Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(1):397-403
(http://derpharmachemica.com/archive.html)

Synthesis and anticancer activity of some new 2-[(4-methy-2-oxo-2H- chromen-7-yl)-oxy]acetamide derivatives

Nirav N. Shah[1,3], Shubhangi S. Soman*[1], Pranav Buch[2] and B. Suresh[2]

[1]*Department of Chemistry, Faculty of Science, the M. S. University of Baroda, Vadodara, India*
[2]*Department of Zoology, Faculty of Science, the M. S. University of Baroda, Vadodara, India*
[3]*M/SGNFC Ltd., Bharuch, India*

ABSTRACT

*Newly designed2-[(4-methy-2-oxo-2H- chromen-7yl) oxy] acetamide derivatives (**4a-4g**) have been synthesized in good yields and characterized by advanced spectroscopic methods. The synthesized coumarinyloxyacetamide derivatives have been studied for their anticancer as well as antimicrobial activities.*

Keywords: Coumarinyloxy acetamides, anticancer activity.

INTRODUCTION

Coumarinheterocycle is one of the major classes of naturally occurring flavanoidor chromen compounds. Coumarin derivatives exhibit wide range of biological activities such as antimicrobial [1,2], anticoagulant [3], antiallergic, anticancer [4], anti-inflammatory [5], antioxidant [6,7] and calcium channel blocking activity [8]. Because of variety of biological activities, both synthetic and naturally occurring coumarin derivatives have been widely studied all over the world. Recently 3-amino coumarin derivatives have been reported as DPP-IV inhibitors from our laboratory [9].

Canceris fatal disease after cardiovascular in terms of morbidity and mortality affecting human health worldwide [10].On other side, multidrug resistance is an increasing concern with current antibacterial agents for treatment of infectious diseases [11]. The coumarincontaining antibiotic, novobiocin is active against grampositive bacteria as potent inhibitor of DNA replication [12].

From our laboratory we have reported amide derivatives of benzodifuran-2-carboxylic acid [13] as antimicrobial agents. We have also reported coumarin derivatives as anticancer agents [14]. It prompted us to combine amide moiety with coumarin derivatives, so that it may show antimicrobial as well as anticancer activity. Thus in continuation of our work on search of new coumarin derivatives, we report herein synthesis characterization and antibacterial as well as anticancer activity of coumarinyloxy amide derivatives.

MATERIALS AND METHODS

Experimental

Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus.IR spectra were recorded as KBr disc on Perkin Elmer RX-1 spectrophotometer.^{1}H NMR and ^{13}C NMR spectral data

were recorded from a Brucker Advance 400 spectrometer (400MHz). TLC was performed on silica gel F 254 plates (Merck).CHN elemental analyses were recorded on Eager Scientific AnalyticalInstrument.

Chemistry

The starting compound 7-hydroxy-4-methyl -2H-chromen-2-one was preparedby the reported method [15].

Synthesis of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate (2)

To the stirred solution of **1** (1 g, 5.0mmol, 1.0 eq) in DMF, ethylchloroacetate (0.9 g, 5 mmol, 1.2 eq) and K_2CO_3 (0.9 g, 6.5 mmol, 1.3eq) were added and the resulting mixture was refluxed for 16 h.After completion of reaction (monitored by TLC), reaction mixture was cooled to room temperature and poured on to crushed ice to give solid. The solid was filtered, washed with water and recrystallized from ethanol to give compound **2**. Yield85 %;m.p: 110-112°C; IR (KBr): 3085, 2950, 2880, 1750, 1705, 1615, 1375, 1210, 950 cm^{-1};^{1}H-NMR($CDCl_3$, 400MHz) δ1.33 (3H, d, *J* = 7.2 Hz), 2.41 (3H, s), 4.30(2H, q, *J* = 7.2 Hz), 4.70 (2H, s), 6.17 (1H, d, *J* = 1.2 Hz), 6.78 (1H, d, *J* = 2.8 Hz), 6.93 (1H, dd, *J* = 8.8, 2.8 Hz), 7.54 (1H, d, *J* = 8.8 Hz); X-ray crystal data (CCDC No. 1000266817) is given in Table-1

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (3)

Compound **2** (2.62 g, 10.0 mmol, 1.0eq) was treated with KOH (5.6 g, 100.0 mmol, 10.0eq) in Ethanol (30 ml). The resulting mixture wasrefluxed at a temperature 100–108 °C for 15 h. After that the reaction mixture was allowed to cool up toroom temperature, the resulting residue waspoured into ice cold water and acidified with Con. HCl to pH 2. The resulting solid was filtered off and washed with cold water. The solid was dissolved in saturated $NaHCO_3$ solution, acidified with conc.HCl to give white solid. The solid was filtered, washed with water, dried and recrystallized from ethanol to give compound **3** as a white solid. % Yield 95%, m.p : 246-248 °C; ^{1}H-NMR (DMSO-d_6, 400MHz) δ2.37 (3H, s), 4.82 (2H, s), 6.20 (1H, s), 6.94 (2H, s), 7.66 (1H, d, *J* = 8.4 Hz); ^{13}C-NMR (DMSO-d_6, 100 MHz)δ18.57, 65.23, 101.89, 111.80, 112.73, 113.95, 126.94, 153.85, 154.95, 160.59, 161.20, 170.11.

General procedure for the synthesis of amides (4a-4g):

A suspension of compound **3** (0.5 g, 2.136mmol) in dichloromethane(DCM) (25 mL) was cooled to 0-5°C. To this oxylyl chloride (0.46 mL, 5.34 mmol, 2.5 eq) was added drop wise at 0-5°C followed by a drop of DMF. The resulting solution was stirred at 0-5°C for30min and at RT for 3 h. The resulting solution was concentrated on rotavapor to give residue. The residue was taken in DCM and concentrated to remove traces of oxylyl chloride. The residue was dissolved in DCM (25 mL) and cooled to 0-5°C. To this cold solution, different amines (1.1 eq.) were added followed by the (TEA)triethylamine (1.5 eq). The resulting mixture was stirred at 0-5°Cfor 30 min and then at RT for the 16 h. The reaction mixture was washed with water (25 mL), sat. $NaHCO_3$(25 mL) and then brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give crude compound.Thecrude product was purified by column chromatography (60–120 mesh) using Pet. ether: EtOAc (70:30 to 20:80) to give corresponding amide (**4a-4g**) in good to excellent yield.

Spectral data:

4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one (4a)

Yield : 82 %; off white solid (EtOH); m.p°C: 160–162 °C;IR (KBr): 3063, 2973, 2875, 1724, 1656, 1612, 1559, 1437, 1331, 1264, 1199, 1155, 1081, 977, 871, 799 cm^{-1};^{1}H-NMR ($CDCl_3$, 400MHz)δ d 1.86–1.92 (2H, m), 2.06–1.99 (2H, m), 3.55-3.49 (4H, m), 4.70 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.80 (1H, d, *J* = 2.4 Hz), 6.96 (1H, dd, *J* = 8.8, 2.4 Hz), 7.51 (1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.68, 23.82, 26.24, 26.48, 67.36, 101.81, 101.91, 112.28, 112.51, 112.59, 114.23, 125.75, 152.52, 155.05, 161.00 161.16, 165.34; MASS: $[M+H]^+$ 288.10; Elemental Analysis for $C_{17}H_{19}NO_4$ Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 66.89;H 5.96 ;N 4.88

4-methyl-7-(2-oxo-2-piperidin-1-yl-ethoxy)-2*H*-chromen-2-one (4b)

Yield : 85 %; off white solid (EtOH); m.p°C: 172–174 °C;IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};^{1}H-NMR ($CDCl_3$, 400MHz)δ1.57–1.69 (6H, m), 2.38(3H, s), 3.44–3.73 (4H, m), 4.77 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.81(1H, d, *J* = 2.4 Hz), 6.94 (1H, dd, *J* = 8.8, 2.4 Hz), 7.50(1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.70, 24.37, 25.48, 26.48, 43.24, 46.20, 67.16, 101.97, 112.29,112.39, 114.22, 125.77, 152.52, 155.03, 161.02, 161.18, 164.98; Elemental Analysis for $C_{17}H_{19}NO_4$ Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 63.36;H 5.65;N 4.61.

4-methyl-7-(2-morpholin-4-yl-2-oxo-ethoxy)-2*H*-chromen-2-one (4c)
Yield: 85 %; white solid (EtOH);m.p:146–148 °C; IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};^{1}H-NMR (DMSO-d_6, 400MHz)δ d2.39 (3H, S), 3.36 (4H, br s), 3.76 (4H, br s), 4.95 (2H, s), 6.22 (1H, s), 6.98 (2H, br s), 7.68 (1H, d, *J* = 9.2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.58, 31.14, 52.40, 65.31, 101.99, 111.95, 112.77, 114.14, 127.00, 153.79, 154.98, 160.51, 161.00, 169.16; Elemental Analysis for $C_{16}H_{17}NO_5$:Calculated,%:C 63.36;H 5.65;N 4.62,Found,% C 67.76;H 6.36;N 4.61.

4-methyl-7-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-2*H*-chromen-2-one (4d)
Yield: 68 %; Off white solid (EtOH); m.p: 98-100 °C;IR (KBr):3476, 3419, 3069, 2924, 2806, 1716, 1644, 1610, 1437, 1391, 1372, 1258, 1207, 1157, 1138, 1079, 861 cm^{-1};^{1}H-NMR (DMSO-d_6, 400MHz)δ 2.27 (3H, s), 2.39(4H, s), 2.5 (3H, s), 3.5 (4H, S), 4.99 (2H, s), 6.21 (1H, d, *J* = 1.2 Hz), 6.97-6.94 (2H, m), 7.67 (1H, dd, *J* = 9.2, 1,2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.60, 41.23, 43.95, 45.66, 66.38, 101.94, 111.67, 113.06, 113.77, 126.79, 153.88, 155.01, 160.59, 161.66, 165.62; Elemental Analysis for $C_7H_{20}N_2O_4$ Calculated,%:C 64.54;H 6.37;N 8.86,Found,% C 64.54;H 6.37;N 8.86.

***N*-(4-methylphenyl)-2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy] acetamides (4e)**
Yield: 74%; off white solid (EtOH); m.p: 214-216 °C; IR (KBr): 3365, 3304, 3038, 2914, 1700, 1681, 1627, 1594, 1534, 1392, 1364, 1296, 1153, 1081, 887, 849, 819 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz)δδ 2.25 (3H, s), 2.40 (3H, s), 4.82 (2H, s), 6.23 (1H, s), 7.07-7.02 (2H, m), 7.11 (2H, d, *J* = 8.4 Hz), 7.51(2H, d, *J* = 8.4 Hz), 7.72 (1H, d, *J* = 8.8, Hz), 10.09 (1H, s); ^{13}C-NMR($CDCl_3$, 100 MHz): δ 18.61, 20.92, 67.74, 102.12, 111.90, 112.86, 114.08, 120.13, 127.02, 129.61, 133.19, 136.25, 153.84, 154.98, 160.53, 161.30, 166.05; Elemental Analysis for $C_{19}H_{17}NO_4$: Calculated,%:C 70.58;H 5.30;N 4.33,Found,% C 70.58;H 5.30;N 4.33.

Anticancer activity Method:
MTT assay
The compounds were tested for their cytotoxic potential on three types of cancer cells, *viz.*, A549 (lung cancer cell-line), MCF7 (breast cancer cell-line) and A375 (melanoma cell-line). The MTT assay was used to determine the effect of each compound on the proliferation of cancer cells.

A549, MCF7 and A375 cultures were purchased from National Centre for Cell Science, Pune, India. All growth media, supplements and reagents were purchased from HiMedia Labs, Mumbai, India. For the assay, cells were seeded at 10^5 cells/ml in a 96-well plate in dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). To each well, test compound was added at six different concentrations of 100µM, 50µM, 10µM, 5µM, 1µM and 0.5µM. Each concentration was tested in triplicates. The cells were incubated with these compounds at 37^{o}C under 5% CO_2 for 48 hours. Following this, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5mg/ml. Cells were incubated with this tetrazolium dye for 4 hours. Subsequently, purple crystals of formazan were observed in each well, formed as a metabolic product of MTT. These crystals were dissolved in Isopropanol and the absorbance in each well was recorded at 570nm in a microplate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability. IC_{50} values were calculated from concentration-response data using Graph Pad Prism software. Results are shown in Table 2.

Antimicrobial activity:
All the synthesized compounds were tested for their antibacterial activity against Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, *BacillusSubtilis*) by cup plate method [16] at 100 ppmconcentration in DMF solvent. Ampicillin was used as standard drug. All compounds did not show activity against all types of Gram positive and Gram negative strains.

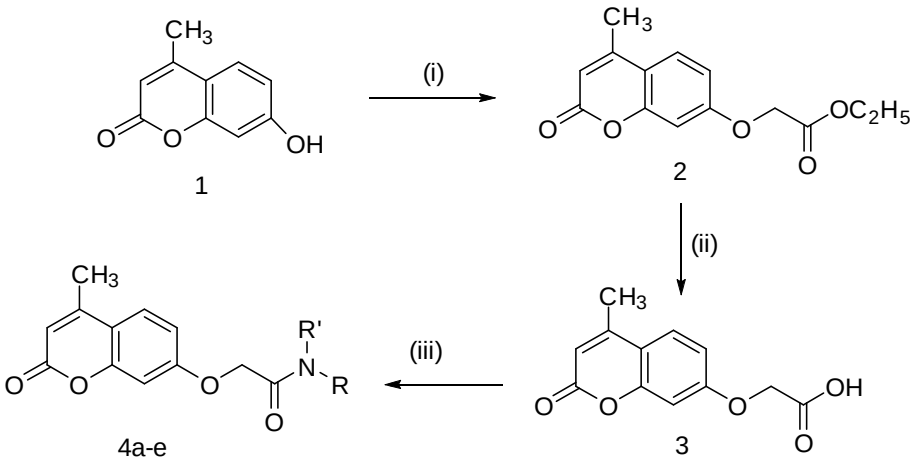
Antibacterial activity of all the synthesized compounds was tested in vitro by (cup plate method) serial agar dilution in which bacterial strains of Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, *Bacillus Subtilis*) were used, using serial agar dilution (cup plate method).The two microorganisms were cultured in petri dishes containing agar medium, cups (8 mm) were put onto the dishes and each synthesized compound dissolved in DMF (0.1 ml of 10 mg/ml) was added into the cups under aseptic condition. Then, the petri dishes were incubated at 37 ^{o}C for 24 h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was

measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments. Results are shown in Table 3.

RESULTS AND DISCUSSION

Chemistry

7-Hydroxy-4-methyl-benzopyran-2[H]-one (**1**) has been prepared by Pechmann condensation of resorcinol with ethyl acetoacetate using concentrated sulfuric acid. 7-Hydroxy-4-methyl coumarin (**1**), on reaction with ethyl chloro acetate in presence of anhydrous potassium carbonate and dry dimethyl formamide (DMF) gave 2-(4-methyl)-2-oxo-2[H]-chromen-7-yloxy) acetate **2**. The structure of **2** was proved from its IR, ^{1}H-NMR and ^{13}C-NMR. Moreover its single crystal was developed from methanol:Pet.ether by slow evaporation technique and proved the structure of **2 by** X-ray Single Crystal. Its CCDC no.is 1439494. It is observed that during single crystal formation transesterification has occurred, and ethyl ester get converted into methyl ester.



Reagents & Conditions: (i) $ClCH_2COOC_2H_5$, K_2CO_3, DMF reflux; (ii) Ethanolic KOH (15 %), reflux; (iii) (1) ClCOCOCl, DMC at 0-5°C, 30 min, RT,4 h; (2) RR'NH, TEA, DCM.

Scheme-1: Synthesis of carbyloxyacetamide derivatives of 4-methyl-7-hydroxy coumarin compounds

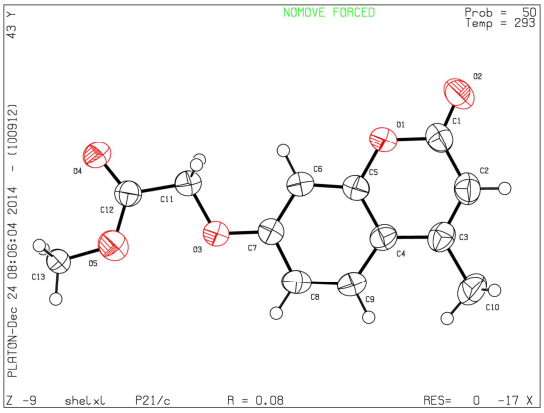


Figure 1: X-ray crystal structure of compound 2 as methyl ester

Table-1 Crystal data and structure refinement parameter for compound 2

Empirical formula	$C_{13}H_{12}O_5$
Formula Weight	248.23
Temperature/K	293(2)
Crystal system	Monoclinic
Space group	$P2_1/c$
a/ Å	10.0839(5)
b/ Å	14.5821(5)
c/ Å	8.4610(4)
α/°	90.00
β/°	112.403(6)
γ/°	90.00
Volume/Å3	1150.23/(9)
Z	4
$p_{Calc}^{mg/mm3}$	1.433
2Θ range for data collection	9.48 to 146.28°
Index range	$-11 \leq h \leq 12, -18 \leq k \leq 16, -10 \leq i \leq 10$
Reflections collected	5597
Independent reflections	2296[R(int)=0.0218]
Peak and hole e $Å^{-3}$	0.58/-0.49

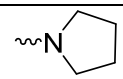
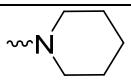
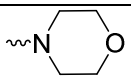
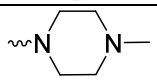
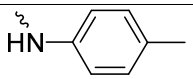
Then ester**2**was hydrolyzed by using aqueous KOH and then acidified withconc.HCl solution to give corresponding acid **3**. The obtained 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetic acid **3** was then converted into acid chloride in situ by the reaction with oxylyl chloride which was immediatelytreated with various amines (aromatic/aliphatic) to give corresponding 7-coumarinyloxyacetamides derivatives(**4a** - **4e**). All the synthesized compounds were purified by recrystallization from ethanol/column chromatography and were characterized by advanced spectral techniques.

The formation of coumarinyloxyacetamides was confirmed by IR spectroscopy. The formation of amide group was confirmed by the amide characteristic peak at 1680-1640 cm^{-1}corresponds to amide group and lactone carbonyl at 1700-1740cm^{-1} In^{1}H-NMR spectra, aromatic protons appeared in region of 6.12-7.68, the methylene group appeared in region of 4.70-4.95 and aliphatic protonsappeared in range of δ1.5-3.7.

Anticancer Activity

All the synthesized compounds were screened againstA549 (lung cancer cell-line) and one of the compound **4d** was screened against A375(melanoma cell-line). IC_{50}(μM) values were determined using Graph Pad prism softwarefor compounds **4a-e** as shown in Table 2.

Table 2:Anticancer activity (IC50, μg/mL) of substituted aminomethylnaphthopyrones4a-i

Compound	-NR^{1}R^2	IC_{50} (μM)[a]	
		A549	A375
4a		9.26	ND
4b		0.66	ND
4c		0.41	ND
4d		NA	0.041
4e		1.1nM	ND

[a]*IC_{50} values were determined using Graph Pad Prism software.*
NA= Not activeND = not determined

From the MTT assay, pyrrolidine compound **4a** showed better activity against A549 with IC_{50}value9.26 μM. On replacement of pyrrolidine with piperidine in compound **4b** resulted in compound with 14 fold higher activity

against A549 with IC_{50}value0.66 µM. Compound **4c** containing morpholine ring showed 22 fold higher activity compared to compound **4a** against A549 cell line with IC_{50}value0.41 µM. *N*-methyl piperazine compound **4d**did not show any activity against A549 cell line, but it showed very good activity against A375 cell line with IC_{50}value0.041µM. Interestingly, p-toluidine substituted compound **4e** showed excellent activity against A549 cell line with IC_{50}value1.1 nM.

Antimicrobial and antifungal activity

Table 3: Antimicrobial and antifungal activity of compounds4a-4e

Compounds	Gram –Ve bacteria		Gram +ve bacteria		Fungi
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus Subtilis*	*Candida Albicans*
4a	>228	>228	>228	>228	>228
4b	>228	>228	>228	>228	>228
4c	>228	>228	>228	>228	>228
4d	>228	>228	>228	>228	>228
4e	>228	>228	>228	>228	>228

Compounds **4a**-**4e** did not show any antimicrobial activity against tested gram –ve bacteria (*E. coli*, *P. aeruginosa)*and gram +ve bacteria (*S.aureus*, *B. subtilis)*. Also compounds **4a**-**4e** found inactive against fungi *C.Albicans*.

CONCLUSION

In conclusion, we have reported here synthesis of 7-coumarinyloxyacetamides derivatives(**4a** - **4e**) and their anticancer activity. Compounds **4a**-**4e** have shown promising anticancer activity against A549 (lung cancer cell-line). Compounds **4b** and **4c** are showing very good activity against A549 (lung cancer cell-line) with IC_{50} values 0.66 and 0.41 µM respectively. Compounds **4e** showed excellent activity against A549(lung cancer cell-line) with IC_{50} value1.1 nM. While compound **4d**is showing very good activity against A375 (melanoma cell-line) with IC_{50}value0.041 µM. Unfortunately all the synthesized compounds didn't show any antimicrobial activity.

Acknowledgment
The authors are thankful to the Heads, Department of chemistry, Department of Zoology, Faculty of science, The M. S. University of Baroda for providing laboratory facility. The authors are thankful to DST-PURSE program in the department of chemistry for X-ray single crystal analysis. One of the author NNS is thankful to M/s GNFC LTD for kind support.

REFERENCES

[1] G. L. Balaji, K. Rajesh, R. Priya, P. Iniyavan, R. Siva, V. Vijayakumar. *Med. Chem. Res.***2013**, *22*, 3185.
[2] T. Smyth, V.N. Ramachandran, W.F. Smyth,*Int. J. Antimicrob. Agents.***2009**, *33*,421.
[3] I. Manolov, C. Maichle-Moessmer, N. Danchev, *Eur. J. Med. Chem.***2006**, *41*, 882.
[4] (a) I.Kostova,*Curr. Med. Chem.*,*Anticancer agents* **2005**,*5*, 29. (b) S. Stanchev, G. Momekov, F. Jensen, I. Monolov, *Eur. J. Med. Chem.***2008**, *43*, 694.
[5] (a) M.Curini, G.Cravotto, F.Epifano, G.Giannone.,*Curr.Med.Chem.* **2006**,*13*, 199. (b) M. Ghate, R. A. Kusanur, M.V. Kulkarni, *Eur. J. Med. Chem.* **2005**, *40*,882.
[6] D. N. Nicolaides, K. C. Fylaktakidou, K. E. Litinas, D. Hadjipavlou-Litina,*Eur. J. Med. Chem.*, **1998**,*33*,715.
[7] K. C. Fylaktakidou, D. Hadjipavlou-Litina, K. E. Litinas, D. N. Nicolaides, *Curr. Pharm. Des.* **2004**, *10*, 3813.
[8] P. Valenti, A. Rampa, R. Budriesi, A. Bisi, A. Chiarnini, Biorg. Med. Chem. **1998,** *6*, 803.
[9] R. Sharma, S. S. Soman, *Pharmanest***2015**, *6*, 2679.
[10] J. B. Gibbs, *Science.* **2000**, *287*, 1969.
[11] World Health Organization, [WHO document WHO/CDS/CSR/RMD/2003] **2003**.
[12] (a) M. Kawase, B. Varu, A. Shah, N. Motohashi, S. Tani, S. Saito, S. Debnath, S. ahapatra, S. G. Dastidar, *Arzneimittelforschung*, **2001**, *51*, 67.
[13] S. S. Soman, T. H. Thaker, R. D. Baloni, *Asian J. Research Chem.* **2011**, *4*, 132.
[14] S.S Soman, J. N. Soni, G. S. Inamdar, G. P. Robertson, *Der PharmaChemica***2013**, *5*, 201.
[15] (a) E. H. Woodruff, *Org. Synth.* **1944**, *24*, 69. (b) K.Hirpara, S.Patel ,A. Joshi ,H. Parekh, *Ind. J. Heterocyclic Chem.***2004**, *13*, 221.

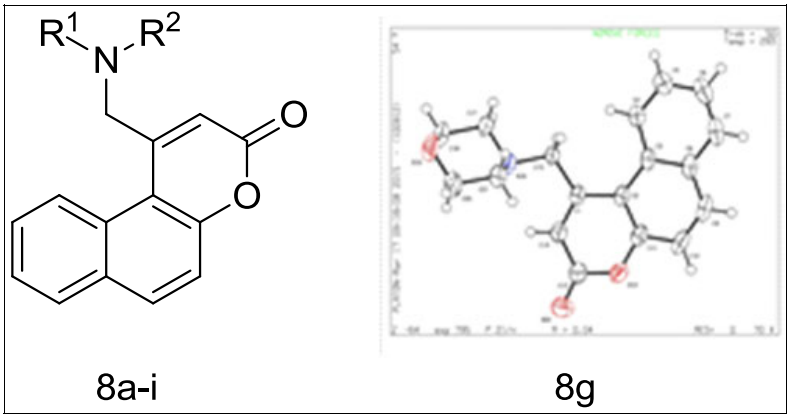
[16] A. L. Barry, **1977**, The antimicrobial susceptibility test principle and practices edited by Illuslea and Febiger, USA, 180. Biol Abstracts

Design, Synthesis, and Anticancer Activity of 3H–benzo[f]chromen-3-one Derivatives

Rina Soni,[a†] Shweta Umar,[b†] Nirav N. Shah,[a,c] Suresh Balkrishnan,[b] and Shubhangi S. Soman[a*]

[a]Department of Chemistry, Faculty of Science, The M. S. University of Baroda, Vadodara 390002, India
[b]Department of Zoology, Faculty of Science, The M. S. University of Baroda, Vadodara 390002, India
[c]GNFC, Bharuch, India
*E-mail: shubhangiss@rediffmail.com
†Both authors contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.
Received October 14, 2016
DOI 10.1002/jhet.2853
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



A series of substituted aminomethylbenzocoumarin derivatives **8a–i** have been synthesized, characterized, and structure of compound **8g** was confirmed by X-ray single crystal analysis. All the synthesized compounds were tested for their anticancer activity against cancer cell lines A549 (lung carcinoma cell line), MCF7 (breast cancer cell line), and A375 (melanoma cell line). Compounds **8a**, **8f**, and **8h** showed excellent growth inhibitory activity against all three cell lines, respectively. Compounds **8a** and **8f** were also found to be quite promising at very low concentration as an anticancer agent against MCF7 and A549 cell lines. Compounds **8g** and **8i** showed excellent antimitotic activity with IC_{50} 0.32 and19.98 n*M* for A549 cell line.

J. Heterocyclic Chem., **00,** 00 (2017).

INTRODUCTION

Coumarin, also known as benzopyrone, is a class of compound found in nature with wide range of applications. It falls under the flavonoid class of plant as a secondary metabolite and found as bioactive compounds in fruits, vegetables, spices, and herbs [1–4]. As coumarins are associated with low toxicity, considerable interest has been increased to find their beneficial effects on human health [1,5]. Coumarins naturally present in many plants showed interesting pharmacological properties like anticoagulant, antimicrobial, antioxidant, anti-inflammatory, and anti-allergic properties [6–8]. Recent studies have created interest in this class of compounds as they have shown diverse biological activities such as anti-human immunodeficiency virus, dyslipidemic, and anticancer [9–13].

Cell division and cell death is the essential requirement for the homeostatic balance of cell; any loss of this balance leads to many fatal diseases such as cancer. Cancer usually affects multiple targets simultaneously and being the leading cause of death, requiring a great attention and adequate medication for its eradication. Available drug either fails to eliminate it completely or has very high side effect on resident cells. Therefore, more potent and drug-specific to only cancer cell is need of the day: hence, scientific researchers and commercial bodies are trying their best to discover anticancer drugs with good potency, safety, and selectivity.

As coumarins have ability to get bind either noncovalently or electrostatically to DNA through intercalation between the base pairs of DNA via major and minor groove through 3,4-position, therefore, it can be utilized for treating rapid proliferating cancer cells with certain modification [14–16].

Lee *et al.* reported a coumarin derivative neotanshinlactone **1**, (Fig. 1) with inhibition for two ER+ human breast cancer cell lines with 20-fold more potency compared with Tamoxifen [17]. Bariwal *et al.* reported 4-substituted coumarin derivatives as cytotoxic agents against MCF7 cell line [18]. 8-Methyl-4-substituted coumarin derivatives **2a** and **2b** (Fig. 1) showed very good

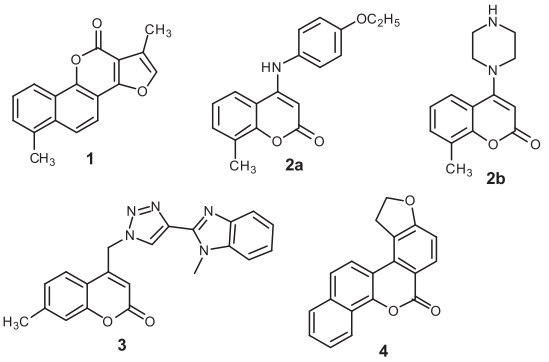


Figure 1. Some potent coumarin derivatives with anticancer activity.

potency as cytotoxic agents with IC_{50} value 6.25 and 6.50 μ*M* respectively against MCF7 breast cancer cell line.

Raić-Malić *et al.* reported triazole-based 4-substituted coumarin as cytotoxic agents. *N*-methyl benzimidazole derivative **3** showed very good cytotoxicity against HepG2 cell line with IC_{50} 0.9 μ*M* [19]. Basak *et al.* synthesized 6H–benzo[c]chromen-6-one derivatives as DNA intercalating agents with moderately good activity [20]. Naphthtyl derivative compound **4** (Fig. 1) induced maximum fluorescence quenching EB-DNA binding assay.

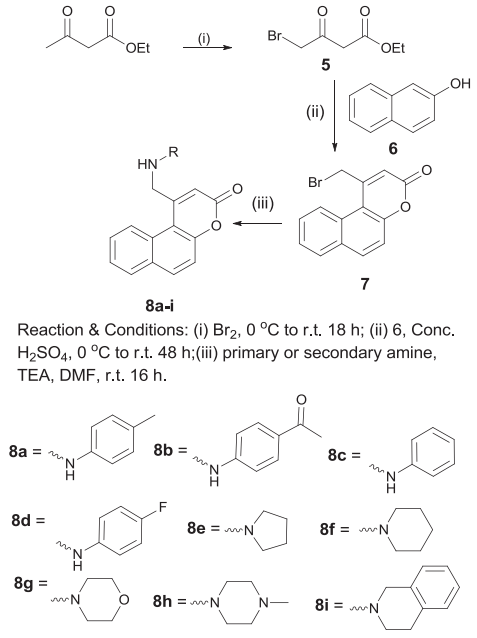
Synthetic coumarin derivatives have been reported with wide range of biological activities along with beneficial effects on human health [21–24]. Since coumarin moiety binds with DNA through 3,4-positions while extended benzene ring increases hydrophobicity and cytotoxicity, these new developments have encouraged us to design new 4-substituted aminomethyl coumarin derivatives that may increase binding ability with DNA along with extended aromatic ring that may increase cytotoxicity. On the basis of these facts and in continuation of our work on synthesis of coumarin derivatives as anticancer and antimicrobial agents [25–27], we have designed 4-aminomethyl substituted coumarin derivatives **8a–i** and report herein their synthesis, characterization, and anticancer activity.

RESULTS AND DISCUSSION

Chemistry. The 1-(substitutedaminomethyl)-3H–benzo[f]chromen-3-one **8a–i** (Scheme1) was synthesized by substitution reaction of 1-(bromomethyl)-3H–benzo[f] chromen-3-one **7** with different amines.

β-Naphthol on Pechmann reaction with ethyl acetoacetate gave 1-methyl-3H-benzo[f]chrome-3-one. Allylic bromination of 1-methyl-3H-benzo[f]chrome-3-one using *N*-bromosuccinimide was failed to give desired product because of solubility problem of starting compound in CCl_4, when reaction was carried out in

Scheme 1. Synthesis of substituted aminomethyl naphthopyrone derivatives **8a–i**.



Reaction & Conditions: (i) Br_2, 0 °C to r.t. 18 h; (ii) 6, Conc. H_2SO_4, 0 °C to r.t. 48 h;(iii) primary or secondary amine, TEA, DMF, r.t. 16 h.

$CHCl_3$ resulted in vinylic bromination instead of desired allylic bromination as reported [28].

In alternate approach, bromination of ethyl acetoacetate using Br_2 gave ethyl 4-bromo-3-oxobutanoate **5** as a red oil [29]. Thus, obtained compound **5** was used as such for Pechmann reaction with β-naphthol **6** in conc. H_2SO_4 to obtain 1-(bromomethyl)-3H–benzo[f]chromen-3-one **7** [30]. ^{1}H-NMR for compound **7** showed presence of singlet at δ 4.90 for two protons indicated presence of –CH_2Br group and all other aromatic protons appeared in the range of δ 6.6–8.5, thereby confirming the formation of **7**. This compound **7** was used to carry out substitution reaction with different amines using triethylamine in dimethylformamide (DMF) to form substituted aminomethyl naphthopyrone derivatives **8a–i**.

The infrared (IR) spectrum of compound **8a** exhibited strong band at 3355 cm^{-1} for the –NH proton, another strong band at 1720 cm^{-1} for lactone carbonyl group of coumarin ring. In the ^{1}H-NMR spectrum of **8a**, all aromatic protons observed at δ 8.40–6.53. The methylene protons were observed as a doublet at δ 4.91 because of the coupling with the amine proton. In the ^{13}C-NMR spectrum, the lactone carbonyl carbon of coumarin ring observed at δ 161, all aromatic carbons observed from δ 155–113, methylene carbon at δ 50, and methyl carbon at δ 20. In the electrospray ionization–mass spectrometry (ESI–MS) spectrum of **8a**, a peak at *m/z* 315.8 for $[M+H]^+$ confirmed its formation.

The structures of substituted aminomethyl naphthopyrones **8a–i** were confirmed by different

analytical techniques such as ^{1}H–NMR, ^{13}C–NMR, IR, and ESI–MS. For compound **8g**, single crystal was developed by using system Pet. Ether: EtOAc (1:1) via slow evaporation of solvents for several days and studied its structure by X-ray single crystal analysis (Fig. 2a) (CCDC no. 1054564). Crystal data and structure refinement parameters for compound **8g** are given in Table 1. Crystal structure analysis of compound **8g** showed presence of such four molecules per unit cell (Fig. 2b), and pi–pi stacking observed along axis a with separation of 3.712 Å (Fig. 2c).

In general, the IR spectra of compounds **8a–i** exhibited one strong band in range of 1724–1711 cm^{-1} for the lactone carbonyl group of coumarin ring. In the ^{1}H-NMR spectra of **8a–i**, peak for the methylene protons observed in range of δ approximately 5.01–3.91 depending on the effect of different amine substitution on it. All these new chemical entities were subjected to in vitro studies.

Biological evaluation. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to screen test compounds **8a–i** (Table 2) for their activity against cancer cell lines, namely, A549 (lung cancer cell line), MCF7 (breast cancer cell line), and A375 (melanoma cell line). IC_{50} (μ*M*) values were determined using GRAPHPAD PRISM software (San Diego, CA) for compounds **8a–i**, and the results are presented in Table 2.

Results from MTT assay were used to assess the growth inhibitory effect of the various compounds on three types of cancer cell lines (A549, MCF7, and A375) and found that most of the compounds are active only in two cell lines viz. A549 and MCF7. Hence, we took forward the only two cell lines for further study with the compounds **8a**, **8f**, and **8h** as these compounds showed activity at lowest conc. IC_{50} values were calculated to determine the concentration of test compound at which 50% of the cells growth is inhibited.

From the MTT assay, it could be deduced that the compound **8a** worked better in all three cell lines as a growth inhibitor against A375 with IC_{50} 4.29 μ*M*, MCF7

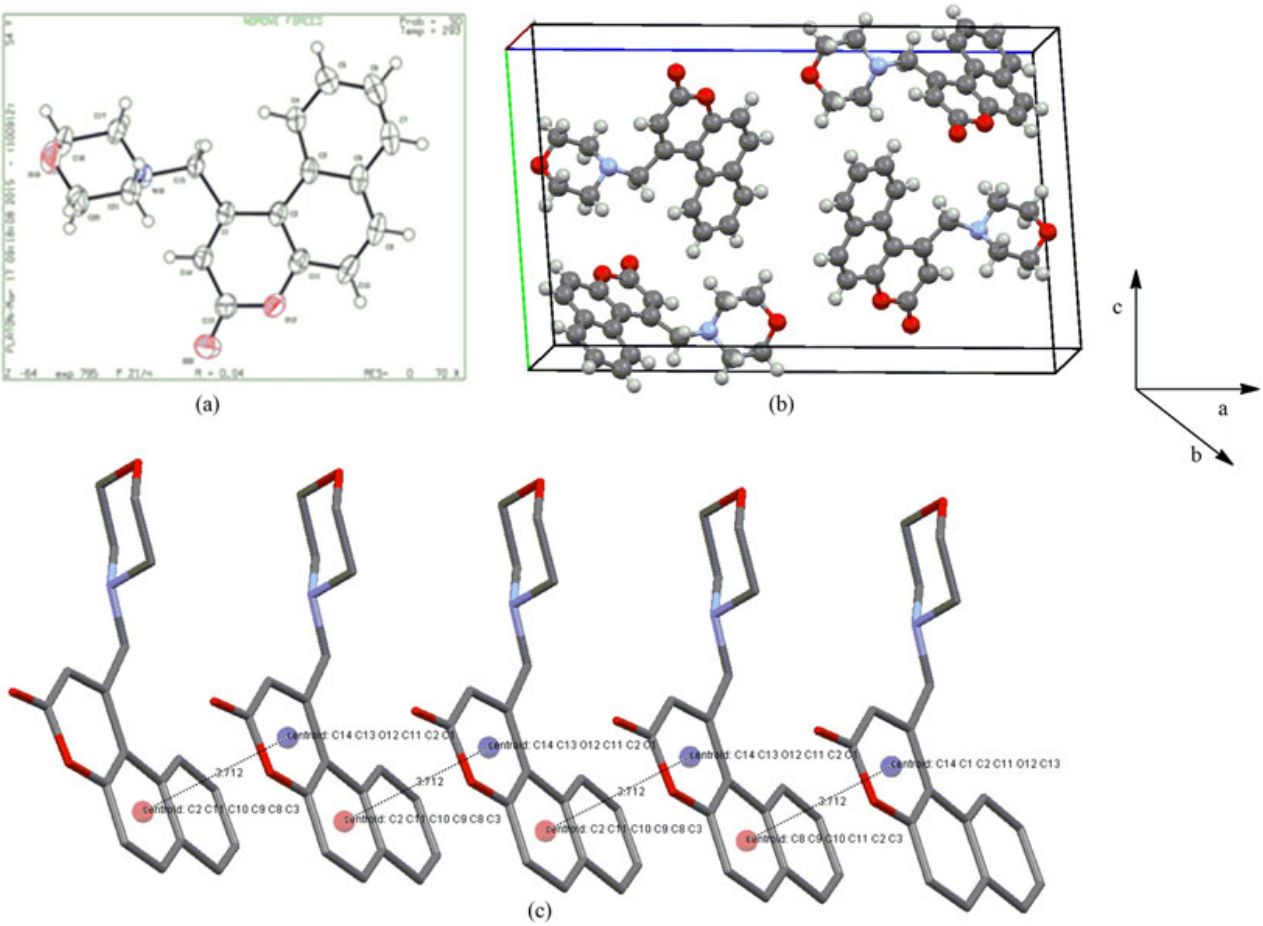


Figure 2. (a) X-ray crystal structure of compound **8g**; (b) molecular packing in unit cell; (c) pi–pi stacking in crystal structure. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1
Crystal data and structure refinement parameters for compound **8g**.

Chemical formula	$C_{19}H_{22}NO_3$
Molecular weight	292.40
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	4.5843(3)
b/Å	13.6007(10)
c/Å	22.9961(15)
α/°	90.00
β/°	95.429(6)
γ/°	90.00
V/Å^3	1427.37(17)
Z	4
ρ_{calc}mg/mm^3	1.361
Θ	6.96 to 58.02°
H	−5 to 5
k	−10 to 17
L	−30 to 28
Total reflections	7373
Independent reflections	3237
Used no. of reflections	3237
R[a]	0.0982
Absorption coefficient (m/Å)	0.082
R_{int}	0.0153
Peak and hole	1.40 and −0.68 Å Å^3

with IC_{50} 5.17 μ*M*, and A549 cell line with IC_{50} 9.02 μ*M*. When methyl substituent on aromatic ring was replaced by acetyl group in compound **8b**, it resulted in loss of activity against MCF7 cell line, but increased activity in A549 cell line with IC_{50} value 2.32 μ*M*. Replacement of methyl group with halogens, compound **8c** with –Cl, and compound **8d** with –F resulted in compounds with poor solubility in dimethyl sulfoxide (DMSO); hence, these compounds were screened against A549 cell line dissolved in DMF. However, both **8c** and **8d** were found ineffective as an anticancer agent against A549 cell line.

Replacement of aromatic amine with pyrrolidine ring as in compound **8e** showed good activity against A549 cancer cell line with IC_{50} 6.20 μ*M*, while no anticancer activity was observed when applied against MCF7 and A375 cell lines. Interestingly, piperidine substituted compound **8f** showed very good activity pattern against all tested cancer cell lines. Compound **8f** gave IC_{50} 1.12 μ*M* for A549 cell line and 0.83 μ*M* for MCF7cell line. Further, replacement of piperidine with *N*-methylpiperazine in compound **8h** resulted in very good growth inhibitory activity against A549 cancer cell line with IC_{50} 0.74 μ*M*, while good activity was observed against MCF7 and A375 cancer cell line with IC_{50} 14.24 and 13.96 μ*M*, respectively. On the other hand, replacement of piperidine moiety with morpholine in compound **8g** and tetrahydroisoquinoline in compound **8i** gave compounds with poor solubility in DMSO; hence, these compounds were screened against A549 cell line using DMF as diluent. Compound **8g** showed excellent antimitotic activity with IC_{50} 0.32 n*M* for A549 cell line; similarly, compound **8i** showed very good activity with IC_{50} 19.98 n*M*. Compounds **8a** and **8f** showed promising inhibitory potential with IC_{50} values falling in micromolar region and good solubility in DMSO; hence, these compounds further investigated for the lactic dehydrogenase (LDH) assay and ethidium bromide/acridine orange (ETBr/AO). The results are shown in Figure 3.

The release of cytosolic LDH enzyme has been used historically to understand the extent of plasma membrane damage that is a hallmark of necrotic cell death. The analysis of the results proved beyond doubt that the selected derivatives induced cell death in cancer cell lines via one of the innocuous mechanisms, namely, apoptosis rather than necrosis that used to inflame the surrounding cells and causes unwanted immunological inflammation. Compound **8f** showed much lower LDH release than **8a** in both the cell lines: however, a dose-dependent increase in membrane damage was noticed, and at higher concentration, these compounds exerted cytotoxicity via necrosis. Further, ETBr/AO assay, which is based on the morphological analysis of cell, confirmed the aforementioned results as shown in Figure 4. AO is a vital dye that stains both live and dead cells; however, ETBr stains only the cells that have lost the membrane integrity. Late apoptotic cells also incorporate ETBr and show condensed and often fragmented nuclei. Necrotic cells nevertheless stain red.

There are many mechanisms by which a compound induces cell death, and it has been reported that the several coumarin derivatives use reactive oxygen species (ROS)-mediated apoptotic pathway for inducing cytotoxicity. In order to understand the mechanism behind apoptosis by these compounds, intracellular ROS was measured using dichlorofluorescein diacetate fluorescence dye. The computational analyses of the images revealed that the intracellular ROS production in coumarin-treated cells was significantly higher compared with that of untreated control cells as shown in Figure 5.

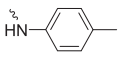
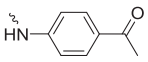
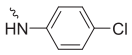
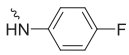
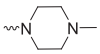
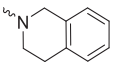
Fluorescence is proportional to the ROS concentration in cell, treated cells were showing higher fluorescence than normal control, fluorescence intensity was analyzed using IMAGE J software (USA).

Therefore, it could be construed that the observed increase in ROS intermediates could be the reason for the induction of apoptosis in coumarin-treated cancer cell lines. It has been reported that the increased ROS production destabilizes the mitochondrial membrane and causes the cytochrome-C release; this in turn activates caspases and downregulate Bcl2 that ultimately leads to apoptosis [31].

It has been observed that when *p*-toluidine, piperidine, morpholine, and tetrahydroisoquinoline were used as substituents on fourth position of coumarin such as in

Table 2

Anticancer activity of substituted aminomethyl naphthopyrones **8a–i**.

Compound	$-NR^1R^2$	IC_{50}[a] A549	IC_{50}[a] MCF7	IC_{50}[a] A375	IC_{50}[b] A549
8a		4.29 μ*M*	5.17 μ*M*	9.02 μ*M*	
8b		2.32 μ*M*	47.80 μ*M*	15.46 μ*M*	
8c		NS	NS	NS	56.75 μ*M*
8d		NS	NS	NS	59.14 μ*M*
8e		6.20 μ*M*	77.90 μ*M*	84.45 μ*M*	
8f		1.12 μ*M*	0.83 μ*M*	5.26 μ*M*	
8g		NS	NS	NS	0.32 n*M*
8h		0.74 μ*M*	14.24 μ*M*	13.61 μ*M*	
8i		NS	NS	NS	19.98 n*M*
5-Fluorouracil		11.13 μ*M*	45.04 μ*M*		

NS, Not soluble in DMSO.
[a]IC_{50} values were determined using GRAPHPAD PRISM software by MTT assay using DMSO.
[b]IC_{50} values were determined using GRAPHPAD PRISM software by MTT assay using DMF.

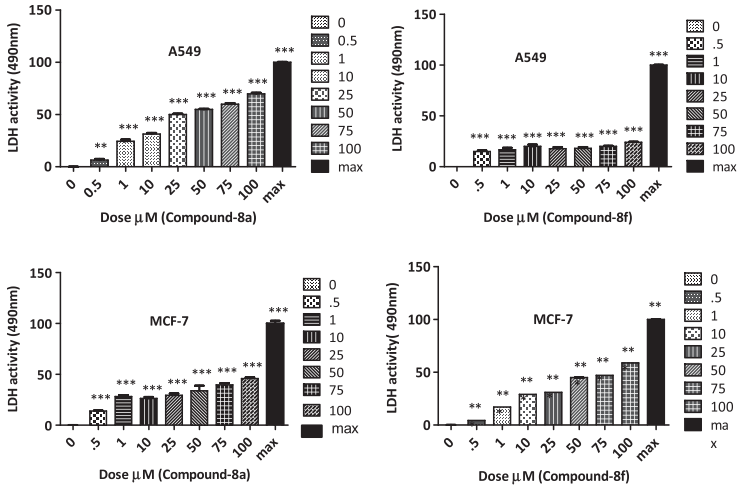


Figure 3. (a) Representation of cytosolic enzyme LDH in A549 cell line; activity of LDH in A549 cell line treated with different concentrations of compounds **8a** and **8f**. Graph plotted against LDH release versus dose. (***$P \leq .001$, **$p < .01$ significance one-way ANOVA (Tukey–Kramer). (b) Representation of cytosolic enzyme LDH in MCF7 cell line; activity of LDH in MCF7 cell line treated with different concentrations of compounds **8a** and **8f**. Graph plotted against LDH release versus dose. (***$P \leq .001$, **$p < .01$ significance one-way ANOVA (Tukey–Kramer). ANOVA, analysis of variance; LDH, lactic dehydrogenase

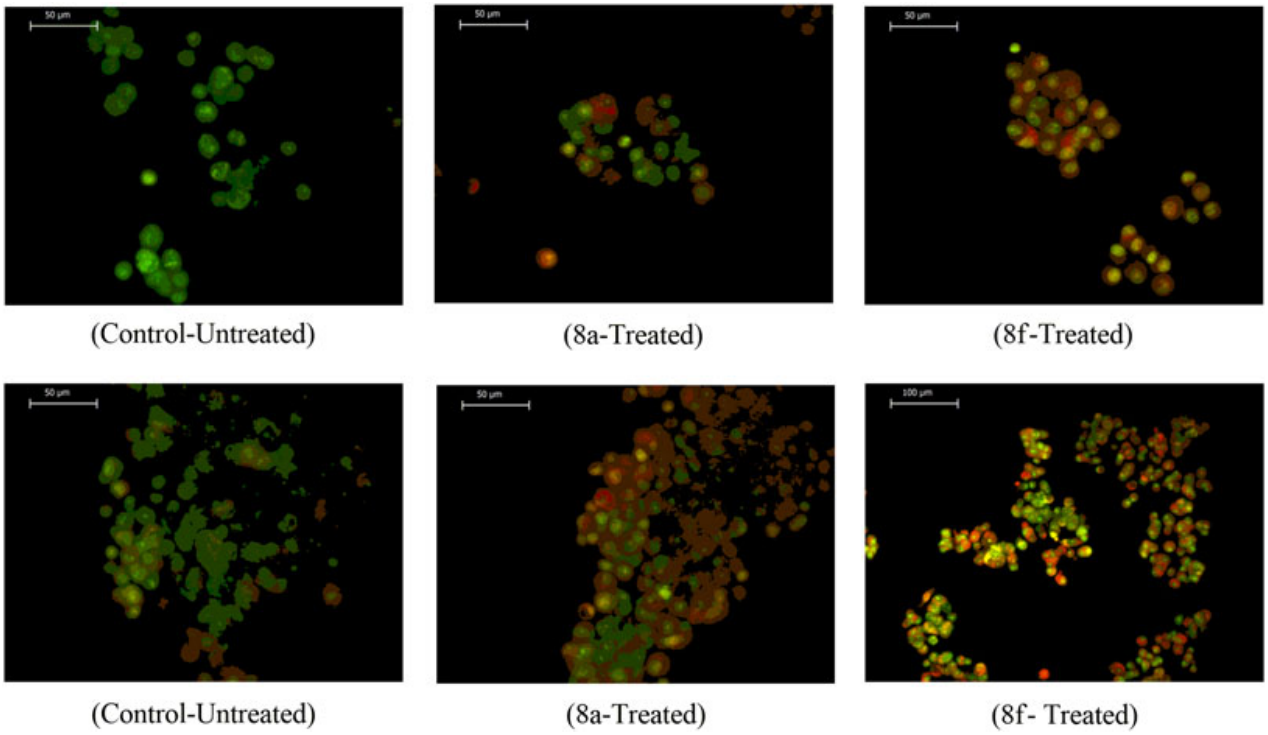


Figure 4. (a) Ethidium bromide/acridine orange staining: induction of apoptosis in A549 cell lines when treated with IC_{50} (μ*M*) concentration of compounds. (b) Ethidium bromide/acridine orange staining: induction of apoptosis in MCF7 cell lines when treated with IC_{50} (μ*M*) concentration of compounds. [Color figure can be viewed at wileyonlinelibrary.com]

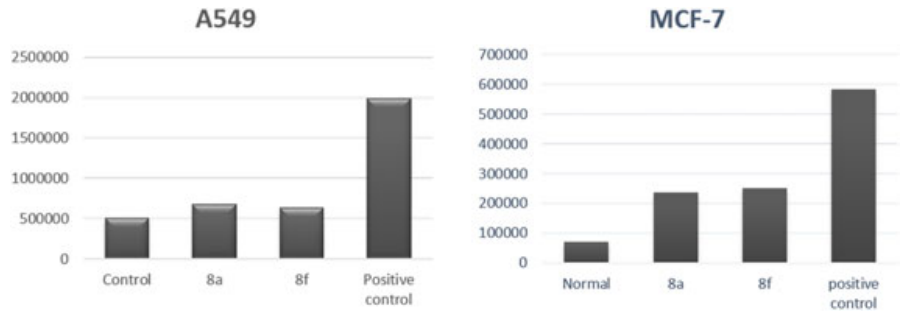


Figure 5. Representation of intracellular reactive oxygen species production in treated cell lines; reactive oxygen species production was measured using 2,7-dichlorodihydrofluorescein diacetate dye. [Color figure can be viewed at wileyonlinelibrary.com]

compounds **8a**, **8f**, **8g**, and **8i,** they showed very good anticancer activity compared with other substituents and even standard drug Fluorouracil. From the LDH assay, ETBr/AO assay, and intracellular ROS production assay, it can be concluded that these derivatives are responsible for apoptosis pathway for anticancer activity.

CONCLUSION

In conclusion, we report here the design and synthesis of substituted aminomethyl naphthopyrone derivatives **8a**–**8i** and their anticancer activity. Compounds **8a, 8f**, and **8g** have shown promising anticancer activity against A549 (lung cancer cell line), MCF7 (breast cancer cell line), and A375 (melanoma cell line). Compound **8f** proved to be very good anticancer agent against A549 and MCF7 with IC_{50} values 1.12 and 0.83 μ*M*, respectively. Both compounds **8g** and **8i** tested against A375 have been excellent in checking cell growth with IC_{50} values of 0.32 and 19.98 n*M*, respectively, after solubilizing in DMF. From the LDH assay and ETBr/AO assay of compounds **8a** and **8f**, it could be deduced that the new coumarin derivatives,

studied here, exert their cytotoxic effect via apoptosis that is known to be less inflammatory to subsiding cells at a lower concentration by altering the redox homeostasis of cell.

EXPERIMENTAL

Chemistry. Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. Thin-layer chromatography was performed on silica gel F254 plates (Merck & Co., Kenilworth, NJ, USA). Acme's silica gel (60–120 mesh) was used for column chromatographic purification. All reactions were carried out in nitrogen atmosphere. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylslane as internal standard. *J* values are in Hz. Mass spectra were determined by ESI–MS, using a Shimadzu LCMS 2020 apparatus (Shimadzu Scientific Instruments, Inc., USA). 2,7-dichlorodihydrofluorescein diacetate, MTT, ETBr, and AO were purchased from Sigma-Aldrich (St. Louis, MO, USA). DMSO, LDH assay kit (Thermo Scientific Pierce) and Dulbecco's modified Eagle's medium, fetal bovine serum, Penicillin–Streptomycin, trypsin–ethylene diamine tetraacetic acid obtained from G.

Preparation of ethyl 4-bromo-3-oxobutanoate (5). To an ice-cold solution of ethyl acetoacetate (25.24 mL), liquid bromine (10.25 mL) added dropwise over a period of 10–15 min. The resulting solution stirred at 0–5°C for 30 min and at room temperature for 24 h. The mixture thus obtained was diluted with ice-cold water and neutralized with saturated sodium bicarbonate (Na_2CO_3) solution in saturated sodium chloride (NaCl) solution. The organic layer separated, filtered through calcium chloride ($CaCl_2$) to give reddish brown oil. The ethyl 4-bromo-3-oxobutanoate **5** thus obtained (25 g) used directly for the next step.

Preparation of 1-(bromomethyl)-3H-benzo[f]chromen-3-one (7). To an ice-cold solution of conc. H_2SO_4 (30 mL), ethyl 4-bromo-3-oxobutanoate **5** (9 mL) was added slowly followed by portionwise addition of β-naphthol **6** (8.5 g) over a period of 10–15 min. Resulting mixture was stirred at room temperature for 48 h. The reaction mixture poured on crushed ice. The solid obtained was filtered and recrystallized from acetic acid to obtain compound **7** as golden yellow crystals. Yield: 79.81%; M.P: 180°C; IR (KBr): 3060, 1725, 1545, 1515, 1210, 1007, 928, 899, 825, 710 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 4.90 (s, 2H), 6.66 (s, 1H), 7.51 (d, *J* = 9.2 Hz, 1H), 7.61 (m, 1H), 7.63 (m, 1H), 7.97 (d, *J* = 9.2 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 8.54 (d, *J* = 8.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): 32.73, 112.36, 117.89, 118.21, 125.35, 125.86, 128.54, 128.76, 129.89, 131.37, 134.46, 151.78, 155.34, 159.97; ESI–MS: 290.11[M+2]$^+$, 287.63 [M]$^+$.

General procedure for the preparation of compounds (8a–8i). 4-Bromomethylnaphthopyrone **7** (500 mg) dissolved in DMF (20–30 mL) and substituted amine (1.1 eq), along with base triethylamine (1.5 eq) was added to it. The resulting mixture was stirred at room temperature for 16 h and then poured into cold water. The aqueous layer thus obtained was extracted using ethyl acetate and/or dichoromethane (checked by TLC) and solvent evaporated to give crude product. The product thus, obtained was purified by column chromatography using pet ether:ethyl acetate.

1-(p-Tolylamino) methyl)-3H-benzo[f]chromen-3-one (8a). Yield: 38.95%; M.P:180°C; IR (KBr): 3859, 3616, 3355, 2840, 2384, 1779, 1720, 1617, 1550, 1519, 1447, 1399, 1373, 1333, 1300, 1256 , 1195, 1130, 1089, 998, 925, 865, 821, 807, 779, 745, 640, 588 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.26 (s, 3H), 4.23 (br s, 1H), 4.90 (d, *J* = 5.2 Hz, 2H), 6.84 (s, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.58 (m, 1H), 7.65 (m ,1H), 7.99 (m, 1H), 8.40 (d, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 20.43, 49.72, 113.03, 113.55, 114.28, 117.93, 125.51, 125.60, 127.95, 128.28, 129.56, 129.88, 129.98, 131.41, 133.85, 144.23, 155.03,160.68; ESI–MS: 315.8 [M]$^+$; *Anal*. Calcd for $C_{21}H_{17}NO_2$: C, 79.98; H, 5.43; N, 4.44. Found: C, 79.95; H, 5.45; N, 4.41%.

1-(((4-Acetylphenyl)amino)methyl)-3H–benzo[f]chromen-3-one (8b). Yield: 53.76%; M.P: 194°C; IR (KBr): 3069, 2969, 2784, 1724, 1676, 1560, 1549, 1458, 1342, 1302, 1269, 1236, 1190, 1149, 987, 919, 858, 825, 738 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 2.40 (s, 3H), 5.05 (d, *J* = 5.3 Hz, 2H), 6.50 (s, 1H), 6.67 (d, *J* = 8.6 Hz, 2H), 7.39 (t, *J* = 5.3 Hz, 1H), 7.65–7.60 (m, 2H), 7.70 (d, *J* = 7.2 Hz, 1H), 7.74 (d, *J* = 8.6 Hz, 2H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 8.51 (d, *J* = 8.8 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 26.43, 47.68, 111.77, 112.97, 113.73, 117.98, 126.14, 126.42, 126.64, 128.82, 129.51, 130.10, 131.02, 131.51, 134.65, 152.26, 154.85, 155.94, 160.00, 195.74; ESI–MS: 342 [M−1]$^+$; *Anal*. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.99; H, 4.95; N, 4.09%.

1-(((4-Chlorophenylamino)methyl)-3H-benzo[f]chromen-3-one (8c). Yield: 42%; M.P:195°C; IR (KBr): 3365, 1692, 1602, 1550, 1506, 1446, 1335, 1270, 1088, 1002, 819, 749 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.84 (d, *J* = 5.3 Hz, 2H), 6.22 (t, *J* = 5.3 Hz 1H), 6.44 (d, *J* = 8.8 Hz, 2H), 6.61 (s, 1H), 6.96 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.14 (m, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 8.00

(d,*J* = 9.2 Hz, 1H), 8.33 (d, *J* = 8.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 53.51, 117.81, 118.41, 118.53, 122.44, 126.06, 130.47, 130.59, 133.15, 133.72, 134.28, 134.66, 136.14, 138.84, 150.96, 159.54, 160.52, 165.28; ESI–MS: 336.8 $[M+1]^+$; *Anal*. Calcd for $C_{20}H_{14}ClNO_2$: C, 71.54; H, 4.20; N, 4.17. Found: C, 71.52; H, 4.18; N, 4.15%.

1-(((4-Fluorophenylamino)methyl)-3H-benzo[f]chromen-3-one (8d). Yield: 54%; M.P: 175°C; IR (KBr): 3369, 2897, 1690, 1550, 1515, 1447, 1336, 1223, 1212, 1002, 818, 746 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.10 (d, *J* = 5.2 Hz, 2H), 5.52 (br t, 1H), 5.75–5.76 (m, 2H), 5.85 (s, 1H), 6.04–6.08 (m, 2H), 6.73 (d, *J* = 8.8 Hz, 1H), 6.80 (t, *J* = 7.4 Hz, 1H), 6.88 (t, *J* = 7.4 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 9.2 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 43.54, 107.49, 107.84, 108.26, 110.11, 110.33, 112.25, 120.39, 120.70, 123.07, 124.03, 124.48, 125.93, 128.81, 138.78, 149.26, 150.88, 154.88; ESI–MS: 320.2 $[M+1]^+$; *Anal*. Calcd for $C_{20}H_{14}FNO_2$: C, 75.22; H, 4.42; N, 4.39. Found: C, 75.19; H, 4.39; N, 4.40%.

1-(Pyrrolidin-1-ylmethyl)-3H-benzo[f]chromen-3-one (8e). Yield: 27.63%; M.P: 128°C; IR (KBr): 3068, 2966, 2784, 1724, 1676, 1654, 1560, 1549, 1518, 1458, 1342, 1236, 1190, 1149, 1112, 987, 919, 858, 825, 738 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 1.86 (br s, 4H), 2.73 (br s , 4H), 4.10 (s, 2H), 6.79 (s, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 8.8 Hz, 1H), 8.66 (d, *J* = 8.8 Hz, 1H); ^{13}C-NMR(100 MHz, $CDCl_3$): δ ppm23.72, 54.04, 60.76, 114.38, 115.97, 117.76, 125.49, 126.52, 127.79, 129.36, 129.76, 131.28, 133.54, 154.84, 154.86, 160.96; ESI–MS: 279.9 $[M+H]^+$; *Anal*. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H 6.13; N, 5.01. Found: C, 77.37; H, 6.09; N, 4.98%.

1-((Piperidine-1-ylmethyl)-3H-benzo[f]chromen-3-one (8f). Yield: 57.7%; M.P:138°C; IR (KBr):3056, 2924, 2838, 2809, 2764, 1711, 1646, 1570, 1516, 1453, 1403, 1373, 1334, 1270, 1236, 1190, 1129, 1108, 1037, 996, 927, 885, 810, 740, 673, 609, 571; ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 1.51 (br s, 2H), 1.65 (m, 4H), 2.59 (m, 4H), 3.91 (s, 2H), 6.84 (s, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.56 (m, 1H), 7.65 (m, 1H), 7.91 (m, 1H), 7.97 (d, *J* = 8.8 Hz, 1H), 8.67 (d, *J* = 8.8 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 24.02, 26.02, 54.68, 63.70, 114.60, 116.15, 117.76, 125.48, 126.64, 127.65, 129.34, 129.77, 131.27, 133.50, 154.23, 154.86, 160.9; ESI–MS: 293.20 $[M]^+$; *Anal*. Calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.63; H, 6.43; N, 4.61%.

1-(Morpholinomethyl)-3H-benzo[f]chromen-3-one (8g). Yield: 57.35%; M.P: 186°C; IR (KBr): 3059, 2955, 2887, 2839, 1712, 1623, 1549, 1515, 1454, 1428, 1373, 1315, 1250, 1192, 1113, 1000, 911, 883, 861, 817, 738 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 2.67 (br s, 4H), 3.78 (br s, 4H), 3.99 (s, 2H), 6.84 (s, 1H), 7.56 (s, 1H), 7.59 (m, 1H), 7.66 (m, 1H), 7.92–7.94 (m, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 8.59 (d, *J* = 8.8 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 53.61, 63.16, 66.91, 114.30, 116.27, 117.77, 125.59, 126.25, 127.77, 129.49, 129.61, 131.29, 133.74, 152.98, 154.96, 160.67; ESI–MS: 294.88 $[M]^+$; *Anal*. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.09; H, 5.72; N, 4.68%. For compound **8g**, single crystal was developed by using system Pet. Ether: EtOAc (1:1) via slow evaporation of solvents for several days and studied its structure by X-ray single crystal analysis (Fig. 2a) (CCDC no. 1054564).

1-((4-Methylpiperazin-1-yl)methyl)-3H-benzo[f]chromen-3-one (8h). Yield: 41.97%; M.P: 124°C; IR (KBr): 3859, 3843, 3444, 3054, 2931, 2836, 2790, 2755, 2700, 2363, 1713, 1621, 1550, 1517, 1455, 1375, 1285, 1193, 1137, 1015, 999, 985, 887, 812, 777, 742, 674, 591, 573 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 2.32 (s, 3H), 2.53 (br s, 4H), 2.71 (brs, 4H), 3.98 (s, 2H), 6.84 (s, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.54–7.58 (m, 1H), 7.65 (m, 1H); 7.91 (d, *J* = 8.0 Hz,1H), 7.97 (d, *J* = 8.8 Hz, 1H), 8.59 (d, *J* = 8.8 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 46.01, 53.015, 55.04, 62.73, 114.42, 116.23, 117.77, 125.54, 126.40, 127.75, 129.42, 129.67, 131.28, 133.64, 153.51, 154.91, 160.78; ESI–MS: 308.75 $[M]^+$; *Anal*. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found: C, 73.96; H, 6.57; N, 9.05%;

1-((3,4-Dihydroisoquinolin-2(1H)-yl)methyl)-3H–benzo[f]chromen-3-one (8i). Yield: 48.57%; M.P: 172°C; IR (KBr): 3022, 2908, 2828, 2799, 1713, 1645, 1570, 1552, 1532, 1452, 1426, 1336, 1268, 1194, 1130, 1095, 1054, 1000, 930, 883, 855, 813, 780, 739 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 2.99 (s, 4H), 3.87 (s, 2H), 4.10 (s, 2H), 6.90 (s, 1H), 7.06 (m, 1H), 7.16 (m, 3H), 7.51, (d, *J* = 8.8 Hz, 1H); 7.52–7.58 (m, 1H), 7.63–7.67 (m, 1H) 7.91 (d, 1H), 8.1 (d, 1H), 8.7 (d, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 29.21, 51.01, 55.90, 62.84, 125.55, 125.82, 126.41, 126.56, 127.83, 128.73, 129.44, 129.71, 131, 133, 134.08, 134.12, 153, 155, 160; ESI–MS: 342.1 $[M+H]^+$; *Anal*. Calcd for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10. Found: C, 80.89; H, 5.58; N, 4.13%.

Biological activity screening. ***3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.*** The compounds were tested for their cytotoxic potential on three types of cancer cells, namely, A549 (lung cancer cell line), MCF7 (breast cancer cell line), and A375 (melanoma cell line). The MTT assay was used to determine the effect of each compound on the proliferation of cancer cells. A549, MCF7, and A375 cultures were purchased from

National Centre for Cell Science, Pune, India. All growth media, supplements, and reagents were purchased from HiMedia Labs, Mumbai, India. For the assay, cells were seeded at 10^5 cells/mL in a 96-well plate in Dulbecco's modified minimum essential medium supplemented with 10% fetal bovine serum. To each well, test compound was added at six different concentrations of 100, 50, 10, 5, 1, and 0.5 μ*M*. Each concentration was tested in triplicates. The cells were incubated with these compounds at 37°C under 5% CO_2 for 48 h. Following this, MTT was added to each well at a final concentration of 0.5 mg/mL. Cells were incubated with this tetrazolium dye for 4 h. Subsequently, purple crystals of formazan were observed in each well, formed as a metabolic product of MTT. These crystals were dissolved in Isopropanol, and the absorbance in each well was recorded at 570 nm in a microplate reader (Metertech Sigma 360). Absorbance at 570 nm directly correlates with cell viability. IC_{50} (μ*M*) values were determined using GRAPHPAD PRISM software.

Lactate dehydrogenase assay. Cytotoxicity was assayed by measuring the activity of cytosolic enzyme LDH that released into culture medium when plasma membrane damage occurs because of necrosis [32]. Cells were seeded on 96-well plate (1×10^6 cells/mL) and allowed to attach for overnight; the next day, cells were treated with various concentrations (0.5, 1, 10, 25, 50, 75, 100 μ*M*) of coumarin derivatives and incubated for 48 h. LDH activity was measured using manufacturer's protocol (Pierce LDH Cytotoxicity Assay Kit). Absorbance was measured at 490 nm, and background was measured at 680 nm. % LDH release was measured using manufacturer's formula.

Acridine orange/ethidium bromide dual staining. Morphological analysis of apoptosis and necrosis was performed using ETBr/AO staining [33]. Cells were plated on 6-well plate and treated with IC_{50} conc for 48 h. A ratio of 1:1 of AO and ETBr (100 μg/mL in PBS) was prepared; 25 μL of cell suspension ($1–2 \times 10^5$ cells/mL) was incubated for 1 min with 1 μL of AO/ETBr. Cell suspension of 10 μL was placed on microscopic slide, and image was taken by fluorescent microscope at 40× (Leica DM2500, LAS EZ V1.6.0 software).

Dichlorofluorescein diacetate staining. Intracellular ROS production was measured with 2′,7′-dichlorodihydrofluorescein diacetate (H2-DCFDA; Sigma-Aldrich). For this, cells were plated on 6-well plate (1×10^5 cells/well) and incubated with IC_{50} concentration of derivatives; after 48 h of incubation, cells were trypsinized, and 10 μ*M* of H2-DCFDA was added, washed with PBS, and incubated: after 30 min, cell suspension was placed on microscopic slide, and image was evaluated, from fluorescent microscope (Leica DM2500, LAS EZ V1.6.0 software). For positive control, H_2O_2 treatment was given.

Acknowledgments. One of the authors (R.S.) is thankful to the Department of Science & Technology, Government of India, for financial support vide reference no. SR/WOS-A/CS-1028/2014 under Women Scientist Scheme to carry out this work. One of the authors (S.U.) is thankful to UGC, Government of India, for UGC-JRF vide reference no. 22/06/2014(i)EU-V. Authors are thankful to The Head, Department of Chemistry and Department of Zoology Faculty of Science, The M. S. University of Baroda for providing laboratory facilities, Zydus Research Centre, Ahmedabad, for the ESI–MS analyses, DST-PURSE for X-Ray crystallography facility. One of the author N.N.S. is thankful to M/S GNFC LTD for kind support.

REFERENCES AND NOTES

[1] Kennedy, R. O.; Thornes, R. D. Coumarins. Biology, Applications and Mode of Action; Wiley: NewYork, 1997.
[2] Almeida, I. M.; Barreira, J. C.; Oliveira, M. B.; Ferreira, I. C. Food Chem Toxicol 2011, 49, 3232.
[3] Barros, L.; Duenas, M.; Carvalho, A. M.; Ferreira, I. C.; Santos-Buelga, C. Food Chem Toxicol 2012, 50, 1576.
[4] Pinela, J.; Barros, L.; Carvalho, A. M.; Ferreira, I. C. Food Chem Toxicol 2012, 50, 829.
[5] Hoult, J. R. S.; Paya, M. Gen Pharmacol 1996, 27, 713.
[6] Kidane, A. G.; Salacinski, H.; Tiwari, A.; Bruckdorfer, K. R.; Seifalian, A. M. Biomacromolecules 2004, 5, 798.
[7] Cheng, J. H.; Hung, C. F.; Yang, S. C.; Wang, J. P.; Won, S. J.; Lin, C. N. Bioorg Med Chem 2008, 16, 7270.
[8] Ma, T.; Liu, L.; Xue, H.; Li, L.; Han, C.; Wang, L.; Chen, Z.; Liu, G. J Med Chem 2008, 51, 1432.
[9] Sashidhara, K. V.; Rosaiah, J. N.; Kumar, A.; Bhatia, G.; Khanna, A. K. Bioorg Med Chem Lett 2010, 20, 3065.
[10] Wang, X.; Nakagawa-Goto, K.; Bastow, K. F.; Don, M. -J.; Lin, Y. -L.; Wu, T.-S.; Lee, K.-H. J Med Chem 2006, 49, 5631.
[11] Myers, R. B.; Parker, M.; Grizzle, W. E. J Cancer Res Clin Oncol 1994, 12, 11.
[12] Yacquot, Y.; Bermont, L.; Giorgi, H.; Refouvelet, B.; Adessi, G.; Daubrosse, E.; Xicluna, A. Eur J Med Chem 2001, 36, 127.
[13] Gibbs, J. B. Science 2000, 287, 1969.
[14] Paul, A.; Bhattacharya, S. Curr Sci 2012, 102, 212.
[15] Wood, A. W.; Huang, M.-T.; Chang, R. L.; Newmark, H. L.; Lehr, R. E.; Yagi, H.; Sayer, J. M.; Jerina, D. M.; Conney, A. H. Proc Natl Acad Sci U S A 1982, 79, 5513.
[16] Li, Y. J.; Wang, C. Y.; Ye, M. Y.; Yao, G. Y.; Wang, H. S. Molecules 2015, 20, 14791.
[17] Dong, Y.; Shi, Q.; Liu, Y.-N.; Wang, X.; Bastow, K. F.; Lee, K.-H. J Med Chem 2009, 52, 3586.
[18] Kaur, P.; Gill, R. K.; Singh, G.; Bariwal, J. J Heterocyclic Chem 2016, 53, 1519.
[19] Kraljević, T. G.; Harej, A.; Sedić, M.; Pavelić, S. K.; Stepanić, V.; Drenjančević, D.; Talapko, J.; Raić-Malić, S. Eur J Med Chem 2016, 124, 794.
[20] Bhattacharya, P.; Mandal, S. M.; Basak, A. Eur J Org Chem 2016 1439.
[21] Chen, Z.; Bi, J.; Su, W. Chin J Chem 2013, 31, 507.
[22] Manidhar, D. M.; Kesharwani, R. K.; Reddy, B. N.; Reddy, S. C.; Misra, K. Med Chem Res 2013, 22, 4146.
[23] Nasr, T.; Bondock, S.; Youns, M. Eur J Med Chem 2014, 76, 539.

[24] Dandriyal, J.; Singla, R.; Kumar, M.; Jaitak, V. Eur J Med Chem 2016, 119, 141.

[25] Soni, J. N.; Soman, S. S. Eur J Med Chem 2014, 75, 77.

[26] Soman, S. S.; Soni, J. N.; Inamdar, G. S.; Robertson, G. P. Der Pharma Chemica 2013, 5, 201.

[27] Soman, S. S.; Thaker, T. H. Med Chem Res 2013, 22, 4223.

[28] Soman, S. S.; Thaker, T. H. J Chem Res 2010, 34, 502.

[29] Sousa, C. M.; Berthet, J.; Delbaere, S.; Coelho, P. J. J Org Chem 2012, 77, 3959.

[30] Dey, B. B.; Sankaranarayan, Y. J Ind Chem Soc 1934, 11, 687.

[31] Chuang, J. Y.; Huang, Y. F.; Lu, H. F.; Ho, H. C.; Yang, J. S.; Li, T. M.; Chang, N. W.; Chung, J. G. In Vivo 2007, 21, 1003.

[32] Danpure, C. I. Cell Biochem 1984, 72, 144.

[33] Kasibhatla, S.; Amarante-Mendes, G. P.; Finucane, D.; Brunner, T.; Bossy-Wetzel, E.; Green, D. R. Cold Spring Harb Protoc 2006 3.

ejpmr, 2018,5(10), 229-241

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DESIGN, SYNTHESIS AND EVALUATION OF ANTIMICROBIAL AND ANTICANCER ACTIVITY OF NOVEL 3-AMINOMETHYL PYRIDIN DERIVATIVES

Nirav Shah[1,2] and Shubhangi Soman[1]*

[1]Department of Chemistry, Faculty of Science, the M. S. University of Baroda, Vadodara 390002, India.
[2]Department of Chemistry, Faculty of Science, the M. S. University of Baroda, India.

***Corresponding Author: Prof. Dr. Shubhangi Soman**

Department of Chemistry, Faculty of Science, the M. S. University of Baroda, India.

Article Received on 29/07/2018 **Article Revised on 18/08/2018** **Article Accepted on 07/09/2018**

ABSTRACT

In search of new nitrogen, oxygen and sulphur containing heterocyclic compounds with improved antimicrobial, antifungal and anticancer activities, we report herein the synthesis of amide derivatives **2a-g** and **5a-f** obtained by reaction of 3-aminomethyl pyridine with phenyl bromo acetamide **1a-f** and (N-substituted benz [d]thiazol-2-yl) 2-bromo acetamide **4a-g** derivatives respectively. All the synthesized compounds were evaluated for their antimicrobial and anticancer activities. Compound **2a** and **2b** showed IC_{50} values 0.2129 µM and 1.186 µM respectively against A549 lungs cancer cell line, while compounds **2c**, **2d** and **5a** showed promising anticancer activity with IC_{50} values 0.51µM, 0.14 µM and 0.73 µM respectively against MOLT3 leukaemia cancer cell lines.

KEYWORDS: Aminomethyl pyridine, benzothiazole, antimicrobial activity, anticancer activity.

INTRODUCTION

Heterocyclic compounds containing nitrogen, oxygen and sulphur heteroatom have been used as drugs for various diseases. Since last few decades, bacterial and fungal resistance to known therapies is a growing threat across the world. An increasing proportion of bacterial growth shows reduced susceptibility to our currently available antibacterial agents. *Staphylococcus aureus is* Methicillin resistant.[1] It can also bind proteins in blood to help evade antibody-mediated immune response.[2] Similarly a bacterium like Escherichia coli is a frequent cause of urinary tract infections[3] and also showed high rate of resistance to amoxicillin and tetracycline. '*Pseudomonas aeruginosa'*, a ubiquitous microorganism, is one of the most relevant pathogens affecting the patients admitted to (ICU).[4] Along with the various types of bacteria, different types of fungus also cause healthcare-associated infections.[5] It is well observed from the literature that the fungal activity depends prominently on electron withdrawing groups as well as its positioning on aromatic ring.[6]

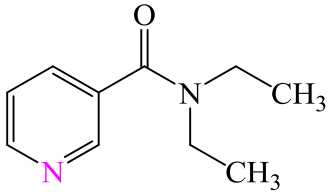
In order to prevent serious medical problems due to drug resistive bacteria the discovery of new types of antibacterial agents is a very important task.[7]

Cancer is considered as fatal disease in terms of morbidity and mortality affecting human health worldwide.[8] It is estimated to further increase of 50% by the end of 2020. The death rates due to lungs cancer and breast cancer in women are very high globally. More than one million cases of lungs cancer are diagnosed every year and is the leading cause of cancer-related death in men and women.[9] Similarly, The incidence of breast cancer has increased intensely in developed countries, however the mortality rate is much higher in developing countries due to lack of early detection of the disease.[10] Despite of substantial research on cancer therapeutics, high toxicity and drug-resistance yet limits the clinical application of some heavy metal containing drugs like Cisplatin which, binds covalently to the N7-guanine of DNA, causing a distortion to the structure of DNA double helix leading to serious side effects, and cell death.[11,13]

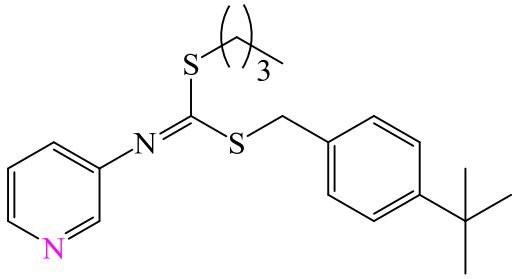
Review of literature indicates that N-containing heterocycles have significant place in the development of pharmacologically important molecules.[14] Likewise Benzothiazole nucleus is also a fertile source of bioactivity in the area of drug discovery because of its varied biological activities viz. Anticancer,[15,16] antimicrobial,[17] and antifungal.[18] Moreover, it has long been known that compounds bearing pyridine ring also occupy a prominent place in medicinal chemistry due to its significant biological activities such as antimicrobial,[19] antiviral,[20] anticancer,[21] and analgesic.[22] Some of the rarely discussed analogue of pyridine like 3-aminomethyl pyridine also showed radical scavenger activity.[23] Several attempts have been made to modify the benzothiazole nucleus to improve their antitumor activities.[24] Various amide derivatives of benzothiazole have potent anticancer property.[25] **Figure-1** and **Figure-2** shows some important Pyridine and benzothiazole containing drugs. Combination of

various amino methyl pyridine and benzothiazole derivatives with acetamide linkage can be a good combination as antimicrobial and anticancer agent.

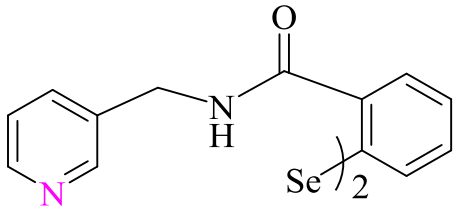
Hence, in continuation of our work on search for potential antimicrobial and anticancer agents,[26,30] we have synthesised compounds **2a-g** and **5a-f** from 3-aminomethyl pyridine which were screened for their antibacterial and antifungal activity using a cup plate method[31] and anticancer activity using MTT assay method.[32]



Nikethamide
1

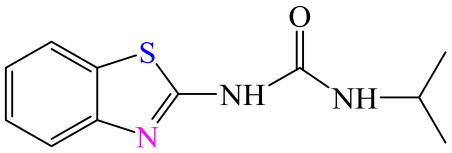


Buthiobate
2

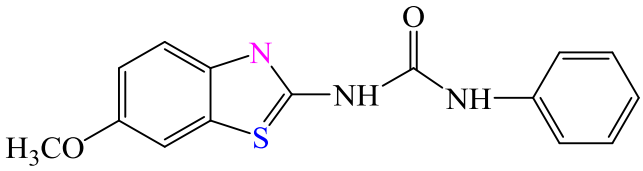


3

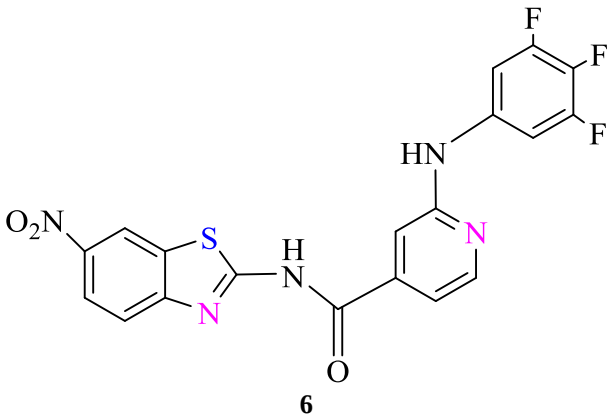
Figure-1: Some 3-substituted pyridine derivatives.



Bentarulam
4



Frentizole
5



6

Figure-2: Some amide derivatives of 2-amino benzothiazole analogues.

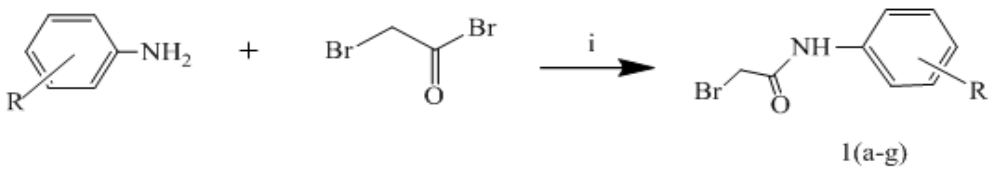
RESULTS AND DISCUSSION

Chemistry

The synthetic routes are depicted in scheme 1 and 2 for the desired target compounds. Compounds **2a-g** as shown in **(scheme-1)** and **5a-f (scheme-2)** were synthesized by reaction of 3-amino methyl pyridine with seven different phenylbromoacetamide of substituted anilines **1a-g** and with six different N-(substituted benzo [d] thiazol-2-yl)-2-bromo acetamide **4a-f** respectively. Compounds 2-bromo-N-substituted phenyl acetamide 1a-g were prepared by reaction of various substituted anilines with bromo acetyl bromide in presence of

catalytic amount of base triethylamine in dichloromethane. Compounds N-(substituted benzo [d] thiazol) **3a-f** were synthesized by the reaction of various substituted anilines with potassium thiocyanate (KSCN) in presence of bromine in acetic acid[33][a,b] further stirring of **3a-f** with bromo acetyl bromide gave N-(substituted benzo [d] thiazol-2-yl)-2-bromo acetamide 4(a-f).[34][a,b] Thus compounds **1a-g** and **4a-f** on substitution reaction with 3-aminomethyl pyridine in dimethylformamide (DMF) in presence of triethylamine (TEA) gave the desired compounds **2a-g** and **5a-f** in good yields respectively. The Structures of all the synthesized compounds were confirmed by its ^{1}H NMR, ^{13}C NMR, IR, Mass Spectra and CHNS analysis. The IR spectrum of compound **2a** exhibited strong band at 3383 cm^{-1} for the characteristic –NH stretching vibrations. Another strong band at 1691 cm^{-1} for the –CO group of amide and another strong band exhibited at 2710 cm^{-1} for –CH stretching vibration, The band at 1612 cm^{-1} indicated C=N stretching vibration. In ^{1}H NMR spectrum of **2a** the two –CH_2 groups were observed as singlet for the two protons each at δ 4.0 and 4.4 for –$COCH_2$ and -CH_2NH respectively. All aromatic protons observed between δ7.20 – 8.40. The amide proton was observed as a singlet at downfield to the aromatic protons i.e at δ 10.5 to 11. In the ^{13}C NMR spectrum of compound **2a** showed two carbons for methylene groups observed at δ 47 and 48.The characteristic –CO carbon was observed at δ 164. All aromatic carbons observed at δ120 to 150. The ESI mass spectrum of compound **2a** showed M^{+} peak at 291.

Scheme-1



Compound	R
2a	4-Cl
2b	4-F
2c	3-Cl
2d	4-CH_3
2e	3-F
2f	3-NO_2
2g	4-$COCH_3$

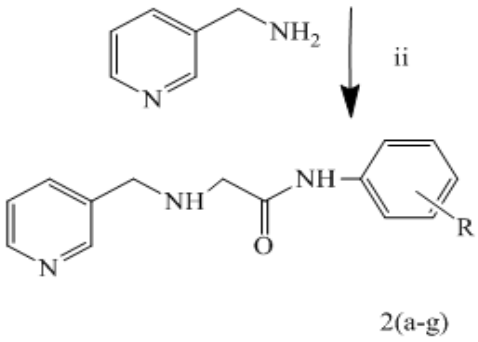
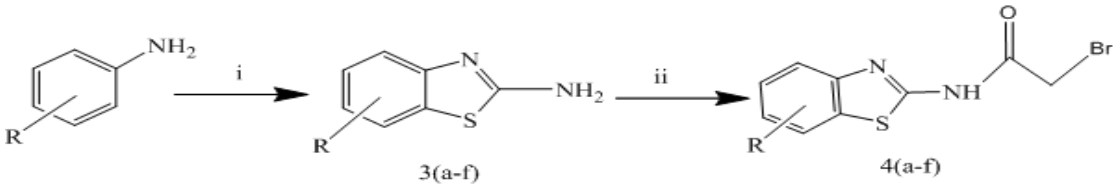


Figure-3(Scheme-1): Synthesis of *N*-substituted phenyl-2-[(pyridin-3-ylmethyl) amino] acetamide.

Reagents & conditions: (i) TEA, Stirring at 0- 5°C 30 min, RT, 2 h, DCM, (ii)TEA, RT Stirring 8h, DMF.

Scheme-2



Compound	R
5a	H
5b	4-CH_3
5c	6-Cl
5d	4-Cl
5e	6-Br
5f	4-CH_3-6-NO_2

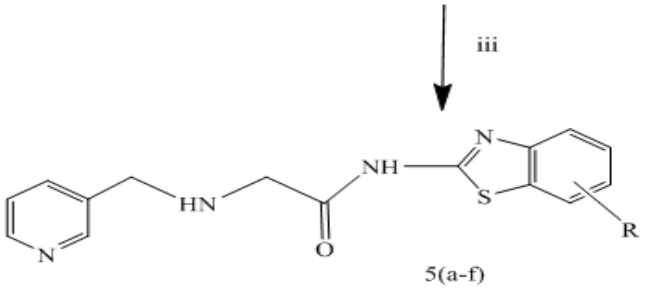


Figure-4(Scheme-2): Synthesis of 3-amino methyl pyridine based acetamide derivatives of substituted 2-amino benzothiazole.

Reagents and Conditions: (i) KSCN, Br_2 in Acetic acid, 0-5°C, room temperature (RT) string 8-10 hrs. Liq. NH_3 (25%). (ii) $BrCOCH_2Br$, TEA, DCM, string at 0-5°C; 30min, room temperature string 10 hrs. (iii) TEA, DMF, 3-aminomethyl pyridine, room temperature String 12 hrs.

Similarly in the IR spectrum of compound **2b** exhibited strong band at 3398 cm^{-1} for the characteristic -NH stretching vibrations. Another strong band at 1689 cm^{-1} for the –CO stretching of amide and another strong band exhibited at 2710 cm^{-1} for –CH stretching and 1620 cm^{-1} for >C=N stretching. In ^{1}H NMR spectrum of **2b** the two $–CH_2$ groups were observed as singlet for the two protons each at δ 4.03 and 4.46 for $-COCH_2$ and $-CH_2NH$ respectively. All aromatic protons observed between δ7.12 to 9.07. The amide protons were observed as two singlets at downfield to the aromatic protons i.e at δ 10.08 and 11.08. In general for all **2a-g**, the IR spectra showed one characteristic band at δ 3380 cm^{-1} for the NH stretching, Strong band at 1690 cm^{-1} for Carbonyl and band at 1612 cm^{-1} for >C=N stretching. In ^{1}H NMR of all **2a-g**, two methylene protons observed around δ 4.0-4.5 as two singlets. All aromatic protons were observed at δ 7.16 -8.82. The –NH protons observed downfield to the all other aromatic protons at δ 10 and 11. In the ^{13}C NMR spectrum of compounds **2a–g** showed two carbons for methylene groups at around δ 47 and 48. The characteristic –CO (Carbonyl) carbon was observed at δ 164. All the other aromatic carbons observed between δ120 to 150. Similarly, for the synthesised compounds **3a-f**, In the IR spectrum of compound **3b** showed two bands at 3433 cm^{-1} and 3285 cm^{-1} indicated free NH_2 group. ^{1}H NMR of compound **3b** showed singlet at δ 2.42 for three protons indicates $–CH_3$ group. Multiplates at 6.88, 7.01 and 7.40 for three protons indicated all three aromatic protons. Downfield Singlet at δ 7.45 for two protons indicated $–NH_2$ protons thus confirmed the structure of compound **3b.** The reaction of **3b** with bromo acetyl bromide at room temperature gave compound **4b.** In the IR spectrum of compound **4b**, showed one band at 3187 cm^{-1} indicated –NH stretching frequency. A sharp band at 1661cm^{-1} indicated –CO stretching frequency of amide. The C=O absorption of amide occurs at lower frequency than the normal carbonyl absorption due to the resonance effect. In ^{1}H NMR of compound **4b,** showed singlet at δ 2.53 for three protons indicated $–CH_3$ group. Another singlet at δ 4.19 for two protons indicated $–CH_2$ group. The multiplet for two protons at δ 7.16 and δ 7.23 indicated two aromatic protons and another doublet at δ 7.74 for one proton indicated third aromatic proton. Broad singlet at δ 12.83 indicated –NH proton. Further ^{13}C NMR of compound **4b,** showed two carbons at δ 17.86 and 28.36 indicated two aliphatic carbons of $–CH_3$ and $–CH_2$ groups respectively. In aromatic region presence of seven carbons between δ 118.96 to δ 156.63 and one carbonyl carbon at δ 165.80 confirmed the structure of compound **4b.** The reaction of compound **4b** with 3-amino methyl pyridine in DMF at room temperature gave compound **5b.** The IR spectrum of compound **5b** showed sharp band at 3320 cm^{-1} indicated –NH stretching vibration. A sharp stretching band at 1691cm^{-1} indicated carbonyl stretching frequency of amide group. The ^{1}H NMR spectrum of compound **5b,** showed singlet at δ 2.36 for three proton of –CH3 group. Two singlets at δ 4.16 and δ 4.48 for two protons each indicated $-CH_2N-$ and $–CH_2-CO-N-$ protons respectively. Six signals in aromatic region for one proton each indicated six aromatic protons. One broad singlet and one sharp singlet at δ 10.15 and 10.98 for one proton each indicated two,-NH protons. In ^{13}C NMR spectrum of compound **5b** showed three aliphatic carbons at δ 19.42, 46.92 and 47.79 for one $–CH_3$ and two $–CH_2$ carbons. The remaining eleven carbons in aromatic region between δ 123.13 to δ 146.66 and one carbonyl carbon at δ 164.26 confirmed the formation of compound **5b.** Further mass spectrum of compound **5b** showed M^+ peak at 312 confirmed the structure of compound**5b.**

In general, the IR spectra of compounds **5a-f** exhibited one strong band in range of 1690-1710 $cm^{-1}$for the carbonyl stretching frequency of amide group and another broad band observed approximately at 3275cm^{-1} to 3300cm^{-1} indicates the –NH stretching vibrations. In the ^{1}H-NMR spectra of **5a–f**, two separate singlet peaks for the two methylene protons observed in range of approximately δ 3.95 to 4.65 and the aromatic protons in the range of δ 6.99 to 8.93. In the ^{13}C NMR spectrum of **5a-f** all the aromatic carbons exhibited in the range of approximately δ 122 to δ 147, Carbon atom at position 5 of the thiazole ring showed signal around δ 110 to δ 112. One carbonyl carbon observed in the range of approximately δ 160 to δ 165 confirms the general structure of the synthesized compounds **5a-f**. Mass spectra showing specific molecular ion peak further confirmed the synthesis of desired products. All these new chemical entities were subjected to *in vitro* studies.

Biological Evaluation

Antimicrobial and antifungal activity

The antibacterial activity of compounds **2a-g** and compounds **5a-f** was evaluated and compared with standard drugs. All the synthesized compounds were screened for their antibacterial activity against two *Gram positive* bacterial strains (*Staphylococcus aureus, Bacillus Subtilis*) and two *Gram negative* bacterial strains (*Escherichia coli*, and *Pseudomonas aeruginosa*) and all the compounds were also tested for their antifungal activity against one fungal strain (*C. albicans)* by cup plate method at 0-250 µg concentration in DMF as a solvent. Ciprofloxacin and Flucanazole were used as standard drugs for determining the antimicrobial and antifungal activity respectively. **Table-1** shows antimicrobial activity of all the newly synthesized compounds **2a-g** and **5a-f**.

Table 1: *In Vitro* antibacterial and antifungal activity of compounds 2a-g and 5a-f.

MIC of antibacterial and antifungal agent (µM)							
Sr. No	**Compound**	**-R**	**S. aureus**	**B. Subtilis**	**E. Coli**	**P. aeruginosa**	**C. albicans**
01	2a	4-Cl	100	100	100	200	100
02	2b	4-F	200	250	100	150	100
03	2c	3-Cl	50	50	200	250	100
04	2d	4-CH_3	200	150	150	200	100
05	2e	3-F	50	50	200	>250	50
06	2f	3-NO_2	>250	150	150	150	150
07	2g	4-$COCH_3$	200	250	150	250	100
08	5a	-H	150	300	>300	>300	>300
09	5b	-CH_3	80	>300	>300	>300	>300
10	5c	6-Cl	80	150	>300	>300	>300
11	5d	4-Cl	>300	>300	>300	>300	>300
12	5e	6-Br	80	300	>300	>300	>300
13	5f	4-CH_3-6-NO_2	300	>300	>300	>300	>300
14	Ciprofloxacin		15	05	20	10	-
15	Flucanazole		-	-	-	-	10

Table 1: Antimicrobial and antifungal activity of compound 2(a-g) and 5(a-g).

S. aureus *= Staphylococcus aureus,* ***B. Subtilis*** *=Bacillus Subtilis,* ***E. coli*** *=Escherichia coli,* ***P. aeruginosa*** *= pseudomonas aeruginosa,* ***C. albicans*** *=Candida albicans.*

Compounds **2(a-g) and 5(a-f)** were screened for their antimicrobial and antifungal activities. Both of the Compounds **2c** and **2e** showed promising antibacterial activity at 50 µM concentrations against tested bacteria (*S. aureus and B. Subtilis)* and Compound **2e** showed promising antifungal activity at 50 µM against fungi *C. albicans.* Compound **2a** showed moderate activity at 100 µM against all the four pathogenic bacterial strains. Compound **2b** remained moderate active against *E. coli* and *P. aaeruginosa.* Both the compounds **2a** and **2b** showed moderate activity against fungi *C. albicans.* Compound **2f** and **2g** showed some activity against fungi *C. albicans.* Among the synthesized compounds **5a-f** Compound **5b**, **5c**, and **5e** remained moderately active at 80 µg concentrations against tested bacteria (*S. aureus*) while compounds **5a**, **5d** and **5f** remained inactive against all the bacterial strains and fungi C. *albicans.* In general it is also concluded that when phenyl ring of amine is substituted at 3rd position plays important role in showing antibacterial as well as antifungal activity. The structure variations such as methyl and halo groups at *meta* and *para* positions of phenyl ring bearing amide linkage resulted in promising antibacterial and antifungal activity.

Anticancer Activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to screen only five test compounds **2a**, **2b**, **2c**, **2d** and **2f** from seven synthesized compounds **2a-g** for their cytotoxic potential against lung cancer cell line A549. Among the tested five compounds of **2a-g**; only three Compounds 2a, 2b and 2f showed promising anticancer activity against A549 cell line with IC_{50} 0.2129 µM, 1.863 µM and 32.63 µM while compounds **2c** and **2d** remained inactive. Three compounds namely compounds 2a, 2e and **2f** were tested against breast cancer cell line MCF7 from all the synthesized compounds **2a-g** as presented in **Table-2** among which only two compounds **2a** and **2f** showed cytotoxic potential with IC_{50} 950.1µM and 90.78 µM while compound **2e** remained inactive against MCF7 cancer cell line. For various amide derivatives of various substituted anilines with amino methyl pyridine it has been observed that 3rd position Cl substitution showed very good anticancer activity against all the three leukaemia cancer cell lines. Similarly a bar chart representation for the anticancer activity of four compounds **5a**, **5c**, **5d** and **5f** from the synthesized benzothiazole derivatives against A549 (lung cancer cell line) showed % inhibition at 5 different concentrations ranging from 0-200µM **Figure-5.** All the synthesized compounds **2a-g** and **5a**,**5c**,**5d** and **5f** were screened for their efficacy as anticancer agent against three leukaemia cancer cell lines namely K562 (Human Chronic Myelogenous leukaemia cell line), KG1 (Human acute Myeloid Leukaemia cells) and MOLT-3 (Human Acute Lymphoblastic Leukaemia cell line). IC_{50}µM values for compounds **2a-g** and **5a**, **5c**, **5d** and **5f** are summarized in **Table-3**. Compound **2c** showed very good cytotoxic potential with IC_{50} 2.351 µM against K562 cell line compared to all other compounds. Likewise, compound **2c** and **2d** also showed very good anticancer activity with IC_{50} 0.51 µM and 0.14 µM against MOLT3 cell line compared to other Compounds. Similarly, Compound **2c** showed better cytotoxic potential with IC_{50} 0.374 µM against KG1 cell line. All the compounds showed good anticancer activity against two leukemia cancer cell line (MOLT3 and KG1) with IC_{50} potential ranging from 1.0 µM to 50 µM except **5c** and **5f**. The bar chart presentation for compounds **2a-g** on K562, KG 1 and MOLT 3 cell growth and determining their % cell viability are shown in **Figure-5**.Similarly bar chart presentation for compounds **5a**, **5c**, **5d** and **5f** on K562,

KG 1 and MOLT 3 cell growth and determining their % cell viability are shown in Figure-**6**. In benzothiazole derivatives it has been observed that without any substitution on benzene ring of benzothiazole showed good activity and 6-chloro substitution showed better activity.

Inhibition (%) = (Absorbance of blank-Absorbance of test/Absorbance of blank) X 100

Table 2: IC_{50} (µ/mL) values for Lung and Brest cancer cell lines for compounds 2(a-g).

No.	Compounds Code	-R	A549	MCF7
			IC_{50} (µM)	IC_{50} (µM)
01	2a	4-Cl	0.2129	950.1
02	2b	4-F	1.186	ND
03	2c	3-Cl	NA	ND
04	2d	4-CH_3	NA	ND
05	2e	3-F	ND	NA
06	2f	3-NO_2	32.63	90.78
07	2g	4-$COCH_3$	ND	ND

ND=**Not Done,** NA=**Not Active.**

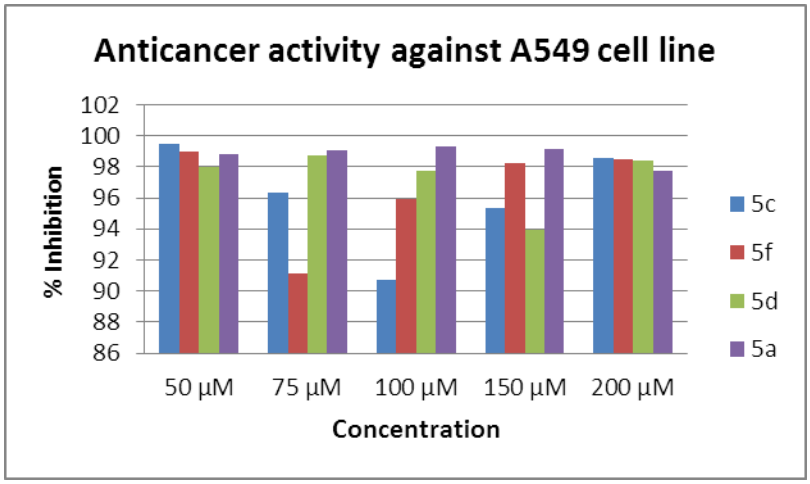


Figure 5: Bar chart representation for the anticancer activity of four compounds from 5(a-f) against A549 (lung cancer cell line) showing % inhibition at 5 different concentrations ranging from 0-200µM.

Table 3: IC_{50} (µM) values for three different Leukaemia cancer cell lines.

No.	Compounds	-R	K 562	Cancer cell lines MOLT 3	KG 1
			IC_{50}(µM)	IC_{50}(µM)	IC_{50}(µM)
01	2a	4-Cl	50.19	3.881	6.228
02	2b	4-F	16.32	1.25	9.73
03	2c	3-Cl	2.35	0.51	0.374
04	2d	4-CH_3	20.47	0.14	4.968
05	2e	3-F	55.94	14.73	3.437
06	2f	3-NO_2	8.304	3.094	3.883
07	2g	4-$COCH_3$	13.57	3.64	4.499
08	5a	-H	284.3	0.73	53.67
09	5c	6-Cl	244.2	67.68	2.34
10	5d	4-Cl	225.2	8.82	0.50
11	5f	4-CH_3-6-NO_2	225.0	96.35	20.36

Table-3: Anticancer activity of compounds **2a-g** and compounds **5a**, **5c**, **5d** and **5f** against three different Leukemia cell lines. Data are reported as IC_{50} values (concentrations of complexes required to inhibit cell viability by 50%) determined by MTT assay after 48h of continuous exposure to each compound. The data represent the mean values ± SEM (standard error of mean) of at least three independent experiments.

Figure-6

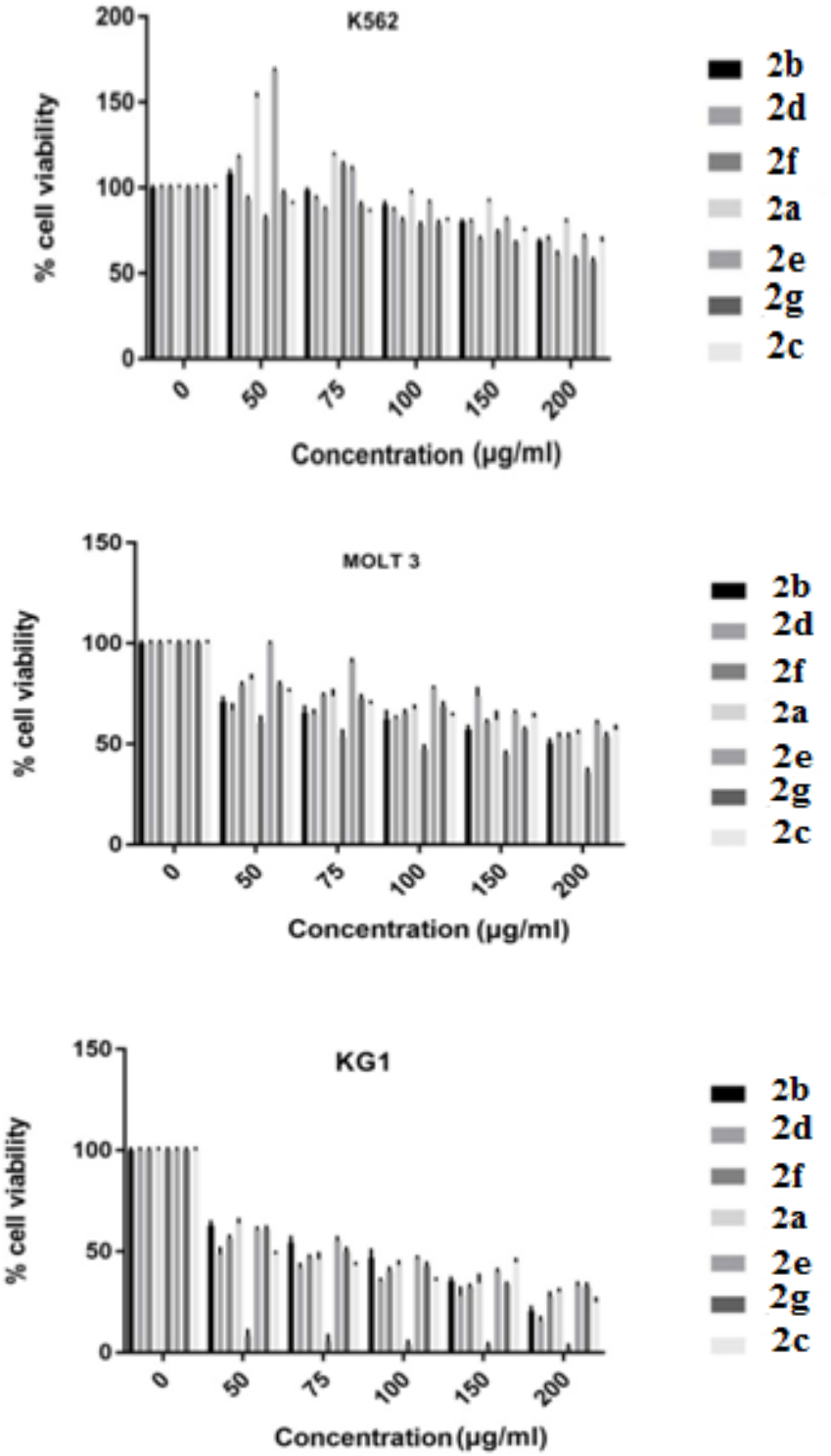


Figure 6: Bar chart representation for the effect of compounds 2a-g on K562, KG 1 and MOLT 3 cell growth and determining % cell viability.

Figure-7

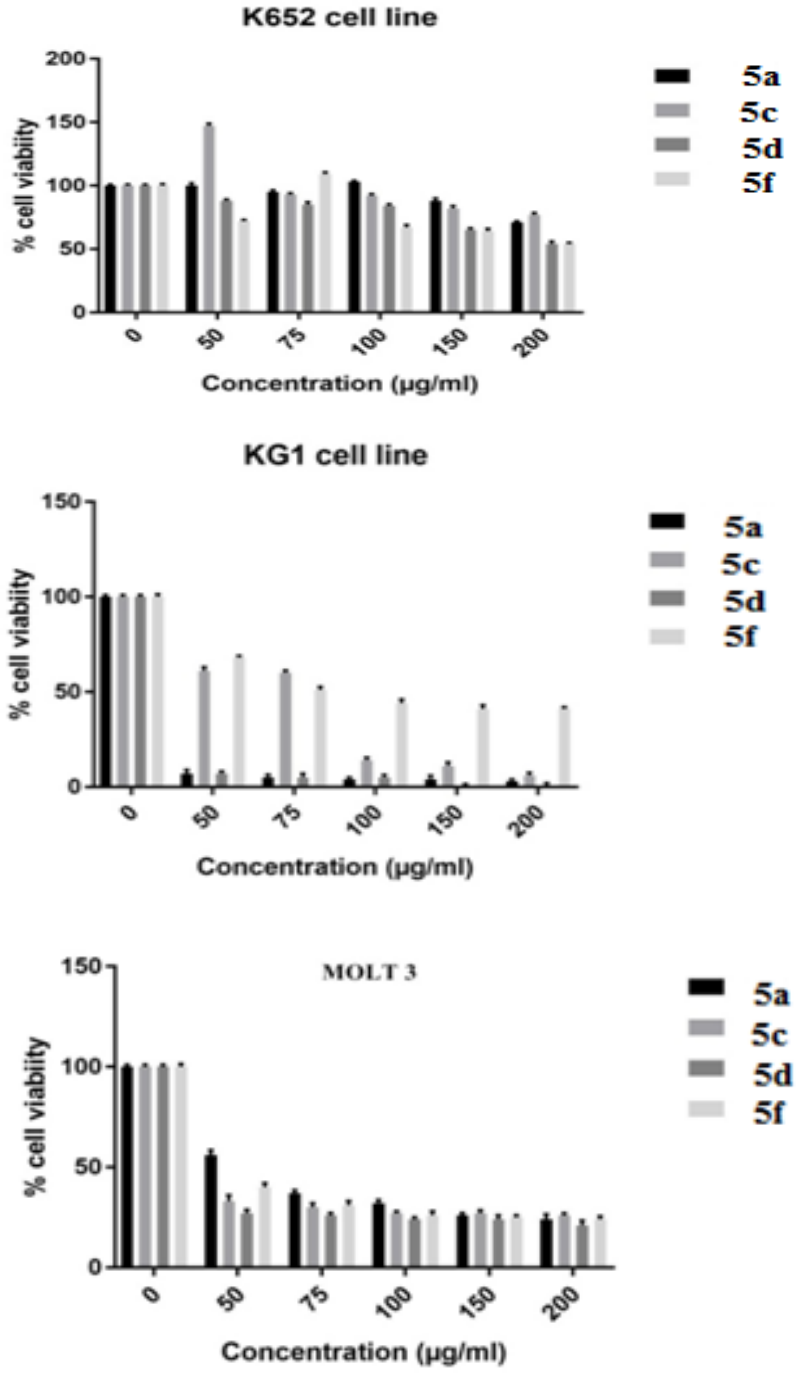


Figure-7: Effect of compounds 5a, 5c, 5d and 5f on K562, KG 1 and MOLT 3 cell growth and determining % cell viability. Cells were cultured and treated with DMSO and 5-Flouro Uracil and % viability was determined by MTT assay test, Experiments were conducted in triplicate and repeated thrice. The value represents the mean ±SD.

MATERIALS AND METHOD

EXPERIMENTAL

Melting points are uncorrected and measured in open capillary using a Rolex melting point apparatus. Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. 3-amino methyl pyridine was purchased from M/s TCI chemicals; Japan. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. All reactions were carried out in nitrogen atmosphere. IR spectra were recorded as KBr pellets on Perkin Elmer RX-1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400

MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI-MS, using a Shimadzu LCMS 2020 apparatus. Elemental analyses were recorded on Eager Xperience Element Analyser.

Chemistry

General procedure for Synthesis of 2-amino substituted benzothiazole 3(a-f) by reported method[33a,b]

The appropriate various substituted aniline derivatives (0.1mol) and equimolecular amount of potassium thiocyanate (KSCN) were added to 100 mL glacial acetic acid with cooling of the reaction mixture in ice bath. The temperature of the ice bath is maintained at 0-5°C. The mixture was left at this temperature up to 20 minute. Then bromine (0.1mol) in glacial acetic acid was added very slowly so that the temperature of the reaction mixture maintained below 10°C, then the mixture was stirred at room temperature for 4-6 h to furnish the hydro bromide (HBr) salt. The salt was then isolated by filtration, washed with acetic acid, dried in vacuum oven and then dissolved in sufficient aqueous ammonia solution to ensure the PH was 11.0. The solid precipitate thus formed was filtered, washed with water and dried in vacuum oven to yield the intermediates **3(a-g).** The progress of the reaction was monitored by TLC with Ethyl acetate-Petroleum ether(3:7) as mobile phase.

General procedure for the Synthesis of Compounds 1(a-g) and 4(a-f) by reported method[34a,b]

To a well stirred solution of substituted aniline 1.0eq. or substituted 2-amino benzothiazole 3(a-f) derivatives in dichloromethane, tri ethyl amine (2.09 mmol,1.01eq.) was added slowly and allowed to stir at 0-5°C for 30 minute. To this bromo acetyl bromide (1.0eq.) added slowly and the reaction mixture was stirred at room temperature for 6-8 hrs. The completion of the reaction monitored on TLC and then the reaction mixture was extracted with ethyl acetate. The extract was washed with water, dilute HCl and again washed with water and dried over anhydrous sodium sulphate and concentrated under vacuum. The yellow precipitates obtained were crystallized from ethanol to give **1(a-g)** and **4(a-f)** as off-white solid.

General Procedure for the Synthesis of final Compounds 2 a-g and 5a-f

To a well stirred solution of **1(a-g)** and **4(a-f)** 1.0eq. in dimethylformamide 15 mL, tri ethyl amine (2.09 mmol,1.01eq.) was added slowly and allowed to stir at 0-5°C for 30 minute. To this 3-amino methyl pyridine (1.0eq.) was added slowly and the reaction mixture was stirred at room temperature for 10-12 hrs. The completion of the reaction monitored on TLC and then the reaction mixture was poured on crushed ice. The solid thus obtained was filtered and washed with excess of water and extracted with ethyl acetate. The extract was washed with excess of water, and dried over anhydrous sodium sulphate and concentrated under vacuum. The precipitates obtained were crystallized from ethanol to give **2(a-g)** and **5(a-f)** as off-white solid.

***N-(4-chlorophenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide* 2a.**

Yield 75%; m.p: 210-212°C; IR(KBr): 3383, 3063, 2968, 2710, 1691, 1612, 1550, 1492, 1400, 1313, 1292, 1251, 1087, 939, 827, 794, 688.cm^{-1};^{1}H-NMR (DMSOd_6, 400MHz) δ 4.026 (S, 2H), 4.405 (S, 2H), 7.39 (d, *J* = 8.0Hz, 2H), 7.66 (d, *J*=8.0Hz,2H), 7.83 (S,1H), 8.46 (S,1H), 8.81(S,1H), 8.97(S,1H), 10.04(S,1H) 11.21 (s ,1H) (amidic proton)^{13}C NMR: δ 47.26, 48.29, 121.25, 125.78, 127.94, 129.28, 130.23, 137.63, 143.81, 146.16, 147.63, 164.24 Molecular weight:275.73g/mol; Mol. Formula: $C_{14}H_{14}ClN_3O$; Elemental analysis; (C,H,N), (Cal: found.), (60.98, 5.12, 15.24: 61.00, 5.14, 15.25). EI MS: 276(m+1).

N-(4-flouro phenyl)-2-[(pyridin-3-ylmethyl) amino acetamide 2b

Yield 75%; m.p: 222-224°C;IR(KBr): 3383, 3063, 2968, 1689, 1564, 1510, 1502, 1410, 1377, 1315, 1259, 1220, 1192, 1116, 1014, 912, 827, 793, 685.cm^{-1};^{1}H-NMR (DMSOd_6,400MHz) δ 4.026 (S,2H), 4.462 (S,2H),7.12-7.21 (m, *J*=16Hz,2H),7.64-7.66(m, *J*=7.6.Hz,2H), 7.83(S,1H), 8.024 (d, *J* = 6.8Hz, 1H) 8.67(S, *J*=6.8.Hz,1H), 8.92 (S,1H), 9.07 (s,1H), 10.09(s,1H) 11.082 (s,1H) (amidic proton).^{13}CNMR:46.96, 48.27, 115.89, 116.11, 121.68, 126.66, 131.68, 135.02, 144.08, 146.28, 157.57, 159.96, 163.94., Molecular weight: 259.23 g/mol; Molecular Formula:$C_{14}H_{14}FN_3O$; Elemental analysis; (C,H,N), (Cal:Obs.), (64.85,5.44,16.21: 64.87,5.42,16.23). EI MS(m/z): 260(m+1).

N-(3-chlorophenyl)-2-[(pyridin-3-yl methyl) amino] acetamide 2c.

Yield 64%; m.p : 204 -206°C; IR (KBr) :3392, 3246, 3065, 2928, 2812, 1691, 1608, 1597, 1546, 1477, 1414, 1375, 1286, 1246, 1192, 1166, 1076, 918, 869, 788, 756, 711, 682cm^{-1}; ^{1}H-NMR(DMSO d_6, 400MHz) δ 3.99 (S, 2H), 4.33(S,2H), 7.17 (d, *J* = 8.0 Hz, 1H) 7.37 (d, *J* = 8.0Hz,1H), 7.49(d, *J* = 8.0 Hz, 1H),7.64(t, *J*=13 Hz,3H),7.80 (S,1H), 8.21(d, *J*=8.0Hz,1H) 8.70 (d, *J*=4Hz,1H), 8.83(S,1H), 9.86(S,1H), 11.14(S,1H) ^{13}C NMR δ:47.60, 48.23, 118.15, 119.17, 124.17, 124.83, 128.97, 131.17, 133.62, 140.03,140.93,148.65,149.91,164.54. Molecular weight: 259.23g/mol; Mol. Formula: $C_{14}H_{14}ClN_3O$; Elemental analysis; (C, H, N), (Cal: Obs.), (60.98, 5.12, 15. 24:60.96, 5.32, 15.26),. EI mass (m/z): 275(m+1).

2-[(pyridin-3-yl methyl) amino] –N-P-tolyl acetamide 2d.

Yield 71%; m.p:208-210°C; IR (KBr): 3412, 3213, 3176, 3055, 2991, 2781, 1690, 1577, 1510, 1483, 1427, 1410, 1388, 1334, 1307, 1292, 1255, 952, 918, 821, 802, 769, 729.cm^{-1};^{1}H-NMR (DMSO d_6, 400MHz) δ2.25(S,3H), 3.94(S, 2H), 4.29 (S,2H), 7.14 (d, *J* = 8.0 Hz, 2H) 7.48-7.54(m,3H), 8.09 (d, *J*=8.0Hz,1H), 8.63 (d, *J*=8.0Hz,

2H), 8.76 (S,1H), 10.75(S,1H).^{13}C NMR δ:20.93,45.72, 48.10, 119.68, 124.25, 129.75, 133.38, 139.27, 149.96, 151.60, 163.80.Molecular weight; 255.14g/mol, Molecular formula;$C_{15}H_{17}N_3O$ Elemental analysis (C,H,N) (Cal: Obs.), (70.56,6.71,16.41:70.73,6.69,16.43) .EI MS(m/z):256(m+1).

N-(3-flouro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide 2e.

Yield 62%; m.p:199-201°C; IR (KBr):3421, 3255, 3200, 3124, 3084, 2972, 2858, 2723, 1691, 1610, 1493, 1481, 1317, 1274, 1257, 1244, 1234, 1190, 1142, 1141, 1074, 1030, 916, 866, 806, 775, 709, 677cm^{-1}; ^{1}H-NMR (DMSOd_6, 400 MHz) δ 3.95 (S,2H) ,4.27 (S,2H),6.94 (t, *J* = 16Hz , 1H) 7.46 (S,1H) , 7.33-7.41(m, J=16Hz,8Hz, 2H) 7.46-7.49 (m,1H) ,7.57-7.60 (d,1H) 8.01 (d, *J* =8Hz,1H), 8.60(d, *J*=8Hz,1H).8.72(S,1H),10.05(S,1H), 11.06(S,1H),^{13}C NMR (DMSOd_6 :100MHz) δ47.95, 48.32, 106.67, 111.02, 115.52, 124.05, 128.30, 131.18, 138.56, 140.23, 140.34, 150.47, 151.64, 164.77. Molecular weight 259.27g/mol, Molecular Formula: $C_{14}H_{14}FN_3O$; Elemental analysis: (C, H, N) (Cal: Obs.), (64.85, 5.44, 16.21:64.87, 5.45, 16.23).EI Ms(m/z) : 260(M+1).

N-(3-Nitro phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide 2f

Yield 59%; m.p:208-210°C; IR (KBr) : 3435, 3244, 3178, 3057, 3012, 2929, 2872, 1693, 1670, 1600, 1545, 1535, 1481, 1365, 1357, 1321, 1271,1257, 1201, 1174, 1030, 962, 937, 908, 840, 802, 707, 609 cm^{-1}; ^{1}H-NMR (DMSOd_6, 400MHz) δ 4.02 (S, 2H), 4.29 (S, 2H),7.49 (m,1H), 7.76 (d, *J* = 8.4Hz, 2H),7.95(d, J=8.4 Hz, 2H), 8.05 (d, *J* = 7.6Hz,1H), 8.61 (d, 1H), 8.74 (s, 1H), 9.84 (s,1H) 11.30 (s,1H).^{13}C NMR (DMSOd_6,100MHz) δ:45.73, 47.92, 119.03, 124.04, 128.20, 130.04, 132.70, 138.66, 142.92, 150.45, 151.67, 164.90, 197.04. Molecular weight: 286.28g/mol, Molecular formula: $C_{14}H_{14}N_4O_3$ Elemental analysis (C, H, N) (Cal: Obs.), (58.73, 4.93, 19.57:58.76, 4.91, 19.57). ESI MS (m/z): 287(m+1).

N-(4-acetyl phenyl)-2-[(pyridin-3-ylmethyl) amino] acetamide 2g.

Yield 68 %; m.p:216-218°C; IR (KBr) : 3412, 3213, 3176, 3055, 2991, 2781, 1690, 1577, 1510, 1483, 1427, 1410, 1388, 1334, 1307, 1292, 952, 918, 821, 802, 769, 729.cm^{-1};^{1}H-NMR (DMSOd_6, 400MHz) δ 2.50(S,3H), 3.82 (S,2H), 4.15 (S,2H), 7.42-7.61(m, J=8.0Hz,2Hz,1H),7.62-7.66 (m, J=8.0 Hz, 2Hz 1H) 7.87-7.90 (m, 3H),7.93-7.99 (m,1H), 82.41-8.42 (d, J=2Hz, 1H) 8.56-8.67(m, J=16Hz, 2Hz,2H),11.29 (S,1H).^{13} C NMR (DMSO d_6,100 MHz)δ: 8.9, 48.62, 49.52, 113.84, 118.74, 123.99, 125.73, 130.49, 130.81, 137.92, 139.91, 148.41, 149.93, 151.18, 166.99. Molecular weight: 283g/mol, Molecular formula: $C_{16}H_{17}N_3O_2$, Elemental analysis :(C, H, N) (Cal: Obs.),(67.83, 6.05, 14.83:67.86, 6.03, 14.85).ESI Ms(m/z):284(M+1).

N – (Benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 5a

Yield 42%, Light yellow coloured solid , mp: 95-97 °C: IR (KBr, cm- 1):3318, 3167, 3048, 2898, 1917, 1702, 1402, 1251,1144, 958, 863, 762, 747, 712, 665; 1H NMR (400 MHz, DMSO-d6) δ : 4.20(s,2H), 4.42(s, 2H), 6.99-7.18(m, 2H), 7.29-7.45(m, 2H), 7.70-7.76(m, 1H), 7.89-7.95(m, 1H), 8.38-8.50(dd, *J*=8.8Hz 1H), 8.74-8.97(d, *J*=8.8 Hz 1H).13C NMR (75 MHz, DMSO-d6) δ : 45.39, 47.08, 121.61, 123.82, 124.80, 126.17, 129.04, 130.85, 131.34, 135.60, 142.11, 143.14, 148.17, 165.22, 172.48.Elemental analysis For C15H14N4OS: (Anal: Cal), (C, H, N) C 60.40; H 4.69; N 18.79, C 60.42; H 4.73: N 18.74.M.W=298 g/mol.

N – (4-Methyl benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 5b

Yield 55 %, Light yellow coloured solid , mp: 146-148 °C: IR (KBr, cm-1):3320, 3172, 3051, 2896, 2855, 2724, 1691, 1598, 1564, 1479, 1408, 1286, 1262, 1146, 960, 865, 767, 749, 713, 666; 1H NMR (400 MHz, DMSO-d6) δ : 2.40(s, 3H), 4.23(s, 2H), 4.55(s, 2H), 8.09(s,2H),8.18(s, 1H),8.31(s, 1H), 8.79(s, 1H), 8.97(s, 1H), 9.17(s, 1H), 10.18(s, 1H), 10.96(s, 1H).13C NMR (75 MHz, DMSO-d6) δ : 19.06, 46.49, 47.50, 123.13, 124.94, 125.57, 126.74, 131.26, 140.26, 140.93, 143.96, 145.56, 146.54, 146.66, 164.26.Elemental analysis For C16H16N4OS: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.58; H 5.09: N 17.99M.W=312 g/mol.

N-(6-Chloro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 5c

Yield 45%, Light yellow coloured solid, mp: 176-178°C:IR (KBr, cm-1):3171, 3066, 2978, 1702, 1664, 1698, 1560, 1441, 1267, 1107, 812, 760; 1H NMR (400 MHz, DMSO-d6) δ:3.50(s,2H), 3.81(s, 2H), 7.32-7.40(m, 2H), 7.41-7.44(m, 1H), 7.68-7.78(m, 1H), 8.05(s,1H), 8.45(d, *J* =3.2 Hz, 1H), 8.55(s, 1H). 13C NMR (75 MHz, DMSO-d6) δ 49.69, 51.02, 121.18, 121.54, 123.21, 126.26, 127.62, 133.14, 135.35, 135.64, 147.34, 147.97, 149.36, 158.35, 171.35.Elemental analysis For C15H13N4OClS: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.58; H 5.09: N 17.99 .M.W=332 .81g/mol.

N-(4-Chloro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 5d

Yield 45%, Light yellow coloured solid, mp: 176-178°C: IR (KBr, cm-1):3171, 3066, 2978, 1702, 1664, 1698, 1560, 1441, 1267, 1107, 812, 760; 1H NMR (400 MHz, DMSO-d6) δ : 3.98(s, 2H), 4.04(s, 2H), 6.91-6.95(m, 1H), 7.21-7.32(m, 1H), 7.44-7.46(d, *J=8.*4 Hz, 1H),7.52-7.54(d, *J=8.4* Hz, 1H), 7.86-7.88(d, *J* = 8.0 Hz 1H) 8.41-8.42(d, *J* =4Hz, 1H), 8.51(s,1H)^{13}C NMR (75 MHz, DMSO-d6) δ: 49.64, 51.01, 119.51, 121.28, 123.22, 124.48, 125.40,126.03, 132.12, 133.06, 145.41, 149.41, 158.64, 167.27, 171.44. Elemental analysis ForC15H13N4OClS: (Anal: Cal), (C, H, N) C 61.54; H 5.13; N 17.95, C 61.51; H 5.10: N 17.92. M.W=332 .81g/mol.

N-(6-bromo benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide. 5e

Yield 39 %, light brown coloured solid, mp: 185-187°C: IR (KBr,cm1): 3325, 3164, 3066, 2908, 2837, 1703, 1668, 1597, 1557, 1478, 1439, 1391, 1330, 1266, 1197,1140, 1085, 1048, 957, 896, 810, 751, 710, 639; 1H NMR (400 MHz, DMSO-d6) δ : 3.95(s, 2H), 4.73(s, 2H), 7.23-7.39(m, 2H), 7.45-7.55(m, 1H) 7.64-7.78(m, 1H), 8.15(s, 1H),8.45- 8.50(m, 1H), 8.50-8.66(m, 1H).^{13}C NMR (75 MHz, DMSO-d6) δ: 47.02, 48.19, 121.94, 123.47, 123.97, 128.93, 132.49, 133.65, 135.65, 147.64, 147.94, 148.48, 149.33, 158.31,171.34 Elemental analysis For C15H13BrN4OS: (Anal: Cal), (C, H, N) C 47.71; H 3.44; N 14.84, C 47.69; H 3.42: N 14.81.M.W=377.26 g/mol.

N-(4-methyl-6-Nitro benzo [*d*]thiazol-2-yl)-2-((pyridine-3-yl methyl) amino) acetamide 5f

Yield 59%, light brown coloured solid, mp: 195-197°C: IR (KBr, cm1):3539, 3473, 3224, 3073, 2959, 2935, 2726, 2601, 1689, 1636, 1609, 1550, 1518, 1468, 1448, 1349, 1267,1230, 1120, 1097, 1064, 980, 879, 804, 744, 680.; 1H NMR (400 MHz, DMSO-d6) δ :2.36(s,3H), 4.16(s,2H), 4.47(s,2H), 8.03(s,1H), 8.21(d, *J*= 1.6Hz, 1H),8.33(d, *J*=1.6Hz,1H), 8.67(d, *J* =6.0Hz, 1H) 8.93(s,1H), 9.07(s,1H), 10.15(s,1H), 10.99(s,1H).^{13}*C* NMR 75 MHz, DMSO-d6) δ: 19.06, 46.49, 47.50, 122.66, 124.28, 124.94, 126.50, 131.03, 139.74, 140.41, 142.83, 144.53, 146.04, 146.86, 163.68. EI MS (m/z):356.49 (m-1). Elemental analysis For C16H15N5O3S: (Anal: Cal), (C, H, N) C 47.71; H 3.44; N 14.84, C47.69; H 3.42: N 14.81. M.W=357 g/mol.

Biological Activity Screening

Antimicrobial Activity

Method: Cup-plate agar diffusion using nutrient agar.[31] Antibacterial activity of all the synthesized compounds was tested in vitro by (cup plate method) serial agar dilution in which bacterial strains of Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus, Bacillus Subtilis*) were used, using serial agar dilution (cup plate method). The two microorganisms were cultured in petri dishes containing agar medium, (four bacterial species and one fungi) cups (8 mm) were put onto the dishes and each synthesized compound dissolved in DMF (0.1 ml of 10 mg/ml) was added into the cups under aseptic condition. Then, the petri dishes were incubated at 37^{o}C for 24 h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments. The antimicrobial activity of tested compounds is shown in **Table-1.**

Anticancer activity

Method: MTT Assay for Anticancer activity.[32]

A549, MCF7, K562, MOLT3 and KG1 cell line cultures were purchased from National Centre for Cell Science, Pune, India. All growth media, supplements and reagents were purchased from HiMedia Labs, Mumbai, India. For the assay, cells were seeded at 10^5 cells/ml in a 96-well plate in dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). To each well, test compounds were added at six different concentrations of 100µM, 50µM, 10µM, 5µM, 1µM and 0.5µM particularly for A549 and MCF7 cell line and other concentrations ranging from 50µM, 100µM, 150µM, 200µM and 250µM particularly for K562, KG1 and MOLT3 cell lines. Each concentration was tested in triplicates. The cells were incubated with these compounds at 37^{o}C under 5% CO_2 for 48 hours. Following this, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5mg/ml. Cells were incubated with this tetrazolium dye for 4 hours. Subsequently, purple crystals of formazan were observed in each well, formed as a metabolic product of MTT. These crystals were dissolved in Isopropanol and the absorbance in each well was recorded at 570nm in a microplate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability. Cells were cultured and treated with DMSO and 5-Flouro Uracil and % viability was determined. Experiments were conducted in triplicate. The values represent the mean ±SD. Data are reported as IC_{50} values i.e. (concentrations of complexes required to inhibit cell viability by 50%) The IC_{50} (µM) values were determined using Graph Pad prism software. The data represent the mean values ± SEM (standard error of mean) of at least three independent experiments.

Statistical analysis

All determinations were performed at least in triplicate, means and standard deviations were determined. Discovery determined using the Two-stage linear step up procedure of Benjamin, Krieger and Yekutieli[35], with Q=1%. Each raw was analysed individually, without assuming a consistent Standard deviation (SD). The Multiple t-test statistical analysis was performed using Graph Pad PRISM® (biostatistics software version 7.0.)

CONCLUSION

In conclusion, we have reported synthesis of compounds **2a-g** and **5a-f** with good yields and screened for their antimicrobial, antifungal and anticancer activities. The compounds of series **2a-g**, two compounds namely **2c** and **2e** showed good antimicrobial activity against *S. aureus and B. Subtilis* and fungi *C. albicans* at 50µg concentration respectively. The screening of compounds **2a-g** and **5a-f** gave very promising results with IC_{50} values 0.51µM, 0.14 µM and 0.73 µM for compounds **2c**, **2d** and **5a** respectively against MOLT3 leukemia cancer cell line. In general it is also concluded that when phenyl ring of amine is substituted at 3rd position plays important role in showing antibacterial as well as antifungal activity. The structure variations such as methyl and halo groups at *meta* and *para* positions of phenyl ring bearing amide linkage resulted in promising

antibacterial and antifungal activity. Furthermore it can be concluded that the designing of amide derivatives of various aromatic amines and 2-amino benzothiazoles with 3-amino methyl pyridine gave the biologically active molecules that can lead to discovery of potential drug candidate.

ACKNOWLEDGMENT

Authors are thankful to the Department of chemistry, Faculty of Science, The M.S. University Baroda for carrying out research work .Authors are thankful to The Head, Department of Chemistry and Department of Zoology Faculty of Science, The M. S. University of Baroda for providing laboratory facilities, One of the author (N.N.S) is thankful to Dr. Rina Soni for her support and thankful to M/s GNFC LTD.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Andria LB, Bowers G, Russell H, Brain JS, Methicillin-Resistant Staphylococcus aureus Infections in Collegiate Football Players, Med. Sci. Sports Exercise, 2008; 40(8): 1362–1367.
2. Foster TJ, Höök M, Surface protein adhesions of *Staphylococcus aureus*. Trends Microbiol, 1998; 6: 484–488.
3. Kennedy KJ, Roberts JL, Collignon PJ, Escherichia coli bacteraemia in Canberra: incidence and clinical features. *Med J Aust*, 2008; 188: 209-213.
4. Vincent JL. Nosocomial infections in adult intensive-care units. Lancet, 2003; 61: 2068–2077.
5. Delaloye J, Calandra T, Invasive candidias is as a cause of sepsis in the critically ill patient. Virulence, 2014; 5: 1, 161-169.
6. Dhanaraj JC, Nair SM, Synthesis, characterization, and antimicrobial studies of some Schiffbase metal(II) complexes. J Co ord Chem, 2009; 62: 4018-4028.
7. Leeb M, News feature, *Nature*, 2004; 431: 892-893.
8. Gibbs JB. Mechanism-Based Target Identification and Drug Discovery in cancer research. *Science*, 2000; *287*: 1969-1973.
9. Parkin DM, Bray F, Ferlay J, Pisani P, Global cancer statistics. CA Cancer J Clin, 2005; 55: 74–108.
10. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin, 2011; 61(2): 69-90.
11. Jung YW, Lippard SJ. Direct Cellular Responses to Platinum-Induced DNA Damage Chem. Rev, 2007; 107: 1387–1407.
12. Abu-Surrah AS, Kettunen M, Platinum Group Antitumor Chemistry: Design and development of New Anticancer Drugs Complementary to Cisplatin Curr. Med. Chem, 2006; 13(11): 1337–1357.
13. Jamieson ER, Lippard SJ, Structure, Recognition, and Processing of Cisplatin-DNA Adducts. Chem. Rev, 1999; 99: 2467–2498.
14. Altaf AA, Shehzad A, Gul Z, Rasool A, Badsha A, Lal B, Khan E A Review on the Medicinal Importance of Pyridine Derivatives. Journal of Drug Design and Medicinal Chemistry, 2015; 1(1): 1-11.
15. Kamal A, Reddy KS, Khan M, Shetti R, Ramaiah M, Pushpavalli SN, Srinivas C, Bhadra MP, Chourasia M, Sastry GN, Juvekar A, Zingde S, Barkume M. Synthesis, DNA-binding ability and anticancer activity ofbenzothiazole/benzoxazole–pyrrolo[2,1 c][1,4]benzodiazepine conjugates. Bioorganic and medicinal chemistry, 2010; 18: 4747-4761.
16. Shi XH, Wang Z, Xia Y, Ye TH, Deng M, Xu YZ, Wei YQ, Yu LT. Synthesis and Biological Evaluation of Novel Benzothiazole-2-thiol Derivatives as Potential Anticancer Agents Molecule, 2012; 17: 3933-3944.
17. Suresh CH, Venkateshwara RJ, Jayaveera KN, Synthesis of 4–(2'–substituted benzothiazoles)–5–mercapto–3–(substituted)-1,2,4-traizole derivatives for possible Antimicrobiological activities Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1(4): 635-640.
18. Alessia C, Alessia C, Ivana D, Marilena M, Antonio C, Françoise VB, Antonio R, Filomena C, Carlo F. 2-Aminobenzothiazole derivatives: Search for new antifungal agents, European Journalof Medicinal Chemistry, 2013; 64: 357-364.
19. Patel NB, Agrawat SN, Shaikh FM, Synthesis and anti microbial activity of new pyridine derivatives. Med. Chem. Res, 2011; 20: 1033-1041.
20. Cobo J, Sanchez A, Nogueras M. Synthesis and antiviral evaluation of pyridine fused heterocyclic and nucleosidic derivatives. Tetrahedron, 1997; 53(24): 8325-8336.
21. Ali A, Bansal D, Kaushik N K, Kaushik N, Choi EH, Gupta R.Synthesis characterization, and anti-cancer activities of pyridine-amide based compounds containing appended phenol or catachol groups. J. Chem. Sci, 2014; 126(4): 1091-1105.
22. Nigade G, Chavan P, Deodhar M, Synthesis and analgesic activity of new pyridine based heterocyclic derivatives. Med chem res, 2012; 21: 27-37.
23. Adriana M. Neves, *a* Auri R. Duval, *a* Gabriele A. Berwaldt, *a* Daniela P. Gouvêa, *a* Natália P. Flores, *b* Pâmela G. da Silva, *b* Francieli M. Stefanello*b* and Wilson Cunico, Thiazolidin-4-ones from 3-(Aminomethyl)pyridine, Arenealdehydes and Mercaptoacetic Acid: Synthesis and Radical Scavenger Activity. J. Braz. Chem. Soc, 2015; 26(2): 381-388.
24. Singh M, Singh SK. Benzothiazoles: How Relevant in Cancer Drug Design Strategy Anticancer Agents Med Chem, 2014; 14: 127-146.
25. Singh M, Modi A, Narayan G, Singh SK. Benzothiazol derivatives bearing amide moiety: Potential cytotoxic and apoptosis-inducing agent

against cervical cancer. Anti cancer drugs, 2016; 27(6): 519-532.
26. Soman SS, Thaker T H, Baloni RD. Synthesis of Amino acetyl coumarins and amino acetyl nepthocoumarines and their antibacterial activity study. Asian J. Res. Chem, 2011; 4: 132-136.
27. Soman SS, Thaker T H. Synthesis of tetracyclic aza -/ oxa-naphthalen-8-ones andtheir antimicrobial activity. Med. chem. Res, 2013; 22: 4223-4227.
28. Soni JN, Soman SS, Synthesis and antimicrobial evaluation of amide derivatives of benzodifuran -2-carboxylic acid Eur. J. Med. chem, 2014; 75: 77-81.
29. Shah NN, Soman SS, Buch P, Balkrishnan S, Synthesis and anticancer activity of some new2-[(4-methyl-2-oxo-2H-chromen-7-yl)-oxy]acetamide derivatives. Der Pharma Chemica, 2016; 8(1): 397-403.
30. Soni. R., Umar. S. Shah N. Balkrishnan. S. Soman. S. Design, Synthesis, and Anticancer Activity of 3H–benzo[f]chromen-3-one Derivatives *J*. het. Chem, (00) 2017.
31. National committee for clinical laboratory standards, Performance standard for Antimicrobia susceptibility testing. 8th informational supplement. M100S12, National committee for Clinical standards, Villanova, Pa, 2002.
32. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J. Immunol. Meth, 1983; 65: 55–63.
33. 33.(a) Vankatesh P. and Pandeya S.N., Int. J. Chem. Res, 2009; 1(4): 1354-1358. (b) Irena, C. A.; Marijeta, K.; Marko, M.; Branimir, B.; Sanja, T.; Gordana, P.; Kresimir, P. and Grace, K. Z.; *Analysis Journal of Medicinal Chemistry*, 2009; *52*: 1744.
34. (a) Nadjet Rezki, Molecules, 2016 Apr 18; 21(4): 505. (b) Akella, S.S.; Kilambi, N.; Lakshmanan, M.; Sriram, R.; Thangapazham, S.; Gaddam, R. Preparation of Acylated Piperazines as Histone Deacetylase (HDAC) Inhibitors for Treating Cancer, Psoriasis and Related Diseases. US Patent 20070088043, 19 April 2007.
35. Benjamini Y, Krieger AM, Yekutieli D Adaptive linear stet-up procedures that control the false discovery rate. *Biometrika*, 2006; 93(3): 491.