0 g, 9.4 mmol), methanol (2 ml) and acetonitrile (4 ml) was added sodium cyanoborohydride (0.4 g) at 0 °C for 0.5 h followed by removal of the ice bath. Stirring was continued for 18 h. The solution was diluted with ethyl acetate and washed with water. The organic layer was evaporated and purified on silica using methanol/ethyl acetate/triethylamine (1:10:0.1, v/v) as eluent to give the reductive product, which was diluted with a small

amount of methanol and 5 N HCl with stirring. The HCl salt was collected and triturated with acetone to give the title compound (0.5 g, 56%) as the HCl salt. Mp 215–217 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.99 (m, 2H, NH^+), 8.47 (m, 2H), 8.10 (m, 2H), 7.93 (s, 2H), 7.65 (d, J = 9.5 Hz, 2H), 7.63 (m, 4H), 7.41 (d, J = 9.5 Hz, 2H), 7.30 (m, 3H), 4.37(s, 2H), 4.17 (m, 4H), 3.94 (s, 6H), 3.27 (m, 4H), 2.51 (m, 4H); The free base **9** was liberated by neutralization with 10% potassium carbonate aqueous solution. ^{13}C NMR and element analysis of the free base **9** is as follows: ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 178.6, 155.8, 153.1, 142.6, 138.7, 138.2, 129.4, 128.7, 127.7, 127.3, 126.3, 125.6, 123.5, 121.4, 115.0, 111.4, 102.3, 59.4, 56.0, 51.7, 48.0, 28.1; MS (70 eV) *m*/*z* (%) 704.1 ($M^+(2Cl^{35})$+1, 40), 612.1 (100); Anal. ($C_{41}H_{39}Cl_2N_5O_2\cdot0.25H_2O$) C, H, N.

The preparation of N-substituted triamine-linked acridine was similar to the former procedure except that a different aldehyde (i.e., arylaldehyde or heteroarylaldehyde or heterocyclic carboxaldehyde) or the acridine dimer homologue was employed. The yields and analytical data are reported as follows.

4.3.15. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-phenethyl-propane-1,3-diamine (10). Yield: 68%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.94, (s, 2H), 7.87 (d, J = 9.5 Hz, 2H), 7.85 (d, J = 9.5 Hz, 2H), 7.28 (m, 2H), 7.21 (m, 2H), 7.14 (s, 2H), 7.00–7.10 (m, 5H), 3.07 (s, 6H), 3.70 (t, J = 6.4 Hz, 4H), 2.78 (m, 2H), 2.70 (m, 2H), 2.67 (t, J = 6.4 Hz, 4H), 1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.9, 150.7, 147.4, 145.5, 140.0, 135.8, 130.0, 128.9, 126.9, 126.7, 124.9, 124.3, 124.2, 117.2, 114.7, 101.1, 56.7, 56.0, 52.7, 49.8, 32.8, 28.5; MS (70 eV) *m*/*z* (%) 718.4 ($M^+(2Cl^{35})$+1, 40), 626.1 (30), 556.1 (100), 517.4 (55); Anal. ($C_{42}H_{41}Cl_2N_5O_2\cdot0.2H_2O$) C, H, N.

4.3.16. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(3-methoxy-benzyl)-propane-1,3-diamine (11). Yield: 66%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ : 8.00 (s, 2H), 7.93 (d, J = 9.3 Hz, 2H), 7.82 (d, J = 9.3 Hz, 2H), 7.35 (d, J = 9.3 Hz, 2H), 7.14–7.18 (m, 5H), 6.81 (s, 1H), 6.80 (m, 2H), 3.82 (s, 6H), 3.71 (t, J = 6.5 Hz, 4H), 3.65 (s, 2H), 3.57 (s, 2H), 2.58 (t, J = 6.5 Hz, 4H), 1.82 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 160.1, 156.0, 150.3, 147.8, 140.8, 140.2, 135.4, 130.4, 129.6, 127.3, 125.3, 124.3, 121.8, 117.4, 115.4, 114.7, 113.2, 112.7, 100.4, 59.2, 56.1, 55.6, 52.1, 49.2, 28.6; MS (70 eV) *m*/*z* (%) 734.5 ($M^+(2Cl^{35})$, 20), 612.3 (100); Anal. ($C_{42}H_{41}Cl_2N_5O_3\cdot0.1H_2O$) C, H, N.

4.3.17. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(3-methoxy-benzyl)-butane-1,4-diamine (12). Yield: 65%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.02 (s, 2H), 7.96 (d, J = 9.3 Hz, 2H), 7.85 (d, J = 9.3 Hz, 2H), 7.35 (d, J = 9.3 Hz, 2H), 7.05–7.30 (m, 6H), 6.70–6.85 (m, 2H), 3.90 (s, 2H), 3.84 (m, 5H), 3.67 (m, 5H), 3.54 (s, 2H), 2.57 (m, 2H), 2.47 (m, 2H), 1.84 (m, 2H), 1.67 (m, 2H), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ : 160.0, 156.3, 155.9, 151.0, 150.8, 147.3, 147.1, 144.9, 140.7, 140.6, 137.1, 135.9, 129.8, 129.6, 126.8,126.7, 125.0, 124.9, 124.8, 124.6, 124.2, 121.7, 121.5, 117.5, 116.9, 115.4, 115.1, 114.5, 113.5, 112.6, 101.0, 100.3, 59.3, 56.0, 56.1, 55.9, 54.0, 52.2, 50.3, 49.6, 29.8, 28.5, 24.5; MS (70 eV) *m*/*z* (%) 747.4 ($M^+(2Cl^{35})$, 23), 626.4 (20), 489.3 (100); Anal. ($C_{43}H_{43}Cl_2N_5O_3\cdot0.1H_2O$) C, H, N.

4.3.18. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(2-methoxy-benzyl)-propane-1,3-diamine (13). Yield: 66%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.95 (s, 2H), 7.90 (d, J = 9.4 Hz, 2H), 7.76 (d, J = 9.4 Hz, 2H), 7.25–7.30 (m, 4H), 7.11 (s, 2H), 6.97 (m, 2H), 6.85 (m, 1H), 6.72 (m, 1H), 3.70–3.73 (m, 10 H), 3.62 (s, 2H), 3.46 (s, 3H), 2.63 (m, 4H), 1.88 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 163.0, 158.2, 155.8, 151.6, 145.7, 143.0, 136.6, 131.9, 129.5, 128.0, 125.6, 125.3, 125.0, 121.0, 120.8, 116.2, 113.5, 111.0, 101.6, 56.1, 55.3, 53.8, 52.7, 49.8, 27.6; MS (70 eV) *m*/*z* (%) 734.4 ($M^+(2Cl^{35})$+1, 8), 612.3 (100); Anal. ($C_{42}H_{41}Cl_2N_5O_3\cdot0.25H_2O$) C, H, N.

4.3.19. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(4-methoxy-benzyl)-butane-1,4-diamine (14). Yield: 64%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.80–8.00 (m, 6H), 7.10–7.40 (m, 6H), 7.09 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 3.88 (s, 2H), 3.82 (m, 5H), 3.75 (s, 3H), 3.66 (m, 2H), 3.50 (s, 2H), 2.57 (m, 2H), 2.44 (m, 2H), 1.83 (m, 2H), 1.67 (m, 2H), 1.56 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 164.0, 159.1, 156.2, 155.8, 154.0, 151.7, 151.5, 146.6, 143.2, 143.0, 136.2, 136.1, 130.6, 130.4, 128.8, 126.0, 125.6, 125.2, 124.7, 124.4, 124.2, 123.5, 123.8, 121.2, 117.3, 116.5, 114.8, 114.0, 101.7, 100.9, 58.5, 56.2, 56.0, 55.6, 53.8, 51.8, 50.0, 49.7, 29.5, 28.0, 24.4; MS (70 eV) *m*/*z* (%) 747.0.($M^+(2Cl^{35})$, 25), 637.3 (100), 626.6 (40), 488.9 (43); Anal. ($C_{43}H_{43}Cl_2N_5O_3\cdot0.15H_2O$) C, H, N.

4.3.20. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(2,3-dimethoxy-benzyl)-propane-1,3- diamine (15). Yield: 60%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.96 (d, J = 9.3 Hz, 2H), 7.82 (d, J = 9.3 Hz, 2H), 7.35 (dd, J = 9.3, 2.4 Hz, 2H), 7.14–7.18 (m, 4H), 6.89–6.93 (m, 1H), 6.81–6.83 (m, 2H), 4.13 (s, 3H), 3.83 (s, 6H), 3.78 (s, 3H), 3.74 (m, 4H), 3.68 (s, 2H), 2.62 (m, 4H), 1.87 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.0, 155.8, 153.0, 150.8, 148.0, 147.8, 135.4, 131.8, 130.5, 127.3, 125.1, 124.6, 124.2, 124.1, 122.9, 117.4, 115.2, 111.8, 100.9, 61.0, 56.0, 55.9, 52.2, 52.0, 49.5, 28.4; Ms (70 eV) *m*/*z* (%) 764.7 ($M^+(Cl^{35}Cl^{37})$+1, 40), 709.3 (40), 692.1 (40), 612.4 (100); Anal. ($C_{43}H_{43}Cl_2N_5O_4\cdot0.36H_2O$) C, H, N.

4.3.21. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(3,4,5-trimethoxy-benzyl)-propane-1,3-diamine (16). Yield: 55%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.03 (s, 2H), 7.97 (d, J = 9.5 Hz, 2H), 7.86 (d, J = 9.5Hz, 2H),

7.38 (m, 2H), 7.16–7.20 (m, 4H), 6.49 (s, 2H), 3.80, 3.82 (each s, 9H), 3.73 (t, J = 6.6 Hz, 4H), 3.71 (s, 6H), 3.55 (s, 2H), 2.61 (t, J = 6.6 Hz, 4H), 1.84 (m, 4H); MS (70 eV) m/z (%) 793.1 ($M^+(3Cl^{35})$, 15), 778.3 (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.0, 153.6, 150.7, 146.5, 144.7, 137.5, 136.0, 134.1, 129.1, 126.2, 125.0, 124.8, 124.7, 117.0, 115.6, 106.3, 100.7, 59.8, 56.3, 56.2, 56.1, 52.1, 49.3, 28.2; Anal. ($C_{44}H_{45}Cl_2N_5O_5 \cdot 0.3H_2O$) C, H, N.

4.3.22. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(4-chloro-benzyl)-propane-1,3-diamine (17). Yield: 67%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.96 (s, 2H), 7.89 (d, J = 9.4 Hz, 2H), 7.77 (d, J = 9.4 Hz, 2H), 7.30 (m, 2H), 7.17 (d, J = 9.4 Hz, 2H), 7.00–7.16 (m, 6H), 3.77 (s, 6H), 3.62 (t, J = 6.6 Hz, 4H), 3.46 (s, 2H), 2.48 (t, J = 6.6 Hz, 4H), 1.76 (m, 4H); MS (70 eV) m/z (%) 737.1 ($M^+(3Cl^{35})$, 5), 612.3 (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.0, 151.8, 145.1, 141.2, 137.0, 136.8, 133.7, 130.8, 129.0, 128.0, 125.4, 124.9, 124.5, 124.0, 116.2, 113.3, 101.6, 58.7, 56.1, 52.0, 48.8, 28.3; Anal. ($C_{41}H_{38}Cl_3N_5O_2 \cdot 0.25H_2O$) C, H, N.

4.3.23. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(2,4-difluoro-benzyl)-propane-1,3- diamine (18). Yield: 70%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.98 (s, 2H), 7.92 (d, J = 9.40 Hz, 2H), 7.80 (d, J = 9.40 Hz, 2H), 7.34 (m, 4H), 7.00–7.15 (m, 5H), 6.71 (m, 2H), 3.79 (s, 6H), 3.64 (m, 4H), 3.58 (s, 2H), 2.52 (m, 4H), 1.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.8, 151.4, 145.7, 142.7, 136.5, 132.8, 128.0, 125.3, 125.1, 124.7, 124.5, 123.9, 116.4, 113.7, 111.6, 104.6, 104.3, 104.1, 101.3, 55.9, 52.0, 51.7, 48.9, 28.4; MS (70 eV) m/z (%) 740.4 ($M^+(2Cl^{35})$ +1, 8), 612.3 (100); Anal. ($C_{41}H_{37}Cl_2F_2N_5O_2 \cdot 0.15H_2O$) C, H, N.

4.3.24. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-furan-2-ylmethyl-propane-1,3-diamine (19). Yield: 66%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.07(s, 2H), 7.90–8.00 (m, 4H), 7.46 (m, 2H), 7.40 (s, 1H), 7.30–7.20 (m, 4H), 6.40 (s, 1H), 6.26 (s, 1H), 3.95 (s, 6H), 3.87 (t, J = 6.5 Hz, 4H), 3.84 (s, 2H), 2.76 (t, J = 6.50 Hz, 4H), 1.97 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.8, 151.4, 150.4, 147.9, 145.9, 142.8, 135.4, 130.6, 127.4, 124.7, 124.4, 124.1, 117.3, 115.0, 110.7, 109.9, 100.7, 55.9, 53.0, 50.8, 49.9, 28.5; MS (70 eV) m/z (%) 693.4 ($M^+(2Cl^{35})$, 6), 614.3 (60), 612.3 (100); Anal. ($C_{39}H_{37}Cl_2N_5O_3 \cdot 0.24H_2O$) C, H, N.

4.3.25. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-furan-2-ylmethyl-butane-1,4-diamine (20). Yield: 63%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.85–8.10 (m, 6H), 7.10–7.50 (m, 7H), 6.39 (s, 1H), 6.26 (s, 1H), 3.98 (m, 2H), 3.96 (s, 6H), 3.80 (m, 2H), 3.79 (s, 2H), 2.75 (m, 2H), 2.63 (m, 2H), 1.94 (m, 2H), 1.82 (m, 2H), 1.75 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 179.2, 164.7, 156.2, 155.7, 153.0, 151.8, 151.3, 150.8, 147.2, 146.9, 144.9, 144.7, 142.7, 135.9, 129.7, 126.5, 125.2, 125.0, 124.9, 124.5, 124.3, 123.9, 117.4, 116.6, 114.8, 114.2, 110.7, 109.7, 101.6, 100.4, 56.0, 55.9, 54.4, 53.1, 50.9, 50.6, 50.1, 29.8, 27.8, 24.8; MS (70 eV) m/z (%) 707.1 ($M^+(2Cl^{35})$, 4), 626.4 (2), 436.2 (100); Anal. ($C_{40}H_{39}Cl_2N_5O_3 \cdot 0.1H_2O$) C, H, N.

4.3.26. *N*-(5-Bromo-furan-2-ylmethyl)-*N*′-(6-chloro-2-methoxy-acridin-9-yl)-*N* -[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine (21). Yield: 65%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.02 (s, 2H), 7.95 (d, J = 9.5 Hz, 2H), 7.87 (d, J = 9.5 Hz, 2H), 7.36 (d, J = 9.5 Hz, 2H), 7.22 (d, J = 9.5 Hz, 2H), 7.14 (s, 2H), 6.87 (d, J = 3.65 Hz, 1H), 6.56 (d, J = 3.65 Hz, 1H), 3.86 (s, 6H), 3.71 (s, 2H), 3.68 (t, J = 6.6 Hz, 4H), 2.56 (t, J = 6.6 Hz, 4H), 1.80 (m, 4H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 176.6, 155.8, 153.7, 153.0, 142.8, 138.5, 138.0, 126.3, 125.3, 124.3, 123.5, 121.6, 115.1, 112.6, 112.3, 111.6, 102.4, 56.0, 52.4, 51.2, 48.6, 27.8; MS (70 eV) m/z (%) 771.1 ($M^+(2Cl^{35}Br^{79})$, 10), 625.2 (100), 612.1 (60); Anal. ($C_{39}H_{36}BrCl_2N_5O_3 \cdot 0.2H_2O$) C, H, N.

4.3.27. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(5-ethyl-furan-2-ylmethyl)-propane-1,3- diamine (22). Yield: 62%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.08, (s, 2H), 8.01 (d, J = 9.5 Hz, 2H), 7.97 (d, J = 9.5 Hz, 2H), 7.42 (d, J = 9.5 Hz, 2H), 7.27 (s, 2H), 7.20 (d, J = 9.5 Hz, 2H), 6.12 (d, J = 3.0 Hz, 1H), 5.94 (d, J = 3.0 Hz, 1H), 3.89 (s, 6H), 3.83 (t, J = 6.5 Hz, 4H), 3.77 (s, 2H), 2.74 (t, J = 6.5 Hz, 4H), 2.48 (t, J = 8.0 Hz, 2H), 1.95 (m, 4H), 1.34 (m, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 159.4, 157.0, 155.2, 150.8, 142.0, 138.5, 136.5, 134.4, 129.2, 125.4, 122.1, 120.2, 119.1, 117.1, 109.9, 105.8, 105.2, 56.4, 51.6, 50.2, 48.7, 28.6, 21.5, 12.8; MS (70 eV) m/z (%) 721.0 ($M^+(2Cl^{35})$, 10), 625.2 (100), 612.1 (60); Anal. ($C_{41}H_{41}Cl_2N_5O_3 \cdot 0.15H_2O$) C, H, N.

4.3.28. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-furan-3-ylmethyl-propane-1,3-diamine (23). Yield: 70%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.99 (s, 1H), 7.97 (d, J = 9.5 Hz, 2H), 7.80–7.90 (m, 5H), 7.30–7.40 (m, 4H), 7.16 (m, 2H), 6.24 (s, 1H), 3.82 (s, 6H), 3.73 (m, 4H), 3.55 (s, 2H), 2.59 (t, J = 6.6Hz, 4H), 1.85 (m, 4H). MS (70 eV) m/z (%) 693.2 (100), 614.1 (63), 611.8 (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 154.4, 151.8, 142.4, 141.0, 140.2, 136.9, 136.6, 124.9, 123.9, 122.5, 122.0, 119.9, 119.5, 113.5, 110.3, 109.9, 101.2, 54.6, 50.4, 47.7, 47.1, 26.4; MS (70 eV) m/z (%) 693.2 ($M^+(2Cl^{35})$; Anal. ($C_{39}H_{37}Cl_2N_5O_3 \cdot 0.2H_2O$) C, H, N.

4.3.29. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-furan-3-ylmethyl-butane-1,4-diamine (24). Yield: 68%, amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.80–8.00 (m, 6H), 7.10–7.40 (m, 8H), 6.23 (s, 1H), 3.86 (s, 6H), 3.82 (m, 2H), 3.65 (m, 2H), 3.50 (s, 2H), 2.57 (m, 2H), 2.45 (m, 2H), 1.81 (m, 2H), 1.67 (m, 2H), 1.57 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20 :1 (v/v), 125 MHz) δ : 156.4, 156.1, 155.7, 151.2, 150.9, 147.8,

146.5, 146.2, 143.4, 141.1, 135.4, 135.3, 130.5, 130.2, 127.2, 126.9, 125.4, 125.3, 124.8, 124.3, 124.1, 123.6, 121.3, 117.9, 117.0, 116.0, 114.6, 111.6, 101.3, 100.5, 56.0, 55.9, 53.7, 51.9, 50.3, 49.9, 48.6, 29.6, 28.0, 24.4; MS (70 eV) *m/z* (%): 707.5 ($M^+(2Cl^{35})$, 100), 626.1 (20), 636.4 (60), 545.3 (40), 522.0 (27), 493.1(60), 466.0 (80), 450.1 (100); Anal. ($C_{40}H_{39}Cl_2N_5O_3 \cdot 0.1H_2O$) C, H, N.

4.3.30. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-thiophen-3-ylmethyl-propane-1,3-diamine (25). Yield: 63%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.00 (s, 2H), 7.94 (d, J = 9.4 Hz, 2H), 7.84 (d, J = 9.40Hz, 2H) 7.35 (m, 2H), 7.22 (m, 2H), 7.16 (m, 2H), 7.03 (s, 1H), 6.91 (s, 1H), 3.81 (s, 6H), 3.70 (t, J = 6.3 Hz, 4H), 3.65 (s, 2H), 2.57 (t, J = 6.3 Hz, 2H), 1.81 (m, 4H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 160.6, 155.4, 152.8, 150.8, 148.4, 146.2, 140.0, 135.4, 132.2, 129.9, 129.1, 128.7, 125.6, 122.3, 119.9, 116.4, 105.6, 60.7, 56.8, 54.3, 53.4, 33.1; MS (70 eV) *m/z* (%) 709.0 ($M^+(2Cl^{35})$, 10), 612.3 (10), 450.8 (30), 438.2 (60), 410.1 (100); Anal. ($C_{39}H_{37}Cl_2N_5O_2S \cdot 0.16H_2O$) C, H, N.

4.3.31. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-thiophen-2-ylmethyl-butane-1,4-diamine (26). Yield: 63%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.00 (d, J = 3.5 Hz, 1H), 7.99–7.80 (m, 4H), 7.36 (m, 2H), 7.26–7.00 (m, 6H), 6.91 (s, 1H), 3.87 (m, 8H), 3.79 (s, 2H), 3.63 (m, 2H), 2.57 (m, 2H), 2.47 (m, 2H), 1.81 (m, 2H), 1.67 (m, 2H), 1.55 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 156.3, 155.9, 150.6, 150.4, 148.3, 148.2, 146.4, 141.8, 135.3, 131.1, 131.0, 127.9, 127.8, 127.1, 126.6, 125.3, 125.0, 124.8, 124.6, 124.5, 124.1, 118.1, 117.5, 115.9, 115.2, 100.8, 99.9, 55.9, 53.7, 53.0, 51.9, 50.6, 49.5, 29.8, 28.5, 24.6; MS (70 eV) *m/z* (%) 723.2 ($M^+(2Cl^{35})$, 57), 626.0 (58), 464.9 (100); Anal. ($C_{40}H_{39}Cl_2N_5O_2S \cdot 0.26H_2O$) C, H, N.

4.3.32. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-thiophen-3-ylmethyl-butane-1,4-diamine (27). Yield: 61%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.09 (m, 2H), 8.03 (m, 2H), 7.96 (m, 2H), 7.25–7.50 (m, 7H), 7.10 (s, 1H), 7.00 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.83 (m, 2H), 3.67 (m, 2H), 3.65 (s, 2H), 2.59 (m, 2H), 2.48 (m, 2H), 1.84 (m, 2H), 1.70 (m, 2H), 1.58 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 161.5, 156.2, 155.8, 151.3, 151.0, 147.4, 147.2, 139.2, 135.8, 135.7, 129.8, 128.7, 126.9, 126.6, 126.2, 125.4, 125.3, 125.0, 124.5, 124.4, 123.9, 123.4, 118.0, 117.8, 116.9, 115.3, 114.5, 101.4, 100.6, 56.0, 54.0, 53.5, 52.1, 50.3, 49.9, 29.7, 28.2, 24.5; MS (70 eV) *m/z* (%) 723.4 ($M^+(2Cl^{35})$, 47), 626.5 (20), 465.2 (100); Anal. ($C_{40}H_{39}Cl_2N_5O_2S \cdot 0.2H_2O$) C, H, N.

4.3.33. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(5-bromo-thiophen-2-ylmethyl)-propane-1,3-diamine (28). Yield: 62%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.00 (s, 2H), 7.93 (d, J = 9.4 Hz, 2H), 7.85 (d, J = 9.4 Hz, 2H), 7.35 (m, 2H), 7.10–7.25 (m, 4H), 6.86 (d, J = 3.6 Hz, 1H), 6.55 (d, J = 3.6 Hz, 1H), 3.84 (s, 6H), 3.70 (t, J = 6.6 Hz, 4H), 2.54 (t, J = 6.6 Hz, 4H), 1.78 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 179.0, 171.6, 164.1, 156.0, 150.5, 147.2, 143.6, 135.8, 129.0, 127.5, 125.2, 124.4, 122.7, 117.3, 114.9, 109.7, 100.6, 56.0, 53.1, 51.8, 48.8, 28.8; MS (ESI) *m/z* (%) 786.5 ($M^+(2Cl^{35}Br^{79})$, 100); Anal. ($C_{39}H_{36}BrCl_2N_5O_2S \cdot 0.16H_2O$) C, H, N.

4.3.34. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(4-bromo-thiophen-2-ylmethyl)-propane-1, 3-diamine (29). Yield: 63%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.95 (s, 2H), 7.88 (d, J = 9.5 Hz, 2H), 7.77 (d, J = 9.5 Hz, 2H), 7.30 (m, 2H), 7.12 (m, 2H), 7.00–7.15 (m, 3H), 6.72 (s, 1H), 3.79 (s, 6H), 3.70 (s, 2H), 3.64 (t, J = 6.5 Hz, 4H), 2.53 (t, J = 6.5 Hz, 4H), 1.77 (m, 2H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 177.0, 164.0, 156.1, 147.0, 143.7, 139.8, 137.0, 128.9, 124.8, 124.6, 124.5, 122.6, 120.0, 117.7, 115.5, 109.6, 100.6, 56.0, 53.1, 51.8, 49.0, 28.9; MS (70 eV) *m/z* (%) 786.5 ($M^+(2Cl^{35}Br^{79})$, 10), 612.1 (15), 517.8 (60), 490.0 (100); Anal. ($C_{39}H_{36}BrCl_2N_5O_2S \cdot 0.23H_2O$) C, H, N.

4.3.35. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-(3-methyl-thiophen-2-ylmethyl)-propane-1, 3-diamine (30). Yield: 62%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.01 (s, 2H), 7.94 (d, J = 9.2 Hz, 2H), 7.82 (d, J = 9.2 Hz, 2H), 7.35 (m, 2H), 7.10–7.20 (m, 4H), 7.08 (d, J = 5.1 Hz, 1H), 6.76 (d, J = 5.1 Hz, 1H), 3.83 (s, 6H), 3.71 (t, J = 6.5 Hz, 4H), 3.68.(s, 2H), 2.58 (t, J = 6.5 Hz, 4H), 2.10 (s, 3H), 1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.1, 150.0, 148.5, 146.9, 142.0, 135.5, 135.2, 131.6, 130.6, 128.4, 124.6, 124.5, 124.4, 123.7, 118.0, 115.9, 100.1, 55.9, 52.1, 51.7, 49.1, 29.1, 14.3; MS (70 eV) *m/z* (%) 724.2 ($M^+(2Cl^{35}+1)$, 10), 612.4 (100); Anal. ($C_{40}H_{39}Cl_2N_5O_2S \cdot 0.2H_2O$) C, H, N.

4.3.36. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-pyridin-2-ylmethyl-propane-1,3-diamine (31). Yield: 61%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.51 (s, 1H), 8.06 (s, 2H), 7.90–8.00 (m, 4H), 7.57 (m, 1H), 7.40 (m, 2H), 7.00–7.20 (m, 6H), 3.84 (s, 6H), 3.83 (s, 2H), 3.73 (t, J = 5.4 Hz, 4H), 2.73 (t, J = 5.7 Hz, 4H), 1.92 (m, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 160.1, 155.8, 151.3, 149.3, 148.8, 148.6, 146.5, 136.7, 134.3, 131.2, 127.5, 127.3, 124.8, 123.1, 122.6, 117.8, 115.2, 101.5, 60.3, 56.3, 51.9, 48.5, 28.7; MS (70 eV) *m/z* (%) 704.6 ($M^+(2Cl^{35})$, 50), 612.1 (100); Anal. ($C_{40}H_{38}Cl_2N_6O_2 \cdot 0.3H_2O$) C, H, N.

4.3.37. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′-pyridin-3-ylmethyl-propane-1,3-diamine (32). Yield: 65%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.46 (s, 2H), 7.92 (s, 2H), 7.90 (m, 2H), 7.80 (m, 2H), 7.50–7.00 (m, 8H), 3.79 (s, 6H), 3.63 (t, J = 6.70 Hz, 4H),

3.52 (s, 2H), 2.51 (t, J = 6.70 Hz, 4H), 1.80(m, 4H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 = 20:1 (v/v), 125 MHz) δ: 155.6, 151.4, 150.0, 148.4, 146.8, 144.0, 136.4, 135.3, 134.2, 128.7, 126.0, 125.7, 124.5, 123.3, 123.2, 117.1, 114.3, 101.2, 55.8, 55.7, 51.1, 48.3, 28.1; MS (70 eV) m/z (%) 705.3 ($M^+(2Cl^{35})$ +1, 17), 621.1 (100), 612.3 (60), 462.1 (55); Anal. ($C_{40}H_{38}Cl_2N_6O_2\cdot0.35H_2O$) C, H, N.

4.3.38. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′(2,2-dimethyl-1,3-dioxolan-4-ylmethyl-propane-1,3-diamine (33). The title compound was prepared by the reductive amination of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine and (*R*)-2,3-isopropylideneglyceraldehyde. Yield: 64%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.90–8.10 (m, 6H), 7.40 (m, 2H), 7.10–7.25 (m, 4H), 4.28 (m, 1H), 4.00 (m, 1H), 3.86 (s, 6H), 3.76 (s, 4H), 3.51 (m, 1H), 1.33 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 156.6, 156.2, 154.3, 127.0, 126.7, 126.4, 125.8, 124.2, 123.8, 114.7, 109.7, 103.3, 101.3, 78.0, 56.3, 54.2, 52.7, 45.9, 27.6, 27.3, 25.8; MS (70 eV) m/z (%) 727.4 ($M^+(2Cl^{35})$, 10), 475.3 (20), 113.2 (100); Anal. ($C_{40}H_{43}Cl_2N_5O_4\cdot0.3H_2O$) C, H, N.

4.3.39. 3-{Bis-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-amino}-propane-1,2-diol (34). The title compound was prepared from compound **33** by hydrolysis (90% CF_3CO_2H), room temperature), followed by neutralization. Yield: 72%, amorphous powder. ^{1}H NMR (methanol-d_4, 200 MHz) δ: 8.00 (d, J = 9.2 Hz, 2H), 7.40–7.51 (m, 4H), 7.10–7.20 (m, 4H), 6.98–7.03 (d, J = 9.2 Hz, 2H), 3.82 (m, 4H), 3.78 (s, 6H), 3.63 (m, 1H), 3.50 (m, 2H), 2.60–2.80 (m, 4H), 1.90 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 162.9, 156.6, 151.0, 132.5, 130.4, 127.5, 126.6, 125.4, 125.2, 124.6, 118.3, 105.2, 99.8, 78.0, 71.0, 62.9, 56.2, 46.1, 35.6, 31.5; MS (70 eV) m/z (%) 677.3 ($M^+(2Cl^{35})$, 60), 465.2 (80), 421.2 (100); Anal. ($C_{37}H_{39}Cl_2N_5O_4\cdot0.43H_2O$) C, H, N.

4.3.40. *N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-*N*′(2,2,2′,2′-tetramethyl-[4,4′]bi[1,3]-dioxolanyl)-5-ylmethyl)-propane-1,3-diamine (35). The title compound was prepared by the reductive amination of *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-propane-1,3-diamine and a mannitol-derived diketalaldehyde (2,2,2′,2′-tetramethyl-[4,4′]bi[1,3]-dioxolanyl)-5- carbaldehyde). The diketal aldehyde is described in Wu and Wu, 1993.[34] Yield: 55%, amorphous powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.09 (m, 2H), 7.88–8.06 (m, 4H), 7.30 (m, 2H), 7.18 (m, 2H), 7.06–7.12 (m, 2H), 4.09–4.14 (m, 2H), 3.92 (m, 3H), 3.79 (s, 6H), 3.73 (m, 4H), 3.43 (m, 1H), 2.92 (m, 1H), 2.72 (m, 4H), 1.86 (m, 4H), 1.34, 1.30, 1.22, 1.16 (each s, 12 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.7, 150.7, 147.4, 135.5, 130.0, 126.8, 125.3, 124.3, 123.9, 117.1, 114.7, 110.1, 101.1, 80.0, 79.1, 73.0, 71.6, 70.8, 77.3, 68.3, 62.0, 56.0, 53.5, 49.7, 28.3, 27.7, 27.5, 27.4, 27.1; MS (ESI) m/z (%) 828 ($M^+(2Cl^{35})$+1, 25), 415 (100); Anal. ($C_{45}H_{51}Cl_2N_5O_6\cdot0.4H_2O$) C, H, N.

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References and notes

1. Arlin, Z. *Cancer Treat. Rep.* **1983**, 967–970.
2. (a) Atwell, G. J.; Cain, B. F.; Seelye, R. N. *J. Med. Chem.* **1972**, *15*, 611–615; (b) Cain, B. F.; Atwell, G. L. *Eur. J. Cancer* **1974**, *10*, 539–549.
3. Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. *Drugs* **1991**, *41*, 400–449.
4. Bridewell, D. L.; Finlay, G. L.; Baguley, B. C. *Anti-Cancer Drug Des.* **2001**, *16*, 317–324.
5. Caponigro, F.; Dittrich, C.; Sorensen, J. B.; Schellens, J. H.; Duffaud, F.; Paz Ares, L.; Lacombe, D.; de Balincourt, C.; Fumoleau, P. *Eur. J. Cancer* **2002**, *38*, 70–74.
6. Lown, J. W.; Nelson, J. M.; Plowman, J.; Jackson, R. C. *Cancer Res.* **1985**, *45*, 5532–5539.
7. Huan, S. D.; Natale, R. B.; Steward, D. J.; Sartiano, G. P.; Stella, P. J.; Roberts, J. D.; Symes, A. L.; Finizio, M. *Clin. Cancer Res.* **2000**, *6*, 1333–1336.
8. Adjei, A. A. *Invest. New Drugs* **1999**, *17*, 43–48.
9. Capps, D. B.; Dunbar, J.; Kesten, S. R.; Shillis, J.; Werbel, L. M.; Plowman, J.; Ward, D. L. *J. Med. Chem.* **1992**, *35*, 4770–4778.
10. (a) Antonini, I.; Cola, D.; Polucci, P.; Bontemps-Gracz, M.; Borowski, E.; Martelli, S. *J. Med. Chem.* **1995**, *38*, 3282–3286; (b) Antonini, I.; Polucci, P.; Magnano, A.; Martelli, S. *J. Med. Chem.* **2001**, *44*, 3329–3333; (c) Antonini, I.; Polucci, P.; Magnano, A.; Gatto, B.; Palumbo, M.; Menta, E.; Pescalli, N.; Martelli, S. *J. Med. Chem.* **2003**, *46*, 3109–3115.
11. Brana, M. F.; Castellano, J. M.; Perron, D.; Maher, C.; Conlon, D.; Bousquet, P. F.; George, J.; Qian, X.-D.; Robinson, S. P. *J. Med. Chem.* **1997**, *40*, 449–454.
12. Charmantray, F.; Demeunynck, M.; Carrez, D.; Croisy, A.; Lansiaux, A.; Bailly, C.; Colson, P. *J. Med. Chem.* **2003**, *46*, 967–977.
13. Chen, Q.; Deady, L. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1994**, *37*, 593–597.
14. Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; Djurickovic, D. B.; Michejda, C. J. *J. Med. Chem.* **1995**, *38*, 3043–3052.
15. Denny, W. A.; Roos, I. A. G.; Wakelin, L. P. G. *Anti-Cancer Drug Des.* **1986**, *1*, 141–147.
16. Krapcho, P. A.; Petry, M. E.; Getahun, Z.; Landi, J. J.; Stallman, J.; Polsenberg, J. F.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P. *J. Med. Chem.* **1994**, *37*, 828–837.
17. Stanslas, J.; Hagan, D. J.; Ellis, M. J.; Turner, C.; Carmichael, J.; Ward, W.; Hammonds, T. R.; Stevens, M. F. G. *J. Med. Chem.* **2000**, *43*, 1563–1572.
18. Su, T. L.; Chou, T. C.; Kim, J. Y.; Huang, J. T.; Ciszewska, G.; Ren, W. U.; Otter, G. M.; Sirotnak, F. M.; Watanabe, K. A. *J. Med. Chem.* **1995**, *38*, 3226–3235.
19. Wakelin, L. P. G.; Bu, X.; Eleftheriou, A.; Parmar, A.; Hayek, C.; Stewart, B. W. *J. Med. Chem.* **2003**, *46*, 5790–5802.
20. Gamage, S. A.; Spicer, J. A.; Atwell, G. L.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1999**, *42*, 2383–2393.
21. Igarashi, K.; Koga, K.; He, Y.; Shimogori, T.; Ekimoto, H.; Kashiwagi, K.; Shirahata, A. *Cancer Res.* **1995**, *55*, 2615–2619.

22. Chen, S. F.; Behrens, D. L.; Behrens, C. H.; Czerniak, P. M.; Dexter, D. L.; Dusak, B. L.; Fredericks, J. R.; Gale, K. C.; Gross, J. L.; Jiang, J. B.; Kirshenbaum, M. R.; McRipley, R. L.; Papp, L. M.; Patten, A. D.; Perrella, F. W.; Seitz, S. P.; Stafford, M. P.; Sun, J. H.; Sun, T.; Wuonola, M. A.; Von Hoff, D. D. *Anti-Cancer Drugs* **1993**, *4*, 447–457.
23. Nitiss, J. L.; Zhou, J.; Rose, A.; Hsiung, Y.; Gale, K. C.; Osheroff, N. *Biochemistry* **1998**, *37*, 3078–3085.
24. O'Reilly, S.; Baker, S. D.; Sartorius, S.; Rowinsky, E. K.; Finizio, M.; Lubiniecki, G. M.; Grochow, L. B.; Gray, J. E.; Pieniaszek, H. J.; Donehower, R. C. *Ann. Oncol.* **1998**, *9*, 101–104.
25. Bousquet, P. F.; Brana, M. F.; Conlon, D.; Fitzgerald, K. M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X. D. *Cancer Res.* **1995**, *55*, 1176–1180.
26. Hernandez, L.; Cholody, W. M.; Hudson, E. A.; Resau, J. H.; Pauly, G.; Michejda, C. J. *Cancer Res.* **1995**, *55*, 2338–2345.
27. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
28. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989.
29. Le Pecq, J. B.; Le Bret, M.; Barbett, J.; Roquest, B. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915–2919.
30. Qin, Y.; Pang, J.-Y.; Chen, W.-H.; Cai, Z.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2006**, *14*, 25–32.
31. Edwards, M. L.; Snyder, R. D.; Stemerick, D. M. *J. Med. Chem.* **1991**, *34*, 2414–2420.
32. Whitmore, F. C.; Mosher, H. S.; Adams, R. R.; Taylor, R. B.; Chapin, E. C.; Weisel, C.; Yanko, W. *J. Am. Chem. Soc.* **1944**, *66*, 725.
33. Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. *Tetrahedron Lett.* **1969**, *10*, 4555.
34. Wu, W.-L.; Wu, Y.-L. *J. Org. Chem.* **1993**, *58*, 3586–3588.