Enhanced Conversion of DNA Radical Damage to Double Strand Breaks by 1,2,4-Benzotriazine 1,4-dioxides linked to a DNA Binder compared to Tirapazamine

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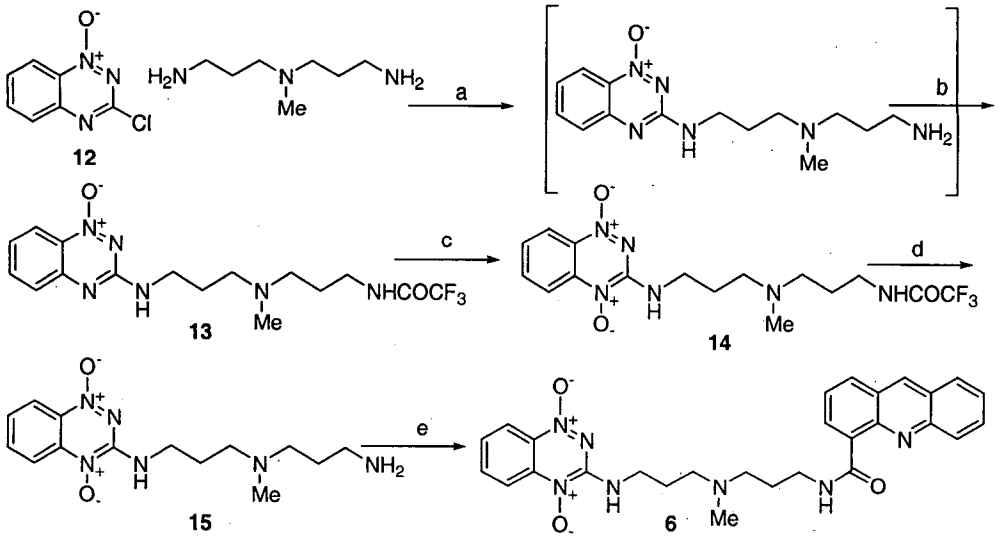
Experimental for compounds 6–9.

General procedures. Elemental analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. IR spectra were recorded on a Midac FT-IR as KBr discs, unless otherwise stated. NMR spectra were obtained on a Bruker Avance-400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in $CDCl_3$ unless otherwise specified, and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Assignments were determined using COSY, HSQC, and HMBC two-dimensional experiments.

Mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. High-resolution spectra were obtained at nominal resolutions of 3000, 5000, or 10000 as appropriate. All spectra were obtained as electron impact (EI) using PFK as the reference unless otherwise stated.

Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F_{254}) with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel, (Merck 230–400 mesh). All compounds designated for testing were analyzed at >99% purity by reverse phase HPLC using an Agilent 1100 liquid chromatograph, an Alltima C_{18} (5 μ) stainless steel column (150 mm × 3.2 mm i.d.) and an Agilent 1100 diode array detector. Chromatograms were run using various gradients of aqueous (0.045 M ammonium formate and formic acid at pH 3.5) and organic (80% MeCN/MilliQ water) phases. DCM refers to dichloromethane; DME refers to dimethoxyethane, DMF refers to dry dimethyl formamide; ether refers to diethyl ether; EtOAc refers to ethyl acetate; EtOH refers to ethanol; MeOH refers to methanol; pet. ether refers to petroleum ether, boiling range 40-60 °C; THF refers to tetrahydrofuran dried over sodium benzophenone ketyl. All solvents were freshly distilled.

***N*-{3-[{3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}(methyl)amino]propyl}-4-acridinecarboxamide (6).**



Reagents:

a) Et_3N, DCM; b) $(CF_3CO)_2O$, DCM, 43% from **12**; c) MCPBA, $NaHCO_3$, DCM, 27% + 24% SM; d) NH_4OH, MeOH, quant.; e) acridine 4-carboxylic acid, CDI, DMF; **15**, DCM, 66%.

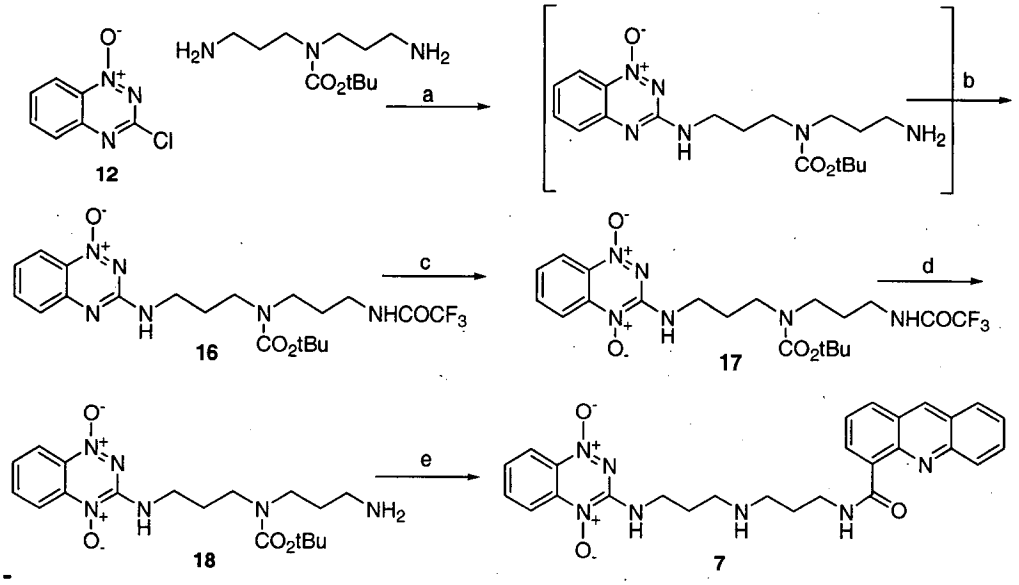
2,2,2-Trifluoro-*N*-[3-(methyl{3-[(1-oxido-1,2,4-benzotriazin-3-yl)amino]propyl}amino)propyl]acetamide (13). A solution of chloride **12** (2.07 g, 11.4 mmol), N^1-(3-aminopropyl)-N^1-methyl-1,3-propanediamine (3.31 g, 22.8 mmol) and Et_3N (3.2 mL, 22.8 mmol) in DCM (200 mL) was stirred at 20 °C for 2 d. The solvent was evaporated and the residue dissolved in MeCN (150 mL). Ethyl trifluoroacetate (5.4 mL, 45.6 mmol) and water (0.8 mL, 45.6 mmol) added and the solution heated at reflux temperature for 16 h. The solvent was evaporated, and the residue purified by chromatography, eluting with a gradient (0–1%) of Et_3N/(0–10%) MeOH/DCM, followed by further chromatography, eluting with 10% MeOH/DCM, to give 1-oxide **13** (1.89 g, 43%) as a yellow solid, mp (DCM) 111–115 °C; ^{1}H NMR δ 9.04 (br s, 1 H, NH), 8.25 (dd, J = 8.7, 1.4 Hz, 1 H, H 8'), 7.70 (ddd, J = 8.4, 7.1, 1.4 Hz, 1 H, H-6'), 7.57 (d, J = 8.4 Hz, 1 H, H-5'), 7.29 (ddd, J = 8.7, 7.1, 1.1 Hz, 1 H, H-7'), 6.17 (br s, 1 H, NH), 3.58 (dd, J = 6.6, 5.8 Hz, 2 H, CH_2N), 3.49 (br t, J = 6.0 Hz, 2 H, CH_2N), 2.52–2.58 (m, 4 H, 2 × CH_2N), 2.27 (s, 3 H, NCH_3), 1.84–1.90 (m, 2 H, CH_2), 1.75–1.82 (m, 2 H, CH_2); ^{13}C NMR δ 158.9, 157.3 (q, J = 36 Hz), 148.8, 135.6, 130.8, 126.4, 124.9, 120.4, 116.1 (q, J = 288 Hz), 57.1, 56.4, 41.3, 40.3 (2), 26.3, 24.4; MS (FAB$^+$) *m/z* 387 (MH^+, 100%), 371 (8), 338 (30); HRMS (FAB$^+$) calcd for $C_{16}H_{22}F_3N_6O_2$ (MH^+) *m/z* 387.1756, found 387.1765. Anal. calcd for $C_{16}H_{21}F_3N_6O_2 \cdot ½MeOH$: C, 49.2; H, 5.8; N, 20.9; found: C, 49.1; H, 5.5; N, 20.7%.

***N*-{3-[{3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}(methyl)amino]propyl}-2,2,2-trifluoroacetamide (14).** Trifluoroacetic anhydride (4.13 mL, 29.2 mmol) was added to a stirred solution of 1-oxide **13** (1.13 g, 2.92 mmol) in $CHCl_3$ (50 mL) and the solution stirred at 20 °C for 30 min. The solution was cooled to –10 °C and 70% H_2O_2 (2 mL) (CAUTION) added dropwise. The solution was stirred at 20 °C for 30 d, partitioned between $CHCl_3$ (50 mL) and sat. aqueous $KHCO_3$ (50 mL). The aqueous fraction was extracted with $CHCl_3$ (3 × 30 mL), the combined organic fraction dried and the solvent evaporated (CAUTION: safety shield). The residue was purified by chromatography, eluting with 10% MeOH/DCM, to give (i) starting material **13** (275 mg, 24%) and (ii) 1,4-dioxide **14** (319 mg, 27%) as a red gum, ^{1}H NMR [$(CD_3)_2SO$] δ 9.44 (br s, 1 H, NH), 8.45 (t, J = 5.9 Hz, 1 H, NH), 8.20 (d, J = 8.8 Hz, 1 H, H-8'), 8.12 (d, J = 8.6 Hz, 1 H, H-5'), 7.93 (ddd, J = 8.6, 7.1, 1.2 Hz, 1 H, H-6'), 7.57 (ddd, J = 8.8, 7.1, 1.3 Hz, 1 H, H-7'), 3.42–3.47 (m, 2 H, CH_2N), 3.21–3.25 (m, 2 H, CH_2N),

2.39 (t, J = 6.7 Hz, 2 H, CH_2N), 2.32 (t, J = 6.9 Hz, 2 H, CH_2N), 2.16 (s, 3 H, NCH_3), 1.72–1.80 (m, 2 H, CH_2), 1.61–1.68 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 155.9 (q, J = 36 Hz), 149.7, 138.1, 135.4, 129.8, 126.7, 121.0, 116.7, 115.9 (q, J = 288 Hz), 54.9, 54.6, 41.4, 39.5, 37.6, 25.9, 25.8; MS (FAB^+) m/z 403 (MH^+, 25%), 387 (5); HRMS (FAB^+) calcd for $C_{16}H_{22}F_3N_6O_3$ (MH^+) m/z 403.1706, found 403.1695.

***N*-{3-[{3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}(methyl)amino]propyl}-4-acridinecarboxamide (6).** A solution of trifluoroacetamide **14** (175 mg, 0.44 mmol) and NH_4OH (5 mL) in MeOH (20 mL) was stirred at 30 °C for 4 h. The solvent was evaporated and the residue dried to give N^1-(3-aminopropyl)-N^3-(1,4-dioxido-1,2,4-benzotriazin-3-yl)-N^1-methyl-1,3-propanediamine (**15**) as a red gum, ^1H NMR [$(CD_3)_2SO$] δ 8.43 (br s, 1 H, NH), 8.21 (d, J = 8.5 Hz, 1 H, H-8'), 8.13 (d, J = 8.4 Hz, 1 H, H-5'), 7.94 (ddd, J = 8.4, 7.1, 1.2 Hz, 1 H, H-6'), 7.75 (br s, 2 H, NH_2), 7.57 (ddd, J = 8.7, 7.2, 1.3 Hz, 1 H, H-7'), 3.45 (t, J = 6.8 Hz, 2 H, CH_2N), 3.20–3.25 (m, 2 H, CH_2N), 2.88 (dd, J = 7.4, 7.2 Hz, 2 H, CH_2N), 2.40–2.46 (m, 2 H, CH_2N), 2.20 (s, 3 H, NCH_3), 1.77–1.83 (m, 2 H, CH_2), 1.68–1.75 (m, 2 H, CH_2); MS (FAB^+) m/z 307 (MH^+, 2%), 291 (5); HRMS (FAB^+) calcd for $C_{14}H_{23}N_6O_3$ (MH^+) m/z 307.1883, found 307.1883. The amine **15** was dissolved in DCM (5 mL) and added to a stirred solution of 4-(1*H*-imidazol-1-ylcarbonyl)acridine (125 mg, 0.46 mmol) in THF (20 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0–1%) of Et_3N/(0–15%) MeOH/DCM, to give compound **6** (146 mg, 66%) as a red solid, mp (EtOAc/DCM) 169–171 °C; ^1H NMR [$(CD_3)_2SO$] δ 11.41 (t, J = 5.3 Hz, 1 H, CONH), 9.31 (s, 1 H, H-9), 8.69 (dd, J = 7.0, 1 4 Hz, 1 H, H-3), 8.43 (t, J = 5.6 Hz, 1 H, NH), 8.38 (d, J = 7.4 Hz, 1 H, H-1), 8.32 (d, J = 8.8 Hz, 1 H, H-5), 8.21 (d, J = 8.4 Hz, 1 H, H-8), 8.16 (d, J = 8.7 Hz, 1 H, H-8'), 8.09 (d, J = 8.7 Hz, 1 H, H-5'), 7.96 (ddd, J = 8.7, 7.1, 1.1 Hz, 1 H, H-6'), 7.91 (dd, J = 8.8, 7.5 Hz, 1 H, H-6), 7.74 (dd, J = 7.4, 7.0 Hz, 1 H, H-2), 7.69 (br dd, J = 8.7, 7.1 Hz, 1 H, H-7'), 7.55 (dd, J = 8.4, 7.5 Hz, 1 H, H-7), 3.60–3.65 (m, 2 H, CH_2N), 3.42–3.48 (m, 2 H, CH_2N), 3.39 (s, 3 H, NCH_3), 3.00–3.08 (m, 2 H, CH_2N), 2.60–2.68 (m, 2 H, CH_2N), 2.02–2.08 (m, 2 H, CH_2), 1.92–1.98 (m, 2 H, CH_2); MS (FAB^+) m/z 512 (MH^+, 25%), 496 (10); HRMS (FAB^+) calcd for $C_{28}H_{30}N_7O_3$ (MH^+) m/z 512.2410, found 512.2424.

***N*-[3-({3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}amino)propyl]-4-acridinecarboxamide (7).**



Reagents:

a) Et_3N, DCM; b) $(CF_3CO)_2O$, DCM, 22% from **12**; c) MCPBA, $NaHCO_3$, DCM, 8% + 65% SM; d) K_2CO_3, MeOH, H_2O, 74%; e) acridine 4-carboxylic acid, CDI, DMF; **18**, DCM, 67%; HCl, MeOH, 90%.

***tert*-Butyl 3-[(1-oxido-1,2,4-benzotriazin-3-yl)amino]propyl{3-[(trifluoroacetyl)amino]propyl}carbamate (16).** A solution of chloride **12** (1.34 g, 7.41 mmol) in DCM (50 mL) was added dropwise to a stirred solution of *tert*-butyl bis(3-aminopropyl)carbamate (2.57 g, 11.1 mmol) and Et_3N (1.55 mL, 11.1 mmol) in DCM (200 mL) at 20 °C. The solution was stirred at 20 °C for 3 d. The solvent was evaporated and the residue purified by chromatography, eluting with 50% EtOAc/acetone, to give a crude oil (2.31 g). Trifluoroacetic anhydride (3.5 mL, 24.3 mmol) was added dropwise to a stirred solution of crude amine in pyridine (50 mL) at 5 °C. The solution was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (30–50%) of EtOAc/pet. ether, to give trifluoroacetamide **16** (0.51 g, 22%) as a yellow solid, mp (EtOAc/pet. ether) 89–90 °C; ^{1}H NMR δ 8.22-8.26 (m, 2 H, H-8, NH), 7.71 (br dd, J = 8.4, 7.0 Hz, 1 H, H-6), 7.59 (d, J = 8.4 Hz, 1 H, H-5), 7.29 (br dd, J = 8.5, 7.0 Hz, 1 H, H-7), 5.45 (br s, 1 H, NH), 4.12 (br dd, J = 6.6, 6.5 Hz, 2 H, CH_2N), 3.26–3.37 (m, 6 H, 3 × CH_2N), 1.84–1.95 (m, 2 H, CH_2), 1.71–1.77 (m, 2 H, CH_2), 1.48 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 158.9, 157.3 (q, J = 37 Hz), 156.8, 148.0, 135.6, 130.9, 126.5, 125.1, 120.4, 116 (q, J = 288 Hz), 80.8, 44.5, 43.0, 38.8, 35.8, 29.7, 28.3 (3), 27.1; MS (FAB$^+$) *m/z* 473 (MH^+, 60%), 457 (10), 373 (100); HRMS (FAB$^+$) calcd for $C_{20}H_{28}F_3N_6O_4$ (MH^+) *m/z* 473.2124, found 473.2136. Anal. calcd for $C_{20}H_{27}F_3N_6O_4$: C, 50.8; H, 5.8; N, 17.8; found: C, 50.5; H, 5.7; N, 17.8%.

***tert*-Butyl 3-[(1,4-dioxido-1,2,4-benzotriazin-3-yl)amino]propyl{3-[(trifluoroacetyl)amino]propyl}carbamate (17).** A solution of MCPBA (2.12 g, 8.6 mmol) in DCM (50 mL) was added dropwise to a stirred solution of 1-oxide **16** (3.13 g, 6.6 mmol) in DCM (250 mL) and $NaHCO_3$ (1.1 g, 13.2 mmol). The mixture was stirred at 20 °C for 16 h, partitioned between DCM (200 mL) and sat. aqueous $KHCO_3$ solution (100 mL). The organic fraction was dried and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–4%) of MeOH/40%EtOAc/DCM, to give (i) starting material **16** (2.04 g, 65%) and (ii) 1,4-dioxide **17** (252 mg,

8 %) as a red solid, ^{1}H NMR δ 8.34 (d, *J* = 8.7 Hz, 1 H, H-8), 8.30 (d, *J* = 8.4 Hz, 1 H, H-5), 8.25 (br s, 1 H, NH), 7.88 (br dd, *J* = 8.4, 7.0 Hz, 1 H, H-6), 7.52 (br dd, *J* = 8.7, 7.0 Hz, 1 H, H-7), 7.20 (br s, 1 H, NH), 3.62 (dt, *J* = 6.8, 6.7 Hz, 2 H, CH_2N), 3.26–3.38 (m, 6 H, 3 × CH_2N), 1.92–1.98 (m, 2 H, CH_2), 1.73–1.79 (m, 2 H, CH_2), 1.49 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 157.3 (q, *J* = 37 Hz), 156.8, 149.8, 138.2, 135.9, 130.5, 127.4, 121.7, 117.4, 116.1 (q, *J* = 288 Hz), 80.9, 44.4, 43.2, 38.9, 31.9, 29.7, 28.4 (3), 22.7; MS (FAB$^+$) *m/z* 489 (MH^+, 10%), 473 (12), 373 (15); HRMS (FAB$^+$) calcd for $C_{20}H_{28}F_3N_6O_5$ (MH^+) *m/z* 489.2073, found 489.2086.

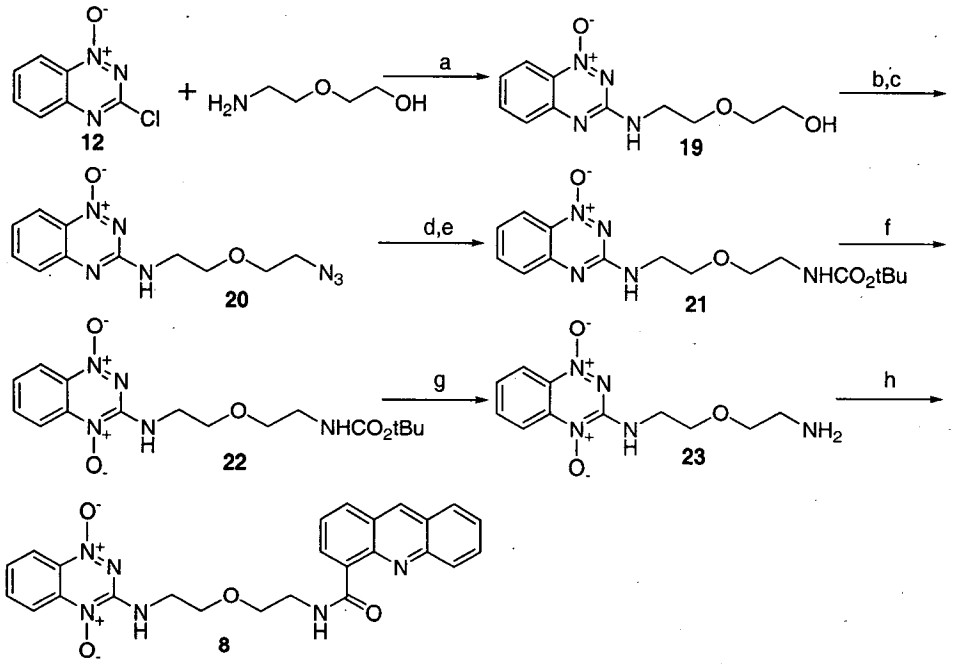
***tert*-Butyl 3-aminopropyl{3-[(1,4-dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}carbamate (18).** A mixture of trifluoroacetamide **17** (541 mg, 1.11 mmol) and K_2CO_3 (0.77 g, 5.54 mmol) in MeOH (20 mL) and water (5 mL) was heated at reflux temperature for 1 h. The mixture was partitioned between $CHCl_3$ (50 mL) and water (30 mL). The aqueous fraction was extracted with $CHCl_3$ (3 × 30 mL), the combined organic fraction dried, and the solvent evaporated to give amine **18** (322 mg, 74%) as a red oil, ^{1}H NMR [$(CD_3)_2SO$] δ 10.50 (br s, 1 H, NH), 8.21 (d, *J* = 8.7 Hz, 1 H, H-8), 8.13 (d, *J* = 8.6 Hz, 1 H, H-5), 7.94 (br dd, *J* = 8.6, 7.5 Hz, 1 H, H-6), 7.56 (br dd, *J* = 8.6, 7.5 Hz, 1 H, H-7), 7.20 (br s, 2 H, NH_2), 3.39 (t, *J* = 6.9 Hz, 2 H, CH_2N), 3.11–3.21 (m, 6 H, 3 × CH_2N), 1.78–1.86 (m, 2 H, CH_2), 1.49–1.58 (m, 2 H, CH_2), 1.39 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR [$(CD_3)_2SO$] δ 154.7, 149.7, 138.1, 135.4, 129.8, 127.9, 121.0, 116.7, 78.3, 44.3, 43.9, 38.8, 38.4, 32.2, 31.6, 27.9 (3); MS (FAB$^+$) *m/z* 393 (MH^+, 15%), 377 (9), 338 (3); HRMS (FAB$^+$) calcd for $C_{18}H_{29}N_6O_4$ (MH^+) *m/z* 393.2250, found 393.2249.

***N*-[3-({3-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}amino)propyl]-4-acridinecarboxamide (7).** A solution of 4-acridinecarboxylic acid (846 mg, 4.35 mmol) and CDI (846 mg, 5.21 mmol) in DMF (20 mL) were stirred at 50 °C for 1 h. The solvent was evaporated and the residue recrystallized from DCM/pet. ether to give 4-(1*H*-imidazol-1-ylcarbonyl)acridine (746 mg, 63%) which was used directly without characterization. A solution of the amine **18** (320 mg, 0.82 mmol) in DCM (10 mL) was added dropwise to a stirred solution of imidazolide (234 mg, 0.86 mmol) in THF (25 mL) at 5 °C and the solution was stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0–5%) of MeOH/DCM, to give *tert*-butyl 3-[(4-acridinylcarbonyl)amino]propyl{3-[(1,4-dioxido-1,2,4-benzotriazin-3-yl)amino]propyl}carbamate (330 mg, 67%) as a red gum, ^{1}H NMR δ 11.92 (br s, 1 H, CONH), 8.98 (dd, *J* = 7.2, 1.5 Hz, 1 H, H-3), 8.89 (s, 1 H, H-9), 8.26–8.32 (m, 3 H, H-5, H-5', H-8'), 8.16 (d, *J* = 8.3 Hz, 1 H, H-1), 8.07 (d, *J* = 8.8 Hz, 1 H, H-8), 7.82–7.89 (m, 3 H, H-3, H-6, H-6'), 7.65–7.69 (m, 1 H, H-7'), 7.58–7.62 (m, 1 H, H-7), 7.48 (br s, 1 H, NH), 3.72 (dt, *J* = 6.6, 6.0 Hz, 2 H, CH_2N), 3.61 (dt, *J* = 6.6, 6.4 Hz, 2 H, CH_2N), 3.38–3.50 (m, 4 H, 2 × CH_2N), 2.04–.08 (m, 2 H, CH_2), 1.88-1.94 (m, 2 H, CH_2), 1.40 [s, 9 H, $C(CH_3)_3$]; MS (FAB$^+$) *m/z* 598 (MH^+, 8%), 582 (6); HRMS (FAB$^+$) calcd for $C_{32}H_{36}N_7O_5$ (MH^+) *m/z* 598.2778, found 598.2772.

HCl saturated MeOH (30 mL) was added to a solution of carbamate (328 mg, 0.55 mmol) in MeOH (30 mL) and the solution stirred at 20 °C for 16 h. The solution was evaporated and the residue dissolved in water (20 mL) the solution neutralized with $KHCO_3$ and extracted with $CHCl_3$ (5 × 50 mL). The combined organic fraction was dried and the solvent evaporated to give compound **7** (247 mg, 90%) as a red solid, ^{1}H NMR [$(CD_3)_2SO$] δ 11.38 (t, *J* = 5.5 Hz, 1 H, CONH), 10.50 (br s, 1 H, NH), 9.28 (s, 1 H, H-9), 8.71 (dd, *J* = 7.1, 1.5 Hz, 1 H, H-3), 8.35 (dd, *J* = 8.4, 1.5 Hz, 1 H, H-1), 8.24 (d, *J* = 8.7 Hz, 1 H, H-5), 8.19 (d, *J* = 8.3 Hz, 1 H, H-8), 8.14 (d, *J* = 8.5 Hz, 1 H, H-8'), 8.03 (d, *J* = 8.5 Hz, 1 H, H-5'), 7.92–7.96 (m, 1 H, H-6), 7.83–7.88 (m, 1 H, H-6'), 7.75 (dd, *J* = 8.3, 7.1 Hz, 1 H, H-2), 7.65–7.68 (m, 1 H, H-7), 7.48–7.54 (m, 1 H, H-7'), 7.38 (s, 1 H, NH), 3.64 (dt, *J* = 6.9, 5.9 Hz, 2 H, CH_2N), 3.46 (t, *J* = 6.7 Hz, 2 H, CH_2N), 2.79 (t, *J* = 6.9 Hz, 2 H, CH_2N), 2.70 (t, *J* = 6.5 Hz, 2 H, CH_2N), 1.88–1.94 (m, 2

H, CH_2), 1.76–1.82 (m, 2 H, CH_2); ^{13}C NMR [$(CD_3)_2SO$] δ 164.7, 149.6, 147.0, 145.4, 138.5, 138.0, 135.3, 134.4, 132.6, 131.8, 129.7, 128.5, 128.4, 128.3, 126.7, 1264.4, 126.3, 125.5, 125.2, 121.0, 116.7, 47.1, 46.9, 39.6, 37.2, 29.3, 28.2; MS (FAB^+) *m/z* 498 (MH^+, 15%), 482 (5); HRMS (FAB^+) calcd for $C_{27}H_{28}N_7O_3$ (MH^+) *m/z* 498.2254, found 498.2258. Anal. calcd for $C_{27}H_{27}N_7O_3 \cdot 2H_2O$: C, 60.8; H, 5.9; N, 18.4; found: C, 60.7; H, 5.6; N, 17.1%.

***N*-(2-{2-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]ethoxy}ethyl)-4-acridinecarboxamide (8).**



Reagents:

a) Et_3N, DCM, 63%; b) MsCl, Et_3N, DCM; c) NaN_3, DMF, 89% from 24; d) propane-1,3-dithiol, Et_3N, MeOH; e) BOC_2O, THF, 93% from 25; f) MCPBA, $NaHCO_3$, DCM, 40% + 50% SM; g) CF_3CO_2H, DCM, 91%; h) acridine 4-carboxylic acid, CDI, DMF; **23**, THF, 97%.

3-{[2-(2-Hydroxyethoxy)ethyl]amino}-1,2,4-benzotriazine 1-oxide (19). A solution of chloride **12** (3.0 g, 16.52 mmol) in DCM (50 mL) was added to a stirred solution of 2-(aminoethoxy)ethanol (2.49 mL, 24.8 mmol) and Et_3N (3.45 mL, 24.8 mmol) in DCM (80 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with 40% EtOAc/DCM, to give 1-oxide **19** (2.62 g, 63%) as a yellow powder, mp (DCM/EtOAc) 131–131.5 °C; ^1H NMR δ 8.25 (dd, *J* = 8.7, 1.2 Hz, 1 H, H-8), 7.68 (ddd, *J* = 8.4, 7.2, 1.5 Hz, 1 H, H-6), 7.57 (d, *J* = 8.4 Hz, 1 H, H-5), 7.28 (ddd, *J* = 8.7, 7.2, 1.3 Hz, 1 H, H-7), 6.02 (br s, 1 H, NH), 3.74–3.80 (m, 6 H, 3 × CH_2O), 3.64–3.67 (m, 2 H, CH_2N), 2.71 (t, *J* = 5.9 Hz, 1 H, OH); ^{13}C NMR δ 158.9, 149.7, 135.5, 130.9, 126.4, 124.9, 120.4, 72.4, 69.5, 61.7, 41.9. Anal. calcd for $C_{11}H_{14}N_4O_3$: C, 52.8; H, 5.6; N, 22.4; found: C, 52.9; H, 5.7; N, 22.6%.

3-{[2-(2-Azidoethoxy)ethyl]amino}-1,2,4-benzotriazine 1-oxide (20). Methanesulfonyl chloride (0.82 mL, 10.6 mmol) was added dropwise to a stirred solution of alcohol **19** (2.41 g, 9.63 mmol) and Et_3N (1.74 mL, 12.5 mmol) in DCM (100 mL) at 5 °C and the solution stirred at 20 °C for 1 h. The solution was diluted with DCM (100 mL) and washed with water (3 × 50 mL), brine (50 mL), dried and the solvent evaporated. The residue was dissolved in DMF (50 mL) and NaN_3 (0.69 g, 10.6 mmol) added. The mixture was heated at 100 °C for 2 h, cooled to 30 °C and the solvent evaporated. The residue was partitioned between EtOAc (100 mL) and water (100 mL). The organic fraction was washed with brine (50 mL), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with 50% EtOAc/pet. ether, to give azide **20** (2.35 g, 89%) as yellow crystals, mp (EtOAc/pet. ether) 102–104 °C; ^{1}H NMR δ 8.27 (dd, J = 8.7, 1.4 Hz, 1 H, H-8), 7.70 (ddd, J = 8.6, 7.1, 1.5 Hz, 1 H, H-6), 7.59 (d, J = 8.6 Hz, 1 H, H-5), 7.29 (ddd, J = 8.6, 7.1, 1.4 Hz, 1 H, H-7), 5.70 (br s, 1 H, NH), 3.71–3.78 (m, 4 H, 2 × CH_2O), 3.69 (dd, J = 5.3, 4.8 Hz, 2 H, CH_2N_3), 3.41 (dd, J = 5.1, 4.9 Hz, 2 H, CH_2N); ^{13}C NMR δ 158.9, 148.7, 135.5, 131.1, 126.5, 125.0, 120.4, 70.0, 69.6, 50.7, 41.1. Anal. calcd for $C_{11}H_{13}N_7O_2$; C, 48.0; H, 4.8; N, 35.6; found: C, 48.3; H, 4.6; N, 35.7%.

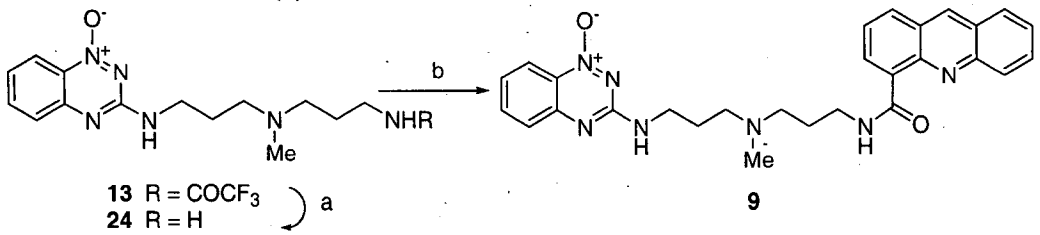
3-{[2-(2-*tert*-Butyloxycarbamoylethoxy)ethyl]amino}-1,2,4-benzotriazine 1-oxide (21). Propane-1,3-dithiol (5.7 mL, 57.0 mmol) was added dropwise to a stirred solution of azide **20** (1.57 g, 5.70 mmol) and Et_3N (7.95 mL, 57 mmol) in MeOH (100 mL) under N_2 and the solution heated at reflux temperature for 8 h. The solution was cooled to 30 °C and partitioned between 1 M HCl (100 mL) and Et_2O (100 mL). The aqueous fraction was adjusted to pH 12 with 7 M NaOH solution and extracted with DCM (3 × 50 mL). The organic fraction was dried and the solvent evaporated. The residue was dissolved in THF (100 mL) and a solution of di-*tert*-butyldicarbonate (1.87 g, 8.55 mmol) in THF (50 mL) added dropwise. The solution was stirred at 20 °C for 16 h, the solvent evaporated and the residue purified by chromatography, eluting with 40% EtOAc/pet. ether, to give carbamate **21** (1.85 g, 93%) as a yellow solid, mp (EtOAc/pet. ether) 134–137 °C; ^{1}H NMR δ 8.26 (dd, J = 8.4, 0.9 Hz, 1 H, H-8), 7.71 (ddd, J = 8.3, 7.1, 1.4 Hz, 1 H, H-6), 7.59 (d, J = 8.3 Hz, 1 H, H-5), 7.29 (ddd, J = 8.4, 7.1, 1.3 Hz, 1 H, H-7), 5.74 (br s, 1 H, NH), 4.93 (br s, 1 H, NH), 3.67–3.73 (m, 4 H, 2 × CH_2O), 3.56 (t, J = 5.2 Hz, 2 H, CH_2N), 3.29–3.36 (m, 2 H, CH_2N), 1.45 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 159.9, 155.9, 148.7, 135.5, 131.0, 126.5, 125.0, 120.4, 79.4, 70.2, 69.2, 41.1, 40.4, 28.4 (3). Anal. calcd for $C_{16}H_{23}N_5O_4$: C, 55.0; H, 6.6; N, 20.1; found: C, 55.3; H, 6.8; N, 20.1%.

3-{[2-(2-*tert*-Butyloxycarbamoylethoxy)ethyl]amino}-1,2,4-benzotriazine 1,4-dioxide (22). A solution of MCPBA (1.57 g, 6.35 mmol) in DCM (50 mL) was added dropwise to a stirred solution of carbamate **21** (1.85 g, 5.29 mmol) in DCM (100 mL) and $NaHCO_3$ (0.89 g, 10.6 mmol) and the mixture was stirred at 20 °C for 6 h. The suspension was filtered through celite, the solvent evaporated and the residue purified by chromatography, eluting with a gradient of (0–5%) MeOH/(40–0%) EtOAc/DCM, to give (i) starting material **21** (926 mg, 50%), spectroscopically identical with an authentic sample, and (ii) 1,4-dioxide **22** (702 mg, 40%) as a red solid, mp (EtOAc) 139–140 °C; ^{1}H NMR δ 8.33 (d, J = 8.7 Hz, 1 H, H-8), 8.30 (d, J = 8.7 Hz, 1 H, H-5), 7.88 (ddd, J = 8.7, 7.2, 1.2 Hz, 1 H, H-6), 7.43–7.50 (m, 2 H, H-7, NH), 5.06 (br s, 1 H, NH), 3.78–3.83 (m, 2 H, CH_2O), 3.69 (dd, J = 5.1, 5.0 Hz, 2 H, CH_2O), 3.56 (dd, J = 5.1, 5.0 Hz, 2 H, CH_2N), 3.29-3.36 (m, 2 H, CH_2N), 1.43 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR δ 156.0, 149.8, 138.5, 135.9, 130.6, 129.5, 121.6, 117.4, 79.4, 70.3, 68.9, 41.3, 40.3, 28.3 (3); MS (FAB$^+$) *m/z* 366 (MH^+, 40%), 350 (5) 310 (20); HRMS (FAB$^+$) calcd for $C_{16}H_{24}N_5O_5$ (MH^+) *m/z* 366.1777, found 366.1767. Anal. calcd for $C_{16}H_{23}N_5O_5 \cdot ½H_2O$: C, 51.3; H, 6.5; N, 18.7; found: C, 51.3; H, 6.2; N, 16.9%.

3-{[2-(2-Aminoethoxy)ethyl]amino}-1,2,4-benzotriazine 1,4-dioxide (23). Trifluoroacetic acid (1.66 mL, 34.6 mmol) was added dropwise to a stirred solution of 1,4-dioxide **22** (632 mg, 1.73 mmol) in DCM (50 mL) and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue partitioned between sat. aqueous $KHCO_3$ solution (100 mL) and $CHCl_3$ (100 mL).The aqueous phase was extracted with $CHCl_3$ (8 × 50 mL), the combined organic fractions dried, and the solvent evaporated. The residue was crystallized from $CHCl_3$ to give the amine **23** (406 mg, 91%) as a red solid, mp ($CHCl_3$) 124 °C (dec.); ^{1}H NMR δ 8.26 (d, *J* = 8.9 Hz, 1 H, H-8), 8.23 (d, *J* = 8.9 Hz, 1 H, H-5), 7.79 (dd, *J* = 8.8, 7.8 Hz, 1 H, H-6), 7.45 (dd, *J* = 8.9, 7.7 Hz, 1 H, H-7), 3.75 (dd, *J* = 5.0, 4.8 Hz, 2 H, CH_2O), 3.66 (dd, *J* = 5.0, 4.9 Hz, 2 H, CH_2O), 3.47 (dd, *J* = 5.1, 5.0 Hz, 2 H, CH_2N), 2.82 (dd, *J* = 5.1, 5.0 Hz, 2 H, CH_2N), NH and NH_2 not observed; ^{13}C NMR δ 149.8, 138.3, 135.8, 130.5, 127.2, 121.6, 117.4, 73.0, 68.9, 41.7, 41.3; MS (FAB$^+$) *m/z* 266 (MH^+, 20%), 250 (5); HRMS (FAB$^+$) calcd for $C_{11}H_{16}N_5O_3$ (MH^+) *m/z* 266.1253, found 266.1230. Anal. calcd for $C_{11}H_{15}N_5O_3 \cdot \frac{1}{4}H_2O$: C, 49.0; H, 5.8; N, 26.0; found: C, 49.0; H, 5.7; N, 24.7%.

***N*-(2-{2-[(1,4-Dioxido-1,2,4-benzotriazin-3-yl)amino]ethoxy}ethyl)-4-acridinecarboxamide (8).** A solution of the amine **23** (54 mg, 0.20 mmol) in THF (2 mL) was added dropwise to a stirred solution of 4-(1*H*-imidazol-1-ylcarbonyl)acridine (58 mg, 0.21 mmol) in THF (5 mL) at 5 °C and the solution stirred at 20 °C for 16 h. The solvent was evaporated and the residue purified by chromatography, eluting with a gradient (0-5%) of MeOH/DCM, to give compound **8** (93 mg, 97%) as a red solid, mp (EtOAc) 98–100 °C; ^{1}H NMR δ 12.14 (s, 1 H, CONH), 8.96 (dd, *J* = 7.1, 1.5 Hz, 1 H, H-3'), 8.82 (s, 1 H, H-9), 8.25 (d, *J* = 8.4 Hz, 1 H, H-8'), 8.16 (d, *J* = 8.4 Hz, 1 H, H-5'), 8.11–8.13 (m, 2 H, H-1, H-5), 7.94 (d, *J* = 8.2 Hz, 1 H, H-8), 7.76–7.84 (m, 2 H, H-6, H-6'), 7.66 (dd, *J* = 8.4, 7.1 Hz, 1 H, H-2), 7.44–7.52 (m, 2 H, H-7, H-7'), 7.36 (br s, 1 H, NH), 3.85–3.95 (m, 8 H, 2 × CH_2O, 2 × CH_2N); ^{13}C NMR δ 166.1, 149.8, 147.2, 146.3, 138.1, 137.6, 135.5, 135.3, 132.4, 131.3, 130.4, 128.8, 128.3, 128.0, 127.1, 126.8, 126.2, 125.8, 125.4, 121.5, 117.3, 70.2, 68.9, 41.1, 39.5; MS (FAB$^+$) *m/z* 471 (MH^+, 5%), 455 (4); HRMS (FAB$^+$) calcd for $C_{25}H_{23}N_6O_4$ (MH^+) *m/z* 471.1781, found 471.1790. Anal. calcd for $C_{25}H_{22}N_6O_4 \cdot \frac{1}{2}H_2O$: C, 62.6; H, 4.8; N, 17.5; found: C, 63.0; H, 4.7; N, 17.5%.

***N*-{3-[{3-[(1-Oxido-1,2,4-benzotriazin-3-yl)amino]propyl}(methyl)amino]propyl}-4-acridinecarboxamide (9).**



Reagents: a) aq. NH_3, MeOH; b) acridine 4-carboxylic acid, CDI, DMF; **24**, THF, 96%.

***N*-{3-[{3-[(1-Oxido-1,2,4-benzotriazin-3-yl)amino]propyl}(methyl)amino]propyl}-4-acridinecarboxamide (9).** A solution of crude **24** (123 mg, 0.42 mmol), prepared by treatment of **13** with aqueous NH_3 in MeOH, and 4-(1*H*-imidazol-1-ylcarbonyl)acridine (273 mg, 1.0 mmol) in THF (15 mL) was stirred at 20 °C for 16 h. The sovent was evaporated and the residue purified by chromatography, eluting with a gradient of (0–1%) aqueous NH_3 /(0–5%) MeOH/DCM, to give

compound **9** (200 mg, 96%) as a soft yellow solid, mp (DCM/pet. ether) 134–137 °C; ^{1}H NMR δ 11.92 (br t, *J* = 5.4 Hz, 1 H, NH), 8.93 (dd, *J* = 7.1, 1.5 Hz, 1 H, ArH), 8.83 (s, 1 H, ArH), 8.21 (d, *J* = 8.7 Hz, 1 H, ArH), 8.08–8.12 (m, 2 H, ArH), 8.00 (d, J = 8.2 Hz, 1 H, ArH), 7.84 (ddd, *J* = 8.7, 7.0, 1.4 Hz, 1 H, ArH), 7.63 (dd, *J* = 8.4, 7.3 Hz, 1 H, ArH), 7.56–7.60 (m, 2 H, ArH), 7.47 (d, *J* = 8.4 Hz, 1 H, ArH), 7.18 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1 H, ArH), 6.65 (br t, *J* = 5.5 Hz, 1 H, NH), 3.76–3.82 (m, 2 H, CH_2N), 3.55–3.60 (m, 2 H, CH_2N), 2.91–2.96 (m, 2 H, CH_2N), 2.82–2.88 (m, 2 H, CH_2N), 2.54 (s, 3 H, CH_3), 2.17–2.25 (m, 2 H, CH_2), 1.98–2.05 (m, 2 H, CH_2); ^{13}C NMR δ 166.3, 158.9, 148.7, 147.5, 146.2, 137.8, 136.4, 136.2, 132.5, 131.5, 130.6, 128.9, 128.0, 127.9, 128.7, 126.4 (2), 125.9, 125.3, 124.5, 120.2, 55.3, 55.1, 41.3, 39.8, 37.5, 26.4, 25.0; Anal. calcd for $C_{28}H_{29}N_7O_2 \cdot ½H_2O$: C, 66.7; H, 6.0; N, 19.4; found: C, 66.6; H, 5.7; N, 19.6%.