Chemistry of Heterocyclic Compounds, Vol. 46, No. 12, March, 2011 (Russian Original Vol. 46, No. 12, December, 2010)

NOVEL SYNTHESIS AND CYTOTOXIC ACTIVITY OF SOME CHROMENO[3,4-*b*]PYRROL-4(3H)-ONES

S. S. Soman[1]*, T. H. Thaker[1], and R. A. Rajput[2]

1-Methylchromeno[3,4-b]pyrrol-4(3H)-one and 1-phenylchromeno[3,4-b]pyrrol-4(3H)-one derivatives have been synthesized by cyclization of amides obtained by condensing α-halo ketones with 3-amino-chromenones, which show cytotoxic activity against lung, colon, and breast cancer cell lines.

Keywords: benzopyrones, halo ketones, chromeno[3,4-*b*]pyrrol-4(3H)-ones, cytotoxic agents.

Benzo-α-pyrones (2H-chromen-2-ones), commonly known as coumarins are reported to possess a wide range of biological activities [1–4]. Many natural and synthetic coumarins are reported to be cytotoxic agents [5]. Coumarins react with DNA by means of intercalation between two base pairs of DNA. There they form cross linkage between two chains of DNA molecule, which is responsible for the carcinogenic and mutagenic effects. 3,4-Substituted coumarins have been reported to inhibit the proliferation of a number of human malignant cell lines in vitro [6–7] and to possess anticoagulant and antithrombotic activities. These properties and our long and continued interest in coumarin chemistry [8–10] encouraged us to synthesize new coumarin derivatives annelated at positions 3 and 4 by the pyrrole ring. Literature survey reveals various methods for synthesis of such five-membered heterocycle using metal-catalyzed reactions [11], which involves use of very costly palladium metal. We report herein a very simple and novel method of synthesis of 3,4-annelated pyrrole derivatives of benzopyrones. To the best of our knowledge, this method has not been used for the synthesis of the pyrrole moiety.

3-Acetamidocoumarin (**1**) was condensed with different aldehydes in the presence of a base to get the corresponding chalcones, but, instead, unreacted starting material was recovered. Therefore, acid hydrolysis of the compound **1** was carried out to obtain 3-amino-2H-chromen-2-one (**2a**) (Scheme 1) [12]. It was then condensed with cinnamoyl chloride in the presence of pyridine to produce the corresponding amide **3a**.

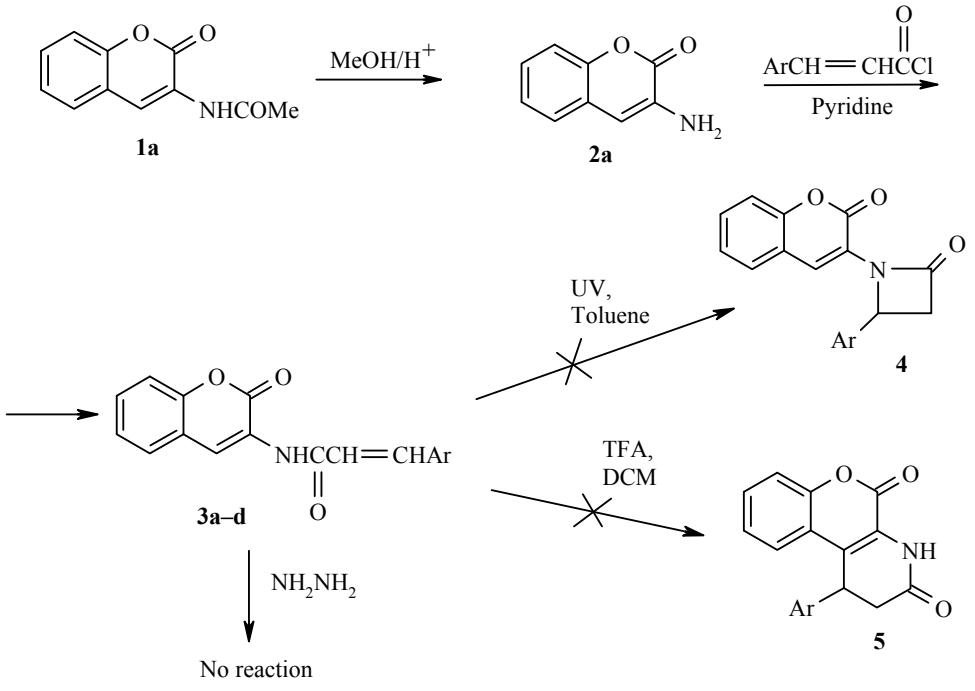
Similarly, compound **2a** was condensed with different derivatives of cinnamoyl chloride in pyridine to give the corresponding 3-(3-arylacryloylamino)chromen-2-ones **3b–d**. The structures of all these compounds were confirmed by their IR and NMR spectra (Scheme 1).

* To whom correspondence should be addressed, e-mail: shubhangiss@rediffmail.com, tirth1982@yahoo.co.in.

[1]Department of Chemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390002, India.
[2]National Institute of Pharmaceutical Education and Research, Thaltej, Ahmedabad, India.

Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 12, pp. 1872-1878, December, 2010. Original article received January 22, 2010, in revised form November 10, 2010.

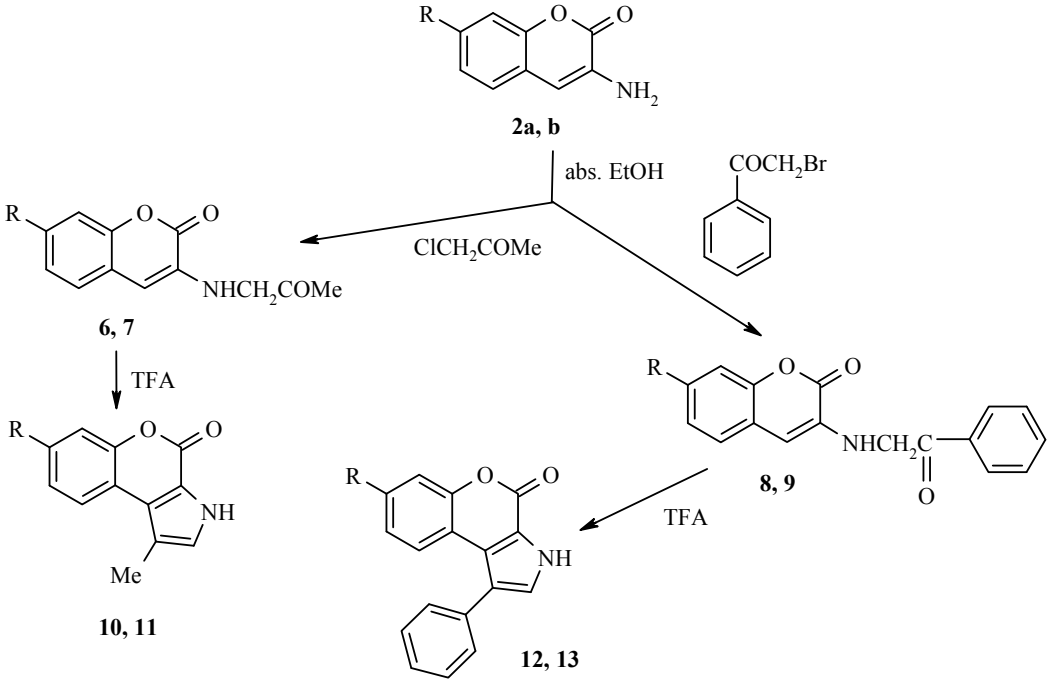
Scheme 1



3–5 a Ar = Ph, **b** Ar = *p*-ClC_6H_4, **c** Ar = *p*-$O_2NC_6H_4$, **d** Ar = *p*-$MeOC_6H_4$

Reaction of compounds **3a-d** with hydrazine or photochemical cyclization [13] in a solvent like toluene to get azetidinones **4** or heterocyclization in trifluoroacetic acid (TFA) [14] to get dihydropyridone **5** failed. Since no desired product was obtained, the new methodology was used to synthesize pyrrole-annelated benzopyrones (Scheme 2).

Scheme 2



2a,6,8,10,12 R = H; **2b,7,9,11,13** R = OH

TABLE 1. Inhibition Compared to Control (Paclitaxel)

Compound	Concentration, ng/ml	Inhibition, %		
		Cell line type		
		Lung	Colon	Breast
10	250	31.8	33.5	38.1
	500	54.3	52.2	53.5
	1000	66.5	61.5	75.7
	2000	85.0	80.2	87.2
11	250	54.3	49.8	56.1
	500	72.2	68.8	77.7
	1000	78.1	79.5	83.4
	2000	91.1	88.2	93.4

Aminochromenones **2a**,**b** were condensed with different α-halo ketones to get 3-(2-oxopropylamino)-chromen-2-one (**6**), 7-hydroxy-3-(2-oxopropylamino)chromen-2-one (**7**), 3-(2-oxo-2-phenylethylamino)chromen-2-one (**8**), and 7-hydroxy-3-(2-oxo-2-phenylethylamino)chromen-2-one (**9**). They were then cyclized in TFA to get chromeno[3,4-*b*]pyrrol-4(3H)-ones **10**–**13** (Scheme 2). The structures of all compounds were confirmed by their IR and NMR spectra. The disappearance of the signal at δ 4.9 for CH_2 proton from the NMR spectra of **10**-**13** clearly indicated that cyclization took place. In conclusion, we found that the new methodology worked well for the synthesis of new pyrrole derivatives of coumarin in good yields.

Cytotoxicity screening models provide important preliminary data to help select plant extracts with potential anticancer properties for future work.

The cytotoxic activity study of compounds **10** and **11** was done using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay against various cell lines of colon, lung, and breast cancer. It was observed that both compounds show good activity against all the cell lines.

To conclude, this paper describes a simple and easy method to synthesize pyrrole derivatives of benzopyrone, which show cytotoxic activity against lung, colon, and breast cancer cell lines.

EXPERIMENTAL

The melting points were taken in scientific open capillaries and are uncorrected. The IR spectra in KBr were recorded on a Shimadzu IR-408 spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on Bruker DRX-400 or Bruker DRX-300 FT NMR instruments using tetramethylsilane as an internal standard. Mass spectra were recorded on a QP 5050A GC-MS spectrometer. Elemental analyses of compounds were done on a Carlo Erba-1108 elemental analyzer. Acme Silica gel (mesh size 60–120) was used for column chromatography.

3-Amino-2H-chromen-2-one (2a) was synthesized from compound **1a** following a published procedure [12].

3-Amino-7-hydroxy-2H-chromen-2-one (2b). A mixture of resorcaldehyde (5.0 g, 0.036 mol), N-acetylglycine (3.6 g, 0.036 mol), acetic anhydride (15 ml, 0.14 mol), and sodium acetate (7.4 g, 0.9 mol) was heated on a steam bath for 6 h to give light-yellow 7-hydroxy-N-(2-oxo-2H-chromen-3-yl)acetamide (**1b**), which was recrystallised from alcohol–water, 1:1; mp 243°C. Refluxing of compound **1b** with methanolic HCl (70 ml, 10%) gave 3-aminocoumarin **2b**, which was recrystallized from alcohol and obtained as a light-yellow solid, mp 227°C, yield 52.2%.

Preparation of Amides 3a-d (General Method). To a mixture of compound **2a** (1.61 g, 10 mmol), triethylamine (1.4 ml, 10 mmol), and a catalytic amount of pyridine in dichloromethane (25 ml), different

cinnamoyl chlorides (10 mmol) were added dropwise at 0–5°C, with constant stirring. The reaction mixture was stirred at 0-5°C for 6 h. The organic phase was then partitioned at first with sat. $NaHCO_3$ and then with water. The organic portions were combined and dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated to give a crude product. It was recrystallized using ethanol–DMF, 8:2, to give different pure cinnamides.

N-(2-Oxo-2H-chromen-3-yl)-3-phenylacrylamide (3a) (1.40 g, 48%) was obtained as a brown solid; mp 226°C. IR spectrum, ν, cm^{-1}: 1205, 1536, 1630, 1674, 1715, 3313. ^1H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.33-7.49 (6H, m, H-α,β, H-5–8); 7.62–7.74 (5H, m, H-2'–6'); 8.79 (1H, s, H-4); 9.94 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 115.5, 119.4, 121.5, 123.4, 124.6, 127.4, 127.6, 128.5, 129.1, 129.5, 134.5, 141.1, 149.4, 157.4, 165.0. Found, %: C 74.11; H 4.51; N 4.92. $C_{18}H_{13}NO_3$. Calculated, %: C 74.23; H 4.46; N 4.81.

3-(4-Chlorophenyl)-N-(2-oxo-2H-chromen-3-yl)acrylamide (3b) (1.69 g, 52%) was obtained as light-brown solid; mp 257°C. Found, %: C 66.23; H 3.51; N 4.52. $C_{18}H_{12}ClNO_3$. Calculated, %: C 66.36; H 3.69; N 4.30.

3-(4-Nitrophenyl)-N-(2-oxo-2H-chromen-3-yl)acrylamide (3c) (1.88 g, 56%) was obtained as a yellow solid; mp 261°C. IR spectrum, ν, cm^{-1}: 1182, 1519, 1537, 1621, 1677, 1708, 3328. ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 7.31–7.35 (2H, m, H-6,8); 7.42–7.46 (1H, d, *J* = 15.6, H-α); 7.48 (1H, m, H-7); 7.56-7.58 (1H, m, H-5); 7.69-7.73 (1H, d, *J* = 15.7, H-β); 7.77–7.79 (2H, d, *J* = 8.7, H-2',6'); 8.24–8.27 (2H, d, *J* = 8.7, H-3',5'); 8.87 (1H, s, H-4); 9.85 (1H, s, NH). Found, %: C 64.16; H 3.52; N 8.40. $C_{18}H_{12}N_2O_5$. Calculated, %: C 64.28; H 3.57; N 8.33.

3-(4-Methoxyphenyl)-N-(2-oxo-2H-chromen-3-yl)acrylamide (3d) (2.02 g, 63%) was obtained as a light-brown solid; mp 271°C. Found, %: C 71.21; H 4.55; N 4.42. $C_{19}H_{15}N_4O$. Calculated, %: C 71.10; H 4.67; N 4.36.

3-(2-Oxopropylamino)chromen-2-one (6). A mixture of compound **2a** (1.61 g, 10 mmol), α-chloro-acetone (0.80 ml, 10 mmol), and a catalytic amount of potassium iodide was refluxed for 5 h and then allowed to reach room temperature. The precipitated crude product was filtered off, dried, and purified by column chromatography using ethyl acetate–petroleum ether, 1:9, as eluent. The title compound **6** (0.89 g, 41%) was obtained as a yellow solid; mp 210°C. IR spectrum, δ, cm^{-1}: 745, 1216, 1357, 1458, 1534, 1629, 1702, 1720, 3329. ^1H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.55 (3H, s, CH_3); 4.24 (2H, s, CH_2); 6.70 (1H, s, H-8); 7.18-7.22 (1H, m, H-6); 7.25-7.30 (2H, m, H-5,7); 8.07 (1H, s, H-4). Found, %: C 66.42; H 5.01; N 6.51. $C_{12}H_{11}NO_3$. Calculated, %: C 66.36; H 5.06; N 6.45.

7-Hydroxy-3-(2-oxopropylamino)chromen-2-one (7). Compound **2b** was used instead of compound **2a**, and the procedure was the same as that for compound **6**. The title compound **7** (1.12 g, 48%) was obtained as an orange solid; mp 232ºC. IR spectrum, ν, cm^{-1}: 1127, 1153, 1165, 1243, 1289, 1460, 1508, 3224, 3349, 3630-2849 (broad). ^1H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 2.64 (3H, s, CH_3); 4.23 (2H, s, CH_2); 6.69-6.79 (2H, m, H-6,8); 7.11-7.13 (1H, d, *J* = 8.4, H-5); 7.38 (1H, s, H-4); 9.33 (1H, s, NH); 11.82 (1H, s, OH). Found, %: C 61.58; H 4.81; N 6.05. $C_{12}H_{11}NO_4$. Calculated, %: C 61.80; H 4.72; N 6.00.

3-(2-Oxo-2-phenylethylamino)chromen-2-one (8). A mixture of compound **2a** (1.61 g, 10 mmol) and phenacyl bromide (1.98 g, 10 mmol) in absolute ethanol was refluxed for 5 h and then allowed to reach room temperature. The precipitated crude product was filtered off, dried, and recrystallized from absolute ethanol. The title compound **8** (1.56 g, 56%) was obtained as a yellow solid; mp 218°C. IR spectrum, ν, cm^{-1}: 1508, 1606, 1628, 1705, 3328, 3403. ^1H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 4.63–4.65 (2H, d, *J* = 4.8, CH_2); 6.36-6.39 (1H, t, *J* = 4.8, NH); 7.25-7.29 (1H, dd, *J* = 2.7, *J* = 8.4, H-8); 7.32–7.39 (3H, m, H-5–7); 7.40–7.55 (5H, m, Ar); 8.07 (1H, s, H-4). Found, %: C 73.06; H 4.69; N 5.28. $C_{17}H_{13}NO_3$. Calculated, %: C 73.11; H 4.66; N 5.01.

7-Hydroxy-3-(2-oxo-2-phenylethylamino)chromen-2-one (9). Compound **2b** was used instead of compound **2a**, and the procedure was the same as that for compound **8**. The title compound **9** (1.80 g, 61%) was obtained as a orange solid; mp 248°C. IR spectrum, ν, cm^{-1}: 1499, 1615, 1684, 1728, 3405, 3652–2880 (broad). ^1H NMR (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 4.63 (2H, d, *J* = 4.8, CH_2); 5.72 (1H, t, *J* = 4.8, NH); 6.43 (1H, s, H-4);

6.76-6.80 (2H, m, H-6,8); 7.17-7.19 (1H, d, *J* = 8.3, H-5); 7.52–7.70 (3H, m, H-3'–5'); 8.04-8.06 (2H, m, H-2',6'); 9.39 (1H, s, OH). Found, %: C 69.03; H 4.44; N 4.82. $C_{17}H_{13}NO_4$. Calculated, %: C 69.15; H 4.40; N 4.74.

Preparation of Compounds 10–13 (General Method). Trifluoroacetic acid was added in catalytic amount to a stirred solution of compounds **6-9** (10 mmol) in acetic acid. The mixture was refluxed for 12 h and poured onto crushed ice, and the crude product was filtered off, dried, and purified by gradient column chromatography using ethyl acetate and petroleum ether as eluent. The pure product was eluted with ethyl acetate–petroleum ether, 1:9.

1-Methylchromeno[3,4-*b*]pyrrol-4(3H)-one (10) (1.35 g, 68%) was obtained as white crystals; mp 252°C. IR spectrum, ν, cm^{-1}: 707, 765, 1247, 1292, 1359, 1451, 1527, 1603, 1682, 1711, 3078, 3337, 3461. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 2.22 (3H, s, CH_3); 7.27-7.75 (4H, m, H-6–9); 8.64 (1H, s, H-2); 9.49 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 23.8, 115.4, 119.3, 123.0, 124.2, 124.4, 127.1, 127.8, 128.8, 149.3, 157.7, 169.9. GC-mass spectrum (CI), *m/z*: 204 $[M+4]^+$. Found, %: C 72.28; H 4.60; N 6.95. $C_{12}H_9NO_2$. Calculated, %: C 72.36; H 4.52; N 7.03.

7-Hydroxy-1-methylchromeno[3,4-*b*]pyrrol-4(3H)-one (11) (0.67 g, 31%) was obtained as a brown solid; mp 258°C. IR spectrum, ν, cm^{-1}: 1378, 1538, 1601, 1704, 3322, 3395, 2851–3751 (broad). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 2.20 (3H, s, CH_3); 6.73-6.81 (2H, m, H-6,8); 7.31–7.33 (1H, d, *J* = 8.4, H-9); 8.55 (1H, s, H-2); 9.10 (1H, s, OH); 9.95 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 23.7, 101.9, 111.2, 113.3, 120.6, 124.9, 128.0, 151.0, 158.1, 159.1, 169.4. GC-mass spectrum (CI), *m/z*: 220 $[M+4]^+$. Found, %: C 66.61; H 4.12; N 6.44. $C_{12}H_9NO_3$. Calculated, %: C 66.97; H 4.18; N 6.51.

1-Phenylchromeno[3,4-*b*]pyrrol-4(3H)-one (12) (1.33 g, 51%) was obtained as a light-brown solid; mp 178°C. Found, %: C 78.29; H 4.12; N 5.11. $C_{17}H_{11}NO_2$. Calculated, %: C 78.16; H 4.21; N 5.36.

7-Hydroxy-1-phenylchromeno[3,4-*b*]pyrrol-4(3H)-one (13). Compound **13** (0.78 g, 28%) was obtained as a red solid; mp 243°C. IR spectrum, ν, cm^{-1}: 1505, 1607, 1733, 3356, 2872–3607 (broad). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 6.78 (1H, s, H-6); 7.41–7.58 (5H, m, H-2,8,3',4',5'); 7.91–7.93 (1H, d, *J* = 8.5, H-9); 8.05–8.07 (2H, d, *J* = 8.7, H-2',6'); 9.42 (1H, s, NH); 10.82 (1H, s, OH). Found, %: C 73.69; H 4.02; N 5.01. $C_{17}H_{11}NO_3$. Calculated, %: C 73.64; H 3.97; N 5.05.

Biological Activity Study. The cell lines were procured from National Centre for Cell Science, Pune, India. The cell lines HCT-15 for colon cancer (DMEM medium), A 549 for lung carcinoma (RPMI medium), and MCF-7 for breast cancer (CMEM medium) were used for cytotoxic activity study.

MTT Assay [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide]. Exponentially growing cancer cells (HCT-15, A549 and MCF-7 cells) were harvested from 75 mm^2 flask, and a stock cell suspension was prepared. Then 5×10^4 cells/ml were seeded in a 96-well flat bottom tissue culture plate with 200 µl of complete medium and incubated for 24 h. The compounds were dissolved in the minimum amount of DMSO and were prepared in complete media without phenol red. All samples were first sterilized using Durapore polyvinylidene difluoride membrane filters of 0.22 µm (Millipore, Ireland). Paclitaxel (5 µM) was used as positive control. Cells were treated with different concentrations of compounds in 100 µl volume prepared in media without phenol red and with positive control (Paclitaxel), and were incubated for 48 h. The cells in the control group received no drug treatment. Each treatment was performed in three-well replicates. After the treatment, drug- containing media was removed and washed twice with 100 µl of phosphate buffered saline. To each well of the 96-well plate, 20 µl of MTT reagent (Merck, India) (stock: 5 mg/ml) was added and the wells incubated for 4 h at 37ºC. Plates were shaken for 10 min and inverted with gentle tapping on tissue paper to remove the media. To solubilize formazan crystals in the wells, 100 µl of 100% DMSO was added to each well. Plates were placed on a rotary shaker (R 100/TW LUCKHAM, England) and agitated for 15-20 min.

The optical density (O.D.) was measured by an Enzyme Linked Immunosorbent assay Bio-Rad plate reader at 570 nm with a reference wavelength of 630 nm. The O.D. of each well was read and expressed as percentage cell viability (absorbance of treated wells / absorbance of control wells × 100). Results were expressed as mean ± S.D. % cell viability values plotted against the crude extract concentrations. From the results, less than 50% survival at exposure time of 48 h was considered to be significant.

The authors are thankful to the Head of Department of Chemistry for providing necessary facilities. The authors are thankful to UGC, New Delhi, for funding. One of the authors (THT) is thankful to UGC, New Delhi, for providing a fellowship.

REFERENCES

1. M. E. Marshall, J. L. Mohler, K. Edmonds, B. Williams, K. Butler, M. Ryles, L. Weiss, D. Urban, A. Beuschen, and M. Markiewicz, *J. Cancer Res. Clin. Oncol*., **120**, 39 (1994).
2. E. Moran, E. Prosser, R. O'Kennedy, and R. D. Thomes, *J. Irish Coll. Physicians Surg*., **22**, 41 (1993).
3. C. P. Siegers and H. C. Bostelmann, *J. Irish Coll. Physicians Surg.*, **22**, 47 (1993).
4. R. B. Myers, M. Parker, and W. E. Grizzle, *J. Cancer Res. Clin. Oncol.*, **120**, 11 (1994).
5. G. Feuer, J. A. Kellen, and K. Kovacs, *Oncology*, **33**, 35 (1976).
6. L. R. Zacharski, M. Z. Wojtukiewicz, V. Constantini, D. L. Omstein, and V. A. Memoli, *Semin. Thromb. Hemost*., **18**, 104 (1992).
7. P. McCulloch and W. D. George, *Br. J. Cancer*, **59**, 179 (1989).
8. S. S. Soman, *Indian J. Chem*., **43B**, 624 (2004).
9. S. S. Soman, *Indian J. Chem*., **38B**, 545 (1999).
10. S. S. Soman, *Indian J. Chem.*, **38B**, 542 (1999).
11. G. Kirsch, S. Hesse, and A. Comel, *Curr. Org. Syn.*, 1, 47 (2004).
12. D. Chakravarty, S. P. Dutta, and A. K. Mitra, *Curr. Sci*., **34**, 177 (1965).
13. O. L. Chapmann and W. R. Adams, *J. Am. Chem. Soc.*, **89**, 4243 (1967).
14. L. Kelin, L. N. Foresee, and J. A. Tunge, *J. Org. Chem.*, **70**, 2881 (2005).