# 3,5-bis(Benzylidene)-1-[3-(2-hydroxyethylthio)propanoyl]-piperidin-4-ones:A novel class of potent tumour-selective cytotoxins

Umashankar Das, Hari N. Pati, Hiroshi Sakagami, Ken Hashimoto, Masami Kawase, Jan Balzarini, Erik De Clercq, James P. Stables, Jonathan R. Dimmock

**Supporting Information**

## Experimental Section

**Chemistry.** Melting points are in degrees Celsius and were recorded using a Gallenkamp apparatus and are uncorrected. The NMR spectra were obtained using a Bruker Avance spectrometer AMX 500 FT machine equipped with a BBO probe (500 MHz for $^{1}$H NMR, 125 MHz for $^{13}$C). Chemical shifts (δ) are reported in ppm. All the compounds have ≥ 95% purity as determined by elemental analysis (CHNS) using an Elementer analyser.

### Synthesis of 3a-e (General Procedure)

A mixture of 4-piperidone hydrochloride monohydrate (0.01 mol), acryloyl chloride (0.012 mol), potassium carbonate (0.02 mol) and tetrabutyl ammonium bromide (0.001 mol) in acetone (75 mL) was stirred at room temperature overnight. The reaction mixture was filtered and after evaporation of the solvent, the residue was dissolved in chloroform and washed with water (100 mL). The organic layer was dried over sodium sulphate, filtered and evaporation of the solvent in vacuo afforded 1-acryloyl-4-piperidone as a viscous oil which was used without any purification. $^{1}$H NMR ($CDCl_3$) : δ 2.49 (br s, 4H), 3.75 (br s, 2H), 3.91 (br s, 2H), 5.75 (dd, 1H), 6.31 (dd,1H), 6.66 (dd, 1H). A solution of 1-acryloyl-4-piperidone vide supra, 2-mercaptoethanol (0.01 mol) and triethylamine (0.01 mol) in dichloromethane (25 mL) was stirred at room temperature overnight. Evaporation of the solvent in vacuo led to the isolation of 1-[3-(2-hydroxyethylthio) propanoyl] piperidin-4-one **6** as a colourless oil. This product was prepared in 83% yield with respect to 4-piperidone hydrochloride monohydrate and used without purification in the synthesis of series **3**.

Hydrogen chloride gas was passed into a solution of **6** (0.0032 mol) and aryl aldehyde (0.0066 mol) in glacial acetic acid (15 mL) and the mixture was stirred at room temperature for 10 h. The solvent was removed in vacuo and the product was purified by passing through a column of silica gel (60-120 mesh) using hexane: ethyl acetate (3:1) as the eluent and then recrystallized from ethanol.

**3E,5E-3,5-bis(Benzylidene)-1-[3-(2-hydroxyethylthio)propanoyl]-piperidin-4-one (3a).**

Yield: 63%; mp 85 ºC; $^{1}$H NMR ($CDCl_3$): δ 7.92 (s, 1H, =CH), 7.87(s, 1H, =CH), 7.48(m,8H, Ar-H), 7.41(t, 2H, Ar-H), 4.97(s, 2H, $NCH_2$), 4.74(s, 2H, $NCH_2$), 3.53(t, 2H, $OCH_2$), 2.74(4H, 2×$SCH_2$, appearing as a quartet), 2.45(t, 2H, $COCH_2$); $^{13}$C NMR ($CDCl_3$): δ 186.64, 169.61, 138.80, 137.55, 134.58, 134.35, 131.57, 131.48, 130.66, 130.11, 129.82, 129.69, 129.05, 128.83, 46.15, 43.73, 42.97, 34.54, 33.43, 27.40. Anal. Calcd for $C_{24}H_{25}NO_3S.\ H_2O$: C, 67.68; H, 5.87; N, 3.29; S, 7.52%. Found: C, 67.69; H, 5.66; N, 3.14; S, 7.35%.

**3E,5E-1-[3-(2-Hydroxyethylthio)propanoyl]-3,5-bis(4-methylbenzylidene)piperidin-4-one (3b).**

Yield: 75%; mp 115 ºC; $^{1}$H NMR ($CDCl_3$): δ 7.89 (s, 1H, =CH), 7.83(s, 1H, =CH), 7.41(d, 2H, Ar-H, J = 7.7Hz)), 7.32(m, 4H, Ar-H), 7.27(d, 2H, Ar-H, J=7.7Hz), 4.96(s, 2H, $NCH_2$), 4.73(s, 2H, $NCH_2$), 3.54(t, 2H, $OCH_2$), 2.74(4H, 2×$SCH_2$, appearing as a quartet), 2.46(m, 8H, 2×$CH_3$, $COCH_2$). $^{13}$C NMR ($CDCl_3$): δ 186.67, 169.59, 140.27, 140.16, 138.71, 137.46, 131.88, 131.57, 130.83, 130.72, 130.24, 129.80, 129.58, 46.21, 43.81, 42.97, 34.53, 33.47, 27.43, 21.53. Anal. Calcd for $C_{26}H_{29}NO_5S.H_2O$: C, 68.78; H, 6.39; N,3.08; S, 7.05%. Found: C, 68.74; H, 6.38; N, 2.97; S, 6.67%.

**3E,5E-1-[3-(2-Hydroxyethylthio)propanoyl]-3,5-bis(4-methoxybenzylidene)piperidin-4-one (3c).**

Yield: 73%; mp 130 ºC; $^{1}$H NMR ($CDCl_3$): δ 7.86 (s, 1H, =CH), 7.82(s, 1H, =CH), 7.48(d, 2H, Ar-H, J = 8.3Hz), 7.38(d, 2H, Ar-H, J=8.2 Hz), 7.02 (dd, 4H, Ar-H, J=8.3 Hz, J=8.3Hz), 4.95(s, 2H, $NCH_2$), 4.74(s, 2H, $NCH_2$), 3.91(s, 3H, $OCH_3$), 3.88(s, 3H, $OCH_3$), 3.54(t, 2H, $OCH_2$), 2.76(4H, 2×$SCH_2$, appearing as a quartet), 2.49 (t, 2H, $COCH_2$). $^{13}$C NMR ($CDCl_3$): δ 186.49, 169.56, 160.82, 138.26, 137.06, 132.75, 132.13, 129.72, 129.49, 127.44, 127.01, 114.57, 114.36, 55.45, 46.26, 43.77, 42.99, 34.58, 33.50, 27.48. Anal. Calcd for $C_{26}H_{29}NO_5S.\ H_2O$: C, 64.25; H, 5.97; N, 2.88; S, 6.59%. Found: C, 64.10; H, 5.97; N, 2.79; S, 6.24%.

**3E,5E-3,5-bis(4-Chlorobenzylidene)-1-[3-(2-hydroxyethylthio)propanoyl]piperidin-4-one (3d).**

Yield: 73%; mp 138 ºC; $^{1}$H NMR ($CDCl_3$): δ 7.85 (s, 1H, =CH), 7.80 (s, 1H, =CH), 7.49 (d, 2H, Ar-H, J = 7.9Hz), 7.44(brs, 4H, Ar-H), 7.35(d, 2H, Ar-H, J=7.9 Hz), 4.92 (s, 2H, $NCH_2$), 4.71(s, 2H, $NCH_2$), 3.56(t, 2H, $OCH_2$), 2.77(4H, 2×$SCH_2$, appearing as a quartet), 2.47(t, 2H, $COCH_2$). $^{13}$C NMR ($CDCl_3$): δ 186.12, 169.61, 137.47, 136.39, 136.08, 135.88, 132.93, 132.66, 131.82, 131.74, 131.36, 129.42,

129.17, 46.19, 43.54, 42.98, 34.65, 33.38, 27.40. Anal. Calcd for $C_{26}H_{29}NO_3S.\ H_2O$: C, 58.25; H, 4.65; N, 2.83; S, 6.47%. Found: C, 58.05; H, 4.54; N, 2.71; S, 6.09%.

**3E,5E-1-[3-(2-Hydroxyethylthio)propanoyl]-3,5-bis(4-nitrobenzylidene)piperidin-4-one (3e)**

Yield: 71%; mp 133 ºC; $^1$H NMR ($CDCl_3$): δ 8.35(dd, 4H, Ar-H, J=7.8Hz, J=7.8Hz), 7.91(2H, 2×=CH, appearing as a doublet), 7.69 (m, 2H, Ar-H), 7.59 (m, 2H, Ar-H), 4.94(s, 2H, $NCH_2$), 4.78(s, 2H, $NCH_2$), 4.13(t, 2H, $OCH_2$), 2.78(t, 2H, $SCH_2$), 2.66(t, 2H, $SCH_2$), 2.48 (t, 2H, $COCH_2$). $^{13}$C NMR ($CDCl_3$): δ 185.57, 170.83, 169.78, 148.11, 147.95, 140.56, 140.32, 136.24, 135.55, 134.03, 133.88, 130.98, 130.79, 124.26, 124.06, 43.35, 43.25, 42.99, 34.78, 33.31, 31.01, 27.42, 20.89. Anal. Calcd for $C_{24}H_{23}N_3O_7S$: C, 57.88; H, 4.62; N, 8.44; S, 6.43%. Found: C, 57.70; H, 4.68; N, 8.24; S, 6.31%.

**Cytotoxicity Assays.** The methodology followed when **3a-e,4,5a-e** and melphalan were evaluated against Molt 4/C8, CEM and L1210 cells has been described previously.[1] In brief, the compounds were incubated at 37˚ C for 72h in RPMI medium except a 48h time period was used in the L1210 bioassay. A literature procedure was followed when **3a-e** and melphalan were examined towards HL-60, HSC-2, HSC-3, HSC-4, HGF, HPC and HPLF cells[2] except the time of incubation at 37˚ C was extended to 48h. The human normal oral cells were prepared from periodontal tissues after obtaining the informed consent of the patients following the guideline A0808 of the Intramural Board of Ethics Committee of Meikai University School of Dentistry, Japan. A literature protocol was followed for the determination of **3c-e,5c,d**, 5-fluorouracil and melphalan against approximately 57 human tissue cell lines.[3] A concentration range of $10^{-4}$ to $10^{-8}$ M was used except quantities of $10^{-2.6}$ to $10^{-6.6}$ M and $10^{-3.6}$ to $10^{-7.6}$ M were employed in the case of 5-fluorouracil and melphalan, respectively. The number of cell lines whose growth was not inhibited by 50% at the maximum concentration used/total number of cell lines are as follows, namely 37/58 (**5c**), 3/58 (**5d**) and 6/50 (5-fluorouracil).

**Effect of 3d and 3e on DNA fragmentation and activation of caspase-3 in HL-60 and HSC-2 cells.** A literature procedure was followed in the evaluation of **3d** and **3e** to induce DNA fragmentation and activation of caspase-3 in HL-60 and HSC-2 cells.[4]

**Toxicity evaluation of 3b-e, 4 and 5a-e in rodents.** The methodology for determining toxicity in rodents has been described previously.[5] In brief, doses of 30, 100 and 300 mg/kg were administered intraperitoneally to mice and the animals observed after 0.5 and 4h for any fatalities while neurotoxicity was evaluated using the rotorod method.[6] A dose of 50 mg/kg of **3b** was administered to 4 rats per os and the animals observed after 0.25, 0.5, 1.2 and 4 h. No deaths or neurological impairment were noted. The animals were fed, housed and handled using the procedures documented in the National Research Council Publication 'Guide for the care and use of laboratory animals'. The rats and mice were euthanized in a manner consistent with the policies of the Institute of Laboratory Resources.

**Table 4**. Evaluation of **3a-e** for drug-like properties[a]

| Compound | logP | MW | HBA | HBD | RB | TPSA | Violations |
|---|---|---|---|---|---|---|---|
| **3a** | 3.44 | 407.53 | 4 | 1 | 7 | 57.61 | 0 |
| **3b** | 4.33 | 435.59 | 4 | 1 | 7 | 57.61 | 0 |
| **3c** | 3.55 | 467.59 | 6 | 1 | 9 | 76.08 | 0 |
| **3d** | 4.79 | 476.42 | 4 | 1 | 7 | 57.61 | 0 |
| **3e** | 3.35 | 497.53 | 10 | 1 | 9 | 149.25 | 2 |
| Drug-like compound | <5 | <500 | <10 | <5 | <10 | <140 | 0 |

[a] The physicochemical properties evaluated are the logarithm of the partition coefficient (logP), molecular weight (MW), the number of hydrogen bond acceptor atoms (HBA), hydrogen bond donor atoms (HBD) and rotatable bonds (RB) as well as total polar surface area (TPSA, $Å^2$). These figures were obtained using the molinspiration Web explorer.[7]

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