Optimization tables, extended write-up, experimental data and details of biological studies
For

Synthesis and anticancer activity studies of α-aminoalkylated conjugated nitroalkenes

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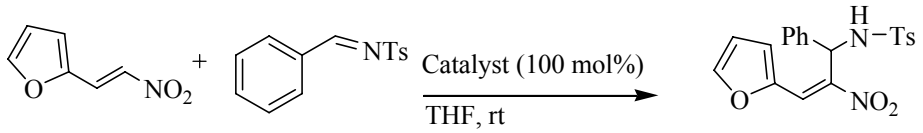
Table of Contents

In order to establish optimum reaction conditions, we selected 2-nitrovinylfuran (2-NVF) **1a** and N-benzylidene-4-methylbenzenesulphonamide **2a** as our model substrates and screened various tertiary amine and tertiary phosphine catalysts (in stoichiometric amounts) in THF (Table S1). However, the desired product **3a** could be isolated, albeit in low yield (18 %), only when imidazole was used as the catalyst (Table S1, Entry 3).

Table S1. The MBH Reaction of 2-NVF **1a** with N-tosylimine **2a** in the presence of 100 mol % of various catalysts.[a]



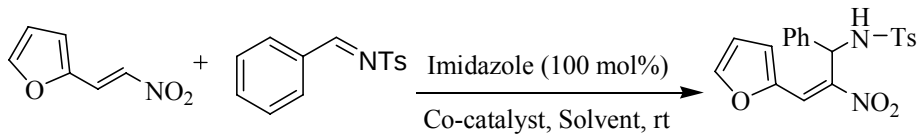
1a **2a** **3a**

Entry	Catalyst	Time	Yield (%)[b] of **3a**
1	DABCO	7 d	None
2	DMAP[c]	7 d	None
3	**Imidazole**[d]	**5 d**	**18**
4	Triethylamine	7 d	None
5	N-Methylimidazole	7 d	None
6	Triphenylphosphine	7 d	None

[a]**1a** and **2a** were taken in 1:1 ratio; [b]Isolated yield after purification by silica gel column chromatography; [c]Ref. 1; [d]Ref. 2.

For further improvement in the yield, we performed the imidazole mediated reactions in various aprotic and protic polar solvents such as THF, DMF, acetonitrile, 1,4-dioxan, methanol, formamide and ethylene glycol (Table S2, Entries 1a-7a). From the solvent screening, DMF proved to be the best solvent providing the desired product in 30 % isolated yield (Table S2, Entry 2a), while acetonitrile and 1,4-dioxan also provided comparable yields viz. 29 % and 23 %, respectively (Table S2, Entries 3a and 4a). Further drop in the yield of the desired MBH product was observed when THF, methanol, formamide, and ethylene glycol were used (Table S2, Entries 1a, 5a-7a). However, since the yields were still low to moderate, various organic co-catalysts such as proline, 2-aminophenol, anthranilic acid and β-cyclodextrin that are capable of activating the substrate and/or electrophile via H-bonding, imine/iminium formation etc and inorganic salts such as LiCl, KPF_6 etc which were shown to possess salting out properties were employed (Table S2, Entries 1b-g to 4b-g). Four of the solvents, namely, THF, DMF, acetonitrile and 1,4-dioxan, that provided relatively higher yields of the MBH adducts in the absence of any co-catalyst were used for investigating the activity of various co-catalysts.

Table S2. The MBH reaction of 2-NVF **1a** with N-tosylimine **2a**[a] in various solvents in the presence of imidazole (100 mol %) and various co-catalysts.[b]



1a **2a** **3a**

Entry	Solvent	Yield (%)[c] of **3a** (Time)						
		None (a)	Proline[d] (b)	2-AMP[e] (c)	AA[f] (d)	β-CD[g] (e)	**LiCl** (f)	KPF_6 (g)
1	THF	18 (5 d)	21 (3 d)	21 (3 d)	22 (5 d)	30 (3 d)	20 (3 d)	40 (5 d)
2	DMF	30 (3 d)	17 (3 d)	30 (3 d)	25 (3 d)	35 (3 d)	17 (1 d)	36 (5 d)
3	CH_3CN	29 (3 d)	32 (3 d)	38 (5 d)	17 (5 d)	37 (3 d)	25 (3 d)	33 (5 d)
4	**1,4-D**	23 (3 d)	27 (3 d)	20 (3 d)	22 (3 d)	10 (3 d)	**50** (3 d)	19 (5 d)
5	MeOH	10 (3 d)	-	-	-	-	-	-
6	FA	17 (12 h)	-	-	-	-	-	-
7	EG	10 (12 h)	-	-	-	-	-	-

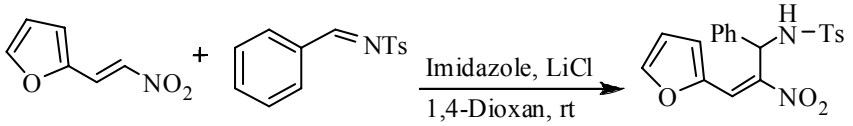
[a] **1a** and **2a** were taken in 1:1 ratio; [b]Organic co-catalysts b-e were 10 mol % and inorganic salts f-g were 0.5 M; [c]Isolated yield after purification by silica gel column chromatography; [d]Ref. 3; [e] 2-aminophenol; [f]anthranilic acid, see Ref. 5a (text); [g]β-cyclodextrin

Results in Table S2 suggest that organic co-catalysts such as proline, 2-aminophenol and anthranilic acid do not have much influence on the imidazole mediated MBH reaction of **1a** with **2a** (Table S2, Entries 1b-d to 4b-d). Some improvement in the yield was observed when β-cyclodextrin was used as the co-catalyst in solvents such as THF, DMF and acetonitrile (Table S2, Entries 1e-3e). Finally, substantial improvement in the yield was achieved when LiCl was used as the co-catalyst in 1,4-dioxan as solvent (Table S2, Entry 4f). Although KPF_6 also provided yields comparable to that obtained from β-cyclodextrin catalyzed reactions, other salts such as LiBr, $LiClO_4$, NaCl, NaI, $MgCl_2$ etc did not have any positive influence on the MBH reaction.

The emergence of imidazole/LiCl in 1,4-dioxan as an effective catalytic system in obtaining the MBH adduct **3a** in 50 % yield prompted us to further optimize the amount of imidazole and LiCl (Table S3).

Finally, 50 mol % imidazole and 0.5 M LiCl in 1,4-dioxan provided the best yield (69 %) of the MBH adduct in acceptable reaction time (Table S3, Entry 7).

Table S3. The MBH reaction of 2-NVF **1a** with N-tosylimine **2a** in 1,4-dioxan in the presence of varying amounts of imidazole and LiCl.[a]



1a **2a** **3a**

Entry	Imidazole (mol %)	LiCl (M)	Time	Yield (%)[b] of **3a**
1	100	3	5 d	63
2	100	2	5 d	65
3	100	1	5 d	53
4	100	0.5	5 d	50
5	100	0.25	5 d	40
6	200	0.5	5 d	45
7	**50**	**0.5**	5 d	**69**
8	50	1	5 d	69
9	50	2	5 d	65
10	25	0.5	5 d	58

[a]**1a** and **2a** were taken in 1:1 ratio; [b]Isolated yield after purification by silica gel column chromatography

Experimental Section: Chemistry

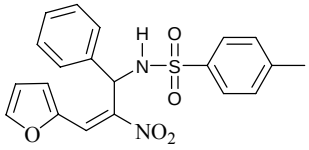
General

The melting points are uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum One FT spectrometer. NMR spectra (^{1}H and ^{13}C) were recorded on an AMX-400 or VXR-300S spectrometer with TMS as the internal standard. ^{1}H-^{1}H COSY and NOESY spectra were recorded on an AMX-400 NMR spectrometer. The coupling constants (*J* values) are given in Hz. High resolution mass spectra were recorded at 60-70 eV on a Waters Micromass Q-TOF spectrometer (ESI). All the nitroalkenes were prepared in the laboratory by standard nitroaldol (Henry) reaction[4] and N-tosylimines **2a-c** were prepared by the method described by Love and co-workers.[5]

General experimental procedure

To a stirred solution of nitroalkene **1** (1 mmol) and tosylimine **2** (1-1.5 mmol) in 1,4-dioxan (4 ml) at room temperature was added imidazole (34 mg, 0.5 mmol) followed by LiCl (80 mg, 0.5 M). After the completion of the reaction (confirmed by TLC analysis), the reaction mixture was acidified with 5 N HCl (5 ml) and the aqueous layer was extracted with ethyl acetate (3 × 10 ml). The combined organic layers were washed with brine (10 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography by eluting with ethyl acetate/pet. ether (0-25 %, gradient elution) to afford pure **3**.

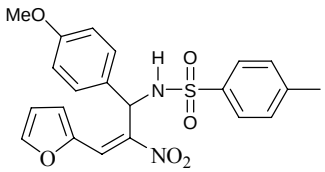
(*E*)-3-(Furan-2-yl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3a)



3a

Yield: 69 %; Yellow crystalline solid; mp 181 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3287s, 1642s, 1505s, 1317s, 1291m, 1169s, 1031m; δ_H (DMSO-d_6) 2.29 (3H, s), 6.55 (1H, d, *J* 8.6), 6.80 (1H, dd, *J* 3.5, 1.6), 7.20 (2H, d, *J* 8.2), 7.28 (1H, dd, *J* 3.5, 0.8), 7.30 (5H, m), 7.57 (2H, d, *J* 8.2), 7.94 (1H, s), 8.05 (1H, dd, *J* 1.6, 0.8), 8.62 (1H, d, *J* 8.6); δ_C ($CDCl_3$) 21.1, 52.8, 113.2, 122.7, 122.8, 125.2, 126.5, 127.4, 128.3, 129.1, 136.9, 137.3, 143.0, 143.7, 146.1, 147.7; *m/z* (ESI) 421 (MNa^+, 100%), 228 (47), 182 (21); HRMS (ESI) calcd. for $C_{20}H_{18}N_2O_5SNa$ (MNa^+) 421.0834, found 421.0824.

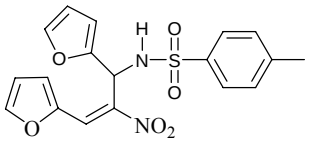
(*E*)-3-(Furan-2-yl)-2-nitro-1-(4-methoxyphenyl)-*N*-tosylprop-2-en-1-amine (3b)



3b

Yield: 25 %; Yellow crystalline solid; mp 156-158 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3333m, 1648s, 1515s, 1337s, 1291s, 1159s, 1037m; δ_H ($CDCl_3$) 2.35 (3H, s), 3.77 (3H, s), 6.15 (1H, d, *J* 10.8), 6.65 (1H, dd, *J* 3.5, 1.4), 6.67 (1H, d, *J* 10.8), 6.82 (2H, d, *J* 8.9), 6.89 (1H, d, *J* 3.5), 7.08 (2H, d, *J* 8.2), 7.24 (2H, d, *J* 8.9), 7.52 (2H, d, *J* 8.2), 7.63 (1H, s), 7.71 (1H, d, *J* 1.4); δ_C ($CDCl_3$) δ 21.5, 52.7, 55.4, 113.6, 114.1, 122.8, 123.0, 126.9 (× 2), 128.8, 129.5, 137.5, 143.6, 144.0, 146.5, 148.0, 159.3; *m/z* (ESI) 451 (MNa^+, 100%), 258 (19), 212 (37), 190 (20); HRMS (ESI) calcd. for $C_{21}H_{20}N_2O_6SNa$ (MNa^+) 451.0940, found 451.0941.

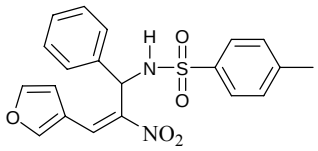
(*E*)-1,3-Di(furan-2-yl)-2-nitro-*N*-tosylprop-2-en-1-amine (3c)



3c

Yield: 23 %; Yellow crystalline solid; mp 127-129 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3282m, 1653s, 1515s, 1332s, 1291m, 1169s, 1026w; δ_H ($CDCl_3$) 2.36 (3H, s), 6.13 (1H, d, *J* 10.6), 6.27-6.31 (2H, m), 6.65 (1H, dd, *J* 3.7, 1.6), 6.75 (1H, d, *J* 10.6), 6.95 (1H, d, *J* 3.7), 7.13 (2H, d, *J* 8.3), 7.23 (1H, d, *J* 0.7), 7.56 (2H, d, *J* 8.3), 7.66 (1H, s), 7.75 (1H, d, *J* 1.6); δ_C ($CDCl_3$) 21.4, 49.0, 107.4, 110.8, 113.4, 123.0, 123.2, 126.9, 129.4, 137.3, 142.0, 142.2, 143.6, 146.3, 148.0, 149.6; *m/z* (ESI) 411 (MNa^+, 39%), 218 (100), 160 (42); HRMS (ESI) calcd. for $C_{18}H_{16}N_2O_6SNa$ (MNa^+) 411.0627, found 411.0613.

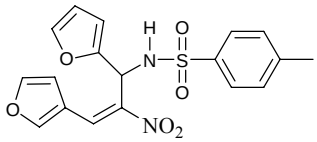
(*E*)-3-(Furan-3-yl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3d)



3d

Yield: 38 %; Yellow crystalline solid; mp 175 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3277m, 1648s, 1515s, 1337s, 1164s, 1087m; δ_H ($CDCl_3$) 2.39 (3H, s), 6.14 (2H, ABq, *J* 10.4), 6.75 (1H, d, *J* 1.6), 7.14 (2H, d, *J* 8.2), 7.28 (5H, m), 7.55 (2H, d, *J* 8.2), 7.57 (1H, dd, *J* 1.6, 0.4), 7.82 (1H, d, 0.4), 7.86 (1H, s); δ_C ($CDCl_3$) δ 21.6, 53.4, 109.1, 117.7, 125.5, 126.8, 128.2, 128.6, 128.9, 129.6, 136.1, 137.4, 143.8, 145.7, 145.8, 148.1; *m/z* (ESI) 437 (MK^+, 16%), 421 (MNa^+, 62%), 228 (100), 182 (12); HRMS (ESI) calcd. for $C_{20}H_{18}N_2O_5SNa$ (MNa^+) 421.0834, found 421.0841.

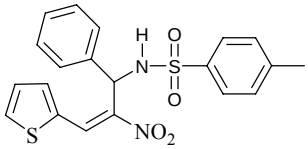
(*E*)-3-(Furan-3-yl)-2-nitro-1-furan-2-yl-*N*-tosylprop-2-en-1-amine (3e)



3e

Yield: 15 %; Yellow crystalline solid; mp 146-148 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3275s, 1647s, 1505s, 1332s, 1166s, 1072m; δ_H ($CDCl_3$) 2.39 (3H, s), 6.10 (2H, ABq, *J* 10.4), 6.23 (1H, d, *J* 3.0), 6.29 (1H, dd, *J* 3.0, 2.0), 6.81 (1H, d, *J* 1.2), 7.17 (2H, d, *J* 8.0), 7.24 (1H, d, *J* 3.0), 7.57 (2H, d, *J* 8.0), 7.60 (1H, d, *J* 1.2, 0.4), 7.85 (1H, s), 7.87 (1H, d, *J* 0.4); δ_C ($CDCl_3$) 21.4, 49.0, 107.8, 109.4, 110.9, 117.4, 126.8, 128.7, 129.5, 137.2, 142.3, 143.8, 144.3, 145.6, 147.7, 149.1; *m/z* (ESI) 411 (MNa^+, 100%), 218 (17), 172 (6); HRMS (ESI) calcd. for $C_{18}H_{16}N_2O_6SNa$ (MNa^+) 411.0627, found 411.0630.

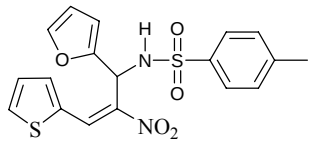
(*E*)-3-(Thien-2-yl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3f)



3f

Yield: 17 %; Yellow crystalline solid; mp 169-170 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3313m, 1632s, 1515s, 1322s, 1164s, 1057w; δ_H ($CDCl_3$) 2.38 (3H, s), 6.14 (1H, d, *J* 10.6), 6.42 (1H, d, *J* 10.6), 7.11 (2H, d, *J* 8.2), 7.22 (1H, dd, *J* 5.2, 3.6), 7.33 (5H, m), 7.42 (1H, d, *J* 3.6), 7.53 (2H, d, *J* 8.2), 7.73 (1H, d, *J* 5.2), 8.14 (1H, s); δ_C ($CDCl_3$) 21.5, 53.4, 125.4, 126.8, 128.2, 128.8, 128.9, 129.5, 130.8, 132.8, 133.8, 135.8, 136.4, 137.2, 143.7, 144.1; *m/z* (ESI) 453 (MK^+, 26%), 437 (MNa^+, 100%), 244 (58), 160 (17); HRMS (ESI) calcd. for $C_{20}H_{18}N_2O_4S_2Na$ (MNa^+) 437.0606, found 437.0603.

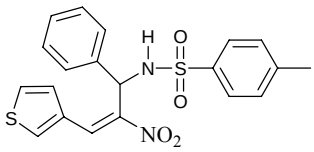
(*E*)-3-(Thien-2-yl)-2-nitro-1-(furan-2-yl)-*N*-tosylprop-2-en-1-amine (3g)



3g

Yield: 35 %; Yellow crystalline solid; mp 136-137 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3249s, 1633s, 1518s, 1416s, 1337s, 1167s, 1068w; δ_H ($CDCl_3$) 2.38 (3H, s), 6.28 (2H, ABq, *J* 10.2), 6.29 (2H, unresolved m), 7.14 (2H, d, *J* 8.5), 7.22 (1H, dd, *J* 5.1, 3.9), 7.25 (1H, unresolved m), 7.49 (1H, d, *J* 3.6), 7.56 (2H, d, *J* 8.5), 7.74 (1H, d, *J* 4.8), 8.12 (1H, s); δ_C ($CDCl_3$) 21.4, 49.2, 108.0, 110.9, 126.9, 128.8, 129.5, 130.7, 132.8, 133.8, 136.3, 137.2, 142.4, 142.8, 143.7, 149.0; *m/z* (ESI) 427 (MNa^+, 89%), 234 (100), 188 (42), 176 (22); HRMS (ESI) calcd. for $C_{18}H_{16}N_2O_5S_2Na$ (MNa^+) 427.0398, found 427.0403.

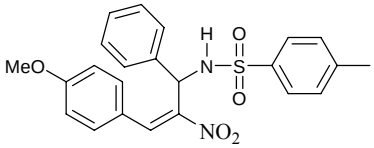
(*E*)-3-(Thien-3-yl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3h)



3h

Yield: 19 %; Yellow crystalline solid; mp 177-178 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3297m, 1637s, 1525s, 1413m, 1332s, 1154w; δ_H ($CDCl_3$) 2.39 (3H, s), 6.07 (1H, d, *J* 10.4), 6.26 (1H, d, *J* 10.4), 7.11 (2H, d, *J* 8.4), 7.23 (1H, dd, *J* 5.1, 1.2), 7.31 (5H, m), 7.48 (1H, d, *J* 5.1), 7.49 (2H, d, *J* 8.4), 7.65 (1H, d, *J* 1.2), 8.0 (1H, s); δ_C ($CDCl_3$) 21.4, 53.3, 125.5, 126.8, 127.1, 127.7, 128.1, 128.9, 129.5, 131.5, 131.6, 131.7, 136.2, 137.2, 143.6, 146.0; *m/z* (ESI) 437 (MNa^+, 16%), 421 (100), 219 (13); HRMS (ESI) calcd. for $C_{20}H_{18}N_2O_4S_2Na$ (MNa^+) 437.0606, found 437.0584.

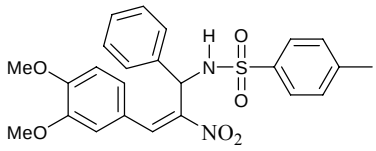
(*E*)-3-(4-Methoxyphenyl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3i)



3i

Yield: 18 %; Yellow crystalline solid; mp 157-158 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3297w, 1648w, 1612m, 1520m, 1332s, 1266s, 1174m; δ_H ($CDCl_3$) 2.40 (3H, s), 3.88 (3H, s), 6.13 (2H, ABq, *J* 10.3), 6.96 (2H, d, *J* 8.7), 7.11 (2H, d, *J* 8.1), 7.28 (2H, d, *J* 8.7), 7.35 (5H, m), 7.43 (2H, d, *J* 8.1), 8.0 (1H, s); δ_C ($CDCl_3$) 21.5, 53.3, 55.5, 115.0, 122.7, 125.7, 126.8, 128.2, 128.9, 129.5, 132.1, 136.6, 137.2, 138.3, 143.5, 145.1, 162.3; *m/z* (ESI) 461 (MNa^+, 77%), 222 (54), 207 (100), 178 (75); HRMS (ESI) calcd. for $C_{23}H_{22}N_2O_5SNa$ (MNa^+) 461.1147, found 461.1150.

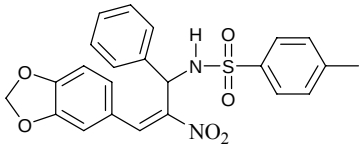
(*E*)-3-(3, 4-Dimethoxyphenyl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3j)



3j

Yield: 21 %; Yellow crystalline solid; mp 135-136 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3313m, 1612w, 1515s, 1459m, 1332s, 1261s, 1159s, 1031m; δ_H ($CDCl_3$) 2.39 (3H, s), 3.76 (3H, s), 3.95 (3H, s), 6.11 (1H, d, *J* 10.4), 6.26 (1H, d, *J* 10.4), 6.87 (1H, d, *J* 1.8), 6.97 (2H, ABq, *J* 8.4, the low field half is further split into d, *J* 1.8), 7.12 (2H, d, *J* 8.2), 7.31 (5H, m), 7.46 (2H, d, *J* 8.2), 8.02 (1H, s); δ_C ($CDCl_3$) 21.6, 53.6, 56.0, 56.1, 111.5, 112.3, 123.0, 124.5, 125.9, 126.8, 128.3, 129.0, 129.6, 136.7, 137.3, 138.4, 143.6, 145.4, 149.5, 152.0; *m/z* (ESI) 491 (MNa^+, 48%), 413 (11), 298 (37), 252 (100), 149 (16); HRMS (ESI) calcd. for $C_{24}H_{24}N_2O_6SNa$ (MNa^+) 491.1253, found 491.1229.

(*E*)- 3-(Benzo-[*d*][1,3]dioxol-5-yl)-2-nitro-1-phenyl-*N*-tosylprop-2-en-1-amine (3k)



3k

Yield: 17 %; Yellow crystalline solid; mp 168-169 °C (CH_2Cl_2-pet. ether 1:3); ν_{max} (KBr)/cm^{-1} 3313m, 1637m, 1500s, 1459s, 1332s, 1266s, 1164s, 1042s; δ_H ($CDCl_3$) 2.41 (3H, s), 6.10 (2H, ABq, *J* 10.7), 6.07-6.09 (2H, m), 6.77 (1H, d, *J* 1.5), 6.87 (2H, ABq, *J* 8.0, the high field half further split into d, *J* 1.5), 7.14 (2H, d, *J* 8.1), 7.33 (5H, m), 7.47 (2H, d, *J* 8.1), 8.0 (1H, s); δ_C ($CDCl_3$) 21.5, 53.3, 102.1, 109.2 (× 2), 124.2, 125.7, 125.9, 126.8, 128.3, 129.0, 129.6, 136.5, 137.3, 138.2, 143.7, 145.7, 148.7, 150.6; *m/z* (ESI) 491 (MK^+, 5%), 475 (MNa^+, 100%), 413 (8), 282 (74), 236 (52), 149 (8); HRMS (ESI) calcd. for $C_{23}H_{20}N_2O_6SNa$ (MNa^+) 475.0940, found 475.0949.

Experimental Section: Biology

Binding measurements

Binding of **3a**, **3f** and **3h** to goat brain tubulin was determined by measuring the intrinsic tryptophan fluorescence quenching of tubulin.[6,7] To specifically excite the tryptophan residues in the tubulin, 295 nm was selected as the excitation wavelength. Tubulin (1 μM) was incubated in the absence (■) and in the presence of 5 and 10 μM of **3a** (□,∗) , **3f** (♦,o) and **3h** (◊,●) in PEM buffer (25 mM Pipes-pH 6.8, 3 mM $MgSO_4$, 1 mM EGTA) for 30 min at room temperature. Fluorescence measurements were taken in a JASCO FP-6500 spectrofluorimeter using a 0.3 cm path length cuvette with excitation and emission wavelengths of 295 and 335 nm, respectively. Data are an average of three independent experiments. Compounds **3a, 3f** and **3h** were found to decrease the intrinsic tryptophan fluorescence of tubulin. The decrease in the tryptophan fluorescence upon binding to **3a, 3f** and **3h** indicated that the binding of these agents to tubulin induced conformational change in the protein.

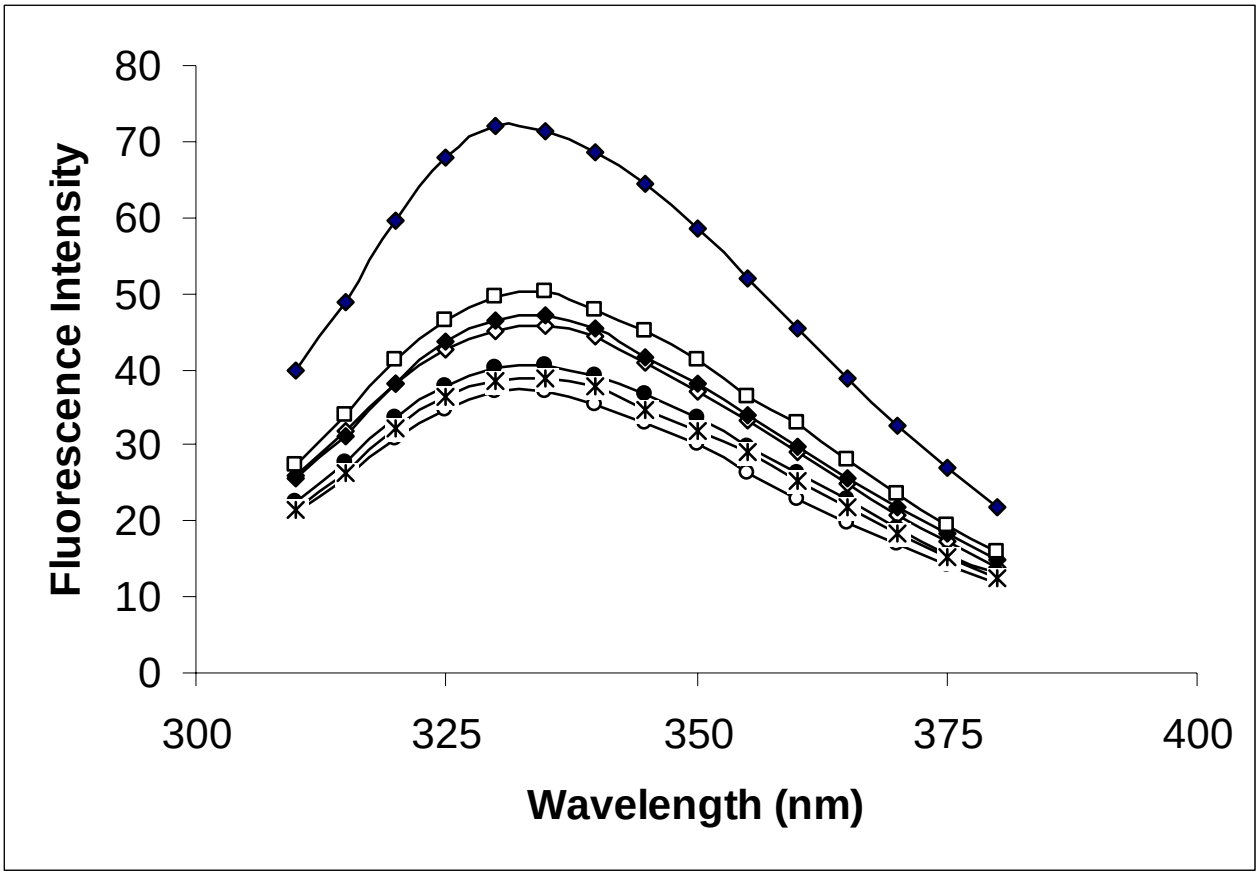


Figure S1. Effect of **3a**, **3f** and **3h** on the intrinsic tryptophan fluorescence of tubulin. Tubulin (1 μM) was incubated in the absence (■) and in the presence of 5 and 10 μM of **3a (**□,∗) , **3f** (♦,o) and **3h** (◊,●) in PEM buffer for 30 min at 25 °C. Fluorescence spectra were recorded using 295 nm as an excitation wavelength.

Effect of 3a, 3f and 3h on tubulin polymerization *in vitro*

Using a standard 90º light scattering assay, we monitored the effects of **3a**, **3f** and **3h** on the polymerization of purified tubulin *in* vitro.[8] Purified goat brain tubulin (1 mg/mL) was incubated in the absence (■) and in the presence of 5 and 10 μM **3a** (●,○)**, 3f** (□,Δ) and **3h** (▲,◊) in assembly buffer (PEM buffer and 1 mM GTP) containing 1M sodium glutamate for 10 min at 0 ºC. Polymerization was initiated by keeping the reaction mixture at 37 ºC in a water bath. The rate and extent of polymerization was monitored by light scattering at 500 nm for 18 minutes in a JASCO FP 6500 fluorescence spectrophotometer using a 0.3 cm path length cuvette. The excitation and emission band passes were 1 and 5 nm, respectively. Buffer blank values were subtracted from all the measurements. 5 and 10 μM of **3a**, **3f** and **3h** decreased the light scattering signal for microtubule assembly suggesting that the three MBH adducts inhibited microtubule assembly *in vitro.*

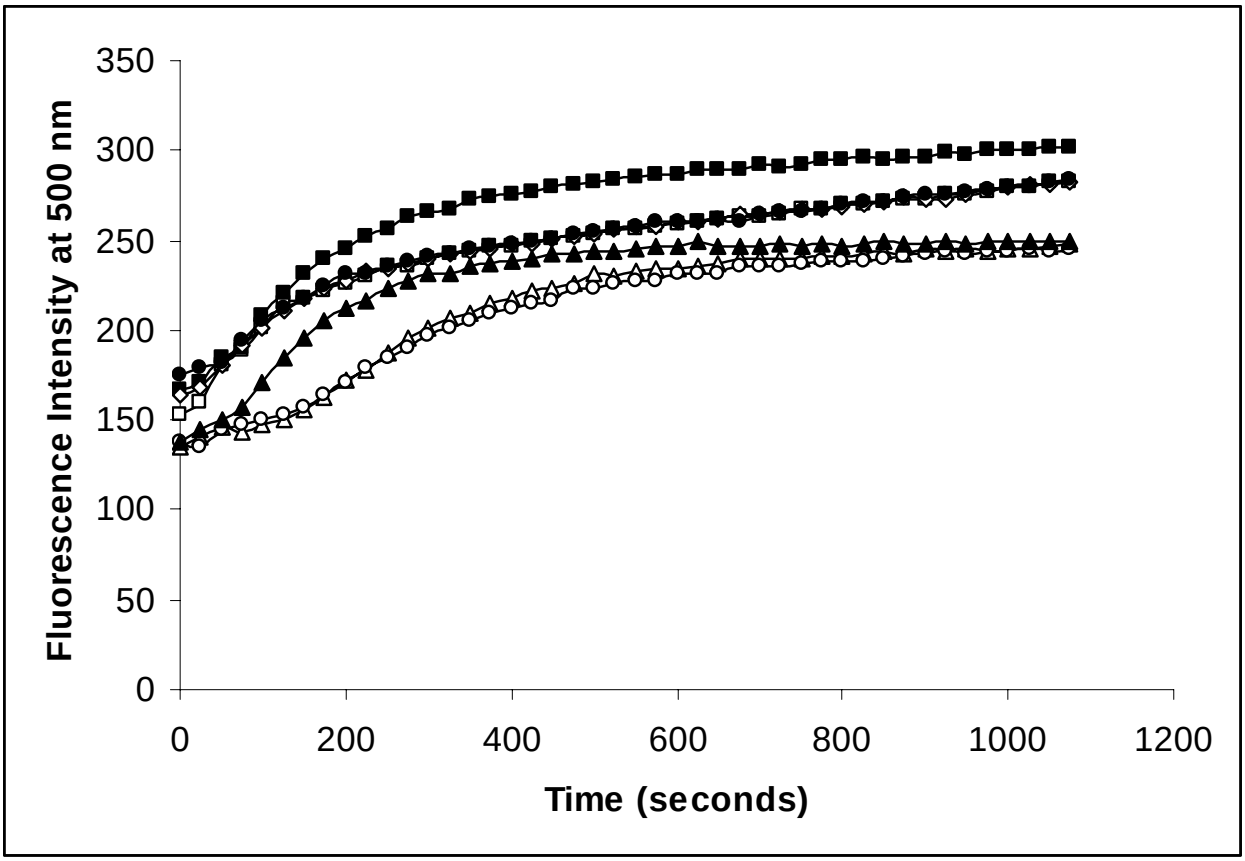
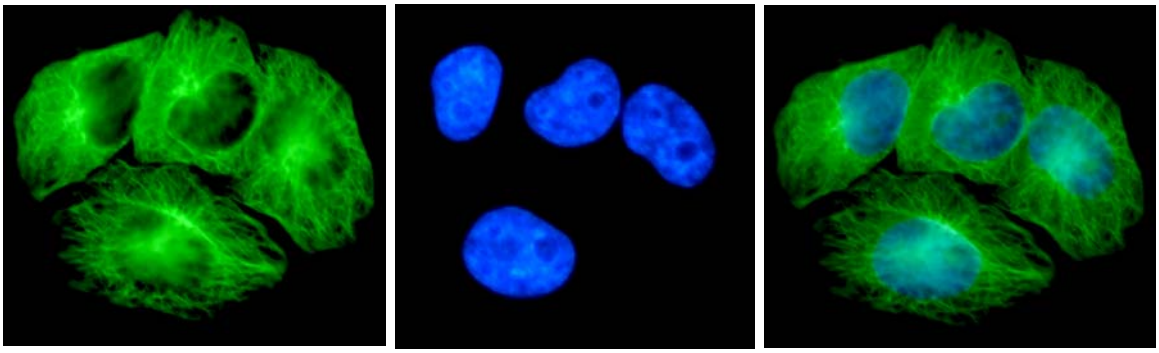


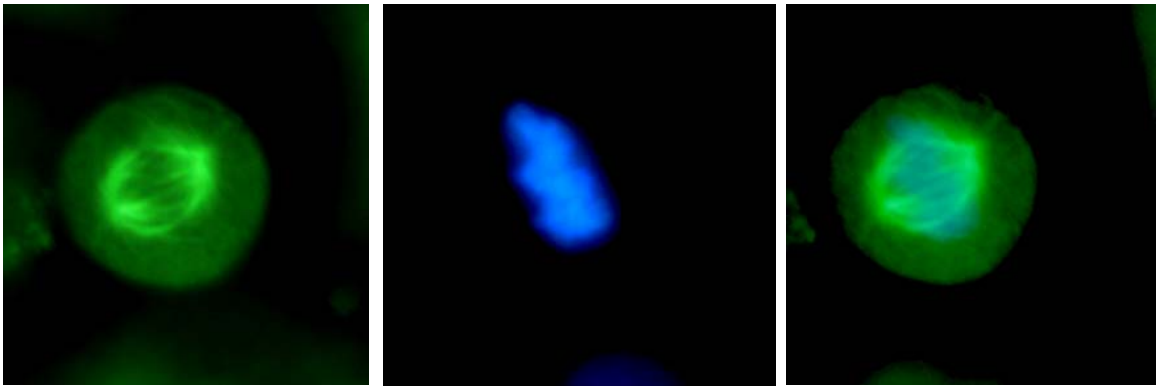
Figure S2. Inhibition of microtubule polymerization in the absence (■) and in the presence of 5 and 10 μM **3a** (●,○)**, 3f** (□,Δ) and **3h** (▲,◊). Tubulin (10 μM) was polymerized in the absence and in the presence of 5 and 10 μM of **3a, 3f** and **3h** and the change in the light scattering intensity at 500 nm was monitored.

Effect of 3a, 3f and 3h on cellular microtubules

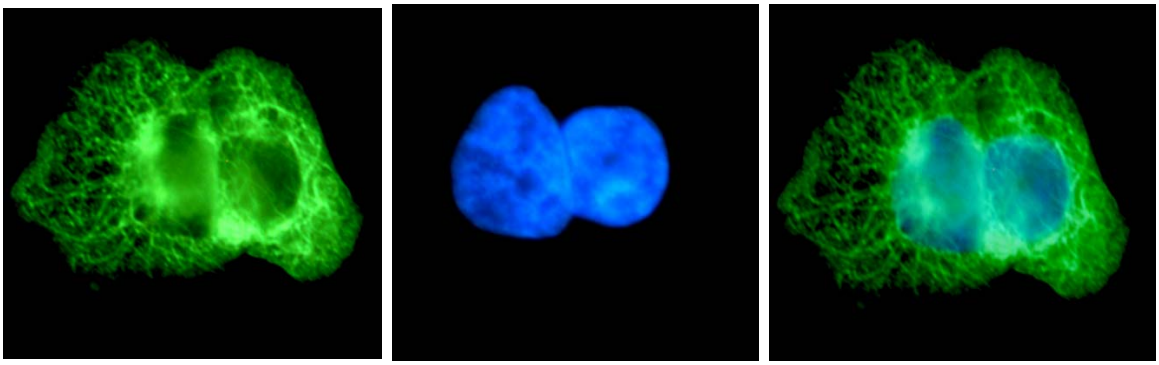
Immunofluorescence microscopic analysis was carried out to analyze the effects of MBH adducts **3a, 3f and 3h** on interphase and spindle microtubules and chromosome organization. Cells (0.6×10^5cells/mL) seeded on poly(L-lysine) coated cover slips were exposed to different concentrations (0, 10 and 20 μM) of **3a, 3f** and **3h** for 20 h at 37 ºC. Cells were fixed in 3.7 % formaldehyde and permeabilized with ice cold methanol for 10 min at -20 ºC. After blocking nonspecific sites with 2 % BSA, cells were stained with mouse monoclonal anti-α-tubulin antibody (1:300 dilution in 2 % BSA) for 2 h at 37 ºC followed by sheep antimouse IgG FITC antibody (1:400 dilution in 2 % BSA) for 1 h at 37 ºC, to visualize microtubules. To visualize nuclei and DNA, cells were stained with 1 μg/ml 4, 6-diamidino-2-phenylindole (DAPI) for 20 seconds at 37 ºC. Cells were washed with 1 × PBS after all incubations. All the cover slips were mounted in 50 % glycerol in PBS containing 1mg/mL ascorbic acid and cells were examined with a Nikon Eclipse 2000-U fluorescence microscope and the images were analyzed with the Image-Pro Plus software.



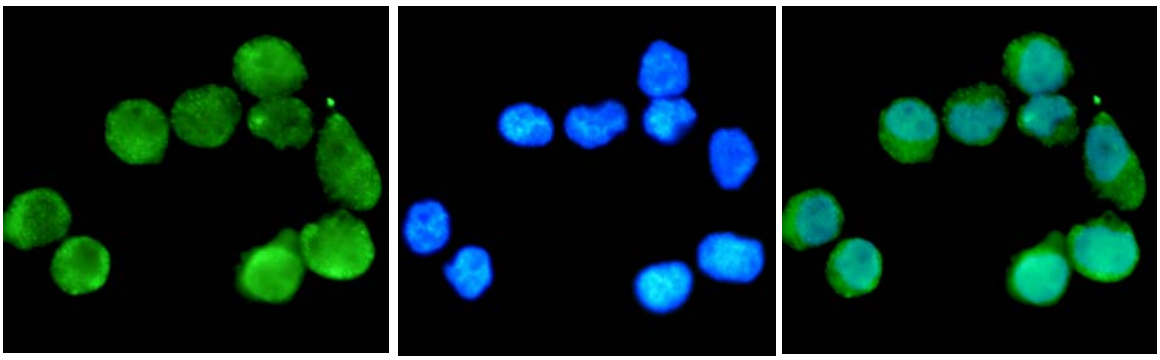
(A) Control



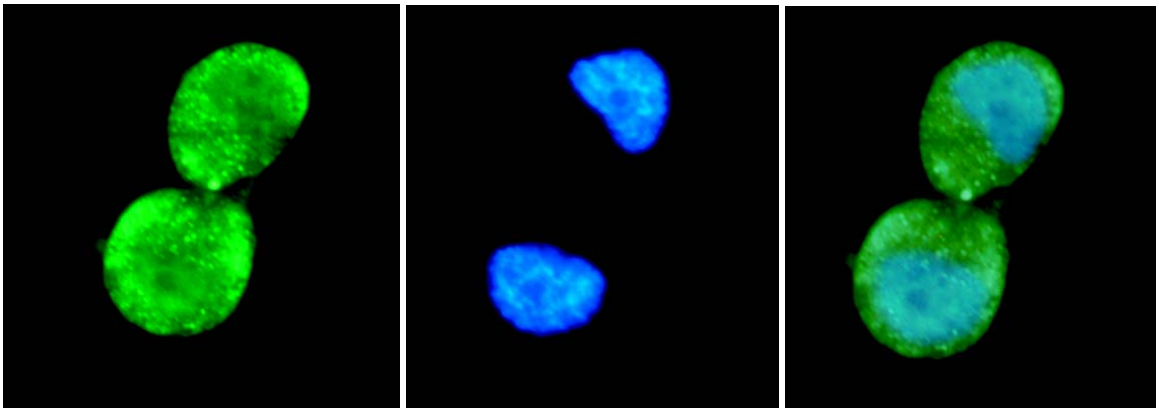
(B) Control



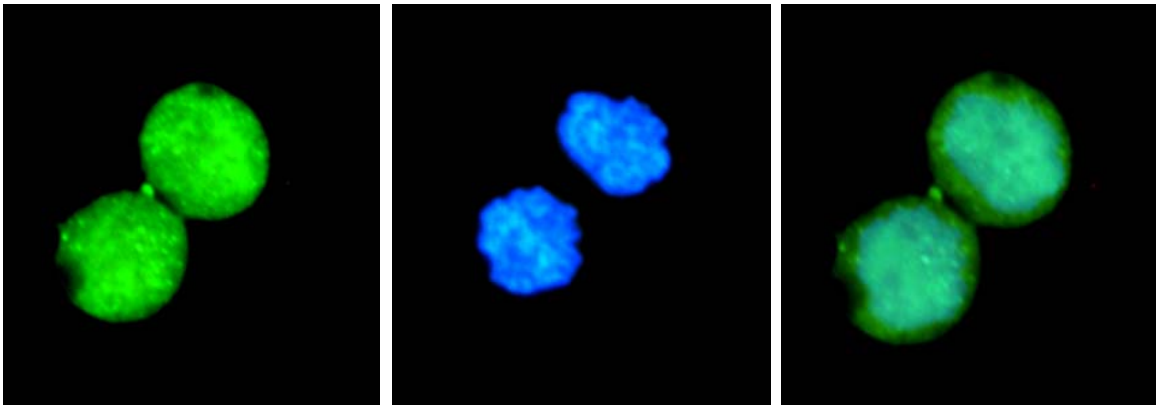
(C) **3f** (10 μM)



(D) **3f** (20 μM)



(E) **3h** (10 μM)



(F) **3h** (20 μM)

Figure S3. Effect of MBH adducts **3f** and **3h** on cellular microtubules. HeLa cells were grown in the absence and in the presence of 10 and 20 μM of **3f** and **3h** for 24 h and stained with the α-tubulin antibody. Microtubules are visualized in green and chromosomes are stained blue with DAPI. (A) control interphase cells with fine network of microtubules; (B) control mitotic cells show normal mitotic spindle with proper chromosome alignment at the metaphase plate (C) 10 μM **3f** induced interphase microtubule depolymerization; (D) In cells treated with 20 μM of **3f,** tubulin aggregates were observed. (E) tubulin aggregates were also observed in cells treated with 10 and 20 μM of **3h.** Altered nuclear morphology was seen in cells treated with 10 and 20 μM of **3h** and 20 μM of **3f.**

Experimental Section: Crystallography

Suitable X-ray quality crystals of **3a** were grown and X-ray diffraction studies were undertaken. Relevant crystallographic data and details of measurements are given in Table S6. X-ray crystallographic data were collected from single crystal samples of volume 0.25 × 0.15 × 0.13 mm^3**.** Unit cell dimensions were obtained using 25 centered reflections in θ range 5.1600-10.4600^o mounted on a Nonius MACH 3 diffractometer equipped with graphite monochromated Mo Kα radiation (0.71073 Å). The intensity data were collected by ω-2θ scan mode, and corrected by Lorentz Polarization and absorption effects using Psi-Scan (ψ-scan). Three standard reflections monitored after every 200 reflections and 3 intensity control reflections monitored every hour showed no significant changes (< 3 %). The structure was solved by direct methods shelxs97 and refined by full-matrix least squares against F^2 using shelxl97 software. Non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were geometrically fixed and allowed to refine using a riding model.

Table S4. Crystallographic data for **3a**

Empirical formula	$C_{40}H_{36}N_4O_{10}S_2$
Formula weight	796.85
Wavelength	0.71073 Å
Temperature, K	293 (2)
Crystal system	Triclinic
Space group	*P*-1
A, Å	8.8738 (8)
B, Å	12.5069 (13)
C, Å	17.5191 (11)
α deg	96.519 (6)
β deg	92.166 (6)
γ deg	96.945 (8)
Volume, $Å^3$	1915.0 (3)
Z	2
Density (calcd), mg/m^{-3}	1.382
Absorption coefficient, mm^{-1}	0.204
F(000)	832
Crystal size, mm	0.25 × 0.15 × 0.13
θ range, deg	1.1700-24.9700 Å
Index ranges	-10<=h<=0, -14<=k<=0, -20<=l<=20
Reflections collected/unique	7049/6708 [R(int) = 0.0518]
Absorption correction	Psi-scan
Max. and min. transmission	0.9750 and 0.9509
Refinement method	Full-matrix least-squares on F^2
No. of data/restraints/parameters	6708/0/507
Goodness-of-fit on F^2	1.002
Final R indices [I>2σ(I)]	R1 = 0.0612 wR2 = 0.1208
R indices (all data)	R1 = 0.1960 wR2 = 0.1538
Largest diff. peak and hole, $eÅ^{-3}$	0.288 and -0.308

References

1. For DMAP and related aminopyridine catalyzed MBH reactions of other activated olefins: (a) F. Rezgui, M. M. El Gaied, *Tetrahedron Lett*., 1998, **39**, 5965: (b) R. Octavio, M. A. de Souza, M. L. A. A. Vasconcellos, *Synth. Commun*., 2003, **33**, 1383; (c) M. Shi, Y.-M. Xu, G. -L. Zhao, X. -F. Wu, *Eur. J. Org. Chem.,* 2002, 3666; (d) K. Y. Lee, J. H. Gong, J. N. Kim, *Bull. Kor. Chem. Soc.,* 2002, **23**, 659; (e) M. Shi, Y. -M. Xu, *Eur. J. Org. Chem*., 2002, **4**, 696; (f) M. Shi, Y. -M. Xu, *Chem. Commun*., 2001, **18**, 1876; (g) H. -T. Chen, S. Huh, J. W. Wiench, M. Pruski, V. S. -Y. Lin, *J. Am. Chem. Soc.,* 2005, **127**, 13305; (h) G.-L Zhao, J. -W. Huang, M. Shi, *Org. Lett.,* 2003**,** **5**, 4737; (i) A. Corma, H. Garcia, A. Leyva, *Chem. Commun.,* 2003, 2806; (k) J.-W. Huang, M. Shi, *Adv. Synth. Cat.,* 2003, **345**, 953; (l) R Murugan, E. F. V. Scriven, *Aldrichimica Acta,* 2003, **36**, 21; (m) R. O. M. A. de Souza, M. L. A. A. Vasconcellos, *Catal. Commun.,* 2004, **5**, 21; (n) N. -F. Yang, H. Gong, W. -J. Tang, Q. -H. Fan, C. -Q. Cai, L. -W. Yang, *J. Mol. Cat.*, 2005, **233**, 55.

2. For MBH reactions of other activated olefins in the presence of imidazole based catalysts: (a) S. Luo, P. G. Wang, J.-P. Cheng, *J. Org. Chem.,* 2004, **69**, 555; (b) J. E. Imbriglio, M. M. Vasbinder, S. J. Miller, *Org. Lett*., 2003, **5**, 3741; (c) M. Shi, J. -K. Jiang, C. -Q. Li, *Tetrahedron Lett*., 2002, **43**, 127; (d) R. Gatri, M. M. El Gaied, *Tetrahedron Lett*., 2002, **43**, 7835; (e) S. Luo, B. Zhang, J. He, A. Janczuk, P. G. Wang, J. -P. Cheng, *Tetrahedron Lett*., 2002, **43**, 7369; (f) S. Luo, X. Mi., P. G. Wang, J.-P. Cheng, *Tetrahedron Lett*., 2004, **45**, 5171; (g) M. Shi, Y. Jiang, *J. Chem. Res. (S)* 2003, 564; (h) Ref. 1m. (i) C. E. Aroyan, M. M. Vasbinder, S. J. Miller, *Org. Lett.,* 2005, **7**, 3849.

3. For proline co-catalyzed MBH reaction: see Ref. 2b-c.

4. Vogel's Text Book of Practical Organic Chemistry, Addison Wesley Longman Ltd., Essex, England, 5th Ed., 1989, p 1035.

5. B. E. Love, P. S. Raje, T. C. Williams II, *Synlett*, 1994, 493.

6. C. J. Lee, D. Harrison, S. N. Timasheff, *J. Biol. Chem.* 1975, **250**, 9272.

7. D. Panda, K. Rathinasamy, M. K. Santra, L. Wison, *Proc. Natl. Acad. Sci U.S.A*, 2005, **102**, 9878.

8. K. Gupta, D. Panda, *Biochemistry*, 2002, **41**, 13029.