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Bioorganic & Medicinal Chemistry Letters 17 (2007) 2443–2447
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological evaluation of novel analogues of dictyostatin

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Received 19 January 2007; revised 9 February 2007; accepted 10 February 2007
Available online 14 February 2007

Abstract—Novel analogues of the microtubule-stabilising agent dictyostatin were designed using existing SAR information from the structurally related discodermolide, synthesised by a late-stage diversification strategy and evaluated in vitro for growth inhibition against a range of human cancer cell lines, including those known to exhibit Taxol-resistance (AsPC-1, DLD-1, PANC-1, NCI/ADR).

The marine sponge-derived macrolide dictyostatin (**1**, Fig. 1) displays powerful growth inhibitory activity against a wide range of human cancer cell lines.[1,2] Its initial isolation by Pettit in 1994 in the Republic of Maldives enabled only a partial assignment of stereochemistry, hindering further synthetic development for nearly a decade.[1a] Upon re-isolation by Wright,[1b] sufficient natural dictyostatin became available in 2004 for a conclusive reassignment of the stereochemistry by detailed NMR analysis combined with molecular modelling.[3] Subsequent total synthesis efforts confirmed these structural revisions,[4] facilitating the production of larger quantities of this otherwise scarce marine macrolide for more extensive biological evaluation. Dictyostatin has been identified as a potent microtubule-stabilising agent (MSA) which like Taxol (**2**) binds to the taxoid binding site on β-tubulin.[1b,2] Its cytotoxicity is more pronounced than that of Taxol and is further retained against (P-glycoprotein efflux-mediated) Taxol-resistant cell lines. Thus, dictyostatin represents a promising antimitotic natural product drug lead for cancer chemotherapy development.

The development of natural product analogues is an appealing goal from a pharmaceutical perspective, which provides exciting opportunities for structural simplification whilst maintaining biological potency.[5] A few

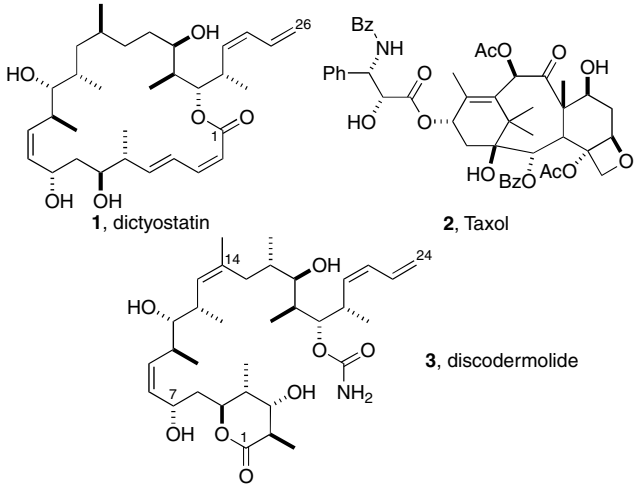

Figure 1. Structures of dictyostatin, Taxol and discodermolide.

dictyostatin analogues have been reported,[6] which, despite displaying potent cytotoxicities against some human cancer cell lines, appear to be much less active against those lines exhibiting Taxol-resistance. Their design was influenced by the structurally related MSA discodermolide (**3**),[7] for which a more substantial number of analogue studies have been reported.[8,9] The resulting SAR information, together with more recent NMR experiments and binding studies, has led to the proposal of a β-tubulin bound bioactive conformation for discodermolide and a common pharmacophore model with the epothilones.[10,11]

Keywords: Macrolide; Anticancer; Tubulin; Total synthesis.
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doi:10.1016/j.bmcl.2007.02.031

Herein, we describe the synthesis of 16-desmethyl- (**4**, Fig. 2), 9-*epi*-16-desmethyl- (**5**), 9-methoxy- (**6**) and 9-*epi*-dictyostatin (**7**), and disclose the cancer cell growth inhibitory activity of these analogues relative to the natural product MSAs dictyostatin, discodermolide and Taxol in both Taxol-sensitive and -resistant cell lines.

Initial efforts were focused towards the synthesis of 16-desmethyl-dictyostatin (**4**), which has also been prepared by the Curran group.[6b] Previously, Smith had shown that removal of the C14 methyl in discodermolide had little effect upon cytotoxicity in non-resistant cell lines, but reduced activity against Taxol-resistant cell lines.[9d,f] We postulated that removal of the corresponding C16 methyl of dictyostatin would produce a similarly active analogue. From a synthetic viewpoint, deletion of the C16 methyl would permit a simplification of the overall synthesis: replacing the Myers alkylation (previously implemented to establish the remote C16 stereocentre) with a Horner–Wadsworth–Emmons/conjugate reduction sequence would access the complete C11–C26 carbon framework (minus the C16 methyl). Completion of the analogue would then follow the same strategy as previously reported for our total synthesis of dictyostatin (Scheme 1).[4a]

Preparation of the C11–C26 fragment started from known aldehyde **8** (Scheme 2).[12] HWE olefination of **8** with triethyl phosphonoacetate and subsequent TBS ether formation provided exclusively the *E*-enoate **9** (90%). Conjugate reduction of the enone using Stryker's reagent[13] and treatment with DIBALH directly afforded aldehyde **10** (73%). A barium hydroxide-mediated HWE olefination[14] of phosphonate **11**[4] with aldehyde **10** provided the *E*-enone, which was similarly reduced with Stryker's reagent.

Reduction of the resulting ketone with $LiAlH(O^tBu)_3$ gave the desired (19*R*)-alcohol **12** with 96:4 *dr* (79%). Protection of this secondary alcohol as its TBS ether, oxidative cleavage of both PMB ethers using DDQ and selective primary oxidation[15] using catalytic TEMPO and $PhI(OAc)_2$ provided aldehyde **13**, in readiness for the Still-Gennari olefination (93%). Synthesis of the fluorinated phosphonate started from the Brown

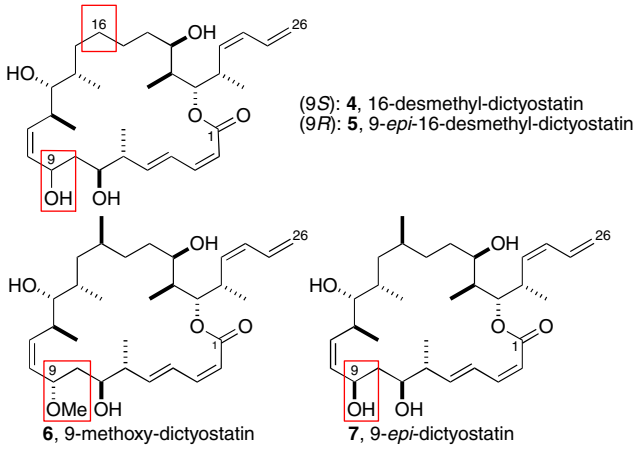
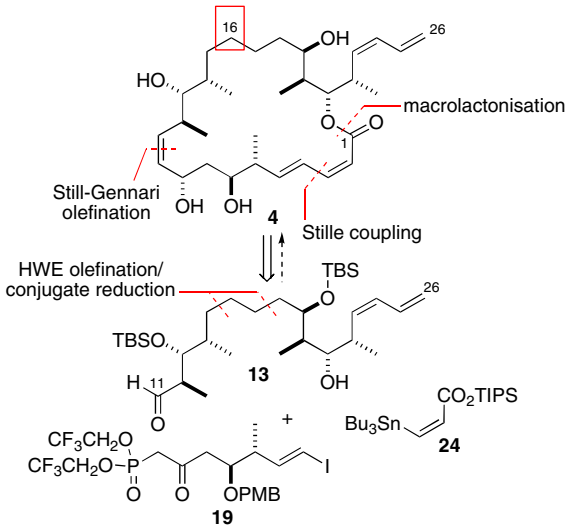
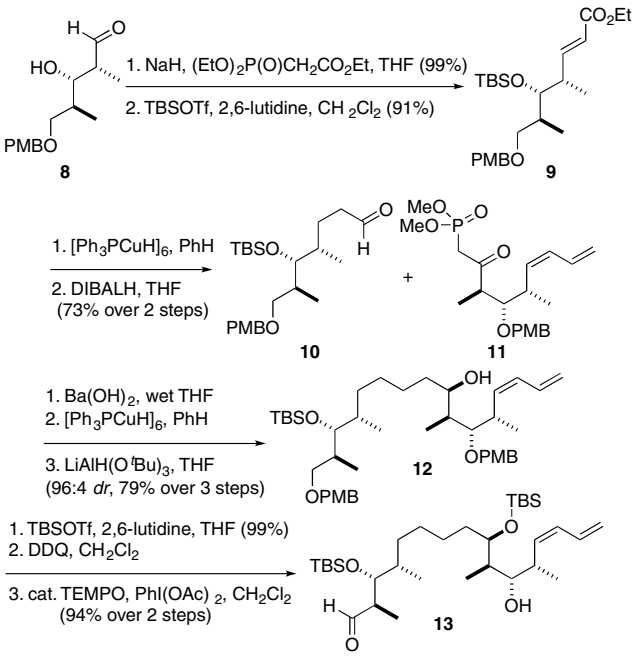


Figure 2. Structures of dictyostatin analogues.



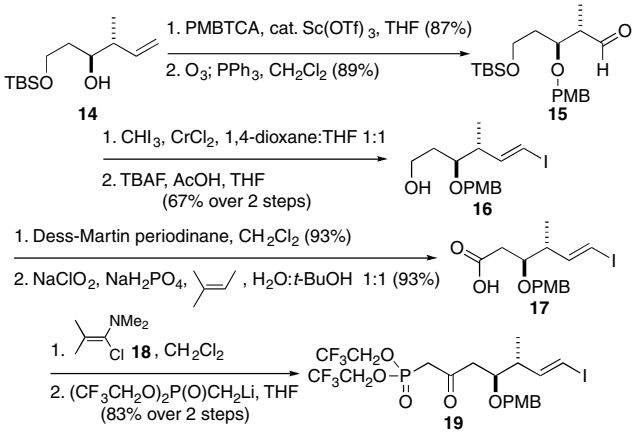
Scheme 1. Retrosynthetic analysis of 16-desmethyl-dictyostatin.



Scheme 2. Synthesis of aldehyde **13**.

crotylation[16] product **14** (Scheme 3).[4a] PMB ether formation followed by ozonolysis provided aldehyde **15** (77%). Takai olefination[17] of **15** effected installation of the *E*-vinyl iodide functionality; subsequent TBAF-mediated silyl ether cleavage and oxidation under Dess-Martin periodinane/Pinnick conditions afforded acid **17** (60% over four-steps). Transformation of **17** into the corresponding acid chloride was performed using the Ghosez reagent **18**.[18] Pleasingly, addition of the lithium anion of $(CF_3CH_2O)_2P(O)CH_3$ at −98°C provided β-ketophosphonate **19** in 83% yield.[19]

Still-Gennari olefination of aldehyde **13** using phosphonate **19** employed K_2CO_3, 18-crown-6 in a mixture of toluene and HMPA (10:1), and gave *Z*-enone **20** in moderate yield with good selectivity (5:1 *Z*:*E*, 41%) (Scheme 4).



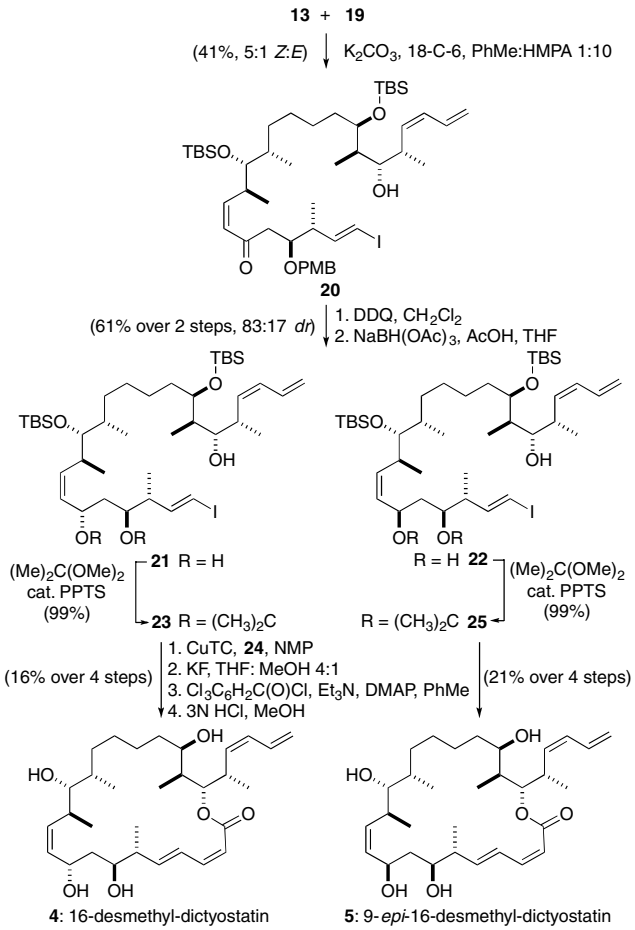
Scheme 3. Synthesis of β-ketophosphonate **19**.

Oxidative PMB ether cleavage (DDQ) and subsequent hydroxyl-directed 1,3-*anti* reduction[20] provided separable diols **21** and **22** (83:17 *dr*, 61%).

After first masking 1,3-*anti* diol **21** as its acetonide **23**, a copper-mediated Stille coupling[21] with *Z*-vinyl stannane **24** smoothly installed the required *E*,*Z*-dienoate. In situ TIPS ester cleavage using KF provided the corresponding *sec*-acid, which afforded the macrocycle under Yamaguchi macrolactonisation conditions (50%) with little or no dienoate isomerisation. Finally, exposure to 3N HCl in MeOH effected concomitant silyl ether and acetonide cleavage to provide 16-desmethyl-dictyostatin **4**. Synthesis of the C9-epimeric analogue **5** could be achieved by progression of 1,3-*syn* diol **22** through the same five-step sequence described above (21%). Both the 16-desmethyl and 9-*epi*-16-desmethyl analogues were purified by HPLC prior to biological evaluation.

It had been observed that discodermolide analogues with modification of the C7 hydroxyl group (removal/methylation/acylation) displayed similar antiproliferative activities to discodermolide.[9b–d] Interestingly, methylation or acylation resulted in comparable and occasionally increased cytotoxicities relative to discodermolide, including in those cell lines resistant to Taxol. Thus, we targeted the synthesis of the corresponding C9-methyl ether dictyostatin congener **6**, anticipating that it may display nanomolar cytotoxicities in both Taxol-sensitive and resistant human cancer cell lines. The synthesis of the (C16-methyl containing) 9-*epi* and 9-methoxy analogues was envisioned via late-stage diversification of known intermediates from our dictyostatin synthesis (Scheme 5).[4a] Direct methylation/deprotection of the (9*S*)-alcohol **26** arising from C9-enone reduction would lead to 9-methoxy-dictyostatin **6**, while deprotection of the minor (9*R*)-alcohol product **27** of the same reduction would afford 9-*epi*-dictyostatin **7**. Both analogues should provide further insight into the importance of the C9 configuration within dictyostatin.

The Still-Gennari olefination of known aldehyde **28**[4a] and phosphonate **29** afforded *Z*-enone **30** (5:1 *Z*:*E*, 75%) (Scheme 6). Its elaboration via a copper-mediated Stille coupling with vinyl stannane **24**, in situ KF TIPS

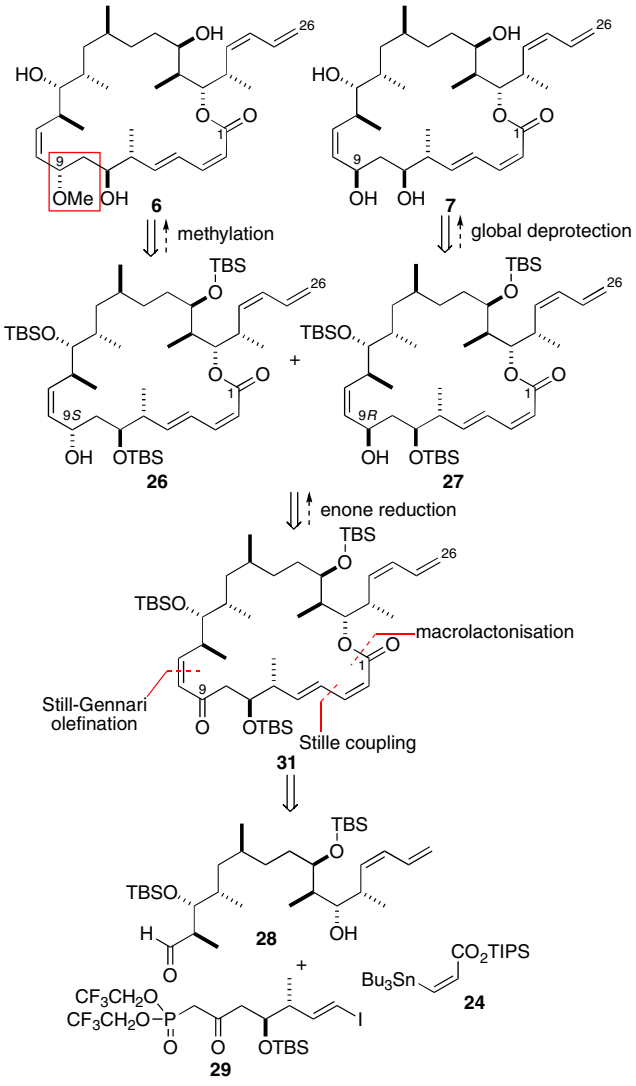


Scheme 4. Completion of the synthesis of 9-*epi*-16-desmethyl- and 16-desmethyl-dictyostatin.

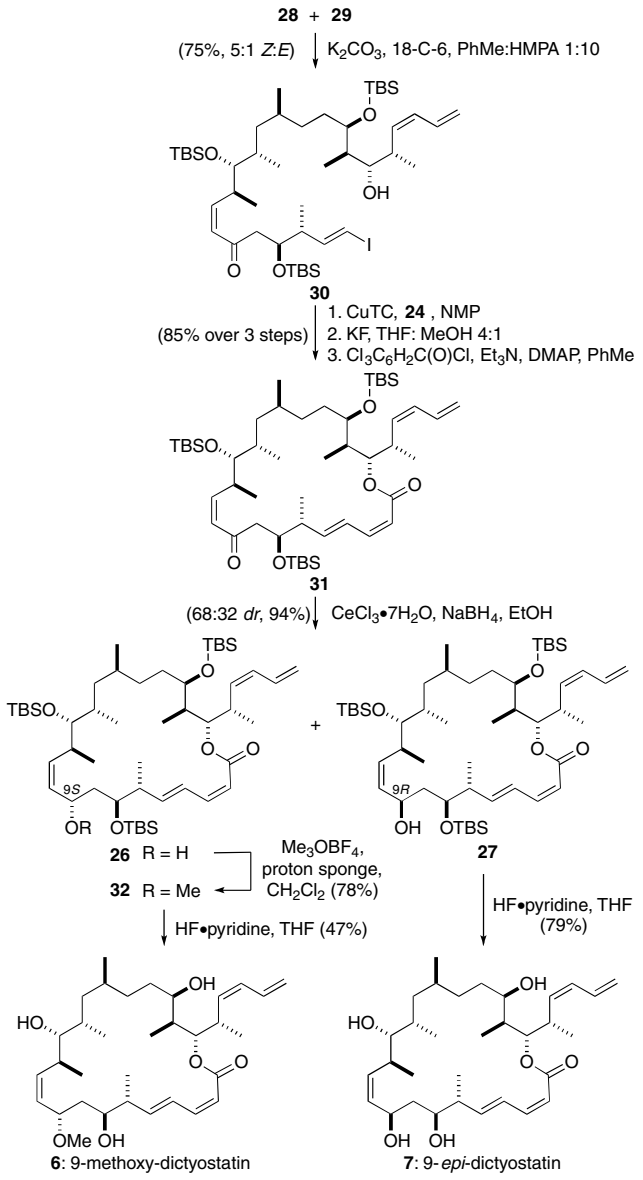
cleavage and macrolactonisation under improved Yamaguchi conditions provided macrolactone **31** (85%). Reduction of enone **31** under Luche conditions provided epimeric alcohols (9*S*)-**26** and (9*R*)-**27** (68:32 *dr*, 94%). Methylation of the (9*S*)-alcohol **26**, using proton sponge and Meerwein's salt, afforded methyl ether **32** (78%). Global deprotection of both **32** and its (9*R*)-epimer **27** was effected cleanly using HFpyridine,[22] which after HPLC purification gave 9-methoxy-dictyostatin **6** (47%) and 9-*epi*-dictyostatin **7** (79%), respectively, in readiness for biological evaluation.

The cell growth inhibitory activities of the dictyostatin analogues **4**, **5**, **6** and **7** were evaluated in vitro, and measured relative to dictyostatin (**1**), Taxol (**2**) and discodermolide (**3**) against four cancer cell lines: AsPC-1 (pancreatic), DLD-1 (colon), PANC-1 (pancreatic) and NCI/ADR (Taxol-resistant) (Table 1). The NCI/ADR cell line contains an overexpressed P-glycoprotein efflux pump within the cell membrane, which is responsible for its resistance to Taxol.

Our synthetic dictyostatin analogues showed a broad spectrum of activities, which correlated well with those of the corresponding discodermolide analogues. Encouragingly, the 16-desmethyl-analogue **4** displayed a moderate level of antiproliferative activity in the



Scheme 5. Retrosynthetic analysis of 9-*epi*- and 9-methoxy-dictyostatin.



Scheme 6. Completion of the synthesis of 9-methoxy- and 9-*epi*-dictyostatin.

non-resistant cell lines, however, this was lost upon the switch to the NCI/ADR resistant cell line. The contrast in these findings to those reported by Curran et al.[6b] can possibly be attributed to differences in cell line selection and experimental parameters. From this result, we can conclude that the C16 methyl group on dictyostatin is both highly important in maintaining low nanomolar cytotoxicity of the natural product, and in helping it to circumvent the P-glycoprotein efflux pump.

Inversion of the C9 hydroxyl stereocentre in both the 9-*epi*-16-desmethyl- and 9-*epi*-dictyostatin analogues (**5** and **7**) resulted in a substantial drop in cytotoxicity relative to both **4** and **1**, respectively. These results reflect the importance of the configuration of this C9 hydroxyl group. We postulate that its configuration may be important in controlling the bioactive conformation of dictyostatin. Most pleasing were the results from the 9-methoxy analogue **6**. As observed with the corresponding discodermolide analogue, low nanomolar cytotoxicities were evident in both the resistant and non-resistant cancer cell lines, making this the most active dictyostatin analogue reported to date. The effect of 'capping' the C9 hydroxyl group with a methyl had an insignificant effect on the binding ability of the analogue. From this finding, it is proposed that the C9 hydroxyl group of dictyostatin does not act as a significant intermolecular hydrogen-bond donor with proximal tubulin residues, and does not form any intramolecular hydrogen bonds, which might stabilise the bioactive conformation.

In conclusion, we have designed and synthesised a small library of active dictyostatin analogues. Notably, 9-methoxy-dictyostatin **6** represents the most active dictyostatin analogue prepared, and, importantly, shows comparable cytotoxicity relative to dictyostatin against a Taxol-resistant cell line. Moreover, we have shown that the dictyostatin framework can withstand structural modification, providing encouragement that simplified analogues with similar dictyostatin-like antiproliferative activity may be synthesised.

Table 1. Cytotoxicity of dictyostatin (**1**), Taxol (**2**), discodermolide (**3**) and dictyostatin analogues **4**, **5**, **6** and **7** in cultured human cancer cells as determined by MTT metabolism following 72-h exposure to the test agent

Compound	IC_{50}/nM[a]			
	AsPC-1 pancreatic	DLD-1 colon	PANC-1 pancreatic	NCI/ADR Taxol-resistant
1	9.0 (±5.6)	0.8 (±3.8)	3.4 (±0.2)	9.8 (±1.5)
2	20 (±23)	11 (±1)	7 (±2)	1000 (±140)
3	98 (±34)	29 (±8)	59 (±34)	160 (±34)
4	170 (±19)	85 (±19)	130 (±19)	1500 (±460)
5	2100 (±210)	790 (±710)	1500 (±140)	2100 (±680)
6	31 (±9.2)	2.4 (±1.1)	9.7 (±1.8)	8.2 (±1.8)
7	410 (±200)	150 (±36)	240 (±2)	1100 (±230)

[a] Values are ± standard deviation (in parentheses) from a minimum of four separate experiments.

Acknowledgments

NIH Grant No. CA-93455 provided financial support for the biological assays and general project support was provided by the EPSRC, AstraZeneca and the EC (Marie Curie Fellowship to K.G.P.). We thank Dr. John Leonard (AstraZeneca) for helpful discussions, Dr. Richard Isbrucker and Dr. Pat Linley (HBOI) for assisting in our biological evaluation and Dr. Stuart Mickel (Novartis) for the gift of chemicals.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.031.

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