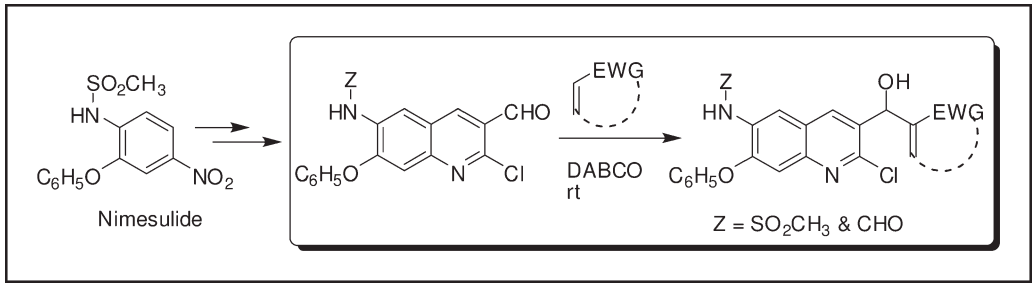
Design and Synthesis of Novel Cytotoxic Agents Based on Combined Framework of Quinoline and Nimesulide

Lingam Venkata Reddy,[a] Mohan Kethavath,[a] Mamatha Nakka,[a] Syed Sultan Beevi,[a] Lakshmi Narasu Mangamoori,[a] Khagga Mukkanti,[a] and Sarbani Pal[b]*

[a]JNT University, Kukatpally, Hyderabad-500072, India
[b]Department of Chemistry, MNR Degree and PG College, Kukatpally, Hyderabad-500072, India
*E-mail: sarbani277@yahoo.com

Received August 26, 2010
DOI 10.1002/jhet.801
Published online 29 August 2011 in Wiley Online Library (wileyonlinelibrary.com).



Functionalization of quinoline aldehydes, derived from nimesulide framework was carried out using Morita–Baylis–Hillman (MBH) chemistry. A number of novel quinoline-based diverse MBH adducts was prepared *via* the reaction of derivatives of 2-chloroquinoline-3-carbaldehyde and various activated alkenes in good yields. Many of these compounds were found to be potent when tested against human prostate cancer (Pc-3) cell line *in vitro*. Among all the compounds tested *N*-(2-chloro-3-(2-cyano-1-hydroxyallyl)-7-phenoxyquinolin-6-yl)formamide (IC_{50} = 1.2 μg mL^{-1}) was identified as the most potent compound in this series.

J. Heterocyclic Chem., **49**, 80 (2012).

INTRODUCTION

The Morita–Baylis–Hillman reaction (MBH) is an attractive method for C—C bond forming reaction affording highly functionalized products containing a new stereocenter known as Baylis–Hillman adducts [1–4]. The reaction, typically catalyzed by tertiary amines or tertiary phosphines, involves a coupling of aldehydes with activated alkenes to give adducts containing α-methylene-β-hydroxy moiety. Substituted quinolines, one of the oldest known heterocyclic pharmaceutical agents, exhibit a wide range of medicinal properties, including antimalarial activities [5]. Not surprisingly, an array of synthetic methods has been developed to access quinoline derivatives, including the classic Skraup, Doebner–vonMiller, Conrad–Limpach, and Knorr syntheses [5,6]. The use of Baylis–Hillman reaction for the preparation of functionalized quinolines has also been reported [7]. Although the use of simple quinoline aldehydes to generate the corresponding MBH adducts has been reported earlier [8–11], the application of this methodology to more complex quinoline system is not common in the literature. As cancer has been a major killer in most developed, developing, and underdeveloped countries, the search for new and effective anticancer agents has become an important area of current pharmaceutical research worldwide. Along with other nitrogen containing heterocyclic moieties quinoline framework has also been explored for this purpose. Recently, cytotoxicity studies of a variety of quinoline derivatives have been reported [12]. Because of our continuing interest in the synthesis and identification of novel cytotoxic agents, we became interested in the synthesis and pharmacological evaluation of quinoline-based small molecules.

Recently, (2-chloro-6-methoxyquinolin-3-yl)methanol **A** (Fig. 1) has been reported to exhibit significant cytotoxic activities when tested *in vitro* (IC_{50} = 42.5 and 47.4 μg mL^{-1} on HeLa and Vero cells, respectively) [13]. On the other hand, compounds containing sulfonamide moiety, for example, 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC236) showed enhanced cytotoxic effects of doxorubicin on HKESC-1 and HKESC-2 cells [14]. Nimesulide [15] **B** (Fig. 1), a sulfonamide based well-known anti-inflammatory agent, is presently in patient's use in certain countries. In our effort to develop novel cytotoxic agents, we designed the structure **C** by (i) introducing appropriately functionalized side chain to the hydroxymethyl carbon of **A** and (ii) incorporating the structural features of nimesulide **B**. We hypothesized that this combination may lead to the identification of potent cytotoxic agents that might possess anti-inflammatory properties as well.

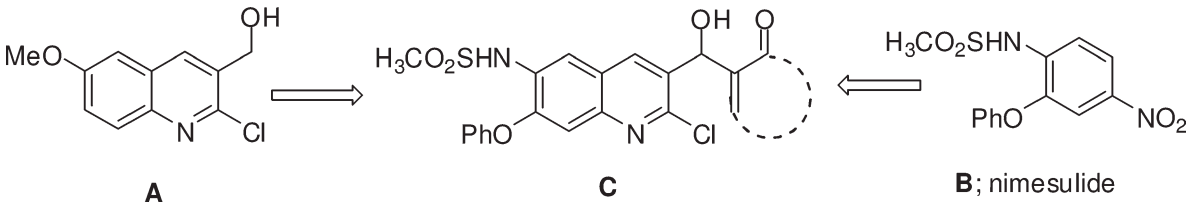


Figure 1. Design of novel cytotoxic agents based on quinoline and nimesulide.

RESULTS AND DISCUSSION

To test our hypothesis, we prepared few simple analogues of **C** *via* applying the MBH reaction according to a known method [16]. Thus, 2-chloro-3-formyl quinoline **1a** (X and Y = H), prepared from acetanilide using $DMF/POCl_3$ [17–19], was treated with activated alkenes **2a–d** electron withdrawing group (EWG) in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) at room temperature under a solvent-free condition to give the desired products **3a–d** (Scheme 1 and Table 1; entries 1–4). Some of these compounds were tested *in vitro* against the human prostate cancer cell line (Pc-3) based on an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [20]. The percentage of cell death was measured for each compound at various concentrations and finally, the IC_{50} (half maximal inhibitory concentration) values were determined to measure the cytotoxic activities (Table 2). Because IC_{50} is inversely proportional to the cytoxicity of a compound (*i.e.*, lower is the IC_{50} value, higher is the activity), compound **3b** was identified as a promising cytotoxic agent.

Encouraged by this observation, we planned to synthesize compounds represented by **C**. Accordingly, nimesulide was converted to *N*-(2-chloro-3-formyl-7-phenoxyquinolin-6-yl)methanesulfonamide (**1b**; X= $NHSO_2Me$ and Y = OPh) and *N*-(2-chloro-3-formyl-7-phenoxyquinolin-6-yl)formamide (**1c**; X = NHCHO and Y = OPh) following the procedure developed by us previously (Scheme 2) [21,22]. Both **1b** and **c** were converted to the corresponding MBH adducts as shown in Scheme 1 and results are summarized in Table 1 (entries 5–9). All the alkenes **2a–d** reacted well with the aldehydes **1b** and **c** at room temperature, affording the desired products **3e–3l**. The methanesulfonamide (—$NHSO_2Me$) and formamide (—NHCHO) group present in the aldehyde **1b** and **c**, respectively, was tolerated during the reaction. The duration of the reaction, however, was longer, that is, 24–36 h, in these cases, compared to **1a** perhaps due to the electron-donating effect of phenoxy group at C-7. Moreover, DABCO-mediated deprotonation of —$NHSO_2Me$ or —NHCHO moiety increases the electron density on the quinoline ring, thereby, decreasing the reactivity of aldehyde group. Nevertheless, all the compounds synthesized were well characterized by spectral and analytical data. The presence of OH and —C=C— was indicated by the appearance of IR absorption in the region 3400–3200 and 1630–1600 cm^{-1}, respectively. The ^1H NMR spectra of two representative compounds **3h** and **3l** (both of which showed a strong IR absorption at 2226 cm^{-1} corresponding to —CN moiety) are shown in Figure 2 and 3, respectively. The presence of olefinic, —C*H*OH— and the $MeSO_2$— protons for compound **3h** was indicated by the appearance of signals at δ 6.32 and 6.28, 5.60, and 3.20, respectively. Similarly, the presence of CHO (δ 8.9), olefinic (δ 6.32 and 6.28) and C*H*OH (δ 5.60) groups can be observed in case of **3l**.

According to the objective of our present study (*i.e.*, identification of novel cytotoxic agents), some of the compounds synthesized were evaluated for their activities *in vitro*. Compounds **3e**, **3f**, **3k**, and **3l** were tested *in vitro* against the human prostate cancer cell line (Pc-3) based on an MTT assay. The percentage of cell death measured for each compound at various concentrations along with IC_{50} values are shown in Table 2. All these compounds showed promising cytotoxic effects and the compound **3l** being the most notable among them as it showed the effect at lower concentration. Although a dramatic increase in its activity was observed at the concentration of 2.0 μg mL^{-1}, further enhancement with the increase of dose was not observed perhaps due to

Scheme 1. Synthesis of quinoline derivatives **3**.

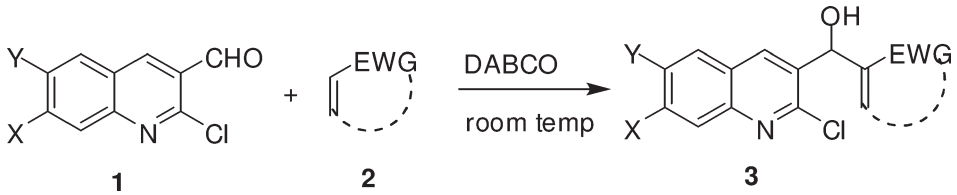
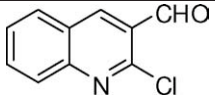
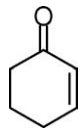
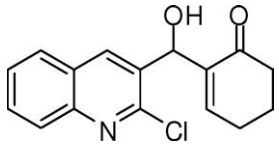
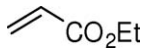
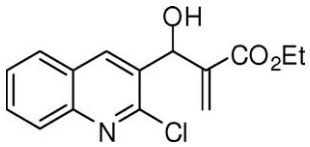
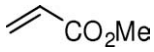
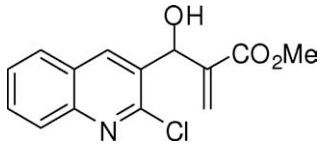
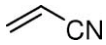
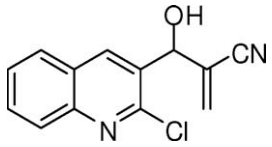
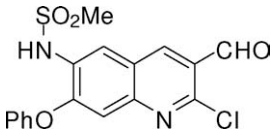
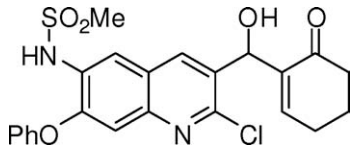
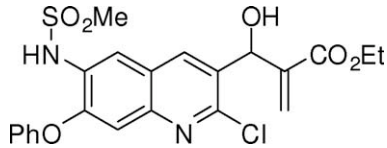
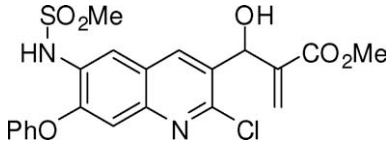
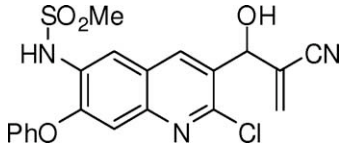
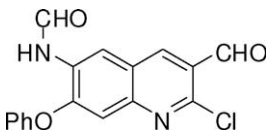
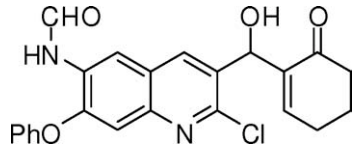


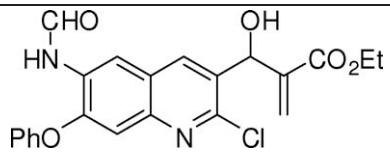
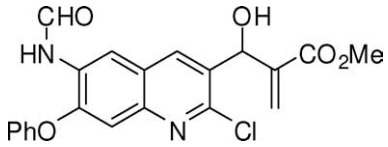
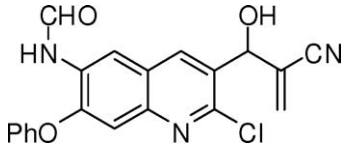
Table 1

Preparation of MBH adducts **3** from 2-chloro-3-formyl quinoline (or its derivatives) **1** and activated alkenes **2**.[a]

Entries	Quinoline **1**	Alkene **2**	Time (h)	MBH adduct **3**	% Yield[b]
1	 **1a**	 **2a**	6.0	 **3a**	55
2	**1a**	 **2b**	5.0	 **3b**	60
3	**1a**	 **2c**	5.3	 **3c**	65
4	**1a**	 **2d**	3.0	 **3d**	65
5	 **1b**	**2a**	24	 **3e**	54
6	**1b**	**2b**	25	 **3f**	60
7	**1b**	**2c**	32	 **3g**	60
8	**1b**	**2d**	24	 **3h**	65
9	 **1c**	**2a**	24	 **3i**	55

(*Continued*)

Table 1
(*Continued*)

Entries	Quinoline **1**	Alkene **2**	Time (h)	MBH adduct **3**	% Yield[b]
10	**1c**	**2b**	28	 **3j**	52
11	**1c**	**2c**	36	 **3k**	55
12	**1c**	**2d**	28	 **3l**	60

[a] Reaction conditions: **1** (10 mmol), alkene **2** (20 mmol), DABCO (1.3 mmol), room temperature.
[b] Isolated yield.

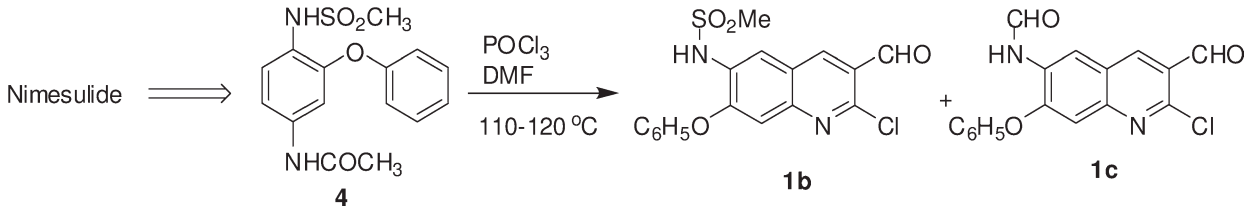
reaching the saturation point at an early dose. Nevertheless, satisfactory dose response curves (DRC) for selected compounds is shown in Figure 4. Studies on the interaction of a number of acrylate esters with biological nucleophiles have shown that these esters interact significantly with cellular glutathione (GSH) rather than deoxyribonucleosides [23]. GSH, a tripeptide, contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Being an antioxidant, it helps to protect cells from reactive oxygen species such as free radicals and peroxides. Notably, the GSH levels were found to be higher in human cancer cell lines than in normal cell [24,25] and, therefore, depletion of GSH in these cells make them more vulnerable to the effects of anticancer drugs or the gene that promotes apoptosis (cell death). Because of the presence of a nucleophilic thiol (—SH) group GSH can interact with acrylate ester *via* addition across the α,β-unsaturated bond (a Michael-type reaction) [26]. Thus, the proposed interaction of GSH with the quinoline derivative **3** can be presented schematically as shown in Figure 5. The path a, based on the known interaction of GSH with acrylate ester may lead to the generation of metabolic product **M1**. However, taking into consideration of (i) the nucleophilicity of amino group of GSH in addition to thiol moiety, (ii) reactivity of chloro group of **3** and (iii) the recent report on the reaction of various amines with the quinoline-

Table 2
Cytotoxic activity of quinoline derivatives for Pc-3 cancer cell line determined by MTT assay protocol.[a]

S. No	Compd	Percentage of cell death at various concentrations (μg mL^{-1})					IC_{50} (μg mL^{-1})
		1.0	2.0	5.0	10	25	
1	**3a**	2.5	21	26	29	64	n.d.
2	**3b**	8	28	45	78	79	3.98
3	**3d**	21	65	73.5	75	77	n.d.
4	**3e**	23	36	48	73	74	4.79
5	**3f**	18	25	36	60	77	7.61
6	**3h**	1.2	9.5	26	78.5	78	n.d.
7	**3i**	5	13	20	34	73	n.d.
8	**3k**	19	25	39	64	77	6.64
9	**3l**	40	76	80	82	83	1.2

n.d. = not done.

Scheme 2. Preparation of derivatives of 2-chloro-3-formyl quinoline **1b** and **1c** from nimesulide.



based MBH adduct [16] an alternative interaction (path b) leading to the generation of product **M2** can be proposed. This path essentially involves a Michael-type addition of GSH with **3** followed by intramolecular cyclization *via* the displacement of chloro group by the secondary amine generated. In order to gain further evidence compound **3g** was treated with *n*-butyl amine in EtOH in the presence of triethylamine at room temperature for 6.0 h and the corresponding adduct, that is, methyl-1-butyl-1,2-dihydrobenzo[*b*][1,8]naphthayridine-3-carboxylate derivative was isolated in 70% yield.

CONCLUSIONS

In conclusion, we have described design, synthesis and *in vitro* cytotoxicities of a number of novel quinoline-based diverse Morita–Baylis–Hillman [27] adducts incorporating the structural features of a well-known anti-inflammatory agent nimesulide. Many of these compounds were found to be potent when tested against human prostate cancer (Pc-3) cell line *in vitro*. Activity of these compounds is thought to be due to the interaction of the carbon–carbon double bond present in compound **3** with the cellular glutathione. Among all the compounds tested *N*-(2-chloro-3-(2-cyano-1-hydroxyallyl)-7-phenoxyquinolin-6-yl)formamide **3l** (IC_{50} = 1.2 μg mL^{-1}) was identified as most potent compound in this series. Further pharmacological studies are ongoing to evaluate anticancer properties of these compounds.

EXPERIMENTAL

General methods. Melting points were all determined by open glass capillary method on a Cintex melting point apparatus and uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer using DMSO-d_6 as a solvent with tetramethylsilane as internal reference (TMS, δ = 0.00). Chemical shift (δ) values are presented as singlet (s), doublet (d), triplet (t), or multiplet (m). Elemental analyses were performed by Varian 3LV analyzer series CHN analyzer. Mass spectra were recorded on a Jeol JMCD-300 instrument. All solvents used were commercially available and distilled before use. All reactions were monitored by TLC on precoated

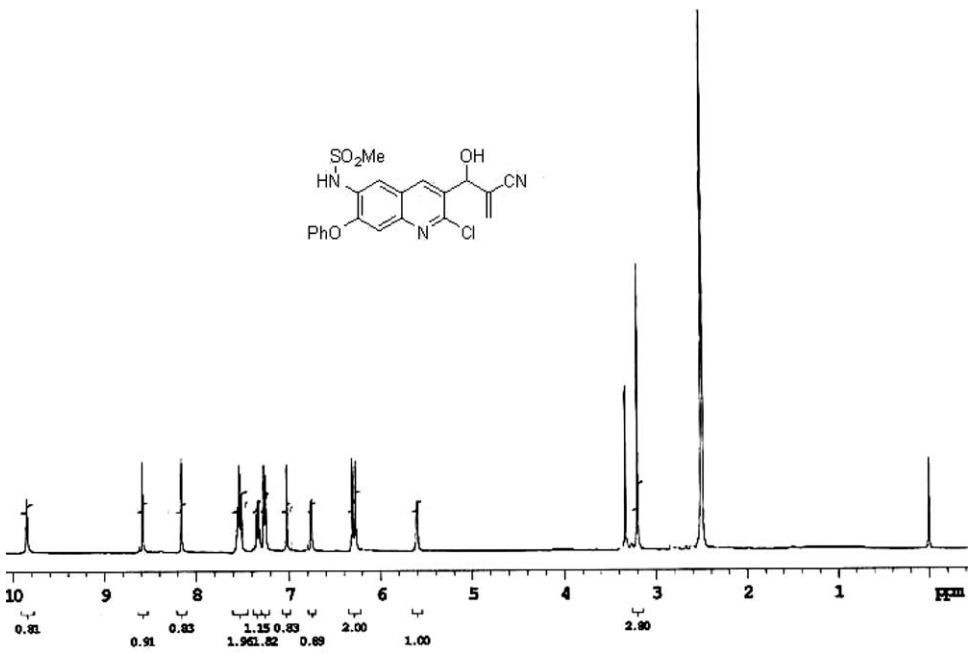


Figure 2. ^{1}H NMR spectra of compound **3h** in DMSO-d_6.

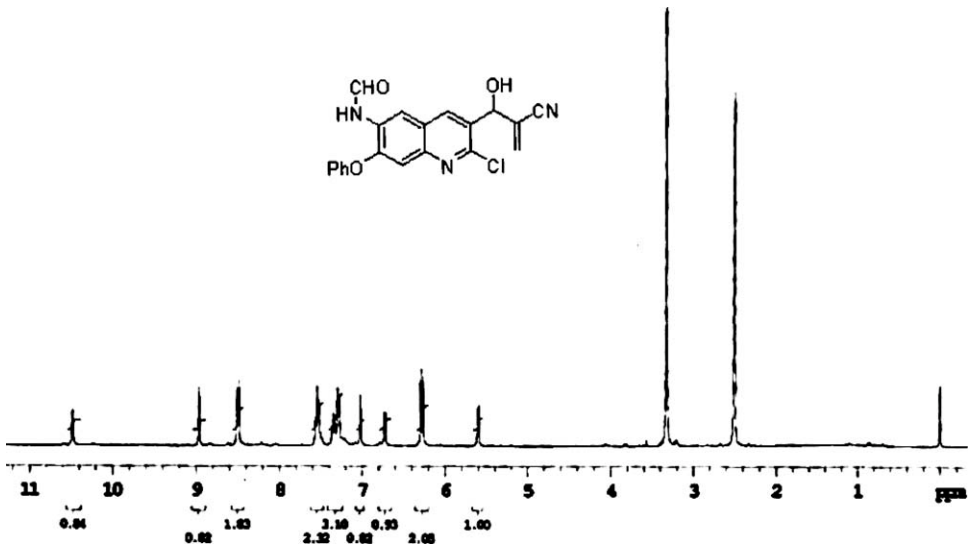


Figure 3. ^{1}H NMR spectra of compound **3l** in DMSO-d_6.

silica gel plates (60 F 254; Merck). Column chromatography was performed on 100–200 mesh silica gel (SRL, India) using 10–20-fold excess (by weight) of the crude product. The organic extracts were dried over anhydrous Na_2SO_4.

General procedure for the synthesis of MBH adduct (3). A mixture of 2-chloro-3-formyl quinoline (or its derivatives) **1** (10 mmol) and activated alkenes **2** (20 mmol) and DABCO (1.3 mmol, 145.8 mg) was stirred at room temperature for the time indicated in Table 1 under solvent-free conditions. After completion of the reaction (indicated by TLC), the mixture was extracted with Et_2O (3 × 15 mL). The organic layers were collected, combined, washed with cold brine (2 × 15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane-EtOAc) to give the desired product.

2-((2-Chloroquinolin-3-yl)(hydroxy)methyl)cyclohex-2-enone (3a). Semi solid; R_f = 0.42 ($CHCl_3$/EtOAc 4:1); MS *m/z* 288.0 (M^+, 100%), 290.0 (M+2, 33%); IR (KBr) ν_{max} (cm^{-1}) 3421, 2925, 2865, 1655; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (1H, s, ArH), 8.10 (1H, d, *J* = 8 Hz, ArH), 7.90 (1H, d, *J* = 8 Hz, ArH), 7.80 (1H, t, *J* = 6 Hz, ArH), 7.65 (1H, t, *J* = 6 Hz, ArH), 6.90 (1H, s, —CH=C), 5.9 (1H, bs, OH), 5.8 (1H, s, C*H*OH), 2.40 (4H, m, $2CH_2$), 1.90 (2H, m, CH_2); Elemental analysis found: C, 66.52; H, 4.91.;N, 4.99; $C_{16}H_{14}ClNO_2$ requires C, 66.79; H, 4.90; N, 4.87.

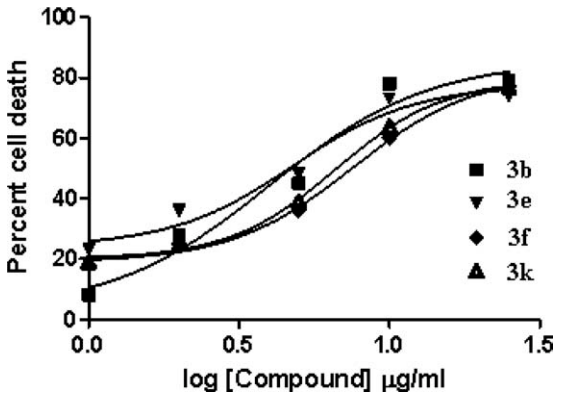


Figure 4. Dose response curve (DRC) of selected compounds.

Ethyl 2-((2-Chloroquinolin-3-yl)(hydroxy)methyl)acrylate (3b). Pale yellow solid; m.p. 75–76°C (lit [10] 75–77°C); R_f = 0.41 ($CHCl_3$/EtOAc 4:1); MS *m/z* 292.0 (M^+, 100%), 294.0 (M+2); IR (KBr) ν_{max} (cm^{-1}) 3401.78, 2923.05, 2871.73,1687.86, 1614.71; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (1H, s, ArH), 8.05 (1H, d, *J* = 8.2 Hz, ArH), 7.95 (1H, d, *J* = 8.2 Hz, ArH), 7.80 (1H, m, ArH), 7.67 (1H, m, ArH), 6.35 (1H, s, CH=C), 6.22 (1H, s, CH=C), 5.95 (1H, s, C*H*OH), 5.67 (1H, s, OH), 4.25 (2H, q, *J* = 7.3 Hz, OCH_2), 1.25 (3H, t, *J* = 7.3 Hz, CH_3).

Methyl 2-((2-Chloroquinolin-3-yl)(hydroxy)methyl)acrylate (3c). Off white solid; m.p. 69–70°C (lit [9] 69–71°C); R_f = 0.40 ($CHCl_3$/EtOAc 4:1); MS *m/z* 278.1 (M^+, 100%), 280.1 (M+2, 33%); IR (KBr) ν_{max} (cm^{-1}) 3250.69, 3050.63, 1641.13; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (1H, s, ArH), 8.10 (1H, d, *J* = 8 Hz, ArH), 7.96 (1H, d, *J* = 8 Hz, ArH), 7.82 (1H, t, *J* = 6 Hz, ArH), 7.66 (1H, t, *J* = 6 Hz, ArH), 6.34 (1H, s, CH=C), 6.18 (1H, s, CH=C), 5.90 (1H, s, C*H*OH), 5.80 (1H, bs, OH), 3.65 (3H, s, OCH_3).

2-((2-Chloroquinolin-3-yl)(hydroxy)methyl)acrylonitrile (3d). Light brown solid; m.p. 118–120°C; R_f = 0.36 ($CHCl_3$/EtOAc 4:1) MS *m/z* 245.2 (M^+, 100%), 247.2 (M+2); IR (KBr) ν_{max} (cm^{-1}) 3233.45, 2923.59, 2226.96, 1589.59; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (1H, s, ArH), 8.20 (1H, d, *J* = 8 Hz, ArH), 8.0 (1H, d, *J* = 8 Hz, ArH), 7.90 (1H, t, *J* = 6 Hz, ArH), 7.60 (1H, t, *J* = 6 Hz, ArH), 6.80 (1H, s, D_2O exchangeable, OH), 6.42 (1H, s, —CH=C), 6.38 (1H, s, —CH=C), 5.70 (1H, s, C*H*OH); Elemental analysis found: C, 63.67; H, 3.58.;N, 11.63; $C_{13}H_9N_2ClO$ requires C, 63.81; H, 3.71; N, 11.45.

N-(2-chloro-3-(hydroxy(6-oxocyclohex-1-enyl)methyl)-7-phenoxyquinolin-6-yl)methanesulfonamide (3e). Pale yellow solid; m.p. 118–120°C; R_f = 0.35 ($CHCl_3$/EtOAc 4:1) MS *m/z* 472.9 (M^+, 100%) 475 (M+2); IR (KBr) ν_{max} (cm^{-1}) 3246.93 (OH), 2927.93 (CH), 1668.07 (CO), 1626.46 (C=C),1589.19;

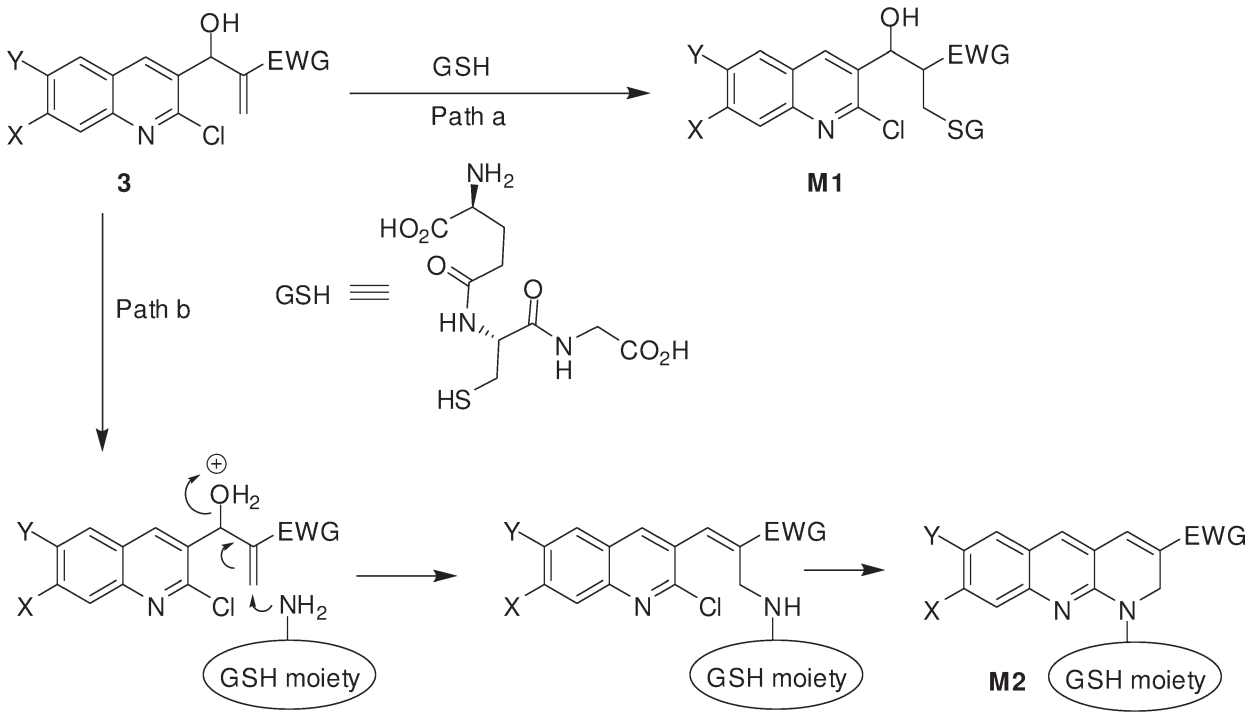


Figure 5. Proposed interaction of MBH adduct with GSH.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (1H, D_2O exchangeable, NH), 8.28 (1H, s, ArH), 7.98 (1H, s, ArH), 7.50 (2H, t, J = 8 Hz, ArH), 7.30 (1H, d, J = 8 Hz, ArH), 7.20 (2H, d, J = 8 Hz, ArH), 7.0 (1H, s, ArH), 6.90 (1H, s, —CH=C), 5.80 (1H, s, —C*H*OH), 5.75 (1H, bs, OH), 3.0 (3H, s, —SO_2CH_3), 2.40 (4H, m, $\overline{2}CH_2$) 2.00 (2H, m, CH_2); Elemental analysis found: C, 58.51; H, 4.36; N, 5.83; $C_{23}H_{21}N_2ClSO_5$ requires C, 58.41; H, 4.48; N, 5.92.

Ethyl 2-((2-chloro-6-(methylsulfonamido)-7-phenoxyquinolin-3-yl)(hydroxy)methyl) acrylate (3f). Cream colored solid; R_f = 0.40 ($CHCl_3$/EtOAc 4:1) m.p. 98–100°C; MS *m/z* 477.1 (M^+, 100%), 479.1 (M+2); IR (KBr) υ_{max} (cm^{-1}) 3205.98 (OH), 2925.72 (CH), 1668.59 (CO),1626.41(C=C), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (1H, D_2O exchangeable, NH), 8.28 (1H, s, ArH), 7.98 (1H, s, ArH), 7.50 (2H, t, J = 8 Hz, ArH), 7.30 (1H, t, J = 8 Hz, ArH), 7.20 (2H, d, J = 8 Hz, ArH), 7.0 (1H, bs, ArH), 6.35 (1H, s, —C*H*OH), 5.82 (1H, s, —CH=C—), 5.78 (1H, s, —CH=C—), 5.70 (1H, s, OH), 4.10 (2H, q, J = 6 Hz, OCH_2), 3.0 (3H, s, SO_2CH_3), 1.20 (3H, t, J = 6 Hz); Elemental analysis found: C, 55.27; H, 4.48.;N, 6.03; $C_{22}H_{21}N_2ClSO_6$ requires C, 55.40; H, 4.44; N, 5.87.

Methyl 2-((2-chloro-6-(methylsulfonamido)-7-phenoxyquinolin-3-yl)(hydroxy)methyl)acrylate (3g). Cream colored solid; R_f = 0.38 ($CHCl_3$/EtOAc 4:1); MS *m/z* 463.1 (M^+, 100%), 465.1 (M+2); IR (KBr) υ_{max} (cm^{-1}) 3246.90 (OH), 2927.95 (CH), 1668.0 (CO),1626.45 (C=C),1589.20; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (1H, D_2O exchangeable, NH), 8.28 (1H, s, ArH), 7.98 (1H, s, ArH), 7.50 (2H, t, J = 8 Hz, ArH), 7.30 (1H, t, J = 8 Hz, ArH), 7.20 (1H, d, J = 8 Hz, ArH), 7.0 (2H, bs, ArH), 6.35 (1H, s, —C*H*OH), 5.82 (1H, s, —CH=C—), 5.78 (1H, s, —CH=C—), 5.67 (1H, s, OH), 3.75 (3H, s, OCH_3), 3.0 (3H, s, SO_2CH_3); Elemental analysis found: C, 54.57; H, 4.08; N, 5.97; $C_{21}H_{19}N_2ClSO_6$ requires C, 54.49; H, 4.14; N, 6.05.

N-(2-Chloro-3-(2-cyano-1-hydroxyallyl)-7-phenoxyquinolin-6-yl)methanesulfonamide (3h). Pale yellow solid; m.p. 120–122°C; R_f = 0.39 ($CHCl_3$/EtOAc 4:1) MS *m/z* 429.9 (M^+, 100%), 432 (M+2); (KBr) υ_{max} (cm^{-1}) 3253.98 (OH), 2927.93 (CH), 2852.30 (CH), 2226.35 (CN), 1626.46 (C=C), 1589.19; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (1H, s, D_2O exchangeable NH) 8.60 (1H, s, ArH), 8.20 (1H, s, ArH), 7.60 (2H, t, J = 8 Hz, ArH), 7.40 (1H, t, J = 6 Hz, ArH), 7.20 (2H, d, J = 8 Hz, ArH), 7.00 (1H, s, ArH), 6.80 (1H, s, D_2O exchangeable, OH), 6.32 (1H, s, CH=C), 6.28 (1H, s, CH=C), 5.60 (1H, s, C*H*OH), 3.20 (3H, s, —SO_2CH_3); Molecular formula: Elemental analysis found: C, 55.51; H, 3.78.; N, 9.93; $C_{20}H_{16}ClN_3O_4S$ requires C, 55.88; H, 3.75; N, 9.77.

N-(2-Chloro-3-(hydroxy(6-oxocyclohex-1-enyl)methyl)-7-phenoxyquinolin-6-yl)formamide (3i). Pale yellow solid; m.p. 110–112°C; R_f = 0.45 ($CHCl_3$/EtOAc 4:1); MS *m/z* 423.2 (M^+, 100%), 425.2 (M+2, 33%); IR (KBr) υ_{max}(cm^{-1}) 3354.36 (OH), 2926.33 (CH), 1674.67 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (1H, s, D_2O exchangeable, NH), 8.90 (1H, s, CHO), 8.50 (1H, s, ArH), 8.30 (1H, s, ArH), 7.55 (2H, m, ArH), 7.30 (3H, m, ArH), 7.0 (1H, s, ArH), 6.90 (1H, s, CH=C), 5.85 (1H, s, C*H*OH), 5.75 (1H, s, OH), 2.85 (2H, m), 2.20 (2H, m), 2.0 (2H, m); Elemental analysis found: C, 65.15; H, 4.58.; N, 6.78; $C_{23}H_{19}ClN_2O_4$ requires C, 65.33; H, 4.53; N, 6.62.

Ethyl 2-((2-chloro-6-formamido-7-phenoxyquinolin-3-yl)-(hydroxy)methyl)acrylate (3j). White solid; m.p. 120–122°C; R_f = 0.45 ($CHCl_3$/EtOAc 4:1); MS *m/z* 427.0 (M^+, 100%), 428.9 (M+2); IR (KBr) υ_{max} (cm^{-1}) 3372.56 (OH), 2925.23 (CH), 1694.0 (CO), 1620 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (1H, s, D_2O exchangeable, NH), 8.85 (1H, s, CHO), 8.45 (1H, s, ArH), 8.25 (1H, s, ArH), 7.60 (3H, m, ArH), 7.25 (2H, m, ArH), 7.0 (1H, s, ArH), 6.40 (1H, s, OH),

6.05 (1H, s, C*H*OH), 5.85 (1H, s, CH=C), 5.80 (1H, s, CH=C), 4.0 (2H, q, J = 3.8 Hz, OCH_2), 1.2 (3H, t, J = 3.8 Hz, CH_3); Elemental analysis found: C, 61.53; H, 4.48.; N, 6.73; $C_{22}H_{19}ClN_2O_5$ requires C, 61.90; H, 4.49; N, 6.56.

Methyl 2-((2-chloro-6-formamido-7-phenoxyquinolin-3-yl)-(hydroxy)methyl)acrylate (3k). Pale yellow solid; m.p. 98–100°C; R_f = 0.41 ($CHCl_3$/EtOAc 4:1); MS m/z 413.3 (M^+, 100%) 415.3 (M+2,33%); IR (KBr) υ_{max} (cm^{-1}) 3372.56 (OH), 2925.23 (CH), 1694.0 (CO), 1620 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (1H, s, D_2O exchangeable, NH), 8.85 (1H, s, CHO) 8.45 (1H, s, ArH), 8.25 (1H, s, ArH), 7.55 (2H, m, ArH), 7.36 (3H, m, ArH), 7.0 (1H, s, ArH), 6.40 (1H, s, OH), 6.10 (1H, s, C*H*OH), 5.85 (1H, s, CH=C), 5.80 (1H, s, CH=C), 3.85 (3H, s, OCH_3); Molecular formula: Elemental analysis found: C, 61.33; H, 4.10.; N, 6.63; $C_{21}H_{17}ClN_2O_5$ requires C, 61.10; H, 4.15; N, 6.79.

N-(2-chloro-3-(2-cyano-1-hydroxyallyl)-7-phenoxyquinolin-6-yl)formamide (3l). Pale yellow solid; m.p. 108–110°C; R_f = 0.38 ($CHCl_3$/EtOAc 4:1); MS m/z 380.0 (M^+, 100%), 381.9 (M+2); IR (KBr) υ_{max} (cm^{-1}) 3253.90 (OH), 2925.98 (CH), 2227.50 (CN), 1689.62 (CO); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.5 (1H, s, D_2O exchangeable, NH), 8.9 (1H, s, CHO), 8.5 (2H, d, J = 6 Hz, ArH), 7.62 (2H, m, ArH), 7.30 (3H, m, ArH), 7.0 (1H, s, ArH), 6.70 (1H, s, OH), 6.32 (1H, s, CH=C), 6.28 (1H, s, CH=C), 5.6 (1H, s, C*H*OH); Elemental analysis found: C, 63.51; H, 3.71.; N, 11.01; $C_{20}H_{14}ClN_3O_3$ requires C, 63.25; H, 3.72; N, 11.06.

Pharmacology. ***Chemicals and reagents.*** Dulbecco's modified eagle medium (DMEM), L-glutamine, streptomycin, and penicillin were obtained from Sigma-Aldrich. Foetal bovine serum was procured from PAA Biotech, Germany. All other fine chemicals/reagents used in this study were of cell culture grade and obtained from Sigma-Aldrich and/or Merck.

Cell line and culture conditions. Pc-3 (human prostate cancer cell line) was obtained from National Centre for Cell Science, Pune, India. The cells were grown in DMEM culture medium supplemented with 2-m*M* L-glutamine, 10% FBS, penicillin (50 IU/mL) and streptomycin (50 μg/mL) at a temperature of 37°C in a humidified incubator with a 5% CO_2 atmosphere and passaged twice weekly to maintain a subconfluent state.

Preparation of test compounds. Test compounds were dissolved in DMSO and were diluted appropriately with culture media before treatment of cells. The final concentration of DMSO used in the culture medium was less than 0.2%.

MTT assay for cytotoxicity. The viability of the cells was assessed by MTT [3,4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide] assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product [12]. Etoposide, a known anticancer drug was used as a reference compound in this assay. Cells (1×10^4) were placed in a 96-well plate. After 24 h, they were treated with different concentration (0 – 10 μ*M*) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media-containing compound were carefully removed by aspiration. 0.4 mg/mL (100 μL) MTT in PBS was added to each well and incubated in the dark for 4 h. 100 μL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent dead cells, whereas absorbance from nontreated control cells was defined as 100% live cells. The percent dead cells was plotted (*Y*-axis) against concentration (*X*-axis) of compounds, where IC_{50} values could be interpolated from the graph.

Acknowledgments. The author (S. Pal) thanks M. N. Raju, the Chairman of M. N. R. Educational Trust for his constant encouragement.

REFERENCES AND NOTES

[1] Basavaiah, D.; Rao, A. J.; Satyanarayana, T. Chem Rev 2003, 103, 811.

[2] Ciganek, E. In Organic Reactions; Paquette, L. A., Ed.; Wiley: New York, 1997; Vol. 51, pp 201–350.

[3] Basavaiah, D.; Rao, P. D.; Hyma, R. S. Tetrahedron 1996, 52, 8001.

[4] Kim, J. N.; Lee, K. Y. Curr Org Chem 2002, 6, 627.

[5] Eicher, T.; Hauptmann, S. The Chemistry of Heterocycles, Georg Thieme Verlag, Stuttgart, 1995, p. 329.

[6] See, for example: Jones, G. In Comprehensive Heterocyclic Chemistry, Boulton, A. J.; McKillop, A, Ed.; Pergamon: Oxford, 1984; Vol. 2, p 395.

[7] Madapa, S.; Singh, V.; Batra, S. Tetrahedron 2006, 62, 8740 and references cited therein.

[8] Porto, R. S.; Amarante, G. W.; Cavallaro, M.; Poppi, R. J.; Coelho, F. Tetrahedron Lett 2009, 50, 1184.

[9] Coelho, F.; Veronese, D.; Pavam, C. H.; de Paula, V. I.; Buffon, R. Tetrahedron 2006, 62, 4563.

[10] Kohn, L. K.; Pavam, C. H.; Veronese, D.; Coelho, F.; De Carvalho, J. E.; Almeida, W. P. Eur J Med Chem 2006, 41, 738.

[11] O'Dell, D. K.; Nicholas, K. M. J. Org Chem 2003, 68, 6427.

[12] Bose, D. S.; Idrees, M.; Jakka, N. M.; Rao, J. V. J Comb Chem 2010, 12, 100.

[13] Roopan, S. M.; Khan, F. R. N. ARKIVOC 2009, xiii, 161.

[14] Yu, L.; Wu, W. K. K.; Li, Z. J.; Liu, Q. C.; Li, H. T.; Wu, Y. C.; Cho, C. H. Mol Pharmacol 2009, 75, 1364.

[15] Roberts, L. J.; Morrow, J. D. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout, in Goodman & Gilman's The Pharmacological Basis of Therapeutics; Hardman, J. G.; Limbird, L. E.; Gilman A. G., Eds.; McGraw-Hill: New York, 2001, pp 687–731.

[16] Zhong, W.; Lin, F.; Chen, R.; Su, W. Synthesis 2009, 2333.

[17] Ali, M. M.; Tasneem, K. C.; Rajanna, P. K.; Prakash, S. Synlett 2001, 251.

[18] Su, W. K.; Zhuang, Y. G.; Wu, D. Z.; Zhong, W. H. Org Prep Proced Int 2007, 39, 195.

[19] Su, W. K.; Li, Z. H.; Zhao, L. Y. Org Prep Proced Int 2007, 39, 495.

[20] Mosmann, T. J. Immunol. Methods 1983, 65, 55.

[21] Reddy, L. V.; Nakka, M.; Suman, A.; Ghosh, S.; Helliwell, M.; Mukkanti, K.; Mukherjee, A. K.; Pal, S. J. Heterocycl. Chem. 2010, in press.

[22] Pericherla, S.; Mareddy, J.; Geetha, R. D. P.; Gollapudi, P. V.; Pal, S. J. Braz. Chem. Soc. 2007, 18, 384.

[23] McCarthy, T. J.; Hayes, E. P.; Schwartz, C. S.; Witz, G. Fundam Appl Toxicol 1994, 22, 543.

[24] Russo, A.; DeGraff, W.; Friedman, N.; Mitchell, J. B. Cancer Res 1986, 46, 2845.

[25] Tew, K. D. Cancer Res 1994, 54, 4313.

[26] Delbressine, L. P. C.; Van Balen, H. C. J. G.; Seutter-Berlage, F. Arch Toxicol 1982, 49, 321.

[27] Basavaiah, D.; Reddy, B. S.; Badsara, S. S. Chem Rev 2010, 110, 5447.