Synthesis and biological evaluation of spongistatin/altohyrtin analogues: E-ring dehydration and C46 side-chain truncation[†]

Ian Paterson,* Jose L. Aceña, Jordi Bach, David Y.-K. Chen and Mark J. Coster

University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: ip100@cam.ac.uk; Fax: +44 1223 336362; Tel: +44 1223 336407

Received (in Cambridge, UK) 23rd December 2002, Accepted 15th January 2003 First published as an Advance Article on the web 23rd January 2003

Simplified analogues of the potent antimitotic marine macrolide spongistatin 1/altohyrtin A were synthesised and evaluated as growth inhibitory agents against a range of human tumour cell lines, including Taxol-resistant strains, revealing that E-ring dehydration leads to enhanced cytotoxicity at the low picomolar level while truncation of the side-chain at C46 results in a drastic decrease in activity.

The spongipyrans are a unique family of bis(spiroacetal) macrolides, obtained from marine sponges in trace amounts by bioassay-guided isolation.^{1,2} They display exceptional cytotoxicity against a wide variety of human cancer cell lines, the mode of action stemming from inhibition of mitosis by binding in the vinca domain of tubulin and blocking microtubule assembly. Spongistatin 1 ($\mathbf{1} \equiv$ altohyrtin A) is typical of the series, as reported by the Pettit^{1*a*-*d*} and Kobayashi groups,^{1*ef*} having subnanomolar growth inhibitory activity in the US National Cancer Institute primary screen.

Due to the extremely scarce natural supply and the painstaking isolation procedure, lack of material has halted the preclinical development of these compounds in cancer chemotherapy. This has stimulated numerous synthetic efforts, culminating in several completed total syntheses,^{2,3} including one by ourselves.^{3a} Despite this interest, relatively little is known regarding structure–activity relationships (SAR) for these architecturally complex marine natural products.^{4–8} In particular, is it possible to design progressively simplified analogues of **1** that retain the exceptional cancer cell growth inhibitory properties whilst increasing their synthetic accessibility?⁵





Smith *et al.* have described two such simplified analogues. bearing a model F ring and retaining the C44-C51 triene sidechain.⁶ These displayed growth inhibitory activity against several cancer cell lines, although only at the micromolar level. Whereas Uckun and co-workers have reported the design, synthesis and surprising level of biological activity of a greatly simplified analogue, based solely on the AB-spiroacetal motif.7 Recently, this claim has been questioned, as the Smith group prepared this same analogue, as well as another AB-spiroacetal mimic, and found neither had significant cytotoxic or antitubulin activity.8 With these contradictory results in mind, we decided to explore less drastic simplifications of 1 to gather SAR data and help identify the spongipyran pharmacophore. Herein, we report the synthesis and in vitro biological evaluation of two novel analogues, where E-ring dehydration at C35, as in 2, leads to enhanced cytotoxicity at the low picomolar level, while truncation of the side-chain at C46, as in 3, results in a drastic decrease in activity.

The first analogue arose out of our recent total synthesis of spongistatin 1 (1),^{3a} where the final deprotection step, performed under acidic conditions (aqueous HF, MeCN), was accompanied by the formation of a minor component. This byproduct was purified by reverse-phase HPLC and showed close homology to that of 1 in its ¹H NMR spectra, with differences only in the regions corresponding to resonances for the E-ring protons. Following mass spectrometry and detailed ¹H NMR analysis (500 MHz, COSY), the structure of this analogue was determined as 2, corresponding to an E-ring dehydrated version of 1, *i.e.* lacking the axial C35 hydroxy group with a double bond between C35 and C36.

The planned synthesis of a side-chain truncated analogue 3 was also undertaken (Scheme 1). This otherwise complete spongipyran was designed to evaluate the effect of deleting the terminal chlorodiene and the C47 hydroxy group. The EF subunit 4^{3a} was first treated with TBSCl, imidazole and Et₃N in DMF to silylate the C35 hydroxy group, followed by displacement of the C29 chloride by exposure to Ph₃P and NaI (*i*Pr₂NEt, MeOH, MeCN) to give 5 (93%). By employing our optimised conditions (LiHMDS, THF, HMPA),^{3a} the challenging Wittig coupling between the side-chain truncated EF phosphonium salt 5 and the ABCD aldehyde 6^{3b} was achieved in 71% yield (>97:3Z:E). Subsequent removal of the three PMB protecting groups (DDQ) and the trichloroethyl ester (TCE) moiety (Zn, THF, NH4OAc) provided seco-acid 7, as a ca. 1:1 mixture of methyl acetal and hemiacetal forms (R = Me, H respectively). Macrolactonisation of 7 under modified Yamaguchi conditions, engaging the C41 hydroxyl group in a completely selective manner, was followed by global deprotection of the resulting 42-membered macrocycle by its exposure to aqueous HF in MeCN, providing the desired side-chain truncated analogue 3.

With spongistatin analogues 2 and 3 in hand, the effects on cytotoxicity of E-ring dehydration and removal of the C47–C51 chlorodiene allylic alcohol were now investigated. Listed in Table 1 are the results of growth inhibition experiments⁹ for paclitaxel/Taxol (8), spongistatin 1 (1),^{3a} E-ring dehydrated analogue 2 and side-chain truncated analogue 3 against a set of five different human cancer cell lines, including the MIP101 colon and 1A9PTX22 ovarian, paclitaxel-resistant strains. In all



Scheme 1 Synthesis of C46 side-chain truncated spongipyran 3. *Reagents and conditions*: (i) TBSCl, Im, Et₃N, DMF, 20 °C, 16 h, 95%; (ii) PPh₃, NaI, *i*Pr₂NEt, MeCN–MeOH, Δ , 20 h, 98%; (iii) LiHMDS, THF–HMPA, -78 °C, 10 min; 6, $-78 \rightarrow 20$ °C, 40 min, 71%; (iv) DDQ, CH₂Cl₂–pH 7 buffer, 0 °C, 90 min, 69%; (v) Zn, THF–NH₄OAc_(aq), 20 °C, 30 min, 62%; (vi) 2,4,6-trichlorobenzoyl chloride, Et₃N, THF, 20 °C, 3 h; DMAP, PhMe, 100 °C, 16 h, 47%; (vii) HF_(aq), MeCN, 0 °C, 30%.

Table 1 Growth inhibition against human cancer cell lines^a

IC ₅₀ values (nM)	8	1	2	3
MIP101 colon (Pgp-1 overexpressing)	200	0.1	0.08	587
HCT116 colon	0.3	0.05	0.02	407
1A9PTX22 ovarian (mutation in β -tubulin)	47	0.03	0.007	>632
1A9 ovarian (parental)	1	0.03	0.007	>632
A549 non-small cell lung	6	0.07	0.04	>632

^{*a*} Cells were plated in 96 well plates at 4 × 10³ cells per well for MIP101, HCT116 and A549 cell lines, and at 2 × 10⁴ cells per well for 1A9 and 1A9PTX22 cell lines. Compounds dissolved in DMSO were added to the wells at 5 fold serial dilutions starting from 1 µg ml⁻¹. No compound was added to control wells. After 72 h incubation, the number of viable cells was assessed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.¹⁰ After processing, the plates were read in a Molecular Devices 96-well plate reader at 540 nm and IC₅₀ values (concentrations in nM causing 50% inhibition of cell growth) were calculated.

cases, 1 was found to be substantially more active (6- to 2000-fold) than paclitaxel, and was particularly effective against the MIP101 colon carcinoma cell line, indicating that it is a poor substrate for the P-glycoprotein (Pgp) drug efflux pump. Given the already exceptional cytotoxicity displayed by $\mathbf{\hat{1}}$, $1^{1a-\hat{f},2}$ we were gratified to find that the E-ring dehydrated analogue 2 was generally (2- to 4-fold) more potent than the parent natural product. Analogue 2 had low picomolar IC_{50} values, in the range 0.007-0.08 nM, against this set of cancer cell lines. This indicates that the C35 hydroxyl of 1 is unnecessary for biological activity, and that its removal leads to an increase in potency. In contrast, the dramatic attenuation of cytotoxicity for analogue 3, against all cell lines employed in these assays (e.g. 0.587 and 0.407 µM against the MIP101 and HCT116 colon carcinoma cell lines), reveals that the C47-C51 chlorodiene allylic alcohol moiety is an essential structural feature. These results suggest that the full C44-C51 triene sidechain is a crucial part of the spongipyran pharmacophore, consistent with the findings of the Smith group⁶ but not with the claims made by Uckun and co-workers.

In conclusion, we have prepared two fully synthetic analogues of the bis(spiroacetal) macrolide spongistatin 1 (1). Evaluation of their growth inhibitory activity *in vitro* against a range of human cancer cell lines reveals that dehydration in the E ring actually leads to enhanced potency—at the low picomolar level—highlighting the extraordinary cytotoxicity of these compounds, particularly against Taxol-resistant cell lines. Additionally, truncation of the side-chain at C46 leads to a drastic decrease in activity ($\geq 5 \times 10^3$ -fold less than 1). While E-ring modification can be tolerated but not F-ring side-chain truncation, the design of simplified synthetic analogues of the spongipyrans, retaining the exceptional antimitotic potency, clearly requires a great deal more SAR work. Financial support was provided by the EPSRC (GR/L41646), EC (Marie Curie Fellowship to J. L. A.), Cambridge Commonwealth Trust (M. J. C.), King's College and the Sims Fund, Cambridge (D.Y.-K. C.). We thank Merck, AstraZeneca and Novartis Pharmaceuticals for generous support, and Dr Kennneth W. Bair, Dr Frederick R. Kinder, Jr., Dr Peter T. Lassota and Dr Erik F. Sorensen at Novartis for providing the biological data.⁹

Notes and references

- (a) G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt and J. N. A. Hooper, J. Org. Chem., 1993, 58, 1302; (b) G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald and M. R. Boyd, J. Chem. Soc., Chem. Commun., 1993, 1166; (c) R. L. Bai, Z. A. Cichacz, C. L. Herald, G. R. Pettit and E. Hamel, Mol. Pharmacol., 1993, 44, 757; (d) R. L. Bai, G. F. Taylor, Z. A. Cichacz, C. L. Herald, J. A. Kepler, G. R. Pettit and E. Hamel, Biochemistry, 1995, 34, 9714; (e) M. Kobayashi, S. Aoki, H. Sakai, K. Kawazoe, N. Kihara, T. Sasaki and I. Kitagawa, Tetrahedron Lett., 1993, 34, 2795; (f) M. Kobayashi, S. Aoki, K. Gato and I. Kitagawa, Chem. Pharm. Bull., 1996, 44, 2142; (g) N. Fusetani, K. Shinoda and S. Matsunaga, J. Am. Chem. Soc., 1993, 115, 3977.
- 2 Review: J. Pietruszka, Angew. Chem., Int. Ed., 1998, 37, 2629.
- 3 For leading references, see: (a) I. Paterson, D. Y.-K. Chen, M. J. Coster, J. L. Aceña, J. Bach, K. R. Gibson, L. E. Keown, R. M. Oballa, T. Trieselmann, D. J. Wallace, A. P. Hodgson and R. D. Norcross, Angew. Chem., Int. Ed., 2001, 40, 4055; (b) I. Paterson, D. J. Wallace and R. M. Oballa, Tetrahedron Lett., 1998, 39, 8545; (c) D. A. Evans, B. W. Trotter, P. J. Coleman, B. Ĉoté, L. C. Dias, H. A. Rajapakse and A. N. Tyler, Tetrahedron, 1999, 55, 8671; (d) M. M. Hayward, R. M. Roth, K. J. Duffy, P. I. Dalko, K. L. Stevens, J. S. Guo and Y. Kishi, Angew. Chem., Int. Ed., 1998, 37, 192; (e) A. B. Smith III, Q. Y. Lin, V. A. Doughty, L. Zhuang, M. D. McBriar, J. K. Kerns, C. S. Brook, N. Murase and K. Nakayama, Angew. Chem., Int. Ed., 2001, 40, 196; (f) M. T. Crimmins, J. D. Katz, D. G. Washburn, S. P. Allwein and L. F. McAtee, J. Am. Chem. Soc., 2002, 124, 5661.
- 4 A C50 chlorine substituent (as in spongistatin 1/altohyrtin A) shows somewhat greater cell growth inhibitory activity than the des-chloro version (spongistatin 2/altohyrtin C), see ref. 2.
- 5 This approach has been used successfully by Kishi and co-workers for the halichondrins, see: M. J. Towle, K. A. Salvato, J. Budrow, B. F. Wels, G. Kuznetsov, K. K. Aalfs, S. Welsh, W. J. Zheng, B. M. Seletsky, M. H. Palme, G. J. Habgood, L. A. Singer, L. V. Di Pietro, Y. Wang, J. J. Chen, D. A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M. J. Yu and B. A. Littlefield, *Cancer Res.*, 2001, **61**, 1013.
- 6 A. B. Smith III, Q. Y. Lin, G. R. Pettit, J.-C. Chapuis and J. M. Schmidt, Bioorg. Med. Chem. Lett., 1998, 8, 567.
- 7 (a) F. M. Uckun, C. Mao, A. O. Vassilev, H. Huang and S.-T. Jan, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 541; (b) H. Huang, C. Mao, S.-T. Jan and F. M. Uckun, *Tetrahedron Lett.*, 2000, **41**, 1699.
- 8 A. B. Smith III, R. M. Corbett, G. R. Pettit, J.-C. Chapuis, J. M. Schmidt, E. Hamel and M. K. Jung, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2039.
- 9 The biological assays were performed by Novartis Pharmaceuticals Corporation, Summit, New Jersey.
- 10 M. C. Alley, C. M. Pacula-Cox, M. L. Hursey, L. R. Rubenstein and M. R. Boyd, *Cancer Res.*, 1991, **51**, 1247.