Antitumor Agents 288. Design, Synthesis, SAR and Biological Studies of Novel Heteroatom-Incorporated Antofine and Cryptopleurine Analogs as Potent and Selective Antitumor Agents

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Compound data (3a-l, 7a-q, and 10a-j):

(*S*)-12*N*-Benzyl-12-aza-antofine (3a): light yellow solid; mp 113-115 ℃; $[\alpha]^{23}_{D}$= -31.7 °(*c* 0.48, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87-7.86 (m, 2H), 7.67 (d, *J* = 9.0 Hz, 1H), 7.47-7.40 (m, 5H), 7.22 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.14 (s, 1H), 4.59 (d, *J* = 14.4 Hz, 1H), 4.31-4.20 (m, 3H), 4.10 (s, 3H), 4.06-3.87 (m, 4H), 4.03 (s, 3H), 4.01 (s, 3H), 3.31-3.25 (m, 1H), 3.07 (m, 2H); ESI MS *m*/*z* 455.15 $(M+H)^+$.

(*S*)-12*N*-Isobutyl-12-aza-antofine (3b): yellow solid; mp 93-95 ℃; $[\alpha]^{23}_{D}$= -84.0 °(*c* 0.37, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.86 (m, 2H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.23 (s, 1H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.54 (d, *J* = 14.7 Hz, 1H), 4.14 (d, *J* = 5.4 Hz, 1H), 4.10 (s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.76 (d, *J* = 15.0 Hz, 1H), 3.62 (d, *J* = 5.1 Hz, 1H), 3.41-3.38 (m, 1H), 3.26-3.19 (m, 1H), 2.95-2.83 (m, 3H), 2.58 (d, *J* = 7.2Hz, 2H), 1.84-1.75 (m, 1H), 1.00-0.98 (d, *J* = 6.0 Hz, 6H); ESI MS *m*/*z* 421.10 $(M+H)^+$.

(*S*)-12*N*-Propyl-12-aza-antofine (3c): light yellow solid; mp 155-157 ℃; $[\alpha]^{23}_{D}$= -75.4 °(*c* 0.50, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7. 90 (s, 1H), 7.88 (d, *J* = 2.7 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.26 (s, 1H), 7.20 (dd, *J* = 9.3 Hz, *J* = 2.4 Hz, 1H), 4.56 (d, *J* = 14.4 Hz, 1H), 4.16 (d, *J* = 5.1 Hz, 1H), 4.11 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.77 (d, *J* = 14.7 Hz, 1H), 3.62 (d, *J* = 4.8 Hz, 1H), 3.44-3.40 (m, 1H), 3.30-3.27 (m, 1H), 3.01-2.80 (m, 3H), 2.72 (t, *J* = 7.5Hz, 2H), 1.64-1.54 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H); ESI MS *m*/*z* 407.15 $(M+H)^+$.

(*S*)-12*N*-(2'-Phenylethyl)-12-aza-antofine (3d): light yellow solid; mp 85-87 ℃; $[\alpha]^{23}_{D}$= -63.2 °(*c* 0.28, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.87 (m, 2H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.34-7.22 (m, 6H), 7.21 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.55 (d, *J* = 14.4 Hz, 1H), 4.23(d, *J* = 5.1 Hz, 1H), 4.10 (s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.78 (d, *J* = 14.7 Hz, 1H), 3.69 (d, *J* = 5.4 Hz, 1H), 3.54-3.52 (m, 1H), 3.29-3.22 (m, 1H), 3.10-3.05 (m, 2H), 2.83-2.78 (m, 5H); ESI MS *m*/*z* 469.20 $(M+H)^+$.

(*S*)-12*N*-(2'-Hydroxyl)ethyl-12-aza-antofine (3e): white solid; mp 210-212 ℃; $[\alpha]^{23}_{D}$= -57.8 °(*c* 0.36, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7. 90-7.89 (m, *2*H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.24-7.22 (m, 2H), 4.62 (d, *J* = 15.0 Hz, 1H), 4.39 (d, *J* = 6.3 Hz, 1H), 4.11 (s, 3H), 4.03 (s, 3H), 4.02 (s, 3H), 3.83 (d, *J* = 15.0 Hz, 1H), 3.81-3.77 (t, *J* = 5.1 Hz, 2H), 3.71 (d, *J* = 5.4 Hz, 1H), 3.64 (m, 1H), 3.41-3.29 (m, 1H), 3.16-3.11 (m, 3H), 3.02-2.95 (m, 2H); ESI MS *m*/*z* 409.15 $(M+H)^+$.

(*S*)-12*N*-Phenyl-12-aza-antofine (3f): white solid; mp 244-246 ℃; $[\alpha]^{23}_{D}$= -121.8 °(*c* 0.34, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.86 (m, 2H), 7.77 (d, *J* = 9.3 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.25 (s, 1H), 7.21 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 6.74 (t, *J* = 7.5Hz, 1H), 6.56 (d, *J* = 8.1 Hz, 2H), 4.73 (d, *J* = 3.6 Hz, 1H), 4.65 (d, *J* = 14.4 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 4.00 (s, 3H), 3.97 (d, *J* = 3.3 Hz, 1H), 3.89 (d, *J* = 14.7 Hz, 1H), 3.74 (dd, *J* = 7.8 Hz, *J* = 5.4 Hz, 1H), 3.43-3.31 (m, 2H), 3.07-3.02 (m, 2H); ESI MS *m*/*z* 441.10 $(M+H)^+$.

(*S*)-12*N*-Cyclopropyl-12-aza-antofine (3g): white solid; mp 204-206 ℃; $[\alpha]^{23}_{D}$= -87.9 °(*c* 0.34, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7. 88 (s, 1H), 7.86 (d, *J* = 2.7 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.25 (s, 1H), 7.18 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.55 (d, *J* = 14.7 Hz, 1H), 4.29 (d, *J* = 5.4 Hz, 1H), 4.09 (s, 3H), 4.04 (s, 3H), 4.00 (s, 3H), 3.73 (d, *J* = 14.7 Hz, 1H), 3.59 (d, *J* = 5.4 Hz, 1H), 3.44 (dd, *J* = 8.7 Hz, *J* = 6.0 Hz, 1H), 3.26 (d, *J* = 15.3 Hz, 1H), 2.99-2.92 (m, 2H), 2.88-2.81 (m, 1H), 2.22-2.19 (m, 1H), 0.53-0.45 (m, 4H); ESI MS *m*/*z* 405.10 $(M+H)^+$.

(*S*)-12*N*-Methyl-12-aza-antofine (3h): white solid; mp 182-184 ℃; $[\alpha]^{23}_{D}$= -45.0 °(*c* 0.34, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.89 (d, *J* = 2.7 Hz, 1H), 7.76 (d, *J* = 9.0 Hz,

1H), 7.26 (s, 1H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 4.57 (d, *J* = 14.7 Hz, 1H), 4.11 (d, *J* = 5.1 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.80 (d, *J* = 15.0 Hz, 1H), 3.62 (d, *J* = 5.1 Hz, 1H), 3.41 (dd, *J* = 8.4 Hz, *J* = 5.1 Hz, 1H), 3.48 (d, *J* = 12.9 Hz, 1H), 3.01-2.95 (m, 2H), 2.85-2.79 (m, 1H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 149.7, 148.7, 130.4, 126.9, 125.8, 124.6, 124.3, 124.0, 123.8, 115.2, 104.9, 104.1, 104.0, 78.0, 61.0, 59.2, 56.2, 56.1, 55.7, 50.4, 43.5, 31.0; ESI MS *m*/*z* 379.05 $(M+H)^+$.

(*S*)-12*N*-Dimethylamino-12-aza-antofine (3i): white solid; mp 113-115 ℃; $[\alpha]^{23}_D$= -71.9 °(*c* 0.31, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.29 (s, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 4.56 (d, *J* = 14.7 Hz, 1H), 4.40 (d, *J* = 6.6 Hz, 1H), 4.11 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.70 (d, *J* = 14.7 Hz, 1H), 3.56 (d, *J* = 6.6 Hz, 1H), 3.51 (dd, *J* = 9.0 Hz, *J* = 5.4 Hz, 1H), 3.32 (m, 1H), 3.07-2.95 (m, 2H), 2.84-2.78 (m, 1H), 2.50 (s, 6H); ESI MS *m*/*z* 408.10 $(M+H)^+$.

(*R*)-12*N*-Benzyl-12-aza-antofine (3j): light yellow solid; mp 95-97 ℃; $[\alpha]^{23}_D$= 28.6 °(*c* 0.28, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.88 (d, *J* = 2.7 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.44-7.42 (m, 2H), 7.38-7.27 (m, 3H), 7.25 (s, 1H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 4.55 (d, *J* = 14.4 Hz, 1H), 4.17 (d, *J* = 5.4 Hz, 1H), 4.10 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 3.96 (s, 2H), 3.78 (d, *J* = 14.4 Hz, 1H), 3.60 (d, *J* = 5.4 Hz, 1H), 3.40-3.38 (m, 1H), 3.29-3.26 (m, 1H), 3.00-2.85 (m, 3H); ESI MS *m*/*z* 455.20 $(M+H)^+$.

(*R*)-12*N*-Dimethylamino-12-aza-antofine (3k): white solid; mp 77-79 ℃; $[\alpha]^{23}_D$= 54.3 °(*c* 0.93, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.76 (d, *J* = 9.2 Hz, 1H), 7.27 (s, 1H), 7.19 (dd, *J* = 9.2 Hz, *J* = 2.8 Hz, 1H), 4.55 (d, *J* = 14.4 Hz, 1H), 4.39 (d, *J* = 6.4 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.69 (d, *J* = 14.8 Hz, 1H), 3.55 (d, *J* = 6.4 Hz, 1H), 3.50 (dd, *J* = 8.8 Hz, *J* = 6.0 Hz, 1H), 3.30 (dd, *J* = 15.2 Hz, *J* = 2.4 Hz, 1H), 3.03 (t, *J* = 9.2 Hz, 1H), 3.01-2.95 (m, 1H), 2.81-2.77 (m, 1H), 2.50 (s, 6H); ESI MS *m*/*z* 408.05 $(M+H)^+$.

(*R*)-12*N*-Methyl-12-aza-antofine (3l): light yellow solid; mp 147-149 ℃; $[\alpha]^{23}_D$= 60.3 °(*c* 1.17, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (s, 2H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.17 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.11 (s, 1H), 4.47 (d, *J* = 14.7 Hz, 1H), 4.22 (d, *J* = 6.0 Hz, 1H), 4.08 (s, 3H), 4.01 (s, 3H), 4.00 (s, 3H), 3.70-3.64 (m, 2H), 3.58-3.55 (m, 1H), 3.14 (d, *J* = 11.4 Hz, 1H), 2.98-2.83 (m, 3H), 2.75 (s, 3H); ESI MS *m*/*z* 379.15 $(M+H)^+$.

(*S*)-13-aza-cryptopleurine dihydrochloride (7a): light yellow solid; mp 228 ℃ (dec.); $[\alpha]^{23}_D$= -66.7 °(*c* 0.77, DMSO); ^{1}H NMR (400 MHz, free amine, $CDCl_3$): δ 7.89 (s, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.20 (s, 1H), 7.19 (dd, *J* = 8.8 Hz, *J* = 2.8 Hz, 1H), 4.41 (d, *J* = 15.2 Hz, 1H), 4.17 (d, *J* = 5.4 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 4.00 (s, 3H), 3.96 (s, 2H), 3.67 (d, *J* = 15.2 Hz, 1H), 3.33 (dd, *J* = 12.0 Hz, *J* = 2.0 Hz, 1H), 3.20-3.08 (m, 3H), 3.01 (dd, *J* = 16.0 Hz, *J* = 2.8 Hz, 1H), 2.85-2.78 (m, 2H), 2.61-2.48 (m, 2H); ESI MS *m*/*z* 379.10 $(M+H)^+$.

(*S*)-13*N*-Ethyl-13-aza-cryptopleurine (7b): light yellow solid; mp 83-85 ℃; $[\alpha]^{23}_D$= -100.5 °(*c* 0.61, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.90 (d, *J* = 2.7 Hz, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 7.23 (s, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 4.47 (d, *J* = 14.7 Hz, 1H), 4.10 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.70 (d, *J* = 14.7 Hz, 1H), 3.24 (m, 2H), 3.07 (m, 2H), 2.93-2.84 (m, 2H), 2.71-2.63 (m, 2H), 2.54 (q, *J* = 7.2 Hz, 2H), 2.39-2.32 (m, 1H), 2.11 (t, *J* = 10.5 Hz, 1H), 1.18 (t, *J* = 6.9 Hz, 3H); ESI MS *m*/*z* 407.15 $(M+H)^+$.

(*S*)-13*N*-Methoxycarbonyl-13-aza-cryptopleurine (7c): light yellow solid; mp 99-101 ℃; $[\alpha]^{23}_D$= -110.4 °(*c* 0.28, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.88 (m, 2H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.20 (dd, *J* = 8.7 Hz, *J* = 2.4 Hz, 1H), 7.19 (s, 1H), 4.44 (d, *J* = 15.3 Hz, 1H), 4.10 (s,

3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.76 (s, 3H), 3.65 (d, *J* = 15.0 Hz, 1H), 3.30-3.04 (m, 4H), 3.00-2.77 (m, 3H), 2.62-2.42 (m, 2H); ESI MS *m*/*z* 437.10 $(M+H)^+$.

(*S*)-13*N*-Acetyl-13-aza-cryptopleurine (7d): light yellow solid; mp 123-125 ℃; $[\alpha]^{23}_D$= -105.3 °(*c* 0.17, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$, rotameric at r.t.): δ 7.90 (t, *J* = 3.3 Hz, 2H), 7.79-7.74 (d, *J* = 9.0 Hz, 1H), 7.23-7.18 (m, 2H), 4.84-4.66 (d, *J* = 12.6 Hz, 1H), 4.47 (dd, *J* = 15.6 Hz, *J* = 3.0 Hz, 1H), 4.11 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.88-3.48 (m, 2H), 3.26-2.97 (m, 3H), 2.90-2.70 (m, 2H), 2.58-2.39 (m, 2H), 2.20-2.18 (s, 3H); ESI MS *m*/*z* 421.10 $(M+H)^+$.

(*S*)-13*N*-Methylsulfonyl-13-aza-cryptopleurine (7e): light yellow solid; mp 230 ℃ (dec.); $[\alpha]^{23}_D$= -66.9 °(*c* 0.16, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.88 (d, *J* = 3.0 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 7.15 (s, 1H), 4.45 (d, *J* = 15.6 Hz, 1H), 4.11 (s, 3H), 4.04 (s, 3H), 4.02 (s, 3H), 3.98 (m, 1H), 3.81 (d, *J* = 9.0 Hz, 1H), 3.71 (d, *J* = 16.2 Hz, 1H), 3.26 (d, *J* = 11.7 Hz, 1H), 3.14-3.00 (m, 2H), 2.87 (s, 3H), 2.81-2.60 (m, 4H); ESI MS *m*/*z* 457.05 $(M+H)^+$.

(*S*)-13*N*-Methoxycarbonylmethyl-13-aza-cryptopleurine (7f): white solid; mp 111-113 ℃; $[\alpha]^{23}_D$= -29.2 °(*c* 0.36, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.88 (d, *J* = 3.0 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.21-7.17 (m, 2H), 4.44 (d, *J* = 15.6 Hz, 1H), 4.10 (s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.77 (s, 3H), 3.70 (d, *J* = 14.4 Hz, 1H), 3.34 (s, 2H), 3.26 (d, *J* = 10.8 Hz, 1H), 3.18 (d, *J* = 11.4 Hz, 1H), 3.04 (m, 2H), 2.88-2.70 (m, 3H), 2.64-2.56 (m, 1H), 2.36-2.30 (m, 1H); ESI MS *m*/*z* 451.15 $(M+H)^+$.

(*S*)-13*N*-Cyclopropylmethyl-13-aza-cryptopleurine (7g): white solid; mp 160-162 ℃; $[\alpha]^{23}_D$= -42.6 °(*c* 0.34, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 7.24 (s, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.48 (d, *J* = 15.6 Hz, 1H), 4.10 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.69 (d, *J* = 15.6 Hz, 1H), 3.37 (d, *J* = 10.2 Hz, 1H), 3.19 (t, *J* = 13.2 Hz, 2H), 3.15-3.06 (m, 1H), 2.92-2.83 (m, 1H), 2.78-2.65 (m, 2H), 2.43-2.38 (m, 1H), 2.37 (d, *J* = 6.6 Hz, 2H), 2.14 (t, *J* = 10.5 Hz, 1H), 0.96-0.89 (m, 1H), 0.58 (d, *J* = 7.8 Hz, 2H), 0.19 (d, *J* = 5.4 Hz, 2H); ESI MS *m*/*z* 433.15 $(M+H)^+$.

(*S*)-13*N*-Cyclopropyl-13-aza-cryptopleurine (7h): white solid; mp 115-117 ℃; $[\alpha]^{23}_D$= -104.6 °(*c* 0.28, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (s, 1H), 7.88 (d, *J* = 2.7 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 7.19 (dd, *J* = 9.3 Hz, *J* = 2.7 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 4.10 (s, 3H), 4.04 (s, 3H), 4.00 (s, 3H), 3.69 (d, *J* = 14.4 Hz, 1H), 3.34 (d, *J* = 10.8 Hz, 1H), 3.26-3.18 (m, 1H), 3.13-3.02 (m, 2H), 2.97-2.80 (m, 1H), 2.72-2.57 (m, 3H), 2.52-2.40 (m, 1H), 1.79-1.72 (m, 1H), 0.54-0.52 (m, 4H); ESI MS *m*/*z* 419.10 $(M+H)^+$.

(*S*)-13*N*-Benzoyl-13-aza-cryptopleurine (7i): white solid; mp 203 ℃ (dec.); $[\alpha]^{23}_D$= -66.6 °(*c* 0.50, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (brs, 2H), 7.72 (brs, 1H), 7.51-7.45 (m, 5H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 7.06 (s, 1H), 4.88-4.81 (m, 1H), 4.44 (m, 1H), 4.09-4.00 (s, 9H), 3.85-3.46 (m, 2H), 3.27-3.08 (m, 3H), 2.85-2.70 (m, 2H), 2.58-2.41 (m, 2H); ESI MS *m*/*z* 483.15 $(M+H)^+$.

(*S*)-13*N*-(2'-Hydroxyl)ethyl-13-aza-cryptopleurine (7j): light yellow solid; mp 266 ℃ (dec.); $[\alpha]^{23}_D$= -56.0 °(*c* 0.15, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90-7.88 (m, 2H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.19 (s, 1H), 4.46 (d, *J* = 15.0 Hz, 1H), 4.10 (s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.71 (t, *J* = 5.1 Hz, 2H), 3.67 (d, *J* = 14.7 Hz, 1H), 3.21-3.19 (m, 2H), 3.06-2.97 (m, 2H), 2.90-2.81 (m, 1H), 2.65 (t, *J* = 5.4 Hz, 2H), 2.62-2.47 (m, 3H), 2.25 (t, *J* = 10.5 Hz, 1H); ESI MS *m*/*z* 423.15 $(M+H)^+$.

(*S*)-13*N*-Dimethoxyphosphoryl-13-aza-cryptopleurine (7k): light yellow solid; mp

98-100 ℃; $[\alpha]^{23}_{D}$= -92.4 °(*c* 0.25, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.88 (m, 2H), 7.76 (d, *J* = 9.3 Hz, 1H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.18 (s, 1H), 4.44 (d, *J* = 15.6 Hz, 1H), 4.09 (s, 3H), 4.04 (s, 3H), 4.01 (s, 3H), 3.77 (d, *J* = 3.3 Hz, 3H), 3.73 (d, *J* = 3.0 Hz, 3H), 3.67 (m, 2H), 3.58 (m, 1H), 3.24-3.13 (m, 2H), 3.06 (dd, *J* = 16.2 Hz, *J* = 3.3 Hz, 1H), 2.94-2.75 (m, 2H), 2.62-2.46 (m, 2H); ESI MS *m*/*z* 487.15 $(M+H)^+$.

(*S*)-13*N*-Methyl-13-aza-cryptopleurine (7l): white solid; mp 179-181 ℃; $[\alpha]^{23}_{D}$= -112.2 °(*c* 0.3, $CHCl_3$) ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87-7.86 (m, 2H), 7.74 (d, *J* = 9.2 Hz, 1H), 7.19 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.16 (s, 1H), 4.45 (d, *J* = 15.2 Hz, 1H), 4.10 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.90 (m, 1H), 3.75-3.65 (m, 1H), 3.68 (d, *J* = 15.6 Hz, 1H), 3.26-3.15 (m, 2H), 3.09-3.04 (m, 1H), 2.95-2.85 (m, 2H), 2.90 (s, 6H), 2.67-2.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.6, 149.6, 148.5, 130.3, 126.6, 125.6, 124.2, 123.8, 123.7, 123.6, 115.0, 104.9, 104.0, 103.9, 62.1, 56.3, 56.2, 56.0, 55.7, 55.3, 55.3, 54.8, 46.3, 31.3; ESI MS *m*/*z* 450.15 $(M+H)^+$.

(*S*)-13*N*-Benzyl-13-aza-cryptopleurine (7m): white solid; mp 94-96 ℃; $[\alpha]^{23}_{D}$= -177 °(*c* 0.21, $CHCl_3$) ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.89 (d, *J* = 2.8 Hz, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 7.42-7.34 (m, 4H), 7.31-7.29 (m, 1H), 7.20 (s, 1H), 7.19 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 4.47 (d, *J* = 15.3 Hz, 1H), 4.09 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 3.70 (d, *J* = 14.8 Hz, 1H), 3.66 (d, *J* = 12.8 Hz, 1H), 3.57 (d, *J* = 13.2 Hz, 1H), 3.20-3.13 (m, 2H), 3.04-2.97 (m, 2H), 2.88-2.81 (m, 1H), 2.72-2.63 (m, 2H), 2.45 (dt, *J* = 11.2 Hz, *J* = 2.4 Hz, 1H), 2.14 (t, *J* = 10.4 Hz, 1H); ESI MS *m*/*z* 469.20 $(M+H)^+$.

(*R*)-13*N*-Dimethylcarbamyl-13-aza-cryptopleurine (7n): yellow solid; mp 206-208 ℃; $[\alpha]^{23}_{D}$= 139 °(*c* 0.3, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.88 (m, 2H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 7.19 (dd, *J* = 9.0 Hz, *J* = 2.1 Hz, 1H), 4.46 (d, *J* = 15.6 Hz, 1H), 4.09 (s, 3H), 4.03 (s, 3H), 4.00 (s, 3H), 3.71 (d, *J* = 15.2 Hz, 1H), 3.34 (d, *J* = 11.2 Hz, 1H), 3.23 (d, *J* = 11.2 Hz, 1H), 3.15 (d, *J* = 10.8 Hz, 1H), 3.08-3.05 (m, 1H), 2.85-2.82 (m, 2H), 2.76 (dt, *J* = 12.0 Hz, *J* = 2.4 Hz, 1H), 2.61 (dt, *J* = 12.0 Hz, *J* = 2.4 Hz, 1H), 2.56 (s, 3H), 2.37-2.32 (m, 1H); ESI MS *m*/*z* 393.10 $(M+H)^+$.

(*R*)-13*N*-Isobutyl-13-aza-cryptopleurine (7o): white solid; mp 175-177 ℃; $[\alpha]^{23}_{D}$= 98.2 °(*c* 1.1, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87-7.86 (m, 2H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.18 (s, 1H), 4.53 (d, *J* = 15.3 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.80 (m, 1H), 3.37-3.28 (m, 2H), 3.18-3.09 (m, 2H), 2.95-2.86 (m, 2H), 2.63-2.60 (m, 2H), 2.42 (d, *J* = 6.6 Hz, 2H), 2.41 (m, 1H), 1.98 (m, 1H), 1.01 (s, 3H), 0.99 (s, 3H); ESI MS *m*/*z* 435.10 $(M+H)^+$.

(*R*)-13*N*-Benzyl-13-aza-cryptopleurine (7p): white solid; mp 86-88 ℃; $[\alpha]^{23}_{D}$= 31.9 °(*c* 3.3, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (s, 2H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.42-7.30 (m, 5H), 7.18 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.15 (s, 1H), 4.45 (d, *J* = 15.3 Hz, 1H), 4.08 (s, 3H), 4.01 (s, 3H), 4.00 (s, 3H), 3.70 (d, *J* = 16.5 Hz, 1H), 3.67 (d, *J* = 12.9 Hz, 1H), 3.56 (d, *J* = 12.9 Hz, 1H), 3.21-3.12 (m, 2H), 3.00-2.96 (m, 2H), 2.87-2.78 (m, 1H), 2.70-2.63 (m, 2H), 2.49-2.42 (m, 1H), 2.14 (t, *J* = 10.2 Hz, 1H); ESI MS *m*/*z* 469.15 $(M+H)^+$.

(*R*)-13*N*-cyclopropylmethyl-13-aza-cryptopleurine (7q): white solid; mp 156-158 ℃; $[\alpha]^{23}_{D}$= 50 °(*c* 0.72, $CHCl_3$) ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 2H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.15 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.04 (s, 1H), 4.36 (brs, 1H), 4.06 (s, 3H), 4.03 (s, 3H), 4.00 (s, 3H), 3.95 (brs, 1H), 3.68-3.60 (m, 2H), 3.26 (brs, 1H), 3.13-2.86 (m, 6H), 2.67 (m, 2H), 1.15 (m, 1H), 0.80 (m, 2H), 0.42 (m, 2H); ESI MS *m*/*z* 433.15 $(M+H)^+$.

(*S*)-12*N*-(2'-Hydroxylethyl)-12-aza-cryptopleurine (10a): white solid; mp 138-140 ℃;

$[\alpha]^{23}_{D}$= -116.1 °(*c* 0.28, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.87 (m, 2H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 7.18 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.34 (d, *J* = 14.7 Hz, 1H), 4.09 (m, 4H), 4.04 (s, 3H), 4.00 (s, 3H), 3.75-3.69 (m, 1H), 3.65 (t, *J* = 4.5 Hz, 1H), 3.57 (d, *J* = 14.7 Hz, 1H), 3.13-3.06 (m, 3H), 2.95-2.89 (m, 1H), 2.84-2.81 (m, 2H), 2.65-2.46 (m, 2H), 1.92-1.86 (m, 2H); ESI MS *m*/*z* 423.10 $(M+H)^+$.

(*S*)-12*N*-Phenyl-12-aza-cryptopleurine (10b): white solid; mp 225-227 ℃; $[\alpha]^{23}_{D}$= -85.6 °(*c* 0.25, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (s, 2H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 2H), 7.22-7.18 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.87 (t, *J* = 6.9 Hz, 1H), 4.77 (d, *J* = 11.1 Hz, 1H), 4.44 (d, *J* = 15.3 Hz, 1H), 4.09 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 3.85 (d, *J* = 12.9 Hz, 1H), 3.63 (d, *J* = 15.3 Hz, 1H), 3.57 (d, *J* = 10.8 Hz, 1H), 3.19-3.06 (m, 2H), 2.93-2.84 (m, 1H), 2.60 (m, 1H), 1.95-1.87 (m, 2H); ESI MS *m*/*z* 455.10 $(M+H)^+$.

(*S*)-12*N*-Isobutyl-12-aza-cryptopleurine (10c): light yellow solid; mp 177-179 ℃; $[\alpha]^{23}_{D}$= -83.7 °(*c* 0.30, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (s, 2H), 7.70 (d, *J* = 9.3 Hz, 1H), 7.18 (dd, *J* = 9.0 Hz, *J* = 2.7 Hz, 1H), 7.17 (s, 1H), 4.42 (d, *J* = 15.9 Hz, 1H), 4.08 (s, 3H), 4.02 (s, 3H), 3.99 (s, 3H), 3.65 (d, *J* = 14.7 Hz, 1H), 3.12-2.87 (m, 5H), 2.50-2.37 (m, 2H), 2.36 (d, *J* = 7.2 Hz, 2H), 1.94-1.81 (m, 3H), 0.96 (d, *J* = 6.6 Hz, 6H); ESI MS *m*/*z* 435.15 $(M+H)^+$.

(*S*)-12*N*-Benzyl-12-aza-cryptopleurine (10d): white solid; mp 211-213 ℃; $[\alpha]^{23}_{D}$= -80.5 °(*c* 0.41, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.87 (m, 2H), 7.72 (d, *J* = 9.0 Hz, 1H), 7.44-7.27 (m, 5H), 7.23(s, 1H), 7.16 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.32 (d, *J* = 15.3 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 4.01 (m, 1H), 3.99 (s, 3H), 3.70 (s, 2H), 3.56 (d, *J* = 15.0 Hz, 1H), 3.12-3.05 (m, 2H), 2.96-2.91 (m, 1H), 2.86 (d, *J* = 9.0 Hz, 1H), 2.44-2.29 (m, 2H), 1.94-1.83 (m, 2H); ESI MS *m*/*z* 469.15 $(M+H)^+$.

(*S*)-12*N*-Cyclopropyl-12-aza-cryptopleurine (10e): white solid; mp 164-166 ℃; $[\alpha]^{23}_{D}$= -105.4 °(*c* 0.50, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88-7.87 (m, 2H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.21 (s, 1H), 7.18 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.38 (d, *J* = 14.4 Hz, 1H), 4.16 (d, *J* = 9.0 Hz, 1H), 4.08 (s, 3H), 4.04 (s, 3H), 3.99 (s, 3H), 3.59 (d, *J* = 15.3 Hz, 1H), 3.20 (m, 1H), 3.09-3.01 (m, 2H), 2.94-2.85 (m, 1H), 2.58-2.41 (m, 2H), 1.99-1.94 (m, 1H), 1.87-1.84 (m, 2H), 0.57-0.49 (m, 4H); ESI MS *m*/*z* 419.10 $(M+H)^+$.

(*S*)-12*N*-Dimethylamino-12-aza-cryptopleurine (10f): white solid; mp 225-227 ℃; $[\alpha]^{23}_{D}$= -86.4 °(*c* 0.50, $CHCl_3$) ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.88 (m, 2H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.23 (s, 1H), 7.20 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 4.42 (d, *J* = 14.7 Hz, 1H), 4.20 (d, *J* = 7.8 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 4.00 (s, 3H), 3.65 (d, *J* = 14.7 Hz, 1H), 3.18-3.06 (m, 2H), 3.04 (d, *J* = 8.1 Hz, 1H), 2.88 (dd, *J* = 15.6 Hz, *J* = 10.5 Hz, 1H), 2.52 (s, 6H), 2.48 (m, 1H), 2.31-2.24 (m, 1H), 2.01-1.83 (m, 2H); ESI MS *m*/*z* 422.15 $(M+H)^+$.

(*R*)-12*N*-Isobutyl-12-aza-cryptopleurine (10g): light yellow solid; mp 175-177 ℃; $[\alpha]^{23}_{D}$= 85.0 °(*c* 0.38, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (m, 2H), 7.64 (d, *J* = 9.0 Hz, 1H), 7.22 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 7.16 (s, 1H), 4.85 (d, *J* = 15.3 Hz, 1H), 4.56 (d, *J* = 9.3 Hz, 1H), 4.09 (s, 3H), 4.04 (s, 3H), 4.00 (s, 3H), 3.76 (d, *J* = 9.9 Hz, 1H), 3.34-3.17 (m, 3H), 3.03 (m, 2H), 2.79 (m, 2H), 2.21 (m, 1H), 2.04-1.89 (m, 2H), 1.00-0.98 (s, 6H); ESI MS *m*/*z* 435.20 $(M+H)^+$.

(*R*)-12*N*-Dimethylamino-12-aza-cryptopleurine (10h): light yellow solid; mp 204-206 ℃; $[\alpha]^{23}_{D}$= 90.0 °(*c* 0.86, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (m, 2H), 7.69 (d, *J* = 9.2 Hz, 1H), 7.19 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.11 (s, 1H), 4.48 (d, *J* = 15.2 Hz, 1H), 4.23 (d, *J* = 8.0 Hz, 1H), 4.10 (s, 3H), 4.01 (s, 3H), 3.99 (s, 3H), 3.72 (d, *J* = 15.2 Hz, 1H), 3.18 (m, 1H), 3.16 (d,

J = 8.0 Hz, 1H), 3.07 (dd, *J* = 16.0 Hz, *J* = 3.2 Hz, 1H), 2.83-2.77 (m, 1H), 2.57-2.54 (m, 1H), 2.52 (s, 6H), 2.45 (m, 1H), 2.00-1.96 (m, 1H), 1.81-1.78 (m, 1H); ESI MS *m*/*z* 422.15 $(M+H)^+$.

(*R*)-12*N*-2'-hydroxylethyl-12-aza-cryptopleurine (10i): white solid; mp 175-177 ℃; $[\alpha]^{23}_D$= 112 °(*c* 0.16, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.87-7.86 (m, 2H), 7.70 (d, *J* = 9.2 Hz, 1H), 7.20 (dd, *J* = 9.2 Hz, *J* = 2.8 Hz, 1H), 7.18 (s, 1H), 4.48 (d, *J* = 14.8 Hz, 1H), 4.33 (d, *J* = 8.8 Hz, 1H), 4.09 (s, 3H), 4.05 (s, 3H), 4.00 (s, 3H), 3.81-3.76 (m, 2H), 3.70 (d, *J* = 15.2 Hz, 1H), 3.30-3.27 (m, 2H), 3.16 (m, 1H), 3.00-2.94 (m, 3H), 2.75-2.66 (m, 2H), 2.00-1.90 (m, 2H); ESI MS *m*/*z* 423.10 $(M+H)^+$.

(*R*)-12*N*-Cyclopropylmethyl-12-aza-cryptopleurine (10j): white solid; mp 80-82 ℃; $[\alpha]^{23}_D$= 82.9 °(*c* 0.17, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 7.27 (s, 1H), 7.20 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 4.39 (d, *J* = 15.2 Hz, 1H), 4.24 (d, *J* = 9.2 Hz, 1H), 4.10 (s, 3H), 4.06 (s, 3H), 4.01 (s, 3H), 3.62 (d, *J* = 15.2 Hz, 1H), 3.27-3.24 (m, 1H), 3.13 (dd, *J* = 16.4 Hz, *J* = 3.2 Hz, 1H), 2.93 (dd, *J* = 16.0 Hz, *J* = 10.0 Hz, 1H), 2.86 (d, *J* = 9.2 Hz, 1H), 2.53-2.34 (m, 4H), 1.95-1.89 (m, 2H), 1.00-0.96 (m, 1H), 0.58-0.54 (m, 2H), 0.19-0.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.6, 149.6, 148.5, 130.2, 126.7, 125.5, 124.5, 124.3, 123.9, 123.5, 115.0, 104.9, 104.0 (2×C), 60.3, 56.8, 56.2, 56.1, 55.7, 52.3, 52.2, 34.1, 31.4, 9.1, 4.2, 3.9; ESI MS *m*/*z* 433.20 $(M+H)^+$.

Cell growth inhibition assay. The sulforhodamine B assay was used according to the procedures developed and validated at NCI. The in vitro anticancer activities are expressed as IC_{50} values, which is the test compound concentration (μg/ml) that reduced the cell number by 50% after 72 h treatment. The values were interpolated from dose-response data. Each test was performed in triplicate with a variation of less than 5%. The IC_{50} values determined in each of the independent tests varied less than 10%. Compound stock solutions were prepared in DMSO with the final solvent concentration ≤1% DMSO (v/v), a concentration without effect on cell replication. The cells were cultured at 37 ℃ in RPMI-1640 supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), 2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum, and 100 μg/mL kanamycin in a humidified atmosphere containing 5% CO2.

DNA microarray analysis. Total RNA was extracted from the cells incubated with or without drugs using RNAzol B solution (Life Tech, Gaithersburg, MD), and the mRNA was extracted using a mRNA isolation kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Five μg of mRNA from each sample was used in each array. The microarray images were scanned, digitized, and analyzed using a flatbed scanner (PowerLook 3000; UMAX, Taipei, Taiwan) and GenePix 3.0 software (Axon Instruments, Union City, CA). When designing the microarray experiments, we adhered to the guidelines of the Microarray Gene Expression Data Society (www.mged.org/Workgroups/MIAME/miame_checklist.html).

Identification of pathways using KEGG and BioCarta databases. Gene identification was performed to determine which biochemical pathways were altered during treatment with antofine. Having identified genes on the basis of the cDNA microarray data, we were also interested in determining whether any of these genes were part of the same pathway. Accordingly, we searched the by Genespring, Ingenuity, and MetaCore biochemical pathway database using the genes selected from cDNA microarray analysis.

RT-PCR. SuperScript reverse transcriptase (Gibco; Invitrogen) was used to synthesize cDNA according to the protocol provided by the manufacturer. Expression levels of target genes were detected by a PCR assay.

Western blot. Equal amounts (50 μg) of cell lysate were separated by 10% SDS–PAGE, and transferred to a polyvinylidene membrane (Millipore, Billerica, MA). The membrane was probed with antibodies against ORC1, CDT1, CDC6, MCM2, MCM4 (Santa Cruze Biotechnology). Antibodies were diluted in TBS (pH 7.5) containing 0.05% (v/v) Tween 20 and 5% (w/v) dried milk. Blots were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Uppsala, Sweden). Bound antibodies were visualized by electrochemical luminescence staining with autoradiographic detection using Kodak X-Omat Blue film (PerkinElmer Life Science, Boston, MA).

***In vivo* study.** Viable human HT-29 colorectal adenocarcinoma cells were injected subcutaneously in the right flank of mice at a dose of 1.5×10^7 cells in 0.2 mL of cell suspension. When the tumor grew and reached 50 mm^3 in volume (designated as D1), mice were sorted into control and treatment groups (eight animals in each group). All doses were administered at a volume of 10 mL/kg, scaled to the body weight of each mouse. Control Group 1 mice received vehicle, i.v./i.p. on a bid to end schedule (twice every day to endpoint). Groups 4 received YXM74, i.v. at 20 mg/kg on a qd to end schedule. Group 5 received YXM74, i.v./i.p. at 20 mg/kg, on a bid to end schedule. Animals were weighed daily for the first five days, and then twice weekly until

the completion of the study (29 days). The mice were examined frequently for overt signs of any adverse, drug-related side effects. Tumor weight (mg) was estimated according to the formula length × $(\text{width})^2$ × 0.5 in mm^3, assuming its specific gravity to be 1. Treatment efficacy was determined from tumor growth delay (TGD), which is defined as the increase in the median TTE for a treatment group compared to the control group: TGD = T – C, expressed in days, or as a percentage of the median TTE of the control group: %TGD = (T-C)/C × 100, where: T = median TTE for treatment group, C = median TTE for control group.

HPLC analysis of the final compounds.

Compound's purity was determined by two different HPLC conditions.

System: Shimadzu LC-20AT prominence liquid chromatography

Detector: Shimadzu SPD-M20A at 254 nm

Column: Alltima 2.1 mm x 150 mm C-18 5u

Flow rate: 0.200 mL/min

Compound	MeOH%	Purity	Retention time (min)	ACN%	Purity	Retention time (min)
3a	85	99.5	9.29	85	97.2	8.26
3b	95	98.9	5.59	100	99.9	7.32
3c	85	100	12.22	100 (MeOH/ACN=4/6)	99.9	9.11
3d	90	99.2	5.83	85 (MeOH/ACN=4/6)	99.5	6.48
3e	80	96	6.66	90 (MeOH/ACN=4/6)	99.8	3.20
3f	90	95.2	6.97	90 (MeOH/ACN=4/6)	100	6.05
3g	85	97.6	6.86	90 (MeOH/ACN=4/6)	95.3	4.79
3h	85	96.8	13.15	100 (MeOH/ACN=4/6)	100	7.54
3i	90	96.1	8.13	90	99.9	13.93
3j	85	99.7	4.98	90	100	3.71
3k	95	98.1	5.55	100 (MeOH/ACN=4/6)	99.2	4.19
3l	90	98.2	12.56	100 (MeOH/ACN=4/6)	99.0	8.66
7a (free amine)	80	100	3.47	80	99.8	3.96
7b	80	95.1	10.77	90	97.3	11.81
7c	75	95.1	7.00	80	95.3	4.50
7d	80	99.0	4.72	70	99.1	3.82
7e	65 (MeOH/ACN=7/3)	99.9	11.76	80	99.3	3.52
7f	85	98.9	4.18	80	98.2	4.11
7g	90	99.4	4.37	100 (MeOH/ACN=4/6)	99.0	5.66
7h	80	97.6	8.89	90	96.7	4.54
7i	75	99.9	7.66	80	95.9	3.37

7j	85	96.9	5.36	75	97.7	5.89
7k	60	99.7	4.62	70	100	3.37
7l	75	97.8	3.26	70	97.7	3.28
7m	75	99.8	9.14	70	99.9	3.35
7n	70	99.7	11.54	80	98.9	4.07
7o	85	97.8	10.86	100	98.6	4.55
7p	95	95.8	5.18	95	98.8	3.49
7q	85	97.7	4.00	70	99.9	3.03
10a	80	96.9	5.77	90 (MeOH/ACN=4/6)	98.2	3.14
10b	90	97.9	6.35	85	98.0	5.95
10c	90	97.5	5.98	95 (MeOH/ACN=4/6)	100	7.15
10d	85	96.4	12.49	100 (MeOH/ACN=4/6)	97.3	4.31
10e	85	95.9	5.22	90	95.8	6.53
10f	80	97.4	8.66	90	97.2	4.71
10g	90	96.2	7.56	95	97.9	14.16
10h	80	98.8	3.11	70	99.7	2.86
10i	50	99.6	3.72	75	100	3.27
10j	75	98.5	8.65	75	99.3	5.07
11	85	99.8	4.78	80	99.2	4.37
13	85	99.4	5.02	85	96.8	4.07
16	60	99.0	10.43	40	97.0	2.97