Synthesis and Antitumor Activity of Fused Tetracyclic Quinoline Derivatives. 1

Masatoshi Yamato,*,† Yasuo Takeuchi,† Kuniko Hashigaki,† Yuji Ikeda,† Chang Ming-rong,† Kyoko Takeuchi,† Mayumi Matsushima,† Takashi Tsuruo,‡ Tazuko Tashiro,‡ Shigeru Tsukagoshi,‡ Yoshinori Yamashita,§ and Hirofumi Nakano§

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka 1-1-1, Okayama 700, Japan, Cancer Chemotherapy Center, Kami-ikebukuro 1-37-1, Toshima-Ku, Tokyo 170, Japan, and Tokyo Research Laboratories, Kyowa Hakko Co. Ltd., Asahimachi 3-6-6, Machida, Tokyo 194, Japan. *Received July 15, 1988*

Several fused tri- and tetracyclic quinolines (I and II) with [2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino or [3-(*N,N*-dimethylamino)propyl]amino side chains were prepared, and their DNA intercalative properties, KB cytotoxicity, antitumor activity (P388 leukemia), and ability to induce topoisomerase II dependent DNA cleavage were investigated. Some compounds having both intercalative ability and KB cytotoxicity were found to be inactive in vivo. However, a positive correlation was seen between the ability to induce topoisomerase II dependent DNA cleavage and antitumor activity in vivo. The indeno- (**13a**), benzofuro- (**21a**), and benzothieno- (**22a**) quinoline derivatives exhibited potent antitumor activities in vitro and in vivo, comparable to those of *m*-AMSA. They also intercalate DNA and induce topoisomerase II dependent DNA cleavage. Extended screening of **13a** showed it to be active against solid tumors such as M5076 sarcoma, B16 melanoma, and colon 38 carcinoma.

DNA-intercalative antitumor drugs such as adriamycin, actinomycin, ellipticine, and amsacrine (*m*-AMSA) represent an important class of antitumor drugs. A large number of studies on their structure–activity relationships have shown that the antitumor properties of these molecules significantly correlate with both strength of DNA binding and the kinetics of binding.[1]

Among them, *m*-AMSA is a synthetic acridine derivative in clinical use, but with a limited antitumor activity spectrum. Denny et al. have reported a large number of studies on the structure–activity relationships of *m*-AMSA analogues,[2] concentrating on structural variations of the substituents on the acridine ring. This led to development of the 4-methyl-5-(methylcarbamoyl) derivative (CI-921),[3] which has a broad spectrum of activity against both leukemia and solid tumors.

Intercalating drugs have been characterized as inhibitors of the template functions of DNA.[4,5] Liu et al. recently showed that *m*-AMSA is a very potent inhibitor of the DNA nicking–closing enzyme topoisomerase II[6,7] and induces DNA strand breaks associated with topoisomerase II.[8]

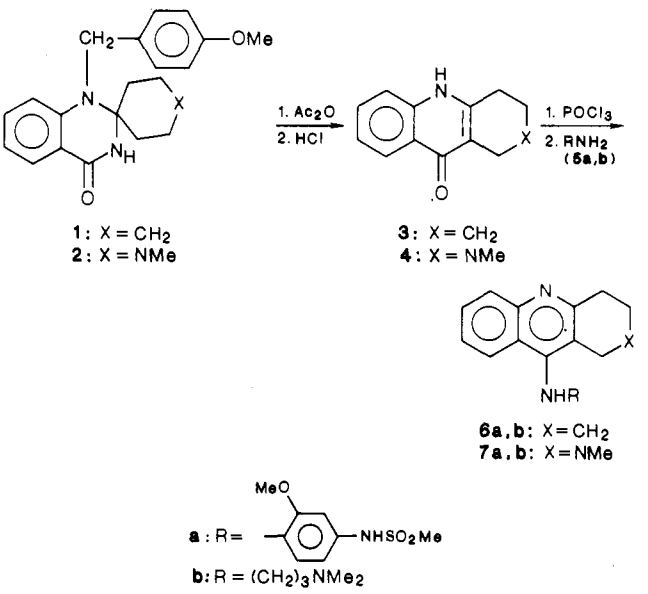
We recently developed a new synthetic method for the preparation of 1,2,3,4-tetrahydroamsacrine (**6a**) and its aza analogue (**7a**)[9] and were interested in the molecular modifications of antitumor-active acridine derivatives in a search for new antitumor agents. Our attention was focused especially on the development of new intercalating chromophores, because the chromophores of antitumor agents undoubtedly play an important role in drug–DNA binding, so that variations of chromophore size, planarity, or linearity and electronics (inclusion of various hetero atoms) must cause variations in antitumor properties.

On the basis of these considerations, we have designed and prepared novel fused tri- and tetracyclic quinolines having [2-methoxy-4-[(methylsulfonyl)amino]phenyl]-amino or [3-(*N,N*-dimethylamino)propyl]amino side chains (the side chains of *m*-AMSA and nitracrine, respectively; see Figure 1). These compounds were evaluated for antitumor activity and DNA-intercalating ability, and several (**13a**, **21a**, and **22a**) were found to have potent antitumor activity.

Chemistry

We have previously reported the preparation of the 9-anilinotetrahydroacridine **6a** and 10-anilinobenzo[*b*]-tetrahydro-1,6-naphthyridine **7a** via the rearrangement of the spiro tetrahydroquinazolinones **1** and **2**.[9] Their [3-(*N,N*-dimethylamino)propyl]amino congeners, **6b** and **7b**, were presently prepared according to a previous method (Scheme I). Treatment of the spiro compound **1** with acetic anhydride afforded the rearranged compound **3**. Chlorination of **3** followed by reaction with 3-(*N,N*-dimethylamino)propylamine (**5b**) gave the desired compound **6b**. Similarly, **7b** was prepared from **2**.

Scheme I

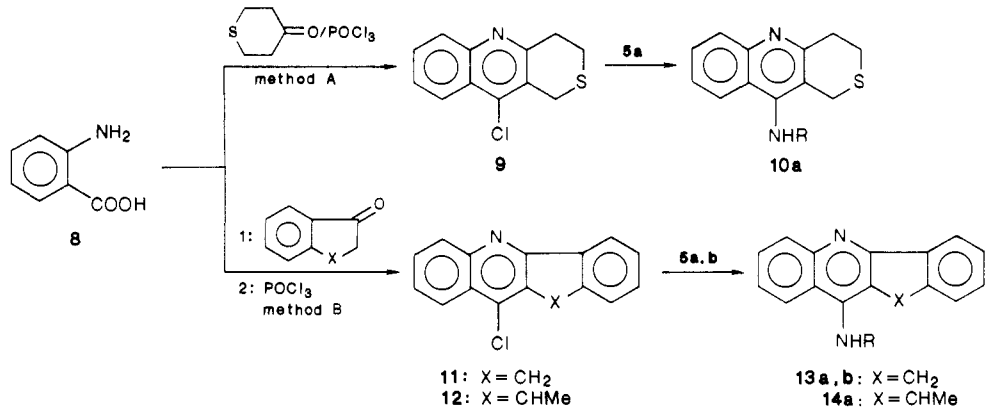


† Okayama University.
‡ Cancer Chemotherapy Center.
§ Kyowa Hakko Co.

(1) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* 1984, *27*, 450.
(2) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In *Molecular Aspects of Anticancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; pp 1–34.
(3) Atwell, G. J.; Baguley, B. C.; Finlay, G. J.; Rewcastle, G. W. *J. Med. Chem.* 1986, 29, 1769.
(4) Sarris, A. H.; Niles, E. G.; Canellakis, E. S. *Biochim. Biophys. Acta* 1977, *474*, 268.
(5) Schwartz, H. *Adv. Cancer Chemother.* 1979, *1*, 1.
(6) Nelso, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* 1984, *81*, 1361.
(7) Yang, L.; Rowe, T. C.; Nelson, E. M.; Liu, L. F. *Cell* 1985, *41*, 127.
(8) Rowe, T. C.; Chen, G. L.; Hsiang, Y. H.; Liu, L. F. *Cancer Res.* 1986, *46*, 2021.
(9) Yamato, M.; Takeuchi, Y.; Ikeda, Y. *Heterocycles* 1987, *26*, 191.

0022-2623/89/1832-1295$01.50/0

Scheme II



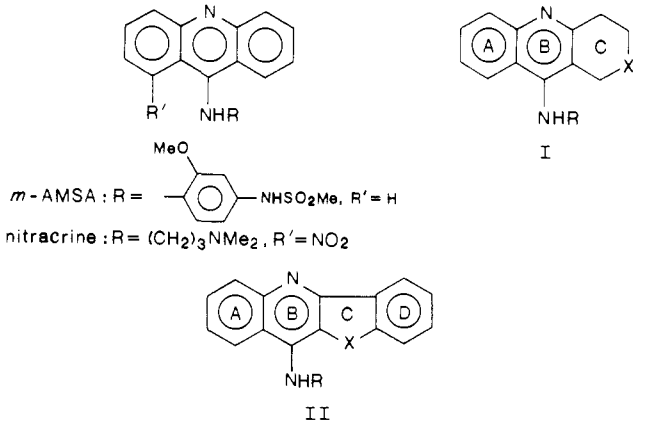
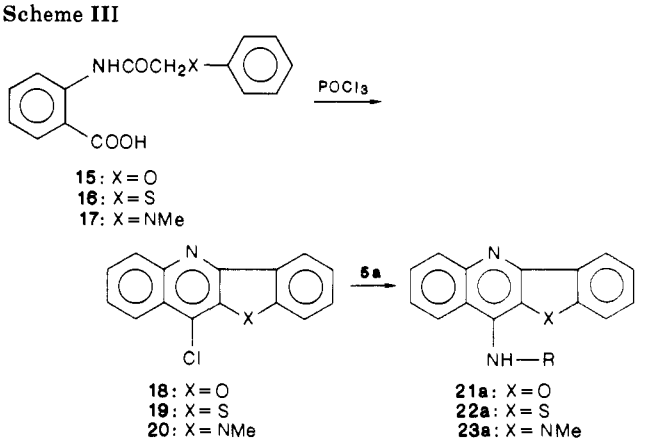


Figure 1.

Scheme III



The sulfur analogue, **10a**, was obtained by an alternative method (Scheme II, method A). Heating of anthranilic acid (8) with tetrahydrothiopyran-4-one in the presence of phosphorus oxychloride afforded the thiopyranoquinoline **9**, which was converted to the anilino analogue **10a** by reaction with 4-[(methylsulfonyl)amino]-2-methoxyaniline (**5a**). Heating of anthranilic acid (8) with indanone or methylindanone gave the tetracyclic indenoquinoline **11** or **12**. Subsequent chlorination followed by reaction with the amines **5a,b** afforded the aminoindenoquinolines **13a,b** or **14a**, (Scheme II, method B), respectively.

The tetracyclic analogues, **21a–23a**, in which the methylene group in the C ring of **13a** was replaced by an oxygen, sulfur, or imino group, were prepared as shown in Scheme III. 2-(Acylamino)benzoic acids **15–17**, prepared by the method previously reported,[10–12] were cyclized to the fused quinolyl chlorides **18–20** by treatment with phosphorus oxychloride, which on reaction with **5a** afforded the desired **21a–23a**.

Antitumor Properties

The compounds were tested for their inhibitory activity against KB cells[13] in vitro. Antitumor activity in vivo was evaluated by use of leukemia P388 in mice. These results are listed in Table I.

In general there was reasonable agreement between KB cytotoxicity and P388 activity. In the case of type I compounds (**6a,b**, **7a,b** and **10a**), all except **6b** were inactive in both the in vitro and in vivo assays. On the other hand, in the case of type II compounds (**13a,b**, **14a**, and **21a–23a**), interesting variations in the activities were observed. For example, both indenoquinolines **13a** and **13b** showed potent KB cytotoxicity. However, in the in vivo assay, **13a** exhibited remarkably potent activity, while **13b** was inactive at the highest dose tested. Moreover, replacement of the methylene group of the C ring in **13a** by an isosteric atom such as oxygen or sulfur leads to compounds (**21a** and **22a**) with almost equal potency in vivo to that of **13a**. In contrast, introduction of a methyl group on the C ring of **13a** (**14a**) produced loss of activity in both assays. Similarly, the aza analogue **23a** with a methyl group on the same position was inactive.

These results imply that slight variations in the structure of the chromophore lead to remarkable changes in antitumor activities. It is likely that the ineffectiveness of tetrahydroacridine analogues **6a**, **7a**, and **10a** as antitumor agents may be due to their nonplanar chromophores. In the case of **14a** and **23a**, the methyl groups in the C ring probably hinder the chromophore from binding to DNA.

The compound most active against P388 leukemia (**13a**) was further examined for antitumor activity against various experimental tumors (Table II). It was found to have a broad spectrum of activity against solid tumors such as M5076 sarcoma, B16 melanoma, and colon 38 carcinoma.

Interaction with DNA

DNA binding affinity was determined by use of a fluorometric assay based on the competition of the test compounds with ethidium bromide[14] (Table I). These results show that the ability to reduce the fluorescence of initially DNA-bound ethidium bromide clearly correlates with KB cytotoxicity. Compounds **6b**, **13a,b**, **21a**, and **22a** signif-

(10) Sunder, S.; Peet, N. P. J. *Heterocycl. Chem.* **1978**, 15.
(11) Gorlitzer, K.; Weber, J. *Arch. Pharm.* **1980**, *314*, 76.
(12) Gorlitzer, K.; Weber, J. *Arch. Pharm.* **1981**, *314*, 852.
(13) Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1979**, *39*, 1063.
(14) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.

Table I. Biological Activities

compd	DNA binding: fluorescence[a]	DNA binding: unwinding[b]	topoisomerase II dependent DNA cleavage act.[c]	inhibn of KB cell growth, IC_{50}, μg/mL	antitumor act. P388 in mice: dose, mg/kg, ip[d]	antitumor act. P388 in mice: % (T/C)
6a	93.30 ± 0.95	–	–	41	inactive[e]	
6b	82.00 ± 1.01	NT[f]	NT	1.3	inactive	
7a	104.30 ± 1.12	NT	NT	>100	inactive	
7b	NT	NT	NT	14	inactive	
10a	106.00 ± 1.27	NT	NT	46	inactive	
13a	67.60 ± 0.81	+++	+	<0.3	400	0
					200	60
					100	80
					50	240
					25	217
					12.5	166
13b	5.45 ± 0.09	+++	–	<0.3	50	101
					25	88
					12.5	110
14a	95.27 ± 0.30	–	–	15	inactive	
21a	81.40 ± 0.41	+	+	<0.3	400	248
					200	168
					100	138
					50	159
					25	125
					12.5	117
22a	79.33 ± 0.93	++	+	<0.3	400	252
					200	238
					100	166
					50	170
					25	170
					12.5	138
23a	93.63 ± 0.97	NT	NT	3.6	inactive	
m-AMSA	63.73 ± 0.97	++	+	<0.3	40	223
					20	198
					10	174

[a] The fluorescence is expressed as a percentage of the control fluorescence of the ethidium bromide–DNA complex. Data are the mean ± SE in three experiments. [b] The reaction mixture as described under Experimental Section, containing PBR322 DNA dimer (20 μg/mL), calf thymus DNA topoisomerase I (200 ng/mL), and drug (5, 25, or 100 μg/mL), was incubated at 37 °C for 30 min: (–) unwinding was not observed at 100 μg/mL of drug; (+) unwinding was observed at 100 μg/mL, (++) at 25 μg/mL, or (+++) at 5 μg/mL. [c] (–) inactive; (+) active; see Figure 2. [d] The dose listed was given once a day at days 1 and 5. [e] Dose: 400 mg/kg. [f] Not tested.

Table II. Antitumor Activity of **13a**

tumor	treatment schedule, day	dose, mg/kg, ip	% (T/C)
P388	1, 5	50	240
B16	1, 5, 8	25	211
		12.5	223
		6.3	184
M5076	1, 5, 9	50	92
		25	111
		12.5	184
		6.3	158
		3.1	193
C38	1, 8, 15, 22	35	150
		25	138
		12.5	133
		6.3	111

icantly reduced fluorescence, and their potencies, except **13b**, were almost equipotent with that of *m*-AMSA. An interesting contrast was observed with **13a** and **13b**. They have the same IC_{50} values (below 0.3 μg/mL), but **13b** completely reduced the fluorescence yet did not have antitumor activity in vivo. On the other hand, **13a**, which showed an equal level of fluorescence to that of *m*-AMSA, has the most potent activity in vivo.

In addition, we determined the intercalative ability of typical compounds in this study by measurement of DNA unwinding in the presence of various amount of those compounds.[15] As shown in Table I, compounds **13a,b**, **21a**, and **22a**, which reduced the fluorescence of DNA-bound ethidium bromide, clearly intercalated. On the other hand, **6a** and **14a** (lacking the ability to reduce the fluorescence) did not intercalate.

Topoisomerase II Dependent DNA Cleavage

It has been suggested that *m*-AMSA interferes with the breakage reunion reaction of mammalian DNA topoisomerase II by stabilizing a reversible enzyme–DNA "cleavage complex".[16] To test the correlation between antitumor activity and topoisomerase II dependent DNA cleavage activity for the compounds of this study, purified topoisomerase II and plasmid PBR 322 DNA were incubated with varying concentrations of the test compounds. Figure 2 shows the following features: all tested compounds with antitumor activity in vivo induced topoisomerase II dependent DNA cleavage in vitro; compound **13b**, lacking antitumor activity, did not cause topoisomerase II dependent DNA cleavage, while **13a** showed a strong intercalation activity and was cytotoxic to KB cell.

These results provide additional evidence that mammalian DNA topoisomerase II dependent DNA cleavage induced by *m*-AMSA and its related fused tetracyclic quinoline derivatives is responsible for their antitumor activity in vivo.

Conclusion

Some fused tri- (I) and tetracyclic (II) quinoline derivatives were prepared, and relationships between their molecular structure, intercalation ability, and antitumor activity were examined.

(15) Chen, G. L.; Liu, Y.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* 1984, *259*, 13560.

(16) Chen, G. L.; Liu, L. F. *Annu. Rep. Med. Chem.* 1986, *21*, 257.

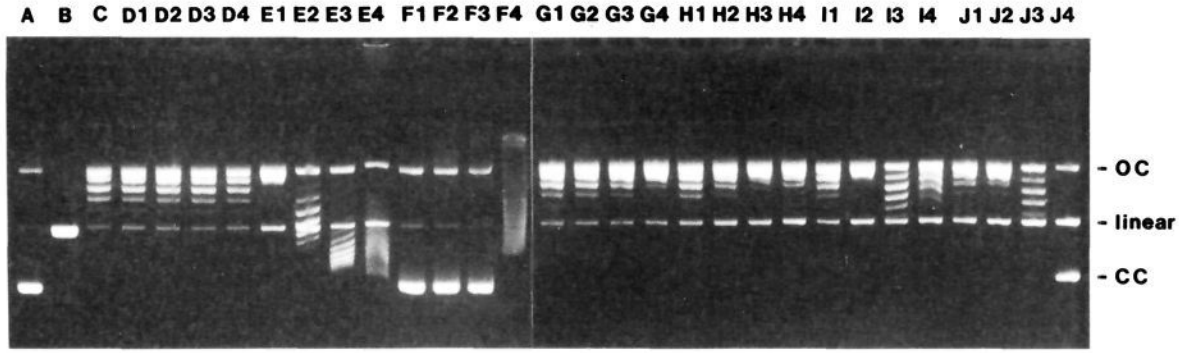


Figure 2. Topoisomerase II dependent DNA cleavage activity of acridine analogues. Lanes: (A) untreated DNA; (B) cleaved with *Pst*I (linear DNA); (C) incubated in reaction mixture without drug; (D1) 1 μg/mL **6a**; (D2) 5 μg/mL **6a**; (D3) 25 μg/mL **6a**; (D4) 125 μg/mL **6a**; (E1–E4) 1–125 μg/mL **13a**; (F1–F4) 1–125 μg/mL **13b**; (G1–G4) 1–125 μg/mL **14a**; (H1–H4) 1–125 μg/mL **21a**; (I1–I4) 1–125 μg/mL **22a**; (J1–J4) 1–125 μg/mL *m*-AMSA. OC: Open circular DNA. Linear: Linear DNA. CC: Closed circular DNA.

The 1,2,3,4-tetrahydroacridine analogues **6a,b** and their isosteric aza and sulfur analogues, **7a,b** and **10a**, did not intercalate and were inactive in the antitumor assays. The most interesting finding of this study was the development of the potent and highly active 10-anilinoindenoquinoline derivative **13a** and its isosteres **21a** and **22a**. They were found to intercalate DNA and to introduce the topoisomerase II dependent DNA cleavage. In addition, **13a** was shown to have a broad spectrum of activity against solid tumors. However, introduction of a methyl group on the C ring in **13a** resulted in complete loss of intercalative ability and antitumor activity. These results imply that a high degree of planarity of the chromophore moiety is essential for efficient intercalation.

Syntheses of other tetracyclic compounds and studies of structure–activity relationships by computer molecular-modeling techniques are in progress.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra run on a Hitachi R-24 spectrometer at 60 MHz, with Me_4Si as an internal standard. Mass spectra were recorded on a Shimadzu LKB-9000 spectrometer and infrared absorption spectra on a JASCO A-102 spectrometer. The elemental analyses were within ±0.4% of the theoretical values.

***N,N*-Dimethyl-*N'*-(1,2,3,4-tetrahydroacridin-9-yl)-1,3-propanediamine (6b).** A mixture of 1,2,3,4,9,10-hexahydroacridin-9-one[9] (2 g, 10 mmol) and $POCl_3$ (20 mL) was heated at reflux for 3 h, and excess $POCl_3$ was evaporated off. The residue was neutralized with 28% aqueous NH_3 solution and extracted with Et_2O. The organic layer was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave the crude product (about 2 g) as a crystal.

A mixture of the crystal, 3-(*N,N*-dimethylamino)propylamine (**5b**; 1.94 g, 19 mmol), phenol (1.89 g, 20 mmol), and NaI (0.07 g, 0.47 mmol) was heated at 130 °C for 1.5 h. The reaction mixture was poured into ice water, made basic with 10% KOH solution, and extracted with Et_2O. The organic layer was shaken with 10% HCl solution, and the aqueous layer was made basic with 10% NaOH solution and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and the solvent was removed. Distillation [bp 130–131 °C, (0.03 mmHg)] of the residue afforded 2.06 g (72%) of **6b** as an oil: IR (Nujol) 3280 cm^{-1}; NMR (CCl_4) δ 1.61–2.01 (m, 6 H, CH_2CH_2N, 2′H, and 3′H), 2.25 (s, 6 H, $(CH_3)_2N$), 2.39 (t, J = 6.4 Hz, 2 H, $CH_2N(CH_3)_2$), 2.5–2.8 (m, 2 H, 1′H), 2.8–3.2 (m, 2 H, 4′H), 3.47 (t, J = 4 Hz, 2 H, CH_2NH), 4.91–5.47 (br, 1 H, NH), 7.0–8.0 (m, 4 H); MS m/z 283 (M^+). Anal. ($C_{18}H_{25}N_3$) C, H, N.

***N,N*-Dimethyl-*N'*-(2-methyl-1,2,3,4-tetrahydrobenzo[*b*]-1,6-naphthyridin-10-yl)-1,3-propanediamine (7b)** was prepared in the similar manner: yield 27%; mp 81–82 °C (from Et_2O–pentane); IR (Nujol) 3260 cm^{-1}; NMR ($CDCl_3$) δ 1.67–2.01 (m, 2 H, CH_2CH_2N), 2.31 (s, 6 H, $(CH_3)_2N$), 2.40 (t, J = 6.0 Hz, 2 H, $CH_2N(CH_3)_2$), 2.53 (s, 3 H, CH_3N), 2.81 (t, J = 7.0 Hz, 2 H, 3′H), 3.21 (t, J = 7.0 Hz, 2 H, 4′H), 3.50–3.80 (m, 4 H, 1′H and CH_2NH), 5.61–5.18 (br, 1 H, NH), 7.2–8.2 (m, 4 H); MS m/z 298 (M^+). Anal. ($C_{18}H_{26}N_4$) C, H, N.

10-Chloro-3,4-dihydro-1*H*-thiopyrano[4,3-*b*]quinoline (9). A mixture of tetrahydropyran-4-one (0.6 g, 5.17 mmol), anthranilic acid (0.71 g, 5.18 mmol), and $POCl_3$ (5 mL) was heated at reflux for 2 h. The excess $POCl_3$ was removed, and the residue was made basic with saturated $KHCO_3$ solution and extracted with AcOEt. The extract was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 0.3 g (43%) of **9**: mp 92–94 °C (from hexane); NMR ($CDCl_3$) δ 3.17 (t, J = 5.0 Hz, 2 H, 4H), 3.46 (t, J = 5.0 Hz, 2 H, 3H), 4.13 (s, 2 H, 1H), 7.6–8.4 (m, 4 H). Anal. ($C_{12}H_{10}ClNS$) C, H, N.

***N*-[4-[(3,4-Dihydro-1*H*-thiopyrano[4,3-*b*]quinolin-10-yl)-amino]-3-methoxyphenyl]methanesulfonamide (10a).** A mixture of **9** (0.65 g, 2.76 mmol), 4-[(methylsulfonyl)amino]-2-methoxyaniline (**5a**; 0.60 g, 2.76 mmol), phenol (0.27 g, 2.87 mmol), and NaI (0.01 g) was heated at 130 °C for 15 min. The reaction mixture was chromatographed on alumina with AcOEt–hexane (1:1) to give 0.74 g (65%) of **10a**: mp 234–235 °C dec; IR (Nujol) 3400 cm^{-1}; NMR (Me_2SO-d_6) δ 2.97 (s, 3 H, CH_3SO_2), 2.95–3.34 (m, 4 H, 3″H and 4″H), 3.69 (s, 2 H, 1″H), 3.91 (s, 3 H, CH_3O), 6.1–8.1 (m, 5 H), 9.19–9.31 (br, 1 H, $NHSO_2$); MS m/z 415 (M^+). Anal. ($C_{20}H_{21}N_3O_3S_2$) C, H, N.

***N*-[4-[(11*H*-Indeno[1,2-*b*]quinolin-10-yl)amino]-3-methoxyphenyl]methanesulfonamide (13a).** A mixture of 10-chloro-11*H*-indeno[1,2-*b*] quinoline[17] (**11**; 0.2 g, 0.8 mmol), **5a** (0.18 g, 0.8 mmol), and 2-ethoxyethanol (10 mL) was heated at reflux for 24 h. The precipitate was collected and washed with MeOH to give 0.24 g (70%) of the hydrochloride of **13a**.

The salt was poured into ice water, made basic with 10% KOH solution, and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and the solvent was removed to give the free base **13a**: mp 232–234 °C (from AcOEt); IR (Nujol) 3380 cm^{-1}; NMR (Me_2SO-d_6) δ 3.04 (s, 3 H, CH_3SO_2), 3.23 (s, 2 H, CH_2), 3.77 (s, 3 H, CH_3O), 6.7–8.6 (m, 11 H). Anal. ($C_{24}H_{21}N_3O_3S$) C, H, N.

***N,N*-Dimethyl-*N'*-(11*H*-Indeno[1,2-*b*]quinolin-10-yl)-1,3-propanediamine (13b).** A mixture of **11** (2.94 g, 11.8 mmol) and **5b** (20 g, 195 mmol) was heated at 130 °C for 24 h, poured into ice water, made basic with 10% KOH solution, and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and the solvent was removed. The residue was chromatographed on alumina with AcOEt–hexane (1:15) to give 2.08 g (56%) of **13b**: mp 81–83 °C (from AcOEt–Et_2O); IR (Nujol) 3270 cm^{-1}; NMR ($CDCl_3$) δ 1.68–2.11 (m, 2 H, NCH_2CH_2), 2.26–2.71 (m, 8 H, $CH_2N(CH_3)_2$), 3.73–4.06 (m, 2 H, $NHCH_2$), 4.18 (s, 2 H, 11′H), 7.3–8.4 (m, 8 H); MS m/z 317 (M^+). Anal. ($C_{21}H_{23}N_3$) C, H, N.

***N*-[3-Methoxy-4-[(11-methyl-11*H*-indeno[1,2-*b*]quinolin-10-yl)amino]phenyl]methanesulfonamide (14a).** A mixture of 3-methyl-1-indanone[18] (5.46 g, 36.9 mmol) and anthranilic acid

(17) Sen, H. K.; Basu, U. J. *J. Indian Chem. Soc.* **1930**, *7*, 435.
(18) Koelsch, C. F.; Hochmann, H.; LeClaire, C. D. *J. Am. Chem. Soc.* **1943**, *65*, 59.

(3.41 g, 24.9 mmol) was heated at 180–190 °C for 2.5 h. The resulting precipitate was collected and washed successively with pyridine and Et_2O.

A mixture of the precipitate and $POCl_3$ (30 mL) was stirred at room temperature for 5 min, poured into ice water, neutralized with aqueous 28% NH_3 solution, and extracted with $CHCl_3$. The extract was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave crude **12**, as a crystal: NMR ($CDCl_3$) δ 1.64 (d, 3 H, J = 8.0 Hz, CH_3), 4.14 (q, 1 H, J = 8.0 Hz, 11H), 7.3–8.5 (m, 8 H).

A mixture of crude **12**, **5a** (5.37 g, 24.9 mmol), and 2-ethoxyethanol (10 mL) was heated at reflux for 14 h. The reaction mixture was poured into ice water, neutralized with 10% KOH solution, and extracted with $CHCl_3$. The extract washed with saturated NaCl solution and then dried over $MgSO_4$. Removal of the solvent gave 1.71 g (15%) of **14a**, which was chromatographed on alumina with CH_2Cl_2: mp 219–222 °C (from Et_2O); IR (Nujol) 3330 cm^{-1}; NMR (Me_2SO-d_6) δ 1.20 (d, J = 8.0 Hz, 3 H, CH_3CH), 2.93 (s, 3 H, CH_3SO_2), 3.56–4.14 (m, 4 H, CH_3 and 11″H), 6.5–8.4 (m, 13 H). MS m/z 445 (M^+). Anal. ($C_{24}H_{21}N_3O_3S$·HCl) C, H, N.

***N*-[4-[(Benzofuro[3,2-*b*]quinolin-11-yl)amino]-3-methoxyphenyl]methanesulfonamide (21a).** A mixture of 11-chlorofuro[3,2-*b*]quinoline[10] (**18**; 0.59 g, 2.3 mmol), **5a** (0.50 g, 2.3 mmol), and 2-ethoxyethanol (5 mL) was heated at reflux for 2.5 h. The reaction mixture was poured into ice water, neutralized with 10% KOH solution, and extracted with $CHCl_3$. The extract was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 0.48 g (48%) of **21a**, which was chromatographed on alumina with CH_2Cl_2: mp 230 °C (from EtOH); IR (Nujol) 3410, 3250 cm^{-1}; NMR (Me_2SO-d_6) δ 3.00 (s, 3 H, CH_3SO_2), 3.83 (s, 3 H, CH_3), 6.7–8.3 (m, 12 H), 9.34–9.62 (br, 1 H, NH); MS m/z 433 (M^+). Anal. ($C_{23}H_{19}N_3O_4S$) C, H, N.

***N*-[4-[(Benzothieno[3,2-*b*]quinolin-11-yl)amino]-3-methoxyphenyl]methanesulfonamide (22a).** A mixture of 11-chlorothieno[3,2-*b*]quinoline[11] (**19**; 1.0 g, 3.7 mmol), **5a** (0.8 g, 3.7 mmol), and 2-ethoxyethanol (15 mL) was heated at reflux for 15 h. The reaction mixture was poured into saturated $KHCO_3$ and extracted with $CHCl_3$. The extract was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 0.80 g (48%) of **22a**: mp 265–266 °C (from Me_2CO); IR (Nujol) 3230 cm^{-1}; NMR (Me_2SO-d_6) δ 3.13 (s, 3 H, CH_3SO_2), 3.68 (s, 3 H, CH_3O), 6.9–8.8 (m, 11 H), 8.91–9.21 (br, 1 H, NH), 9.80–10.08 (br, 1 H, NH); MS m/z 449 (M^+). Anal. ($C_{23}H_{19}N_3O_3S_2$) C, H, N.

***N*-[4-[(10-Methyl-10*H*-indolo[3,2-*b*]quinolin-11-yl)amino]-3-methoxyphenyl]methanesulfonamide (23a).** A mixture of 11-chloro-10-methyl-10*H*-indolo[3,2-*b*]quinoline[12] (**20**; 0.20 g, 0.75 mmol), **5a** (0.17 g, 0.79 mmol), and 2-ethoxyethanol (10 mL) was refluxed for 13 h. The mixture was poured into saturated $KHCO_3$ solution and extracted with CH_2Cl_2. The extract was washed with saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 0.35 g (88%) of **23a**: mp 280–282 °C (from EtOH); NMR ($CDCl_3$) δ 2.90 (s, 3 H, CH_3SO_2), 3.91, 4.02 (each s, 6 H, CH_3N or CH_3O) 5.7–8.1 (m, 11 H), 9.23 (br, 1 H, $NHSO_2$); MS m/z 446 (M^+). Anal. ($C_{24}H_{22}N_4O_3S$) C, H, N.

Compounds **6a,b**, **7a,b**, **10a**, **13a,b**, **14a**, and **21a–23a** were converted to their hydrochlorides, which were tested for biological activity.

Intercalation with DNA. Fluorometric Measurement. The technique used was essentially the same as that reported by Cain et al.[19]

A Hitachi fluorescence spectrometer was used at the maximum sensitivity. Excitation of the buffer solution was achieved by using a 564-nm filter. Fluorescence emission was measured at 595 nm. The buffer contained Hepes (2 mM), EDTA (10 μM), and NaCl (9.4 mM). The pH was adjusted to 7.0 with NaOH. Ethidium bromide (3 μM, Tokyo Kasei), a calf thymus DNA (20 μM, Sigma, highly polymerized type 1), and DMSO (10 μM) were dissolved in the buffer. The fluorescence of the DNA–ethidium bromide complex was measured in the presence (T) and in the absence (C) of drug (10 μM). Percentages in the fluorescence were calculated as $(T/C) \times 100$.

Unwinding Measurement. DNA unwinding effects of intercalators were assayed according to the Chen's method.[15]

Unwinding measurements were done in reaction mixtures (20 μL each) containing Tris (pH 7.5, 40 mM), KCl (100 mM), $MgCl_2$ (10 mM), dithiothreitol (0.5 mM), EDTA (0.5 mM), bovine serum albumin (30 μg/mL), relaxed PBR322 DNA dimer (20 μg/mL), and calf thymus DNA topoisomerase I (200 ng/mL). The reaction mixture was incubated for 30 min at 37 °C at 5, 25, or 100 μg/mL drug concentration.

Agarose Gel Assay for Topoisomerase II Dependent DNA Cleavage. Calf thymus DNA topoisomerases were purified according to Halligan's methods.[20] Proteinase K was from Sigma. Reaction mixtures (20 μL) containing Tris-HCl (pH 7.5, 50 mM), KCl (100 mM), $MgCl_2$ (10 mM), ATP (1 mM), dithiothreitol (0.5 mM), EDTA (0.5 mM), bovine serum albumin (30 μg), PBR322 DNA (0.4 μg), and calf thymus DNA topoisomerase II were incubated at 37 °C. After 60 min, reaction were terminated by the addition of 2 μL of a solution containing 5% SDS and proteinase K (2.5 mg/mL). Following an additional 60-min incubation at 37 °C, the samples were electrophoresed through a 1.2% agarose gel in Tris–borate (pH 8.3, 89 mM)–EDTA (2 mM) buffer containing 0.1% SDS. After electrophoresis, gels were stained with ethidium bromide and photographed.

Culture of KB Cells and Determination of ED_{50} of the Drugs. A clonal KB cell line, established by Dr. M. Green, St. Louis University, and kindly supplied by Dr. K. Fujinaga, Sapporo Medicinal College, was grown in Eagle's minimal essential medium containing 10% calf serum (Grand Island Biological).[13] Cells were grown in plastic dishes (Lux Scientific) at 37 °C in 5% CO_2–95% air. The cells grew exponentially for at least 72 h under the experimental conditions, and the doubling time of the KB cell populations was about 20 h.

The cytotoxic activity of the drugs on cultured KB cells was measured by determining the IC_{50}.[13] KB cells were seeded in plastic dishes (diameter 60 mm; Lux Scientific) at a density of 2100 cells/cm^2 growth surface. At 24 h after inoculation, the medium was changed and the cells were treated with graded concentrations (0.3–100 μg/mL) of the drugs. Two dishes were used for each drug concentration. The cells were cultivated for 48 h in the presence of drugs. The medium was removed and the cell layer was washed with phosphate-buffered saline (PBS) and trypsinized with an aliquot of 0.25% trypsin–EDTA (Grand Island Biological). PBS containing 2% fatal calf serum was added to neutralize the trypsin. The cells were suspended by pipetting and enumerated with a Coulter counter. The IC_{50} of each drug was obtained by plotting the logarithm of the drug concentration vs the growth rate (percentage of control) of the treated cells.

Antitumor Activity. Mouse tumors used in the experiment were P388 leukemia, B16 melanoma, M5076 fibrosarcoma, and colon 38. All of them were kindly supplied by the National Cancer Institute (U.S.). P388 (10^6) cells were transplanted ip into $CD2F_1$ mice (six mice per group). The drugs were dissolved in 0.9% NaCl solution with an addition of one drop of Tween 80 and administered ip on days 1 and 5. The other tumors were transplanted into $B6D2F_1$ mice. B16 was inoculated ip with 0.5 mL of 10% homogenate in Hank's saline of subcutaneous tumor. **13a** was given ip on days 1, 5, and 8. M5076 (10^6) cells were transplanted ip and treated with **13a** on days 1, 5, and 9. Colon 38 as a 1 × 1 × 1 mm^3 fragment was inoculated sc and **13a** was administered ip on days 1, 8, 15, and 22. Antitumor activity was evaluated by the median survival time (MST) of mice and expressed as % (T/C), T and C being the MST of treated and control groups, respectively.

Registry No. **3**, 13161-85-8; **4**, 111232-54-3; **6a**, 111232-55-4; **6a**·HCl, 113798-11-1; **6b**, 81483-75-2; **6b**·HCl, 119971-23-2; **7a**, 111232-56-5; **7a**·HCl, 119971-24-3; **7b**, 119971-25-4; **7b**·HCl, 119971-26-5; **9**, 119971-27-6; **10a**, 119971-28-7; **10a**·HCl, 119971-29-8; **11**, 35639-26-0; **12**, 119971-30-1; **13a**, 115582-71-3; **13a**·HCl, 119971-31-2; **13b**, 119971-32-3; **13b**·HCl, 119971-33-4; **14a**, 11996-93-9; **8**, 118-92-3; **14a**·HCl, 119971-34-5; **15**, 18704-92-2; **16**,

(19) Cain, B. F.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* 1978, *21*, 658.

(20) Halligan, B. D.; Edwards, K. A.; Liu, L. F. *J. Bio. Chem.* 1985, *260*, 2475.

77705-59-0; **17**, 80271-17-6; **18**, 69764-19-8; **19**, 35771-74-5; **20**, 80271-20-1; **21a**, 115582-72-4; **21a**·HCl, 119971-35-6; **22a**, 115582-73-5; **22a**·HCl, 119971-36-7; **23a**, 119971-37-8; **23a**·HCl, 119971-38-9; 1,2,3,4-tetrahydroacridin-9-ol, 56717-04-5; 3-(*N*,*N*-dimethylamino)propylamine, 109-55-7; tetrahydrothiopyran-4-one, 1072-72-6; 4-[(methylsulfonyl)amino]-2-methoxyaniline, 57165-06-7; 11-methyl-4a,10,10a,11-tetrahydro-5*H*-indeno[1,2-*b*]-quinolin-10-one, 119971-39-0; 3-methyl-1-indanone, 6072-57-5.

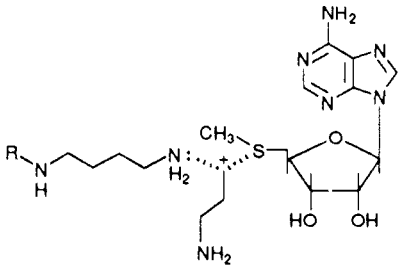
Synthesis and Biological Evaluation of *S*-Adenosyl-1,12-diamino-3-thio-9-azadodecane, a Multisubstrate Adduct Inhibitor of Spermine Synthase

Patrick M. Woster,[†,‡] Alison Y. Black,[†] Keith J. Duff,[†] James K. Coward,*[,†,‡] and Anthony E. Pegg[§]

Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180, Departments of Chemistry and Medicinal Chemistry, The University of Michigan, Ann Arbor, Michigan 48109, and Department of Physiology, Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033.
Received August 22, 1988

As part of a continuing search for specific inhibitors of the enzymes involved in polyamine biosynthesis, we have designed and synthesized a multisubstrate adduct inhibitor, *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD), in which critical portions of the nucleophilic aminopropyl acceptor are covalently linked to critical portions of the electrophilic aminopropyl donor to form a potent and specific inhibitor of spermine synthase. In addition, the corresponding desamino analogue which was designed to lack activity against spermine synthase on the basis of substrate structure–activity data has been synthesized as a control. Preliminary biological results demonstrate that AdoDATAD is a potent and specific inhibitor of mammalian spermine synthase in vitro, while being almost completely devoid of inhibitory activity toward the closely related aminopropyltransferase spermidine synthase. The desamino analogue, as predicted, showed no inhibitory activity against either enzyme. AdoDATAD represents an important addition to the arsenal of specific enzyme inhibitors available for blockade of the polyamine biosynthetic pathway at specific sites.

The polyamines spermidine and spermine are synthesized in vivo by a pair of closely related aminopropyltransferases (APT), spermidine synthase (putrescine aminopropyltransferase, PAPT, EC 2.5.1.16) and spermine synthase (spermidine aminopropyltransferase, SAPT, EC 2.5.1.22).[1] In these reactions, nucleophilic attack by putrescine or spermidine at the electrophilic methylene carbon of decarboxylated *S*-adenosylmethionine (dcAdoMet) leads to transfer of an aminopropyl group to the incoming nucleophile to form spermidine or spermine, respectively. We have recently demonstrated that, in the case of spermidine synthase from *Escherichia coli*, transfer of the aminopropyl group occurs via a ternary complex involving direct nucleophilic attack (single displacement) rather than through an aminopropylated enzyme intermediate (double displacement).[2] Thus, the transition state for the APT-mediated transfer of an aminopropyl group should resemble the structure:



In a previous report, the synthesis and biological evaluation of *S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) were described.[3] This compound is to date the most potent inhibitor known for spermidine synthase, while showing virtually no inhibitory activity against spermine synthase.[4] The high degree of potency and specificity exhibited by multisubstrate adduct inhibitors such as AdoDATO is, in theory, due to their resemblance to the substrate portion of the ternary complex.[5] As part of a research program involving the rational design and synthesis of APT inhibitors exhibiting a high degree of specificity, we now report the synthesis and preliminary biological evaluation of *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD, **1**), the corresponding multisubstrate adduct inhibitor for the spermine synthase reaction. The synthesis and evaluation of the desamino analogue, **2**, predicted to be a very poor inhibitor of spermine synthase on the basis of the known structural requirements for substrate binding to the APT active site,[6] is also described. Preliminary studies using purified mammalian spermine synthase have shown that **1** is a potent and specific inhibitor of this enzyme and offers great potential, especially in combination with AdoDATO and other specific inhibitors of polyamine biosynthesis, for studies of this pathway in mammalian cells.[7]

* Address correspondence to this author at the Departments of Chemistry and Medicinal Chemistry, The University of Michigan, Ann Arbor, MI 48109.
† Rensselaer Polytechnic Institute.
‡ The University of Michigan.
§ The Pennsylvania State University College of Medicine.

(1) Williams-Ashman, H. G.; Pegg, A. E. In *Polyamines In Biology and Medicine*; Morris, D. R., Marton, L., Eds.; Marcel Dekker: New York, 1981; pp 3–73.
(2) Orr, G. R.; Danz, D. W.; Pontoni, G.; Prabhakaren, P. C.; Gould, S. J.; Coward, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 5791–5799.
(3) Tang, K.-C.; Maruizza, R.; Coward, J. K. *J. Med. Chem.* **1981**, *24*, 1277–1284.
(4) Pegg, A. E.; Tang, K.-C.; Coward, J. K. *Biochemistry* **1982**, *21*, 5082–5089.
(5) Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219.
(6) Samejima, K.; Nakazawa, Y. *Arch. Biochem. Biophys.* **1980**, *201*, 241–246.
(7) Pegg, A. E. *Cancer Res.* **1988**, *48*, 759–774.

0022-2623/89/1832-1300$01.50/0