Synthesis and anticancer activity studies of α-aminoalkylated conjugated nitroalkenes†

Namrata Rastogi,[a] Renu Mohan,[b] Dulal Panda,*[b] Shaikh M. Mobin[c] and Irishi N. N. Namboothiri*[a]

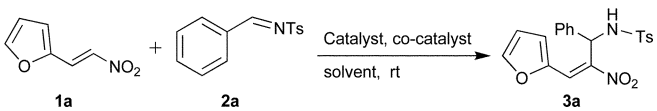
Received 26th May 2006, Accepted 19th July 2006
First published as an Advance Article on the web 28th July 2006
DOI: 10.1039/b607537a

Novel α-aminoalkylated conjugated nitroalkenes which inhibit human cervical cancer (HeLa) cell proliferation by binding to tubulin were synthesized by imidazole/LiCl-mediated reaction of conjugated nitroalkenes with *N*-tosylimines.

Introduction of hydroxyalkyl, aminoalkyl and analogous substituents at a vinylic carbon α to an activating group *via* a one-pot reaction between an activated alkene and an electrophile under the influence of a catalyst, generally a Lewis base, is of considerable current interest.[1–4] This convenient and atom-economical methodology, commonly known as the Morita–Baylis–Hillman (MBH) reaction, has become an attractive strategy for the synthesis of diverse multifunctional molecules.[2,4] Although a variety of activated alkenes has been employed as substrates in more than three decades of the MBH reaction,[2] an olefin activated by a nitro group has received attention only recently.[5] As for the electrophiles, activated imines are the most sought after ones for the MBH reaction, besides aldehydes, by virtue of their electrophilicity and ability to provide aminoalkylated activated alkenes, usually with a new chiral center.[2,6] Among the activated imines, *N*-sulfonylated imine remained particularly attractive ever since it was introduced by Perlmutter and Teo,[7] and aminoalkylation of α,β-unsaturated ketones (both cyclic and acyclic),[8] esters,[9] aldehydes[10] and nitriles[11] mediated by several tertiary amines, phosphines and Lewis acids such as lanthanide triflates, $TiCl_4$, titanium isopropoxide, *etc.*, has been reported.[12]

Although formaldehyde and a variety of 1,2-dicarbonyl compounds reacted well as electrophiles with conjugated nitroalkenes,[5] simple aliphatic and aromatic aldehydes were not amenable for the MBH reaction of nitroalkenes under a variety of conditions. Therefore, we envisaged that such aldehydes, upon transformation to activated imines, would be suitable for aminoalkylation of nitroalkenes thus offering a novel entry into 1,2-diamines, α-amino ketones and several other synthetically useful building blocks.

The reaction conditions for obtaining the best yield of the aminoalkylated products were optimized using 2-nitrovinylfuran (2-NVF) **1a** and *N*-benzylidene-4-methylbenzenesulfonamide **2a** as model substrates in the presence of various tertiary amines and tertiary phosphines in stoichiometric amounts (Scheme 1, see also the ESI†). Various aprotic and protic polar solvents and co-catalysts were also systematically screened to establish optimum reaction conditions. From these experiments, imidazole[13] and LiCl[14] emerged as the best catalyst and co-catalyst, respectively, in dioxan as solvent.[15] Thus, the MBH adduct **3a** was obtained in 69% yield when nitroalkene **1a** and tosylimine **2a** were stirred at room temperature for three days in the presence of 50 mol% imidazole and 0.5 M LiCl in 1,4-dioxan.



Scheme 1

Having optimized the conditions for the reaction of our model substrate 2-NVF **1a** and *N*-tosylimine **2a**, we extended the scope of nitroalkenes **1** and *N*-tosylimines **2** as shown in Table 1. Thus, 2-NVF **1a** was reacted with *N*-tosylimines **2b** and **2c** to afford the MBH adducts **3b** and **3c** in relatively low yields (25 and 23%), respectively (Table 1, entries 2 and 3). Treatment of 3-NVF **1b** with *N*-tosylimines **2a** and **2c** provided the adducts **3d** (38%) and **3e** (15%), respectively (Table 1, entries 4 and 5). Reaction of 2-thienyl nitroethylene **1c** with tosylimines **2a** and **2c** also led to the formation of the novel MBH adducts **3f** and **3g** in low to moderate yields (Table 1, entries 6 and 7). Formation of the MBH adducts **3h**–**3k** in low yield was encountered when 3-thienyl nitroethylene **1d** and substituted nitrostyrenes **1e**–**g** were reacted with tosylimine **2a**.

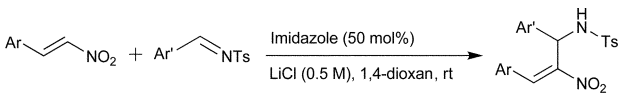
It is discernible from Table 1 that although the model substrates **1a** and **2a** provided the MBH adduct **3a** in good yield (69%, entry 1) under the optimized conditions, *i.e.* 50 mol% imidazole, 0.5 M LiCl in 1,4-dioxan, reactions of other aromatic and heteroaromatic nitroalkenes **1b**–**g** with **2a** and other *N*-tosylimines **2b**,**c** were not impressive (entries 2–11). In fact, satisfactory yields of the MBH adducts were isolated only in cases **1b** + **2a** (38%) and **1c** + **2c** (35%). It is also obvious that these reactions are extremely sluggish, taking 3–9 days for completion, and such long reaction times appeared detrimental to the stability of the substrates, especially *N*-tosylimines **2**, and their MBH adducts **3**. All our attempts to accelerate the reaction and to enhance the chemical yields by employing conditions such as sonication, microwave irradiation and high pressure proved, on the whole, futile.

The structural and stereochemical assignments of **3** were performed by extensive NMR experiments (see ESI†). For instance, analysis of the ^{1}H NMR and ^{1}H–^{1}H COSY spectra of **3a** suggested that the doublets appearing at δ 6.55 and 8.62 (J = 8.6 Hz) were due to the benzylic proton and the adjacent N–H proton,

[a]*Department of Chemistry, Indian Institute of Technology, Bombay, Mumbai 400 076, India. E-mail: irishi@iitb.ac.in*
[b]*School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Mumbai 400 076, India. E-mail: panda@btc.iitb.ac.in*
[c]*National Single Crystal X-ray Diffraction Facility, Indian Institute of Technology, Bombay, Mumbai 400 076, India*

† Electronic supplementary information (ESI) available: Experimental procedures, full characterization data, copies of ^{1}H and ^{13}C NMR spectra and HPLC profiles for all the new compounds; details of biological studies, *viz.* binding experiments, light scattering assays and immunofluorescence analysis also included. See DOI: 10.1039/b607537a

Table 1 MBH reaction of nitroalkenes **1** with *N*-tosylimines **2** in 1,4-dioxan in the presence of imidazole and LiCl



1 2 3

Entry	Nitroalkene **1** (Ar)	*N*-tosylimine **2** (Ar′)	Time/d	MBH adduct **3**	Yield (%)[a]
1	**1a** (2-Furyl)	**2a** (Ph)	3	**3a**	69[b]
2	**1a** (2-Furyl)	**2b** (4-OMePh)	8	**3b**	25[b]
3	**1a** (2-Furyl)	**2c** (2-Furyl)	4	**3c**	23[b]
4	**1b** (3-Furyl)	**2a** (Ph)	3	**3d**	38[c]
5	**1b** (3-Furyl)	**2c** (2-Furyl)	4	**3e**	15[c]
6	**1c** (2-Thienyl)	**2a** (Ph)	4	**3f**	17[b]
7	**1c** (2-Thienyl)	**2c** (2-Furyl)	4	**3g**	35[c]
8	**1d** (3-Thienyl)	**2a** (Ph)	4	**3h**	19[c]
9	**1e** (4-OMePh)	**2a** (Ph)	9	**3i**	18[c]
10	**1f** [3,4-$(OMe)_2$Ph]	**2a** (Ph)	3	**3j**	21[b]
11	**1g** [3,4-(OCH_2O)Ph]	**2a** (Ph)	4	**3k**	17[c]

[a] Isolated yield of **3** after purification by silica gel column chromatography. [b] **1** and **2** were taken in 1 : 1 ratio; no further improvement in the yield when excess **2** was used. [c] **1** and **2** were taken in 1 : 1.5 ratio; the yields were lower when **1** and **2** were taken in 1 : 1 ratio; no further improvement in the yield when >1.5 equiv. of **2** was used; no appreciable amounts of nitroalkene **1** were recovered in any of the cases.

respectively. The singlet appearing at δ 7.94 was assigned to the olefinic proton. All other peaks appearing in the range δ 6.7–8.1 were assigned to the aromatic protons. The ring protons of furyl, phenyl and tolyl groups were further distinguished. While one of the furyl protons overlaps with the five phenyl protons at δ 7.28, the other two appear at δ 6.80 and 8.05. The two doublets appearing at δ 7.20 and 7.57 (J = 8.2 Hz) were assigned to the tolyl protons. In the light of the assignment of all the protons in **3a** the ^{1}H–^{1}H NOESY spectrum was analyzed in order to assign the geometry of the double bond. Thus, positive NOE interactions observed between (a) the furyl protons and the tolyl protons and (b) the furyl protons and the benzylic proton (next to N–H) suggested (*E*) stereochemistry for the double bond. However, the presence of NOE between the olefinic proton and the tolyl proton and the absence of any NOE between the furyl protons and the phenyl protons coupled with the non-availability of the other geometrical isomer for comparison prompted us to resort to X-ray crystallography for further confirmation of the stereochemistry (Fig. 1).[16] There are two independent molecules in the asymmetric unit and the values of the torsion angles which establish the *E*-configuration are −2.1(8) and −2.3(8)°. After having unambiguously established the double bond geometry in **3a** to be (*E*) by single crystal X-ray analysis (a dihedral angle of close to 0° between C13 and C16 was obtained from X-ray data), the (*E*) geometry has been assigned, by analogy, to all other MBH adducts **3b**–**k**.

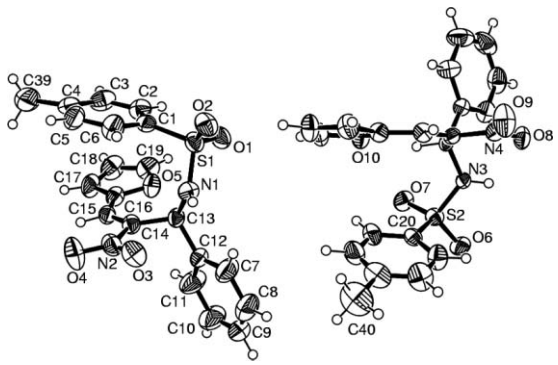


Fig. 1 ORTEP diagram of MBH adduct **3a** (the probability chosen for the ellipsoids is 50%).

The effect of MBH compounds **3a**–**k** on human cervical cancer (HeLa) cells was examined by incubating HeLa cells with 1, 5 and 10 μM of the different aminoalkylated products for 24 h. Some of the tested compounds produced a concentration-dependent inhibition of cell proliferation (Table 2).[17] Among

Table 2 Percentage inhibition of HeLa cell proliferation by MBH adducts **3a**–**k**

Entry	**3** (Ar, Ar′)	Inhibition of HeLa cell proliferation (%)		
		1 μM	5 μM	10 μM
1	**3a** (2-Furyl, Ph)	17 ± 4	44 ± 1	67 ± 3
2	**3b** (2-Furyl, 4-OMePh)	18 ± 1	25 ± 4	34 ± 3
3	**3c** (2-Furyl, 2-Furyl)	20 ± 3	32 ± 5	42 ± 2
4	**3d** (3-Furyl, Ph)	14 ± 2	28 ± 2	77 ± 5
5	**3e** (3-Furyl, 2-Furyl)	19 ± 6	33 ± 3	65 ± 3
6	**3f** (2-Thienyl, Ph)	25 ± 3	49 ± 2	73 ± 5
7	**3g** (2-Thienyl, 2-Furyl)	20 ± 4	28 ± 1	66 ± 1
8	**3h** (3-Thienyl, Ph)	24 ± 3	45 ± 3	86 ± 6
9	**3i** (4-OMePh, Ph)	11 ± 5	23 ± 4	40 ± 5
10	**3j** [3,4-$(OMe)_2$Ph, Ph]	24 ± 2	35 ± 3	83 ± 3
11	**3k** [3,4-(OCH_2O)Ph, Ph]	17 ± 1	29 ± 2	61 ± 2

these, **3a**, **3f** and **3h** were found to be potent inhibitors of HeLa cell proliferation producing >40% inhibition at 5 μM. MBH adducts **3h** and **3j** also produced substantial (>80%) inhibition of cell proliferation at 10 μM concentrations. The antiproliferative activity of these agents are comparable to those of the known anticancer agents like noscapine,[18] estramustine[19] and 2-methoxy estradiol.[20] These agents exert anticancer activity by targeting tubulin/microtubules.[21] MBH compounds **3a**, **3f** and **3h** decreased the intrinsic tryptophan fluorescence of tubulin (Fig. S1, ESI†), indicating that these agents bind to tubulin. Further, **3a**, **3f** and **3h** were found to inhibit microtubule assembly *in vitro* (see Fig. S2, ESI†).

Immunofluorescence studies reveal that compounds **3a**, **3f** and **3h** depolymerized cellular microtubules (Fig. 2, see also Fig. S3 in the ESI†). Untreated cells displayed a regular network of interphase microtubules and a normal mitotic spindle with proper metaphase chromosome alignment in the mitotic cells. Cells treated with 10 μM of **3a** and **3f** showed depolymerization of interphase microtubules. In cells treated with 10 μM of **3h**, interphase microtubules were completely depolymerized and tubulin aggregates were observed in these cells. At 20 μM each of **3a**, **3f** and **3h**, interphase microtubule network was completely absent. DAPI staining reveals that the nuclear morphology of cells treated with 20 μM of the tested compounds was also disorganized.

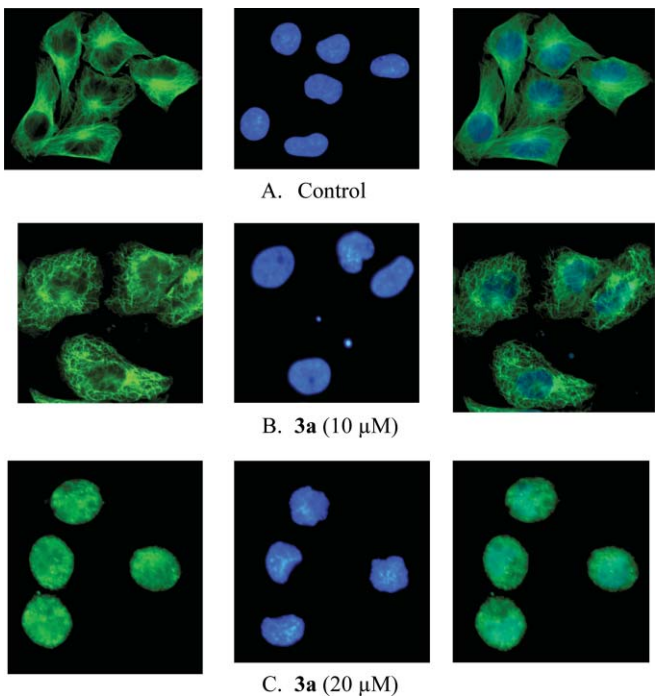


Fig. 2 Effect of MBH adduct **3a** on interphase microtubules.

In conclusion, activated imines, namely, *N*-tosylimines, reacted as electrophiles in the Morita–Baylis–Hillman reaction of conjugated nitroalkenes under the influence of imidazole and LiCl affording novel aminoalkylated nitroalkenes. Some of these products exhibited appreciable inhibition of human cervical cancer (HeLa) cell proliferation at concentrations in the range of 1–10 μM and compounds **3a**, **3f** and **3h** inhibited microtubule assembly both *in vitro* and in cells. The results indicate that compounds **3a**, **3f** and **3h** inhibit HeLa cell proliferation probably by perturbing microtubule assembly through tubulin binding.

I.N.N.N. thanks CSIR (India) and D. P. thanks DBT (India) for financial support. This work was partly supported by a Swarnajayanthi fellowship to D.P. N.R. thanks IIT Bombay and R.M. thanks UGC (India) for a research fellowship. The authors thank SAIF (IIT Bombay) for selected NMR spectra.

Notes and references

1 K. Morita, Z. Suzuki and H. Hirose, *Bull. Chem. Soc. Jpn.*, 1968, **41**, 2815; A. B. Baylis and M. E. D. Hillman, *Ger. Offen.*, 1972, DE 2155113 (*Chem. Abstr.*, 1972, **77**, 34174); M. E. D. Hillman and A. B. Baylis, *US Pat.*, 1973, US 3743669.

2 For reviews, see: (*a*) D. Basavaiah, A. J. Rao and T. Satyanarayana, *Chem. Rev.*, 2003, **103**, 811; (*b*) S. E. Drewes and G. H. P. Roos, *Tetrahedron*, 1988, **44**, 4653; (*c*) D. Basavaiah, P. D. Rao and R. S. Hyma, *Tetrahedron*, 1996, **52**, 8001; (*d*) E. Ciganek, in *Organic Reactions, Vol. 51*, ed. L. A. Paquette, John Wiley & Sons, Inc., New York, 1997, p. 201; (*e*) P. Langer, in *Organic Synthesis Highlights V*, eds. H.-G. Schmalz and T. Wirth, VCH-Wiley, Weinheim, 2003, p. 165; see also (*f*) T. Kataoka and H. Kinoshita, *Eur. J. Org. Chem.*, 2005, 45.

3 For recent mechanistic studies, see: V. K. Aggarwal, S. Y. Fulford and G. C. Lloyd-Jones, *Angew. Chem., Int. Ed.*, 2005, **44**, 1706; L. S. Santos, C. H. Pavam, W. P. Almeida, F. Coelho and M. N. Eberlin, *Angew. Chem., Int. Ed.*, 2004, **43**, 4330; K. E. Price, S. J. Broadwater, B. J. Walker and D. T. McQuade, *J. Org. Chem.*, 2005, **70**, 3980; P. Buskens, J. Klankermayer and W. Leitner, *J. Am. Chem. Soc.*, 2005, **127**, 16762.

4 Selected recent articles: K. Y. Lee, S. GowriSankar and J. N. Kim, *Tetrahedron Lett.*, 2004, **45**, 5485; B. Alcaide, P. Almendros, C. Aragoncillo and R. Rodriguez-Acebes, *J. Org. Chem.*, 2004, **69**, 826; S. Luo, X. Mi, H. Xu, P. G. Wang and J.-P. Cheng, *J. Org. Chem.*, 2004, **69**, 8413; M. Shi and C.-Q. Li, *Tetrahedron: Asymmetry*, 2005, **16**, 1385; R. Sagar, C. S. Pant, R. Pathak and A. K. Shaw, *Tetrahedron*, 2004, **60**, 11399; N. T. McDougal, W. L. Trevellini, S. A. Rodgen, L. T. Kliman and S. E. Schaus, *Adv. Synth. Catal.*, 2004, **346**, 1231; Y. Hayashi, T. Tamura and M. Shoji, *Adv. Synth. Catal.*, 2004, **346**, 1106; T. Kataoka and H. Kinoshita, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2005, **180**, 989; X. Liu, J. Zhao, G. Jin, G. Zhao, S. Zhu and S. Wang, *Tetrahedron*, 2005, **61**, 3841.

5 (*a*) N. Rastogi, I. N. N. Namboothiri and M. Cojocaru, *Tetrahedron Lett.*, 2004, **45**, 4745; (*b*) M. Dadwal, R. Mohan, D. Panda, S. M. Mobin and I. N. N. Namboothiri, *Chem. Commun.*, 2006, 338; (*c*) I. Deb, M. Dadwal, S. M. Mobin and I. N. N. Namboothiri, *Org. Lett.*, 2006, **8**, 1201; (*d*) M. Dadwal, S. M. Mobin and I. N. N. Namboothiri, *Org. Biomol. Chem.*, 2006, **4**, 2525; (*e*) R. Mohan, N. Rastogi, I. N. N. Namboothiri, S. M. Mobin and D. Panda, *Bioorg. Med. Chem.*, 2006, DOI: 1016/j.bmc.2006.07.035.

6 For recent reviews on the nucleophilic addition to imines and related C=N systems, see: T. Vilaivan, W. Bhanthumnavin and Y. Sritana-Anant, *Curr. Org. Chem.*, 2005, **9**, 1315; S. M. Weinreb and R. K. Orr, *Synthesis*, 2005, 1205; P. M. Pihko, *Lett. Org. Chem.*, 2005, **2**, 398.

7 P. Perlmutter and C. C. Teo, *Tetrahedron Lett.*, 1984, **25**, 5951.

8 Selected recent articles: (*a*) M. Shi and L.-H. Chen, *Pure Appl. Chem.*, 2005, **77**, 2105; (*b*) Y.-Li. Shi, Y.-M. Xu and M. Shi, *Adv. Synth. Catal.*, 2004, **346**, 1220; (*c*) Y.-M. Xu and M. Shi, *J. Org. Chem.*, 2004, **69**, 417 (also ester and aldehyde); (*d*) G.-L. Zhao, J.-W. Huang and M. Shi, *Org. Lett.*, 2003, **5**, 4737; (*e*) M. Shi and L.-H. Chen, *Chem. Commun.*, 2003, 1310.

9 Selected recent articles: (*a*) see ref. 8*c*–*e*; (*b*) Y.-L. Shi and M. Shi, *Tetrahedron*, 2006, **62**, 461; (*c*) D. Balan and H. Adolfsson, *Tetrahedron Lett.*, 2003, **44**, 2521; (*d*) X. Liu, Z. Chai, G. Zhao and S. Zhu, *J. Fluorine Chem.*, 2005, **126**, 1215; (*e*) V. Declerck, P. Ribiere, J. Martinez and F. Lamaty, *J. Org. Chem.*, 2004, **69**, 8372; (*f*) I. T. Raheem and E. N. Jacobsen, *Adv. Synth. Catal.*, 2005, **347**, 1701; (*g*) allenic ester: G.-Li. Zhao and M. Shi, *J. Org. Chem.*, 2005, **70**, 9975.

10 See ref. 8*b*,*c*.

11 See ref. 9*c*,*d*.

12 See ref. 2*a*.

13 For a recent imidazole-catalyzed MBH reaction, see: C. E. Aroyan, M. M. Vasbinder and S. J. Miller, *Org. Lett.*, 2005, **7**, 3849.
14 For studies on the salt effect on MBH reactions, see: (*a*) V. K. Aggarwal, D. K. Dean, A. Mereu and R. Williams, *J. Org. Chem.*, 2002, **67**, 510; (*b*) A. Kumar and S. S. Pawar, *Tetrahedron*, 2003, **59**, 5019.
15 Ref. 14*b* showed the catalytic influence of LiCl in the MBH reaction as a salting out agent. See ref. 5*b* for the imidazole/LiCl-mediated MBH reaction of nitroalkenes with other activated alkenes as electrophiles.
16 Selected X-ray crystallographic data for **3a**: $C_{40}H_{36}N_4O_{10}S_2$, M = 796.85, triclinic, space group $P\bar{1}$, $a = 8.8738(8)$, $b = 12.5069(13)$, $c = 17.5191(11)$ Å, $\alpha = 96.519(6)$, $\beta = 92.166(6)$, $\gamma = 96.945(8)°$, $U = 1915.0(3)$ Å^3, $D_c = 1.382$ Mg m^{-3}, $Z = 2$, $F(000) = 832$, $\lambda = 71073$ Å, $\mu = 0.204$ mm^{-1}, total/unique reflections = 7049/6708 ($R_{int} = 0.0518$), $T = 293(2)$ K, θ range = 1.17–24.97°, final R [$I > 2\sigma(I)$]: $R_1 = 0.0612$, $wR_2 = 0.1208$; R (all data): $R_1 = 0.1960$, $wR_2 = 0.1538$. CCDC reference number 609442. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b607537a.
17 For related work on MBH adducts of nitroalkenes with other activated alkenes and formaldehyde, respectively, see: ref. 5*b* and 5*e*. The inhibition of cell proliferation was determined using the sulforhodamine B assay, see: P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesh, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.
18 J. Zhou, K. Gupta, S. Aggarwal, R. Aneja, R. Chandra, D. Panda and C. H. Joshi, *Pharmacol. Mol.*, 2003, **63**, 1.
19 E. von Schoultz, D. Lundblad, J. Bergh, K. Grankvist and R. Henriksson, *Br. J. Cancer*, 1988, **58**, 326.
20 L. T. Tinley, M. R. Leal, D. A. Randall-Hlibek, W. J. Cessac, R. L. Wilken, N. P. Rao and L. S. Mooberry, *Cancer Res.*, 2003, **63**, 1538.
21 M. A. Jordan and L. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 253.

