The Morita–Baylis–Hillman adducts of β-aryl nitroethylenes with other activated alkenes: synthesis and anticancer activity studies†‡

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The Morita–Baylis–Hillman (MBH) adducts of β-aryl nitroethylenes with methyl vinyl ketone (MVK) and acrylate, formed in moderate to good yield when mediated by imidazole/LiCl in THF at room temperature, inhibit HeLa cell proliferation by binding to tubulin.

The coupling of the α-position of activated alkenes with various carbon electrophiles mediated by a tertiary amine or tertiary phosphine, popularly known as the Morita–Baylis–Hillman (MBH) reaction, has emerged as an important C–C bond forming reaction in organic synthesis.[1,2] It provides a simple, convenient and atom-economical methodology for the synthesis of useful multifunctional molecules.[2] Although various activated alkenes have been employed in the MBH reaction,[2] conjugated nitroalkenes[3] have not found a place in more than three decades of the MBH chemistry.[4–7] However, the superior Michael acceptor ability of conjugated nitroalkenes[3] together with the well-recognized status of the nitro group as a synthetic chameleon[8] call for greater attention on nitroalkenes as substrates for the MBH reaction.

The ability of nitroalkenes to exhibit important biological properties and function as key substrates and/or intermediates in the synthesis of many potent drugs and bioactive natural products makes them attractive molecules in the biological domain as well.[9] Among various biological properties, the anticancer properties of nitroalkenes have been scarcely investigated.[10,11]

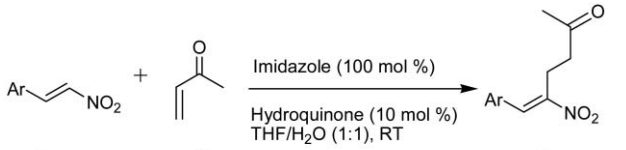
In this report, we describe our results on the successful MBH type reaction of β-aryl nitroethylenes with other activated alkenes such as methyl vinyl ketone (MVK) and acrylate and the preliminary evaluation of the novel MBH adducts in their ability to inhibit human cervical cancer (HeLa) cell proliferation by binding to tubulin.

There is a handful of reports in the literature where activated alkenes underwent self-condensation (dimerization) in the absence of another electrophile.[12,13] These include DABCO catalyzed dimerization of alkyl vinyl ketone and acrylonitrile,[12] and TDAP (tris-dimethylaminophosphine) or DABCO catalyzed dimerization of acrylate.[13] However, to our knowledge, there are only two reports where activated alkenes have been consciously used as electrophiles in the MBH reaction.[14]

At first, the reaction of our model substrate 2-nitrovinyl furan (NVF) **1a** with MVK **2** was carried out in the presence of 10 mol% of various catalysts such as imidazole,[15] DMAP,[16] DABCO, DBU and various other catalysts under a variety of conditions (see supplementary information‡).[2] However, while imidazole and DMAP provided isolable amounts of the desired MBH adduct, all others failed to catalyze the reaction. Subsequently, the amount of imidazole required to obtain the best yields of the MBH adduct was confirmed to be 100 mol%.

Having optimized the amount of imidazole, the co-catalytic activity of a variety of additives which are capable of (a) forming imine/iminium of the enone; (b) activating the enone *via* hydrogen bonding; and/or (c) inhibiting polymerization of the substrate (nitroalkene), was investigated (see supplementary information‡). But, any appreciable improvement in the yield could be achieved only when hydroquinone and *p*-methoxyphenol were used in conjunction with imidazole in the reaction between NVF **1a** and MVK **2**. However, further attempts to improve the yields in these imidazole mediated reactions using hydroquinone as the additive and employing the optimized conditions for the reaction of other nitroalkenes **1b–e** with MVK **2** led to unsatisfactory results (Table 1). This prompted us to examine the effect of salt[17] on our MBH reaction by employing LiCl as the additive.[18]

Table 1 The MBH reaction of β-aryl nitroethylenes **1** with MVK **2** in the presence of 100 mol% imidazole, and 10 mol% hydroquinone in THF/H_2O (1 : 1) at room temperature



1 **2** **3**

Entry	**1**, **3**	Ar	Time (min)	Isolated yield (%) of **3**[a]
1	**a**	2-Furyl	60	39
2	**b**	2-Thienyl	60	41
3	**c**	4-ClPh	120	22
4	**d**	4-OMePh	60	16
5	**e**	Ph	5	Polymerization

[a] Complete consumption of **1** was observed in all the cases.

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† Dedicated to the memory of the late Professor A. K. Lala.

‡ Electronic supplementary information (ESI) available: X-ray data for **3g** in CIF format, experimental procedures, full characterization data, copies of ^1H and ^{13}C NMR spectra and HPLC profiles for all the new compounds. See DOI: 10.1039/b512267h

Although the imidazole mediated reaction of NVF **1a** with MVK **2** in the presence of LiCl in THF/H_2O (1 : 1) was complete in 1 h, it provided only low yield (22%) of the MBH product **3a**. Subsequently, exclusion of water from the reaction mixture led to isolation of the desired product **3a** in satisfactory yield (44%). Other polar aprotic solvents such as DMF, acetonitrile and dioxane provided **3a** only in much lower yields.

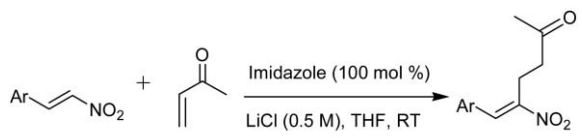
The imidazole–LiCl ratio of 100 mol%/0.5 M was found to be optimum because increasing the amount of imidazole to 200 mol% or LiCl to 1, 2 and 3 M did not have any appreciable influence on the yield. On the other hand, decreasing the amount of imidazole to 50 mol% led to dramatic decrease in the yield (18%). Finally, the optimum ratio of the MBH substrate **1a** and the electrophile **2** was found to be 1 : 3. While decreasing the amount of MVK **2** led to decrease in the yield, increasing the ratio from 1 : 3 to 1 : 4 or 1 : 5 did not affect the yield at all.

In view of the fact that stoichiometric amounts of imidazole in conjunction with 0.5 M LiCl in THF emerged as the best system for the MBH reaction of NVF **1a** with MVK **2**, the scope of the reaction was extended by reacting a variety of aromatic and heteroaromatic nitroalkenes **1b–j** with MVK **2** (Table 2, Entries 2–10). Although the chemical yields were moderate in these cases (28–60%), clean, isomerically pure products **3a–j** were isolated after column chromatography.

Having established the experimental conditions for the reaction between nitroalkenes **1** and MVK **2**, we explored the reactivity of other activated alkenes as electrophiles under these conditions. Thus, selected nitroalkenes **1a** and **1g–i** were reacted with ethyl acrylate **4** which afforded the desired MBH products **5a** and **5g–i**, respectively, though, in low yield (Table 3, Entries 1–4). All our attempts to use other activated alkenes such as acrolein, acrylonitrile, acrylamide and nitroethylene as terminal electrophiles have not been successful.

The geometry of the double bond in products **3** and **5** was confirmed to be *E* by performing 2D-NOESY experiment on a representative compound **3g**. One of the CH_2 groups and the CH_3 group in **3g** have strong NOEs with the Ar protons, but only weak

Table 2 The MBH reaction of β-aryl nitroethylenes **1** with MVK **2** in the presence of 100 mol% imidazole, and 0.5 M LiCl in THF at room temperature

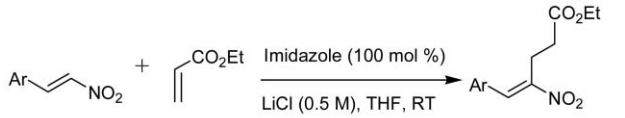


1 **2** **3**

Entry	**1**, **3**	Ar	Time (h)	Isolated yield (%) of **3**[a]
1	**a**	2-Furyl	48	44
2	**b**	2-Thienyl	50	40
3	**c**	3-Furyl	48	42
4	**d**	3-Thienyl	48	41
5	**e**	4-ClPh	72	39
6	**f**	4-OMePh	80	36
7	**g**	Ph	20	34
8	**h**	3,4-(OCH_2O)Ph	48	47
9	**i**	3,4-$(OMe)_2$Ph	56	60
10	**j**	4-CF_3Ph	6	28

[a] 15–23% of **1** was recovered.

Table 3 The MBH reaction of β-aryl nitroethylenes **1** with ethyl acrylate **4** in the presence of 100 mol% imidazole, and 0.5 M LiCl in THF at room temperature



1 **4** **5**

Entry	**1**, **5**	Time (h)	Isolated yield (%) of **5**[a]
1	**a**	56	21
2	**g**	34	18
3	**h**	88	21
4	**i**	90	24

[a] 5–12% of **1** was recovered.

NOEs with the benzylic olefinic proton. In fact, all the reactions provided isomerically pure MBH adducts and there was no evidence for the formation of the other geometrical isomer. The structure was further unambiguously established by single crystal X-ray analysis of **3g** (see also supplementary information‡).[19]

The effect of the MBH adducts on human cervical cancer (HeLa) cells were examined by incubating HeLa cells with 5 μM and 25 μM of the MBH adducts **3a–j**, **5a** and **5g–i** for 24 hours.[20] While most of the MBH adducts produced 70–100% inhibition of HeLa cell proliferation at 25 μM concentrations, three of the MBH adducts, *viz.* **3e**, **3f** and **3g** were found to produce more than 70% inhibition even at 5 μM concentration.‡ MBH adduct **3f** was found to be the most potent inhibitor of HeLa cell proliferation among the tested compounds producing 80% inhibition at 5 μM.

To determine the binding affinity of MBH adducts **3a–j**, **5a** and **5g–i** to tubulin, tubulin (1 μM) was incubated with 10 μM each of all the 14 MBH adducts for 30 min at room temperature.[21] In all the cases, a decrease in the intrinsic tryptophan fluorescence of tubulin was observed which confirmed that all the compounds were binding to tubulin.‡

In conclusion, activated alkenes such as MVK and acrylate reacted as electrophiles in the MBH reaction of a variety of aromatic and heteroaromatic nitroalkenes and provided novel α-substituted nitroalkenes. Normally, β-substituted activated alkenes do not react or react only sluggishly in the MBH type reactions. However, in the case of nitroalkenes, β-substituted ones were found to react satisfactorily. One of the MBH adducts was found to be a potent inhibitor of HeLa cell proliferation at 5 μM concentrations by binding to tubulin. Applications of these MBH adducts for the synthesis of novel 1,4-dicarbonyl compounds, 1,4-amino alcohols, γ-amino acids *etc* will be reported in due course.

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19 Selected X-ray crystallographic data for **3g**. $C_{12}H_{13}NO_3$, M = 219.23, triclinic $P1$, a = 7.2530(10), b = 7.9810(7), c = 10.7050(9), α = 106.198(7), β = 104.883(9), γ = 97.193(9), V = 562.02 (10) Å^3, Z = 2, D_{calc} = 1.295 g cm^{-3}, μ = 0.094 mm^{-1}, 2153 reflections measured, R_1 = 0.0453, R_w = 0.1010. CCDC 283074. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b512267h.
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