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Bioorganic & Medicinal Chemistry 14 (2006) 4639–4644
Bioorganic & Medicinal Chemistry

Design, synthesis, and antitumor evaluation of novel acenaphtho[1,2-*b*]pyrrole-carboxylic acid esters with amino chain substitution

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Received 15 January 2006; revised 5 February 2006; accepted 7 February 2006
Available online 3 March 2006

Abstract—8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters and derivatives were prepared and evaluated for cytotoxicity against A549 and P388 cell lines. Based on a novel chromophore precursor 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1**, the very insoluble **1** was converted to more soluble esters **5** and a series of 3-amino derivatives from **5** were obtained by mild S_NAr^H reaction between **5** and various amines. The biological evaluation indicated that methyl esters **5a** are the most cytotoxic with IC_{50} values of 0.45 and 0.80 μM (against A549 and P388, respectively) among the parent esters **5a**–**5f**, but 3-amino derivatives **4b** and **4c** of **5f** with bromine showed the highest activity (with IC_{50} values of 0.019–0.60 μM) among the 3-amino derivatives.

1. Introduction

Major progress in cancer chemotherapy requires new drugs to eradicate the entire neoplastic diseases in human being.Finding of novel structure leads that may be of use in designing new, potent, selective, and less toxic anticancer agents remains a major challenge for medicinal chemistry researchers.[1–4] Random screening of natural products and synthetics has been the source of new leads in approaches to drug discovery.[5–7] In our ongoing search for the new potential antitumor agents, we therefore aimed to develop series of new synthetic leads which are more accessible and more amenable to optimization through analog synthesis.

In our previous work, we designed and synthesized an excellent acenaphtho-heterocycle chromophoric precursor, 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carbonitrile **1** (see Fig. 1), which was characteristic of a flat highly electron-deficient heteroaromatic system. S_NAr^H reaction could easily occur between the precursor **1** and aliphatic amines in very mild conditions, and obtained amino derivatives 3-amino-8-oxo-8*H*-acenaphtho[1,2-*b*]-pyrrol-9-carbonitrile **A** (see Fig. 1) were typical ICT (intramolecular charge transfer) fluorophores.[8] It is well known that many electron-deficient chromophoric systems containing planar polycycles represent a large number of valuable antitumor agents, such as the anthraquinone ring system in daunomycin, mitoxan-

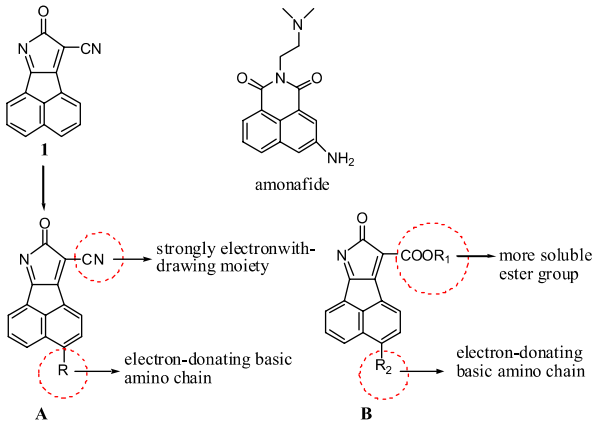

Figure 1. Structure of amonafide and new acenaphtho-heterocyclic derivatives.

Keywords: Acenaphtho[1,2-*b*]pyrrol-carboxylic acid esters; Antitumor; Esterification; Nucleophilic substitution.
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doi:10.1016/j.bmc.2006.02.016

tron, and doxorubicin, naphthalimide in amonafide, and acridine in DACA.[2,9,10] It was noted that **A** resemble some valuable antitumor agents in structure, such as amonafide (Fig. 1), namely they both possessed electron-deficient planar polycycles and basic side chains. The similarity inspired us to assume that the derivatives of **A** could show certain biological activity. Therefore, it was desirable to investigate antitumor activity of amino derivatives of **A** in order to find new structure leads.

However, in view of the fact that poor solubility of precursor 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile and derivatives **A** not only was unfavorable for synthesis and purification of products, but also influenced the performance of biological evaluation and further application, it was necessary for us to attempt structural modification to improve the solubility of precursor and derivatives. Considering highly electron-deficient conjugated planar of precursor **1** to be remained, cyano group was taken into account as a variable site. Esters are usually more soluble in all organic solvents, which is a significant advantage for both the chemical preparation and purification. On the basis of these considerations, we synthesized a series of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters **3** and their amino derivatives **4** as modified analogs of **A** in an attempt to evaluate their cytotoxic activity against cell lines of A549 and P388, and looked forward to the discovery of some potential antitumor leads.

2. Chemistry

Preparation of title compounds is shown in Scheme 1. Precursor **1** was prepared according to our previous publication.[8] Owing to highly electron-deficient features of **1**, it was easily attacked by the nucleophiles occurring in basic medium, which resulted in the appearance of undesirable by-products. Thus, acidic condition was selected to operate the hydrolysis of **1**. Hydrolysis of **1** in 98% H_2SO_4 at room temperature for 10 h afforded 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid **2** in high yield (95%).

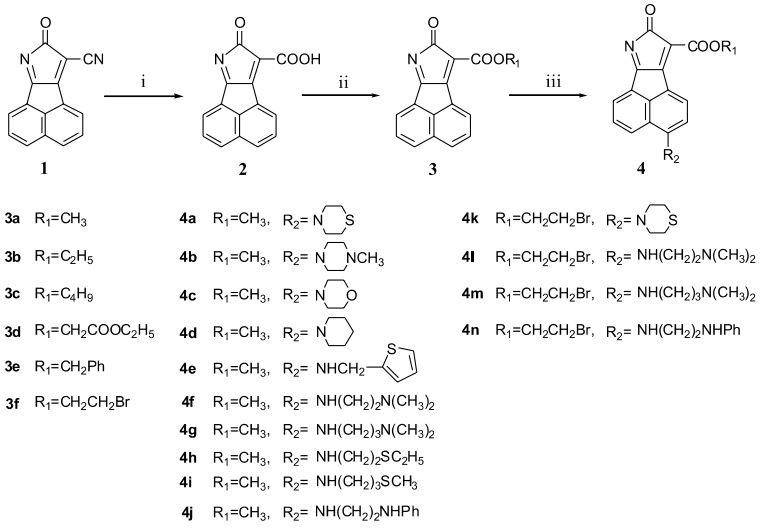
Acid **2** was reacted with corresponding iodide or bromide in CH_3CN in the presence of K_2CO_3 to give ester analogs **3a–f** in acceptable yield, but with an exception, yield of **3f** was very low (18%).

Before preparing amino derivatives of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters, preliminary biological activity of **3a–f** against two cell lines was evaluated. The results are showed in Table 1. Methyl ester **3a** possessed strongest cytotoxicity with IC_{50} values of 0.45 and 0.80 μM (against A549 and P388, respectively). Thus, **3a** was selected as a precursor to be derivatized to give amino derivatives. In addition, due to the presence of a bromine atom in side chain, some derivatives of **3f** were also synthesized in order to investigate the influence of halogen Br on the biological activity.

In our previous report,[10] nucleophilic aromatic substitution of hydrogen (**NASH**) reaction of **1** could easily occur with amines at room temperature due to a strongly electron-deficient feature. The electron-withdrawing ability of ester group was weaker than that of cyano group, which resulted in the decreasing of electron-deficient ability of ester analogs. Thus, the NASH reaction of esters **3a** and **3f** with nucleophilic amines was operated at 50–60 °C in CH_3CN and reaction time was prolonged.

3. Cytotoxic evaluation

The antitumor activities in vitro of these compounds were evaluated by sulforhodamine B (SRB) assay[11]



Scheme 1. Reagents and conditions: (i) 98% H_2SO_4, rt, 10 h; (ii) R_1X (X = Br, I), K_2CO_3, CH_3CN, 35–50 °C (X = I), reflux (X = Br), 12–24 h; (iii) corresponding amines, 50–60 °C, reflux, 10–36 h.

Table 1. Cytotoxicity evaluation against A549 and P388 cell lines

Compound	Cytotoxicity (IC_{50}, μM)	
	A549[a]	P388[b]
3a	0.45	0.80
3b	2.05	1.35
3c	2.98	1.51
3d	9.75	2.20
3e	2.14	2.77
3f	5.51	1.34
4a	10.1	5.65
4b	0.80	0.27
4c	3.41	0.80
4d	31.1	19.7
4e	6.69	11.3
4f	1.24	3.08
4g	4.12	0.95
4h	7.08	1.40
4i	ND[c]	ND[c]
4j	>50	1.03
4k	8.03	1.41
4l	0.60	0.032
4m	0.14	0.019
4n	1.67	3.90

[a] Cytotoxicity against human lung cancer cells (A549) was measured by sulforhodamine B dye-staining method.[11]

[b] Cytotoxicity against murine leukemia cells (P388) was measured by micro-culture tetrazolium-formazan method.[12]

[c] ND = not determined.

against A549 (human lung cancer cell) and MTT tetrazolium dye assay[12] against P388 (murine leukemia cell), respectively (Table 1). The IC_{50} represents the drug concentration (micromolar) required to inhibit cell growth by 50%.

The results revealed that both 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters and amino derivatives of esters possessed desirable antitumor activity. Surprisingly, 3-amino derivatives **4a**–**j** of **3a** displayed weaker activity than that of parent **3a**, and only **4b** showed similar activity to **3a**. Also surprising is considerably high activity of amino derivatives **4l** and **4m** of **3f** bearing Br in ester group chain (0.60 and 0.032 μM for **4l**, 0.14 and 0.019 μM for **4m** against A549 and P388, respectively), which was greatly higher than that of the amino derivatives of methyl esters. Thus, it was speculated that the well-known reactivity of alkyl halide suggested that 2-bromoethyl ester moiety might confer alkylating activity in this series of compounds. The speculation was consistent with previous report,[13,14] excellent leaving groups such as chloride and bromide are required for cytotoxicity in an alkylation mechanism.

Moreover, the derivatives bearing aminoalkyl amine substituent, whether chain aliphatic amines **4f**, **4g**, **4l**, and **4m** or alicyclic amines **4b**, possessed higher activity than that of other amino-substituted derivatives. In fact, the requirement for a basic side chain, such as an N-dialkyl group, in enhancing antitumor activity was demonstrated in naphthalimide, pyridocarbazoles, anthracyclines, acridines, and phenazines, among others.[2,15,16] But unlike naphthalimide compounds, those compounds described without an N-dialkyl group basic side chain unexpectedly showed moderate activity, which suggested that the series of compounds could possess different structure–activity relationship from naphthalimide.

Since most compounds especially **4l** and **4m** showed considerable activity and could be further improved in structure, they are potential leading compounds for finding of valuable antitumor agents. Our research on the novel acenaphtho-heterocycle structural manipulation that is focused on the modification of cyano group and ester group at 9-position and the variance of amino substituents at 3-position and other biological evaluation is still in progress.

4. Conclusion

In summary, a variety of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters and their amino derivatives were readily synthesized and were evaluated for their antitumor activity against cell lines of A549 and P388. The results displayed that most of the compounds showed considerable cytotoxicity. The novel acenaphtho-heterocycle possesses simple structure and can be easily improved structurally. We believe that the discovery has provided a basis for the screening of new antitumor leads with simple structure.

5. Experimental

5.1. General

All the solvents were of analytic grade. ^1H and ^{13}C NMR were obtained with a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$/DMSO-d^6 TMS as internal standard). IR was obtained using a Perkin-Elmer 2000 FTIR instrument. High-resolution mass spectra (HRMS) were obtained on a HPLC-Q-Tof MS (Micro) spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and are uncorrected. Column chromatography was performed using silica gel 200–300 mesh.

5.2. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid (2)

8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1** (10 mmol) was added to 10 mL of 98% H_2SO_4 at room temperature. The reaction mixture was stirred for 10 h and then poured onto crushed ice. The solid precipitate was filtered, washed by water, and dried to give a brown yellow powder. Yield 95%. Mp 245 °C dec. ^1H NMR (400 MHz, DMSO-d^6) δ = 11.41(s, 1H, O*H**), 8.95 (d, 1H, J = 7.2 Hz), 8.55 (d, 1H, J = 8.0 Hz), 8.53 (d, 1H, J = 7.2 Hz), 8.48 (d, 1H, J = 8.0 Hz), 7.96 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.2 Hz), 7.88 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.2 Hz). IR (KBr) cm^{-1}: 3208, 1774, 1700, 1636, 1583, 1570. MS m/z $(M-H)^-$ 248.1(API-ES).

5.3. General procedure for the preparation of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters (3a–c)

8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carboxylic acid **2** (6 mmol) and corresponding iodide (30 mmol) were added to 50 mL CH_3CN in the presence of K_2CO_3 (6.5 mmol). The mixture was stirred for 12–16 h at 35–50 °C, then was filtered and the filtrate was obtained. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel. **3a–c** were separated with CH_2Cl_2/CH_3OH 120:1(v/v) as yellow powders.

5.3.1. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (3a). Yield 98%. Mp 202 °C dec. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (d, 1H, J = 7.2 Hz), 8.75 (d, 1H, J = 7.6 Hz), 8.31 (d, 1H, J = 8.0 Hz), 8.25 (d, 1H, J = 8.0 Hz), 7.87 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.2 Hz), 7.77 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 3.20 ppm (s, 3H, CH^*_3). IR (KBr) cm^{-1}: 3068, 2933, 1765, 1712, 1645, 1586, 1571. HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{16}H_{10}NO_3$ 264.0661, found 264.0672.

5.3.2. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid ethyl ester (3b). Yield 83%. Mp 214–215 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.11 (d, 1H, J = 7.2 Hz), 8.73 (d, 1H, J = 7.6 Hz), 8.29 (d, 1H, J = 8.0 Hz), 8.24 (d, 1H, J = 8.0 Hz), 7.86 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.76 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 3.76 (q, 2H, J = 7.2 Hz, CH$^*_2$CH$_3$), 1.31 (t, 3H, J = 7.2 Hz, CH$_2$CH^*_3). ^{13}C NMR (100 MHz, $CDCl_3$): 178.6, 168.9, 166.5, 142.9, 136.8, 136.0, 133.8, 132.5, 132.0, 131.51, 129.1, 127.8, 127.3, 125.4, 121.3, 39.0, 13.3. IR (KBr) cm^{-1}: 3102, 2978, 1766, 1704, 1647, 1587, 1572. HRMS (ESI) m/z (M+Na)$^+$ calcd for $C_{17}H_{11}NNaO_3$ 300.0637, found 300.0642.

5.3.3. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid butyl ester (3c). Yield 40%. Mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (d, 1H, J = 7.6 Hz), 8.70 (d, 1H, J = 7.6 Hz), 8.27 (d, 1H, J = 8.0 Hz), 8.21 (d, 1H, J = 8.0 Hz), 7.83 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.73 (t, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 3.69 (t, 2H, J = 7.2 Hz, OCH^*_2), 1.68 (m, 2H, J = 7.2 Hz, OCH$_2$CH^*_2), 1.38 (m, 2H, J = 7.2 Hz, CH^*_2CH$_3$), 0.96 ppm (t, 3H, J = 7.2 Hz, CH$_2$CH^*_3). IR (KBr) cm^{-1}: 3096, 2942, 1769, 1710, 1645, 1587, 1572, 1508, 1436. HRMS (ESI) m/z (M+Na)$^+$ calcd for $C_{19}H_{15}NNaO_3$ 328.0950, found 328.0940.

5.4. General procedure for the preparation of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters (3d–f)

8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carboxylic acid **6** (6 mmol) and corresponding bromide (12 mmol) were added to 50 mL CH_3CN in the presence of K_2CO_3 (6.5 mmol). The mixture was refluxed for 16–24 h, then was filtered and the filtrate was obtained. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel. **3d–f** were separated with CH_2Cl_2/CH_3OH 80:1(v/v) as yellow powders.

5.4.1. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid ethoxycarbonylmethyl ester (3d). Yield 93%. Mp 167–168 °C ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.10 (d, 1H, J = 7.6 Hz), 8.75 (d, 1H, J = 7.6 Hz), 8.32 (d, 1H, J = 8.0 Hz), 8.27 (d, 1H, J = 8.0 Hz), 7.87 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.77 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 4.45 (s, 2H, COOCH^*_2COOC$_2$H$_5$), 4.25 (q, 2H, J = 7.0 Hz, COOCH^*_2CH$_3$), 1.30 ppm (t, 3H, J = 7.0 Hz, COOCH$_2$CH^*_3). IR (KBr) cm^{-1}: 3057, 2985, 2924, 1770, 1746, 1712, 1643, 1584, 1571. HRMS (ESI) m/z (M+Na)$^+$ calcd for $C_{19}H_{13}NNaO_5$ 358.0691, found 358.0682.

5.4.2. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid benzyl ester (3e). Yield 51%. Mp 215 °C dec. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.08 (d, 1H, J = 7.6 Hz), 8.72 (d, 1H, J = 7.6 Hz), 8.27 (d, 1H, J = 8.0 Hz), 8.21 (d, 1H, J = 8.0 Hz), 7.84 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.72 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.48 (d, 2H, J = 7.2 Hz), 7.34 (t, 1H, J = 7.2 Hz), 7.29 (d, 2H, J = 7.2 Hz), 4.85 ppm (s, 2H, COOCH^*_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 178.8, 168.9, 166.5, 143.0, 137.1, 136.3, 136.2, 134.1, 132.7, 132.2, 131.6, 129.2, 129.0, 128.9, 128.2, 128.0, 127.4, 125.5, 121.4, 41.8. IR (KBr) cm^{-1}: 1768, 1711, 1641, 1586, 1571. HRMS (ESI) m/z (M+Na)$^+$ calcd for $C_{22}H_{13}NNaO_3$ 362.0793, found 362.0807.

5.4.3. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid 2-bromo-ethyl ester (3f). Yield 18%. Mp 205–206 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.10 (d, 1H, J = 7.6 Hz), 8.74 (d, 1H, J = 7.6 Hz), 8.31 (d, 1H, J = 7.6 Hz), 8.26 (d, 1H, J = 8.0 Hz), 7.86 (dd, 1H, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 7.77 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 4.13 (t, 2H, J = 6.4 Hz, CH^*_2CH$_2$Br), 3.66 (t, 2H, J = 6.4 Hz, CH$_2$CH^*_2Br). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.7, 168.7, 166.3, 142.9, 137.2, 136.5, 134.2, 132.7, 132.3, 131.6, 129.3, 128.1, 127.5, 125.3, 121.3, 39.5, 28.3. IR (KBr) cm^{-1}: 3063, 2921, 1769, 1712, 1647, 1585, 1572. HRMS (ESI) m/z (M+Na)$^+$ calcd for $C_{17}H_{10}BrNNaO_3$ 377.9742, found 377.9730.

5.5. General procedure for the preparation of 3-substituted-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid esters (4a–n)

8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carboxylic acid esters (0.5 mmol) and corresponding amines (2 mmol) in CH_3CN (20 mL) were stirred for 1–2 h at 50–60 °C. The solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel. 3-substituted products **4a–n** were separated with CH_2Cl_2/CH_3OH 20:1(v/v) as dark purple powders.

5.5.1. 3-Thiomorpholin-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4a). Yield 40%. Mp 245 °C dec. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.00 (d, 1H, J = 8.0 Hz), 8.75 (d, 1H, J = 7.2 Hz), 8.43 (d, 1H, J = 8.0 Hz), 7.80 (dd, 1H, J_1 = 8.0 Hz, J_2 = 7.2 Hz), 7.19 (d, 1H, J = 8.0 Hz), 3.72 (br s, 4H, –N(CH^*_2CH$_2$)$_2$S), 3.17 (s, 3H, COOCH^*_3), 3.00 ppm (br s, 4H, –N(CH$_2$CH^*_2)$_2$S). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.1, 169.5, 167.3, 159.8, 142.7, 135.6,

132.9, 132.5, 132.4, 131.2, 127.6, 126.8, 121.8, 116.4, 115.5, 56.1, 28.1, 24.0. IR (KBr) cm^{-1}: 2906, 1766, 1705, 1633, 1571, 1511. HRMS (ESI) *m/z* $(M+Na)^+$ calcd for $C_{20}H_{16}N_2NaO_3S$ 387.0779, found 387.0768.

5.5.2. 3-(4-Methyl-piperazin)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4b). Yield 38%. Mp 210 °C dec. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.01$ (d, 1H, $J = 8.0$ Hz), 8.77 (d, 1H, $J = 7.6$ Hz), 8.46 (d, 1H, $J = 8.4$ Hz), 7.78 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 7.6$ Hz), 7.17 (d, 1H, $J = 8.4$ Hz), 3.55 (br s, 4H, $-N(CH^*_2CH_2)_2NCH_3$), 3.17 (s, 3H, $COOCH^*_3$), 3.46 (br s, 4H, $-N(CH_2CH^*_2)_2NCH_3$), 2.48 ppm (br s, 3H, NCH_3). IR (KBr) cm^{-1}: 2929, 1765, 1702, 1629, 1572, 1509. HRMS (ESI) *m/z* $(M + H)^+$ calcd for $C_{21}H_{20}N_3O_3$ 362.1505, found 362.1449.

5.5.3. 3-Morpholin-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4c). Yield 40%. Mp >300 °C. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.98$ (d, 1H, $J = 8.0$ Hz), 8.72 (d, 1H, $J = 7.6$ Hz), 8.46 (d, 1H, $J = 8.4$ Hz), 7.77 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 7.6$ Hz), 7.16 (d, 1H, $J = 8.4$ Hz), 4.05 (br s, 4H, $-N(CH_2CH^*_2)_2O$), 3.46 (br s, 4H, $-N(CH^*_2CH_2)_2O$), 3.17 ppm (s, 3H, $COOCH^*_3$). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 178.1$, 169.5, 167.3, 158.8, 142.7, 135.7, 133.0, 132.6, 132.4, 131.3, 130.8, 127.2, 126.7, 121.8, 115.4, 66.9, 54.0, 24.0. IR (KBr) cm^{-1}: 2923, 1767, 1702, 1631, 1576, 1519. HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{20}H_{17}N_2O_4$ 349.1188, found 349.1189.

5.5.4. 3-Piperidin-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4d). Yield 36%. Mp 148–149 °C. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 8.84$ (d, 1H, $J = 8.0$ Hz), 8.58 (d, 1H, $J = 7.6$ Hz), 8.51 (d, 1H, $J = 8.0$ Hz), 7.88 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 7.35 (d, 1H, $J = 8.0$ Hz), 4.17 (br s, 4H, $-N(CH^*_2CH_2)_2CH_2$), 2.99 (s, 3H, $COOCH^*_3$), 1.84 (br s, 4H, $-N(CH_2CH^*_2)_2CH_2$), 1.74 ppm (br s, 2H, $-N(CH_2CH_2)_2CH^*_2$). IR (KBr) cm^{-1}: 2923, 1708, 1630, 1573, 1540, 1519. HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{21}H_{19}N_2O_3$ 347.1396, found 347.1386.

5.5.5. 3-[(Thiophen-2-ylmethyl)-amino]-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4e). Yield 41%. Mp 240 °C dec. ^1H NMR (400 MHz, d^6-DMSO): $\delta = 9.71$(br s, 1H, NH^*), 8.87 (d, 1H, $J = 8.0$ Hz), 8.77 (d, 1H, $J = 8.8$ Hz), 8.60 (d, 1H, $J = 7.6$ Hz), 7.86 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 7.46 (d, 1H, $J = 3.6$ Hz), 7.23 (d, 1H, $J = 3.6$ Hz), 7.09 (d, 1H, $J = 8.8$ Hz), 7.02 (dd, 1H, $J_1 = 3.6$ Hz, $J_2 = 3.6$ Hz), 4.98 (s, 2H, CH^*_2), 2.97 ppm (s, 3H, $COOCH^*_3$). IR (KBr) cm^{-1}: 3298, 2923, 1748, 1710, 1693, 1624, 1563, 1525. HRMS (ESI) *m/z* $(M+Na)^+$ calcd for $C_{21}H_{14}N_2NaO_3S$ 397.0623, found 397.0614.

5.5.6. 3-(Dimethylamino-ethylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4f). Yield 38%. Mp 220 °C dec. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 8.84$ (d, 2H, $J = 8.8$ Hz), 8.62 (d, 1H, $J = 7.2$ Hz), 7.85 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 8.0$ Hz), 7.02 (d, 1H, $J = 8.8$ Hz), 3.71 (br s, 2H, $NHCH^*_2CH_2$), 2.98 (s, 3H, $COOCH^*_3$), 2.84 (br s, 2H, $CH^*_2N(CH_3)_2$), 2.41 ppm (s, 6H, $CH_2N(CH^*_3)_2$). IR (KBr) cm^{-1}: 2923, 1765, 1703, 1685, 1654, 1626, 1563; HRMS(ESI) *m/z* $(M+H)^+$ calcd for $C_{20}H_{20}N_3O_3$ 350.1505, found 350.1505.

5.5.7. 3-(3-Dimethylamino-propylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4g). Yield 35%. Mp 186–187 °C. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 8.79$ (d, 2H, $J = 8.8$ Hz), 8.59 (d, 1H, $J = 7.6$ Hz), 7.84 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 6.99 (d, 1H, $J = 8.8$ Hz), 3.61 (br s, 2H, $NHCH^*_2CH_2$), 3.17 (br s, 2H, $(CH_3)_2NCH^*_2CH_2$), 2.98 (s, 3H, $COOCH^*_3$), 2.38 ppm (s, 6H, $N(CH^*_3)_2$), 1.95 (m, 2H, $NHCH_2CH^*_2$). IR (KBr) cm^{-1}: 3047, 2912, 1749, 1702, 1624, 1567, 1545, 1511. HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{21}H_{22}N_3O_3$ 364.1661, found 364.1665.

5.5.8. 3-(2-Ethylsulfonyl-ethylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4h). Yield 40%. Mp 135–136 °C. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 9.21$ (br s, 1H, NH^*), 8.85–8.89 (m, 2H), 8.65 (d, 1H, $J = 7.2$ Hz), 7.87 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 8.4$ Hz), 7.04 (d, 1H, $J = 8.8$ Hz), 3.77 (q, 2H, $NHCH^*_2CH_2$, $J = 6.4$ Hz), 2.99 (s, 3H, $COOCH^*_3$), 2.92 (t, 2H, $NHCH_2CH^*_2$, $J = 6.8$ Hz), 2.64 (q, 2H, SCH^*_2),1.23 ppm (t, 3H, CH^*_3). IR (KBr) cm^{-1}: HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{20}H_{19}N_2O_3S$ 367.1116, found 367.1124.

5.5.9. 3-(3-Methylsulfonyl-propylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4i). Yield 41%. Mp 280 °C dec. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 9.33$ (br s, 1H, NH^*), 8.93 (d, 1H, $J = 8.4$ Hz), 8.84 (d, 1H, $J = 8.8$ Hz), 8.62 (d, 1H, $J = 7.6$ Hz), 7.86 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 8.4$ Hz), 7.04 (d, 1H, $J = 8.8$ Hz), 3.65 (q, 2H, $NHCH^*_2CH_2$, $J = 6.0$ Hz), 2.97 (s, 3H, $COOCH^*_3$), 2.63 (t, 2H, SCH^*_2, $J = 6.8$ Hz), 2.09 (s, 3H, SCH^*_3), 2.00 ppm (t, 2H, $NHCH_2CH^*_2$, $J = 6.8$ Hz). IR (KBr) cm^{-1}: 2925, 2676, 1745, 1708, 1640, 1624, 1593, 1561. HRMS(ESI) *m/z* $(M+H)^+$ calcd for $C_{20}H_{19}N_2O_3S$ 367.1116, found 367.1104.

5.5.10. 3-(2-Phenylamino-ethylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid methyl ester (4j). Yield 32%. Mp 180 °C dec. ^1H NMR (400 MHz, DMSO-d^6): $\delta = 9.24$ (br s, 1H, $NH^*CH_2CH_2NHPh$), 8.85 (d, 1H, $J = 8.8$ Hz), 8.79 (d, 1H, $J = 7.6$ Hz), 8.61 (d, 1H, $J = 8.0$ Hz), 7.86 (dd, 1H, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 7.10 (t, 2H, $J = 8.0$ Hz), 7.01 (d, 1H, $J = 8.8$ Hz), 6.64 (d, 2H, $J = 7.6$ Hz), 6.56 (t, 1H, $J = 7.0$ Hz), 5.85 (br s, 1H, $NHCH_2CH_2NH^*Ph$), 3.74 (br s, 2H, $NHCH^*_2CH_2NHPh$), 3.46 (br s, 2H, $NHCH_2CH^*_2NHPh$), 2.97 ppm (s, 3H, $COOCH^*_3$). IR (KBr) cm^{-1}: 3015, 2919, 1749, 1699, 1624, 1599, 1567, 1505. HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{24}H_{20}N_2O_3$ 398.1505, found 398.1497.

5.5.11. 3-Thiomorpholin-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid 2-bromo-ethyl ester (4k). Yield 35%. Mp 173 °C dec. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.03$ (d, 1H, $J = 8.4$ Hz), 8.78 (d, 1H, $J = 7.2$ Hz), 8.44 (d, 1H, $J = 8.0$ Hz), 7.82 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 8.4$ Hz), 7.20 (d, 1H, $J = 8.0$ Hz), 4.11((t, 2H,

J = 6.4 Hz, OCH^*_2), 3.64 (t, 2H, J = 6.4 Hz, CH^*_2Br), 3.72 (br s, 4H, –N(CH^*_2CH_2)$_2$S), 3.00 ppm (br s, 4H, –N(CH$_2$CH^*_2)$_2$S). IR (KBr) cm^{-1}: 2916, 1753, 1703, 1628, 1601, 1570. HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{18}BrN_2O_3S$ 457.0222, found 457.0211.

5.5.12. 3-(Dimethylamino-ethylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid 2-bromo-ethyl ester (4l). Yield 38%. Mp >300 °C. ^{1}H NMR (400 MHz, *DMSO-d*^{6}): δ = 8.85 (d, 1H, J = 8.0 Hz), 8.78 (d, 1H, J = 8.8 Hz), 8.60 (d, 1H, J = 7.2 Hz), 7.85 (dd, 1H, J_1 = 7.2 Hz, J_2 = 8.0 Hz), 7.03 (d, 1H, J = 8.8 Hz), 3.92 (t, 2H, J = 6.4 Hz, OCH^*_2), 3.71 (t, 2H, J = 6.4 Hz, CH^*_2Br), 3.69 (t, 2H, J = 6.4 Hz, NHCH^*_2), 2.69 (t, 2H, J = 6.4 Hz, CH^*_2N(CH$_3$)$_2$), 2.29 ppm (s, 6H, N(CH^*_3)$_2$). IR (KBr) cm^{-1}: 2928, 1750, 1698, 1625, 1572, 1546. HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{21}BrN_3O_3$ 442.0766, found 442.0753.

5.5.13. 3-(3-Dimethylamino-propylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid 2-bromo-ethyl ester (4m). Yield 35%. Mp 220 °C dec. ^{1}H NMR (400 MHz, DMSO-*d*^{6}): δ = 9.52 (br s, NH), 8.84 (d, 1H, J = 8.0 Hz), 8.83 (d, 1H, J = 8.8 Hz), 8.63 (d, 1H, J = 7.6 Hz), 7.88 (dd, 1H, J_1 = 7.6 Hz, J_2 = 8.0 Hz), 7.05 (d, 1H, J = 8.8 Hz), 3.93 (t, 2H, J = 6.4 Hz, OCH^*_2), 3.71 (t, 2H, J = 6.4 Hz, CH^*_2Br), 3.63 (br s, 2H, NHCH^*_2), 2.67 (br s, 2H, CH^*_2 N(CH$_3$)$_2$), 2.40 ppm (s, 6H, N(CH^*_3)$_2$), 1.96 (m, 2H, CH$_2$CH^*_2CH$_2$). IR (KBr) cm^{-1}: 2938, 2776, 1748, 1698, 1626, 1571, 1546, 1507. HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{22}H_{24}BrN_3O_3S$ 456.0923, found 456.0930.

5.5.14. 3-(2-Phenylamino-ethylamino)-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carboxylic acid 2-bromo-ethyl ester (4n). Yield 30%. Mp 240 °C dec. ^{1}H NMR (400 MHz, DMSO-*d*^{6}): δ = 9.15 (br s, 1H, NH^*CH$_2$CH$_2$NHPh), 8.84 (d, 1H, J = 8.8 Hz), 8.80 (d, 1H, J = 7.6 Hz), 8.60 (d, 1H, J = 8.0 Hz), 7.88 (dd, 1H, J_1 = 7.6 Hz, J_2 = 8.0 Hz), 7.11 (t, 2H, J = 8.0 Hz), 7.03 (d, 1H, J = 8.8 Hz), 6.66 (d, 2H, J = 7.6 Hz), 6.54 (t, 1H, J = 7.0 Hz), 5.82 (br s, 1H, NHCH$_2$CH$_2$NH^*Ph), 3.92 (t, 2H, J = 6.4 Hz, OCH^*_2), 3.75 (br s, 2H, NHCH^*_2 CH$_2$NHPh), 3.70 (t, 2H, J = 6.4 Hz, CH^*_2Br), 3.43 (br s, 2H, NHCH$_2$CH^*_2NHPh). IR (KBr) cm^{-1}: 3299, 3047, 1750, 1699, 1625, 1600, 1569. HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{25}H_{22}BrN_3O_3$ 490.0766, found 490.0760.

5.6. In vitro growth delay assays

The prepared compounds were submitted to Shanghai Institute of Materia Medica and Department of Bioscience and Biotechnology in Dalian University of Technology, respectively, with a view to get their cytotoxicities tested.

Acknowledgments

Financial support by National Basic Research Program of China (2003CB114400) and under the auspices of 863 plan for High-Tech (2003AA2Z3520) is greatly appreciated. This work is also supported by Dalian Excellent Young Scientist Research Fund.

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