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Design and Synthesis of a Novel Water Soluble Benzotetrazepinone

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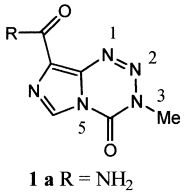
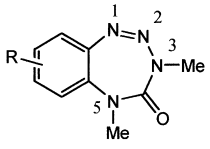
Abstract—In order to confer water solubility to the benzotetrazepinone ring system, the synthesis of **12** was undertaken. The design and synthesis of **12** were based upon previously established structural requirements for the stability of the 1,2,3,5-tetrazepin-4-one ring system. Tetrazepinone **12** was extremely water soluble and was 10-fold more potent than its imidazo-1,2,3,5-tetrazin-4-one counterpart **1a**, against the human MCF-7 breast cancer cell line.

Tetrazepinones of type **2a** are a novel class of anti-tumour agents which are more potent than temozolomide **1a** (a strong alkylating agent) against alkylating-agent-resistant Mer+ tumour cells.[1,2] In contrast to **1a**, Maxam–Gilbert assays show that tetrazepinones are weak alkylating agents that create barely detectable levels of guanine-N7 alkylation in DNA.[3] Alkaline sucrose density–gradient sedimentation studies indicate that despite their weak alkylating activity, tetrazepinones induce significant levels of single strand breaks in human tumour cells. All the results obtained thus far suggest that tetrazepinones may damage DNA by a novel mechanism.



1 a R = NH_2
b R = OH



2a R = H
b R = COOH
c R = OCH_2R'
d R = NHR'

Studies designed to mimic the interactions of tetrazepinones with nucleosides under physiological conditions have been extremely limited by the poor water solubility of the existing compounds. During the development of the chemistry of this novel class of heterocycles,[4–9] the marked instability of the 1,2,3,5-tetrazepin-4-one moiety precluded the synthesis of compounds containing functional group at the 3- and 5-positions: bulky substituents at these positions destabilize the 1,2,3,5-tetrazepin-4-one ring, whereas electron-withdrawing groups at the aromatic ring stabilize it.[5] In addition, tetrazepinones are rapidly converted to their corresponding 2-diazoniumphenyl urea precursors at acidic pH.[7,9] Therefore, benzoic acid-fused tetrazepinones like **2b**, which could be used for coupling with a water soluble amine, cannot be synthesized. In contrast, imidazotetrazinone **1b** is stable enough to be converted to its corresponding acyl chloride for further coupling with amine-containing biomolecules.[10,11] To circumvent the problems associated with the instability of the tetrazepinone ring system, we designed compound **12**, which contains all the structural requirements for a stable molecule. The 3- and 5-positions are substituted with methyl groups and the water soluble 4-aminoethylmorpholine moiety is connected to the aromatic moiety via an electron-withdrawing carboxamido (in order to preserve the electron-withdrawing character of the benzene ring). Ring closure to the 1,2,3,5-tetrazepinone ring was planned to be performed at the last step of the total synthesis.

The preparation of **12** proceeded according to Scheme 1. Acid **3** was treated with aqueous methylamine in a pressure vessel kept at 160 °C to give **4** which was converted to the corresponding acyl chloride **5** by treatment with thionyl chloride. Esterification with trimethylsilyl

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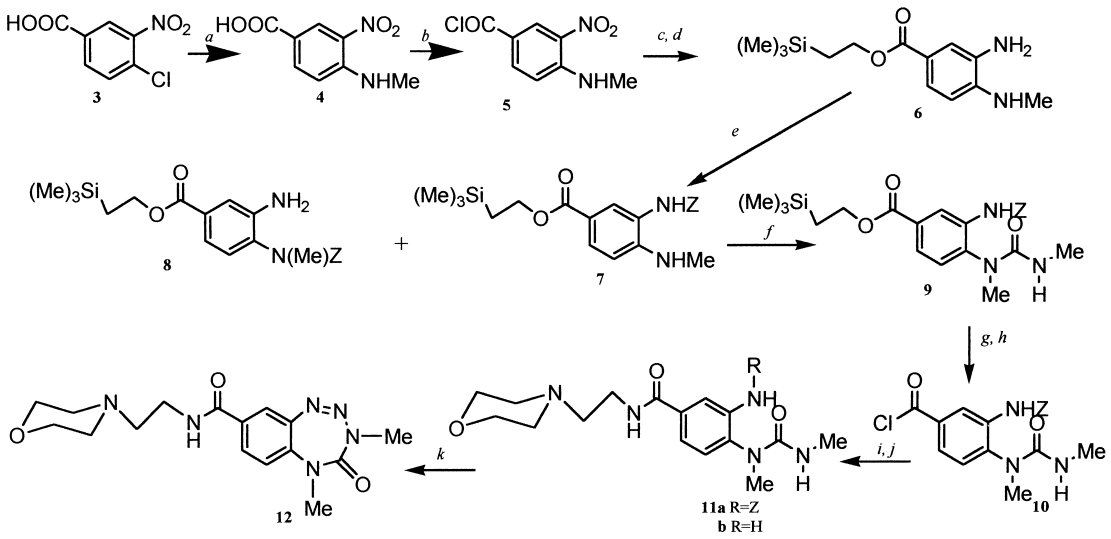

Scheme 1. (a) aq CH_3NH_2 (40%), sealed (160 °C), 70%; (b) $SOCl_2$ (neat) reflux, 100%; (c) 2-(trimethylsilyl)ethanol (1.1 equiv), pyridine (1 equiv), 72%; (d) H_2/Pd–C, methanol, 90%; (e) K_2CO_3, $PhCH_2OCOCl$; (f) CH_3NCO in CH_2Cl_2, 35%; (g) tetrabutylammonium fluoride (1 M in THF), 82%; (h) $SOCl_2$ (neat), reflux, 100%; (i) K_2CO_3, 4-(aminoethyl)morpholine, 98%; (j) H_2/Pd–C, methanol, 90%; (k) $NaNO_2/H^+$ and K_2CO_3, 51%; Z = carbobenzyloxy.

ethanol and hydrogenation gave **6**, which was treated with benzylchloroformate to provide a mixture of **7** and **8** in a 2:1 ratio. Isomers **7** and **8** were extremely difficult to separate by columnn chromatography. Therefore, the mixture was treated with methyl isocyanate to provide the corresponding ureas. Separation of the mixture on silica gel provided the desired urea **9**. After removal of the trimethylsilylethyl group by treatment with tetrabutyl ammonium fluoride (1.5 equiv) in tetrahydrofuran, the resulting acid was refluxed in thionyl chloride for 30 min to give acyl chloride **10** in quantitative yield. Coupling of **10** with 4-(2-aminoethyl)morpholine provided the corresponding amide **11a** which was converted to amine **11b** by catalytic hydrogenation. The latter amine was diazotized with ^{15}N $NaNO_2$ and cyclized at pH 9 to give the desired tetrazepinone **12**.

The structure of **12** was confirmed by ^1H and ^{15}N NMR spectroscopy, chemical ionization (CI) mass spectrometry and elemental analysis.[12] Tetrazepinone **12** was extremely soluble in water. In ^1H NMR, the 3-methyl group appeared as a doublet at 3.4 (^3J $^{15}NH = 2.8$ Hz) in $CDCl_3$ and as a singlet at 3.1 ppm in D_2O. In ^{15}N NMR, N2 appeared at 69.16 ppm in H_2O (Fig. 1a). The peak assignment was based on literature values.[4,9] These data provide the first evidence for a ring-closed tetrazepinone structure in aqueous medium.

The CI mass spectrum showed a minor $MH^+ - 29$ (^{15}NN) peak and a large $MH^+ - MeNCO$ peak. The molecular ion could not be detected by mass spectrometry using chemical ionization, however the characteristic $MH^+ - N_2$ and $MH^+ - MeNCO$ peak were observed.[4,5]

Tetrazepinone **12** was tested against the resistant MCF-7 breast tumour cell line using the sulforhodamine B assay.[13] As shown in Figure 1b, **12** (referred to as MOPH) was 10-fold more potent than temozolomide against the Mer+ breast tumour cell line MCF-7 [IC_{50} (temozolomide): 800 μM, IC_{50} (**12**): 80 μM].

The limitations imposed by the instability of the 1,2,3,5-tetrazepinone ring to the synthesis of conjugated structures can be circumvented by carefully designing a conjugated

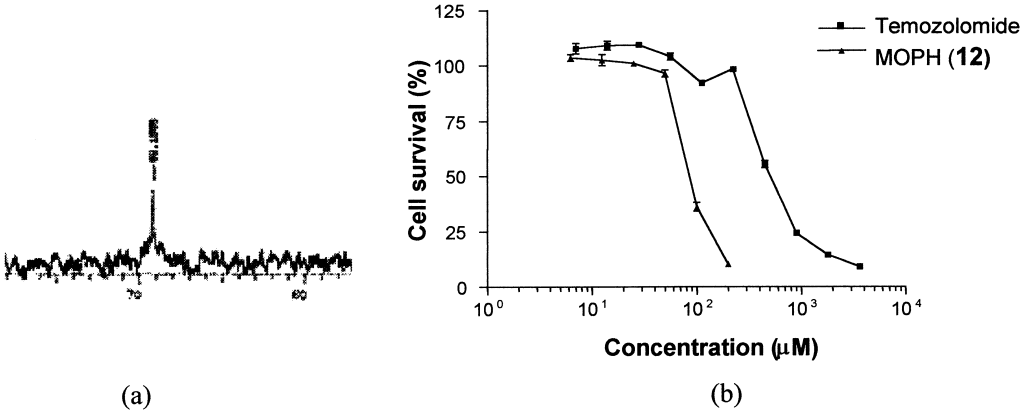

Figure 1. (a) ^{15}N NMR spectrum of **12** in D_2O; (b) dose–response curve of MCF-7 cells exposed to temozolomide and **12** for 72 h.

2-aminoaromatic urea, which can be cyclized by diazotation at the last step of the total synthesis. The availability of this first water soluble tetrazepinone will stimulate further studies on the interactions of tetrazepinones with water soluble biomolecules and thereby significantly improve our understanding of the mechanism of action of this novel class of cytotoxic agents.

Acknowledgements

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References and Notes

1. Jean-Claude, B. J.; Mustafa, A.; Watson, A. J.; Damian, Z.; Vasilescu, D.; Chan, T. H.; Leyland-Jones, B. *J. Pharmacol. Exp. Ther*. **1999**, *288*, 467.
2. Jean-Claude, B. J.; Mustafa, A.; De Marte, J.; Yen, R.; Chan, T. H.; Leyland-Jones, B. *Can. J. Infect. Dis*. **1997**, *6*, 465C.
3. Jean-Claude, B. J.; Mustafa, A.; Damian, Z.; De Marte, J.; Yen, R.; Chan, T. H.; Leyland-Jones, B. *Br. J. Cancer* **1997**, *76*, 467.
4. Jean-Claude, B. J.; Just, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2525.
5. Jean-Claude, B. J.; Just, G. *Heterocycles* **1998**, *48*, 1347.
6. Williams, C. I.; Whitehead, M. A.; Jean-Claude, B. J. *J. Mol. Sruct. (Theochem.)* **1997**, *392*, 27.
7. Williams, C. I.; Whitehead, M. A.; Jean-Claude, B. J. *J. Org. Chem*. **1997**, *62*, 7006.
8. Jean-Claude, B. J.; Britten, J. F.; Just, G. *Acta Crystallogr*. **1993**, *C49*, 1070.
9. Jean-Claude, B. J.; Williams, C. I. *Magn. Res. Chem*. **1998**, *36*, 87.
10. Clark, S. A.; Deans, B.; Stevens, M. F. G.; Tisdale, M. J.; Wheelhouse, R. T.; Denny, B. J.; Hartley, J. A. *J. Med. Chem*. **1995**, *38*, 1493.
11. Horspool, K. R.; Stevens, M. F. G.; Newton, C. G.; Lunt, E.; Walsh, R. J. A.; Pedgrift, B. L.; Baig, G. U.; Lavelle, F. *J. Med. Chem*. **1990**, *33*, 1393.
12. Experimental data for **12**: Urea **11b** (95 mg, 0.2 mmol) was dissolved in 2.4 N HCl (10 mL) and a solution of 27% $NaNO_2$ (1 mL) [50% enriched with ^{15}N $NaNO_2$] was added dropwise at 0 °C. After 30 min, the solution was extracted three times with 25 mL portions of CH_2Cl_2. The aqueous layer was separated and neutralized with saturated sodium bicarbonate, after which it was further re-extracted three times with 25 mL portions of CH_2Cl_2. The organic layer was removed and dried over anhydrous potassium carbonate and evaporated to give **12** (50 mg, 51%) as a pale brown powder: mp 125–130 °C (effervescence); δ_H ($CDCl_3$) 7.95 (1H, d, $J=2$ Hz, Ar), 7.93 (1H, dd, $J=8.6$ Hz, $J=2$, Ar), 7.26 (d, 1H, $J=8.6$, Ar), 7.05 (1H, br s, NHCO), 3.78 (4H, m, $(CH_2)_2$), 3.59 (2H, br q, NCH_2CH_2NHCO), 3.42 (3H, d, ^3J $^{15}NH=2.8$ Hz, $^{15}NNCH_3$), 3.31 (3H, s, $N(CO)CH_3$), 2.64 (2H, t, $J=4.5$ Hz, NCH_2CH_2 NHCO), 2.5 (4H, m, morpholine CH_2N); δ_N ($CDCl_3$): 69.16. δ_C ($CDCl_3$): 165.25, 159.02, 140.82, 130.42, 130.07, 125.63, 125.57, 119.98, 119.76, 66.58, 57.01, 53.29, 35.90, 35.07. CIMS (NH_3) m/z 319 ($MH^+-{}^{15}NN$, 3.3%), 291 (MH^+-CH_3NCO, 100). Anal. calcd for $C_{16}H_{22}N_6O_3$: C, 55.5; H, 6.4; N, 24.3. Found: C, 55.27; H, 6.33; N, 24.5.
13. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, H. S.; Boyd, M. R. *J. Natl. Cancer Inst*. **1991**, *82*, 1107.