DR SHUBHANGI S. SOMAN (Orcid ID : 0000-0002-1405-4064)

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3-Aminomethyl pyridine chalcone derivatives: Design, Synthesis, DNA binding and Cytotoxic studies

Sunil Dutt Durgapal[1], Rina Soni[1], Shweta Umar[2], Balakrishnan Suresh[2], Shubhangi S Soman[1*]

[1]Department of Chemistry, [2]Department of Zoology, Faculty of Science, The M. S. University of Baroda, Vadodara - 390 002, India.

*Corresponding author's e-mail: shubhangiss@rediffmail.com

Ph. No.: +91-265-2795552

Abstract

Herein we report design, synthesis and anticancer activity of compounds **6a-h** and **11a-j**. Compounds **6a-f** were designed based on 3-aminomethyl pyridine attached to different acetamide derivatives and in compounds **6g-h** it was attached to coumarin moiety. Coumarin containing compounds **6g-h** showed very poor anticancer activity against both A549 (Lungs cancer cell line), and MCF-7 (Breast cancer cell line) cell lines in MTT assay. Compounds **11a-j** were designed as derivatives of 3-aminomethyl pyridine and 4-amino chalcones. A series of chalcone derivatives of 3-aminomethyl pyridine **11a-j** have been synthesized and screened for their in vitro anticancer activity and DNA binding affinity. Most of the compounds showed very good antimitotic activity against A549 cell line as compared to fluorouracil. Compounds **11g** and **11i** were selected for DNA-binding studies as they showed excellent activity against cancer cell lines in MTT assay. CT-DNA binding affinity of compounds **11g** and **11i** have been investigated by UV based DNA titration and fluorescence emission study against DNA-EtBr complex. Interestingly, compound **11i** has displayed excellent antiproliferative activity, with IC_{50} 0.0067 ± 0.0002 µM, against MCF-7 cell line. Compound **11i** has been studied for its cytotoxicity using MTT, LDH, as well as EtBr/AO assay and was found to induce apoptosis in the cancerous cell line.

Keywords: Aminomethyl pyridine, Anticancer activity, Cytotoxic studies, Chalcone derivatives, DNA binding.

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1. Introduction

Cancer is second leading cause of death worldwide. According to WHO report, the increase in number of new cases is expected to rise by 70% over next two decades [1]. Cancer progresses *via* multistep carcinogenesis, which involves various physiological processes of human body like uncontrolled growth of cells due to deregulation of essential enzymes, cell signaling and apoptosis. Cancer is extremely complicated to combat [2]. The root cause was found out to be pattern of lifestyle adopted such as excessive use of tobacco, physical inactivity and improper diet [3]. The increase in cancer incidence proves that even today it is not curable with the available therapy and medication. The major downsides of current therapy available to treat cancer include side effects, lack of tumor specificity and multi drug resistance. Hence there is a need of potent anticancer agents to overcome this hurdle. One of the important aspects in anticancer agent is designing of small molecules targeting DNA. The interaction of small molecule with DNA depends not only on ability of molecule to DNA recognition but also on type of interactions such as electrostatic, intercalation, and groove binding [4-5].

α,β-Unsaturated ketones, commonly known as chalcones are important class of natural as well as synthetic products which show variety of biological activities. During last few decades, chalcone derivatives have been reported having potent anticancer activity with low side effects and better solubility for therapeutic applications [6-7]. Simple structural modification in chalcone moiety with heterocycles, polyarene compounds or organometal complexes may lead to new anticancer agents with promising activity [8-9]. Chalcone based small molecules provide advantage over other due to their low toxicity and mutagenicity profile. Yu *et al* showed that 4-(dimethylamino)-4-amino chalcone can interact into base pair and created a new method to determine trace amount of DNA [10].

Interestingly, pyridine derivatives have shown very good effect on anticancer activity when substituted at 3rd position as in Entinostat **1** (MS-275), which is in phase II clinical trials for treatment of various types of cancers [11]. Inci Gul *et al* have reported compound **2** as tumor selective cytotoxin containing both chalcone and pyridine moieties in a molecule [12]. Recently, Wang *et al* have reported pyridine containing compound **3** with very good antitumor activity against xenograft A2780 ovarian cancer [13].

As a part of our ongoing research on anticancer agents [14-15], herein we report design, synthesis and anticancer activity of compounds **6a-h** and **11a-j**. compounds **6a-f** were designed based on 3-aminomethyl pyridine attached to different acetamide derivatives and in compounds **6g-h** it was attached to coumarin moiety. Compounds **11a-j** were designed as derivatives of 3-aminomethyl pyridine and 4-amino chalcones.

2. Experimental

2.1. *Chemistry*

Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. 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. 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 TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI-MS, Mass spectra were determined by ESI–MS, using a Shimadzu LCMS 2020 apparatus (Shimadzu Scientific Instruments, Inc., USA). Elemental analyses were recorded on Thermo Finnigan Flash 11-12 series EA. All reactions were carried out under nitrogen atmosphere. 7-amino-4-methyl coumarin, 3-amino coumarin and chalcones were prepared as reported in literature [16-18].

2.1.1. *Preparation of N-substituted bromoacetamide derivatives (5, 10)*

To an ice-cold solution of substituted amines (10.0 mmol) in dichloromethane (DCM) (25 mL) was added triethylamine (TEA) (15.0 mmol) and stirred for 5-10 min. To this bromoacetyl bromide (12.0 mmol) was added dropwise over a period of 10 min under cooling. The resulting solution was stirred at 0-5 ^{o}C for 30 min and at room temperature for 24 hrs. The reaction mixture was diluted with water and extracted with DCM (2 x 30 mL). The organic layers were combined, washed with 0.5N HCl solution (15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated on a rotavapor to give compounds **5** and **10**. The substituted bromoacetamide **5** and **10** were directly used for next step without any purification

2.1.2. *General procedure for the preparation of compounds 6a-h and 11a-j*

To a cold solution of substituted bromoacetamide **5/10** (1.0 eq) in DMF (20 mL) was added of 3-aminomethyl pyridine **4** (0.5 g, 1.730 mmol) along with base triethylamine (1.5 eq) and stirred for 30 min. The resulting mixture was stirred at room temperature for 14-16 h. The completion of reaction was checked by TLC using DCM:MeOH (9:1). The reaction mixture was poured into ice-cold water. The aqueous layer was extracted using ethyl acetate or dichoromethane (3 x 25 mL). The organic layers were combined, dried over anhy. Na_2SO_4, filtered and concentrated on a rotavapor to give crude product. The crude compound was purified by column chromatography using DCM:MeOH (95:5).
For compounds **6a-f**: To a cold solution of compounds **6a-f** in methanol (15 mL) was added 2M HCl in methanol (1.2 eq). The resulting solution was stirred at 0-5 ^{o}C for 30 min and concentrated on a rotavapor to give solid. The solid was triturated in diethyl ether, filtered and dried to give compounds **6a-f**.
For compounds **6g-h** and **11a-j**: Compounds **6g-h** and **11a-j** were obtained as a solid after column purification.

2.2. Materials and methods for biological assays

2.2.1. Reagent and cell culture

MTT, LDH assay kit (Pierce LDH Cytotoxicity Assay), *N*,*N*-dimethylformamide (DMF), EtBr and Acridine Orange, were purchased from Sigma-Aldrich, India. DMEM, Fetal Bovine Serum (FBS) and trypsin were sourced from (Gibco USA). Human lung carcinoma cell line A549 and human breast cancer cell line MCF-7 were purchased from NCCS, Pune, India. Cell line was maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2 mM l-glutamine and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100g) were adjusted to 1 mL/L. The cells were maintained at 37 ^{o}C with 5% CO_2 in a humidified CO_2 incubator.

Stock solutions of derivatives were prepared in DMF and were diluted with PBS (Phosphate-buffered saline) to achieve working concentration. However, care was taken to maintain the final concentration of DMF not exceed more than 0.5% in any case.

2.2.2. MTT assay

The half minimal inhibitory concentration was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as per standard protocol. Cells were plated in a 96-well plate (1 x 10^4 cells/well) and incubated overnight in 100 µl DMEM media supplemented with 10% FBS. Each compound was added in 0.5, 1, 10, 25, 50, 75, 100 µM conc. and incubated further for 48h. 20 µl of MTT solution (5 mg/mL in PBS) was added and further plate was incubated for 4h. Supernatant solution was removed and the blue formazan was dissolved in 100µl of acidified isopropanol. The absorbance was measured using microplate reader at 570 nm (Metertech Σ960)

Cell viability (%) = (average absorbance of treated groups/average absorbance of control group) × 100%. IC_{50} values were calculated using GraphPad Prism. Each experiment was performed in triplicates.

2.2.3. UV-Based Assay

PerkinElmer Lambda- 35 dual beam UV–Vis spectrophotometer was used for absorption spectral studies. Solution of calf thymus DNA (CT DNA) was prepared in water. The UV absorbance at 260 was found to 0.277 which is used to calculate the DNA concentrations (Ɛ = 6600 M^{-1}) and was expressed in terms of base molarity. UV absorption titrations were carried out by keeping the concentration of compounds **11g** and **11i** (dissolved in DMSO) fixed and by adding a known concentration of CT DNA solution in both the cuvettes in increasing amount until hypochromism saturation was observed. Absorbance values were recorded after each successive addition of DNA solution and equilibration.

2.2.4. Ethidium Bromide Displacement Assay

DNA (100 µL, 8.4 x 10^{-4} M), EtBr (100 µL, 4.2 x 10^{-4} M), and Tris-HCl buffer pH 7.2 was used to make a total volume of 3 mL EtBr displacement fluorescence assay was employed to find DNA intercalation. Fluoroscence emission spectra (λmax= 600 nm, excitation wavelength 546nm, slit width 10nm, 1cm path length) were obtained at 30^{o}C on a JASCO FP-6300 fluorescence spectrophotometer. The assays were performed by using different

concentrations of compounds **11g** and **11i** in buffer solution (3 mL). I_0/I are plotted along with y axis against the concentration of compound, where in I and I_0 are the fluorescence intensities of the DNA-EtBr complex in the presence of and in the absence of compounds, respectively.

2.2.5. Trypan blue

5×10^5 cells per well were seeded in 12 well plate and kept overnight for attachment. Next day cells were treated with IC_{50} conc. of compound **11i**, DMF and TritonX-100 and were incubated for 48 h. DMF treated cells were taken as vehicle control and TritonX-100 as positive control. Following incubation, the supernatant pool was collected and adherent cells were trypsinized and collected. Cell viability was performed by the dye exclusion test with 0.5% trypan blue using a hemocytometer. Each experiment was performed in triplicates.

2.2.6. LDH assay

Lactate dehydrogenase enzyme remains in cytoplasm, however during necrosis due to plasma membrane damage it leaches out. Cells were plated on 96 well plate (1×10^4 cells/well) for 24 h in DMEM media without phenol red, then derivatives were added in the 0.5, 1, 10, 25, 50, 75, 100 µM concentration range. Subsequently, they were incubated for 48 h. Assay was performed according to the manufacture's instruction (Pierce LDH Cytotoxicity Assay, Thermo Scientific, USA). Absorbance was measured at 490 nm in a microplate reader and percentage cytotoxicity was calculated.

2.2.7. Ethidium Bromide/Acridine Orange staining assay

Morphological changes due to apoptosis and necrosis were visualized using EtBr/AO staining technique [19]. Cells were treated with IC_{50} concentration of compound **11i**. For positive control cell were treated with Triton-X 100 and incubated for 48h. Cells were washed with PBS and stained with 1 µl of 1:1 ratio of EtBr and AO (100 µg/ml). Cell to stain ratio was maintained as 1:25 µl. 10 µl of cell suspension was placed on microscopic slide and images were taken using Leica DM 2500 fluorescence microscope fitted with Leica EZ camera.

3. Results and Discussion

3.1. Chemistry

Compounds **6a-h** were synthesized by reaction of bromoacetamide derivative **5** with 3-aminomethyl pyridine **4** (Scheme 1). Amino derivatives were reacted with bromoacetyl bromide to give bromoacetamide derivative **5**, which on reaction with 3-aminomethyl pyridine **4** using triethylamine in anhydrous *N*,*N*-dimethylformamide gave derivatives **6a-h** as shown in scheme 1.

Compounds **6a**-**f** were isolated in the form of hydrochloride salts. The structures of compounds **6a-f** were confirmed by different analytical techniques such as ^{1}H-NMR, ^{13}C-NMR, IR, ESI-MS. In general, the IR spectra of compounds **6a-f** exhibited three strong bands in the range of 3421-3383 cm^{-1}, 3255-

3213 cm^{-1} and 3200-3176 cm^{-1} for secondary amine salt and N-H stretching respectively. Carbonyl stretching of amide was observed in the range of 1691-1670 cm^{-1}. In the ^{1}H NMR spectra of **6a-f**, peak for methylene next to amide group was observed in range of δ ~3.94-4.02 while methylene next to pyridine was observed in range of δ ~4.26-4.46, aromatic protons were observed in range of 6.92-9.67 depending on effect of different substituents on aromatic ring. For compounds **6a-f**, -NH protons of secondary amine salts were observed as broad singlet in range of 9.82-10.09 ppm, while amide -NH proton was observed in range of 10.75-11.30 ppm. In the ^{13}C NMR spectra of **6a-f**, peak for methylene carbons observed around 46.96-47.95 and 48.10-48.37 ppm, aromatic carbons in range of 106-161 ppm, amide carbonyl carbon in range of 164-165 ppm.

All compounds **6a-f** were analyzed by ESI-MS analyses to give $[M+H]^+$/$[M+Na]^+$ peak corresponding to their molecular weight. As modification in compounds **6a-f**, 3-aminomethyl pyridine was attached to comuarin moiety at 7^{th} and 3^{rd} position in compounds **6g** and **6h** respectively. Both compounds **6g** and **6h** were also fully characterized by ^{1}H-NMR, ^{13}C-NMR, IR and ESI-MS analysis and all data complies for formation of desired product.

As a structural modification of compounds **6g-h**, 3-aminomethyl pyridine was linked to 4-amino chalcone derivatives to study the effect of chalcone on activity profile. Compounds **11a-j** were synthesized by reaction of bromoacetamide derivative **10** with 3-aminomethyl pyridine **4** (Scheme 2). 4-Aminoacetophenone **7** on reaction with aldehyde **8** under basic conditions gave 4-amino chalcone derivatives **9** as shown in scheme 2. 4-Amino chalcone **9** on reaction with bromoacetyl bromide gave **10**, which on reaction with 3-aminomethyl pyridine gave various derivatives **11a-j** (Scheme 2).

The structures of compounds **11a-j** were confirmed by different analytical techniques such as ^{1}H-NMR, ^{13}C-NMR, IR, ESI-MS. In general, the IR spectra of compounds **11a-j** exhibited two strong bands in the range of 3333-3236 cm^{-1} and 3290-3169 cm^{-1} for secondary amine and amide N-H stretching vibrations respectively. Carbonyl group of chalcone exhibited stretching frequency in range of 1693-1685 cm^{-1} and that of amide carbonyl in range of 1658-1649 cm^{-1}. In the ^{1}H NMR spectra of **11a-j**, peak for methylene next to amide group was observed in the range of δ ~3.34-4.13 while methylene next to pyridine was observed in range of δ ~3.78-4.50, aromatic protons and chalcone protons observed in range of 6.93-9.11 depending on effect of different substituents on aldehyde. For compounds **11a-j** -NH protons are observed between δ 9.42-11.49 ppm.

In the ^{13}C NMR spectra of **11a-j**, two peak for methylene carbons observed around 46.37-51.51 and 48.03-52.50 ppm, aromatic carbons between δ 113-161 ppm, amide carbonyl carbon around δ 164-171 ppm, chalcone carbonyl carbon between δ 187-189 ppm. All compounds **11a-j** were analyzed by ESI-MS analysis to give $[M+H]^+$/$[M+Na]^+$ peak corresponding to their molecular weight. All these new chemical entities were subjected to *in-vitro* studies for anticancer activity by MTT assay method.

3.2 Biological activity

3.2.1 MTT assay:

Since the 70-80% of total cancer diagnosed all over world are breast cancer and lung cancer, we have selected representative MCF7 and A549 cell lines for our study. 3-Aminomethyl pyridine derivatives **6a-h** were screened for their anticancer activity against A549 (Lungs cancer cell line), and MCF-7 (Breast cancer cell line) cell lines using MTT assay and compared the results with that of standard drug Fluorouracil (Table 1) [20].

Compound **6a** with 4-methyl group found to be inactive against both the tested cell lines. Replacement of 4-methyl to 4-fluoro in compound **6b**, resulted in very good activity against A549 cell lines with IC_{50} 1.186 ± 0.024 µM, but remained inactive against MCF-7 cell line. Similar trend of activity was observed for compound **6c** with 4-chloro group with IC_{50} 0.213 ± 0.0031 µM against A549 cell lines. Interestingly, change in position of halogen from 4th to 3rd position in compounds **6d-e**, resulted in loss of anticancer activity. Compound **6f** with 4-$COCH_3$ group showed moderate activity against tested cell lines. However, coumarin containing compounds **6g-h** showed very poor anticancer activity against both cell lines.

3-Aminomethyl pyridine linked to 4-amino chalcones showed very good activity profile for anticancer activity in MTT assay against tested cell lines. Compounds **11a-b** and **11g-j** showed moderately good activity against MCF-7 cell line as compared to A549 cell line. Compound **11a** showed very good activity against A549 cell line with IC_{50} 6.18 ± 0.11 µM. On placing methyl group at 4th position on chalcone moiety in compound **11b** resulted in loss of activity against tested cell lines. Compound **11c** with methoxy group on 4th position showed very good activity against A549 cell line with IC_{50} 0.269 ± 0.0089 µM, however remained inactive against MCF-7 cell line. On replacement of methoxy group of compound **11c** by nitro group for compound **11f**, showed loss of activity against A549 cell line, while remained inactive against MCF-7 cell line. Compound **11e** having chloro at 4th position, showed very good activity with IC_{50} 5.14 ± 1.07 µM against A549 cell line. Further position change of methoxy group from 4th position in compound **11c** to 3rd position in compound **11g**, resulted in decrease in activity against A549 cell line, however this variation showed excellent activity against MCF-7 cell line with IC_{50} 0.174 ± 0.0076 µM.

On replacement of chloro from 4th to 2nd position in compound **11h** showed loss of activity against both tested cell lines. Interestingly, compound **11i** with chloro on 3rd position showed very good activity against A549 cell line and excellent activity against MCF-7 cell line. Further, compound **11j** containing 2,4-dichloro showed moderate activity against tested cell lines.

Out of all screened compounds **6a-h** (Table 1) and **11a-j** (Table 2), compounds **11g** and **11i** showed excellent activity with IC_{50} 0.174 ± 0.0076 µM and 0.0067 ± 0.0002 µM respectively, as compared to standard drug Fluorouracil against MCF-7 cell line in MTT assay. Hence Compounds **11g** and **11i** were selected to study their DNA binding studies.

3.2.2 DNA binding studies

Compounds **11g** and **11i** were selected for DNA-binding studies as they showed excellent activity against cancer cell lines in MTT assay. To explore mode of interaction of compounds **11g** and **11i** with DNA in cell, DNA binding UV based DNA titration and fluorescence emission study against DNA-EtBr complex were carried out [21]. UV based DNA binding studies gave information for possible interaction taking place between DNA and the lead compounds, while fluorescence based assay, i.e. EtBr displacement assay gave mode of interaction of our lead compounds is through intercalation.

The fixed concentration of compounds **11g** and **11i** was titrated against the known concentration of CT-DNA solution with tris-HCl buffer (pH 7.2) (Figure 3). The strength of binding to CT-DNA was determined through the calculation of intrinsic binding constant K_b which is obtained by monitoring the changes in the absorbance of the compounds with increasing concentration of CT-DNA. Plot of [DNA]/ (ε_A-ε_f) *versus* [DNA] (equation 1) is used to find out K_b. Compound **11g** and **11i** showed the hypochromic shift with the intrinsic binding (k_b) values 2.12 x 10^4 M^{-1} and 1.06 x 10^4 M^{-1} respectively in UV based DNA titrations indicated intercalative mode of binding (λ_{max} = 325 nm) (Figure 3 & Table 3).

$$[DNA]/ (\varepsilon_A-\varepsilon_f) = [DNA]/ (\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_b-\varepsilon_f) \quad \text{(eq 1)}$$

Fluorescence emmission based Ethidium bromide (EtBr) displacement assay was also performed with compound **11g** and**11i**. The emission spectra of DNA-EtBr (λ_{ex} = 546 nm) in the absence and presence of increasing amount of compound **11g** and **11i** were recorded at emission λ_{max} 608 nm**.** (Figure 4). The data were plotted according to the Stern-Volmer equation (eqation 2). Quenching of fluoroscence intensity was observed for compound **11g** and **11i** with K_{sv} values 5.21 x 10^3 M^{-1} and 5.85 x 10^3 M^{-1} respectively (Table 3), which supports the DNA intercalating property of these compounds (Figure 4).

$$I_0/I = 1 + K_{SV}[Q] \quad \text{(eq 2)}$$

3.2.3 Cytotoxic studies:

Compound **11i** induced cytotoxic studies in A549, MCF-7 cancer cell lines and NIH/3T3 non cancer cell line were performed. The cytotoxicity of compound **11i** was estimated using MTT assay and IC_{50} conc. of compound **11i** was measured in both the cancer cell line as well as in non-cancer cell line. Mechanism of cytotoxicity was evaluated by Trypan blue and LDH assay in cancer cell lines. LDH assay is based on the release of cytosolic enzyme into culture medium due to the damage of cell membrane which is a hallmark of necrosis, while MTT assay is based on the activity of mitochondrial dehydrogenase enzyme activity and represent the metabolic rate or percentage viability of cell [22-24].

In MTT assay, the percentage viability of cell line was decreased with increased concentration of compound **11i**. IC_{50} concentration of compound **11i** was estimated using Graphpad Prism 5 software viz. 0.245 ± 0.011, and 0.0067 ± 0.0002 µM in A549 and MCF-7 cell line respectively (Figures 5(a) and 5(b)). Compound **11i** showed IC_{50} 79.31 ± 0.08 µM (Figure 5(c)) for non-cancer mouse fibroblast cell line (NIH/3T3), which was significantly high in compare to IC_{50} values against cancer cell lines A549 and MCF-7. These results prove beyond doubt that when cancer cell will be treated at such lower concentration of compound **11i**, will not be cytotoxic to nearby normal cells, hence, compound **11i** was very specific toward cancer cells. Therefore, it was taken further for the study of mechanism in cancer cell lines against A549 and MCF-7 cell line.

There are several mechanisms by which an anticancer compound exerts its effect on cancer cell *in vitro*. Apoptosis and necrosis are the most preferred mechanisms. To understand the mode of cytotoxicity of compound **11i** for cancer cell lines, LDH Assay was performed in both cancer cell lines. At lower concentration of compound **11i**, the LDH release was very low which revealed that at lower concentration the prevalent mechanism of cytotoxicity was apoptosis but with increased concentration of compound **11i** LDH release increases significantly therefore, at higher concentration treated cells changed its fate towards the necrosis from apoptosis. As IC_{50} value of compound **11i** is very low for both cell lines therefore, it can be deduced that preferred mechanism of cytotoxicity for compound **11i** can be apoptosis (Figure 6 (a)-(b)).

To confirm, that loss of percentage viability was either due to cell death or due to cell proliferation inhibition effect of compound **11i**, Trypan blue assay was performed in both A549 and MCF-7 cell lines. The percentage cell death at IC_{50} concentration of compound **11i** was approx. 36% ± 3% in A549 cell line and 45% ± 2.68% in MCF-7 cell line (Figure 7), from this it can be construed that compound **11i** is cytotoxic towards the MCF-7 whereas it might be cytotoxic or cytostatic toward A549 cell line, further experimental confirmation is required to explain the effect of compound **11i** on cancer cell lines.

In order to reaffirm the findings of LDH assay, the EtBr/AO staining assay was performed with compound **11i**. Acridine orange is a vital dye that stains both live and dead cells however, ethidium bromide stains only the cells that have lost their membrane integrity. EtBr/AO dye stains, necrotic cells red, live cells green, early apoptotic cell's nuclei green but with visible condensation whereas, late apoptotic cell's nuclei Orange with condensation and fragmentation.

Cells were treated with IC_{50} concentration of compound **11i** and it was found that most of the cells of A549 cell line (Figure 8(a)-(c)) were under late apoptosis, there were no cells under necrosis whereas, in MCF-7 cell line (Figure 8(d)-(f)) most of the cells were under early apoptosis with few under necrosis. Number of the cells under apoptosis and necrosis were negligible in control cell lines. Which confirmed the LDH assay finding that compound **11i** exhibits cytotoxic activity in both cell lines *via* apoptosis. To confirm the possible interaction of compound with DNA, compound **11i** was studied for DNA binding activity.

4. Conclusion

In conclusion, a series of chalcone of 3-aminomethyl pyridine derivatives were synthesized and evaluated for their anticancer activity against A549 and MCF-7 cell lines. Based on MTT assay, one of the compounds **11i** has shown very good selectivity for MCF-7 cancer cell line as compared to NIH/3T3 normal cell line (non-cancer mouse fibroblast cell line). DNA binding studies of compound **11i** indicated intercalation mode of binding with CT-DNA. The cytotoxic studies of compound **11i** have shown the apoptosis in MCF-7 cell line using LDH assay and the EtBr/AO assay. From the K_b binding values it can be concluded that compound **11g** has two-fold higher affinity for CT-DNA binding than compound **11i.** Further study for sequence selectivity is undergoing for the lead compounds.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Characterization data and spectral data for compounds **6a-h** and **11a-j** are provided in the supporting Information.

Figure 1: Compounds with anticancer activity.

Figure 2: Designing of hybrid chalcone **11a-j** derived from 3-aminomethyl pyridine and 4-amino chalcones.

Scheme 1: Synthesis of compound **6a-h**. Reagents and Conditions: (i) TEA, DMF, 0-5 ^{o}C, 30 min, r.t 14-16 h.

Scheme 2: Synthesis of compound **11a-j**. Reagents and Conditions: (i) 40% aq NaOH, ethanol, r.t 5-10 h; (ii) bromoacetyl bromide, TEA, DCM, r.t, 16-18 h; (iii) TEA, DMF, 0-5 ^{o}C, 30 min, r.t 14-16 h.

Table 1: Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) for compounds **6a-h**.

[a]IC_{50} values were determined based on MTT assay using GraphPad Prism software. NA = Not active

Table 2: Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) compounds **11a-j**.

[a]IC_{50} values were were determined based on MTT assay using GraphPad Prism software. NA = Not active

Figure 3: Titration plot of compounds 11g and 11i with DNA. Plot of Absorbance *versus* Wavelength (nm) (a) for compound **11g** and (b) compound **11i**. Plot of [DNA]/($Ɛ_A$-$Ɛ_f$) versus [DNA] (c) for compound **11g** and (d).for compound **11i**

Figure 4: Plot of Fluorescence emission intensity I *versus* Wavelength (nm) for DNA-EtBr complex at different concentrations (a) for compound **11g** and (b) for compound **11i**. Stem-Volmer quenching plot of DNA-EtBr (c) for compound **11g** and (i) compound **11i**.

Table 3: K_b and K_{SV} values for compound **11g** and **11i** .

Figure 5: The cytotoxicity of compound **11i** against A549, MCF-7 and NIH/3T3 cell line. **In MTT assay (A-C)** cells were treated with 0.5, 1, 10, 25, 50, 75, 100 µM concentrations of compound **11i** graph was plotted against % viability v/s dose. Data were represented as mean ± SD from three independent experiments. ***P<0.001

Figure 6: In LDH assay (a-b): Representation of cytosolic enzyme LDH (a) activity of LDH in A549 cell line (b) activity of LDH in MCF-7 cell line. Cells were treated with 0.5, 1, 10, 25, 50, 75, 100 µM concentrations of compound **11i**. Graph was plotted against LDH release versus dose. (***P ≤ .001, **p < .01 significance one-way ANOVA (Tukey–Kramer). ANOVA, analysis of variance; LDH, lactic dehydrogenase.

Figure 7: Trypan blue assay- The percentages cell death of A549 and MCF-7 cell line treated with DMF, with IC_{50} concentration of compound **11i** and with positive control was plotted. Data was represented as mean ± SD from three independent experiments.

Figure 8: EtBr/AO assay: EtBr/AO assay was performed with A549 cell line (a,b,c) and MCF-7 (d,e,f). (a,b,c) images represent control, IC_{50} conc. compound **11i** treated and positive control in A549 cell line; (d,e,f) images represent control, IC_{50} conc. compound **11i** treated and positive control in MCF-7 cell line.

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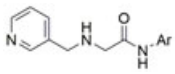
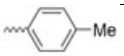
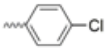
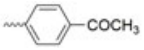
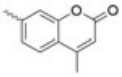
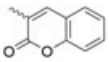
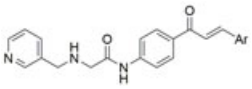
Table 1: Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) for compounds **6a-h**.

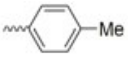
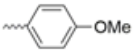
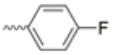
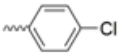
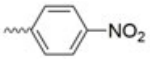
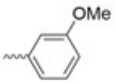
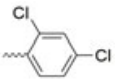


Compound no	Ar	IC_{50} in µM[a]	
		A549	**MCF-7**
6a		NA	NA
6b		1.186 ± 0.024	NA
6c		0.213 ± 0.0031	950 ± 11.87
6d		NA	NA
6e		NA	NA
6f		32.63 ± 2.1	90.78 ± 6.24
6g		177.10 ± 18.2	377.8 ± 29.32
6h		69.1 ± 8.2	298.9 ± 21.2
fluorouracil		11.13 ±0.083	45.04 ±1.02

[a]IC_{50} values were determined based on MTT assay using GraphPad Prism software. NA = Not active

Table 2: Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) compounds **11a-j**.

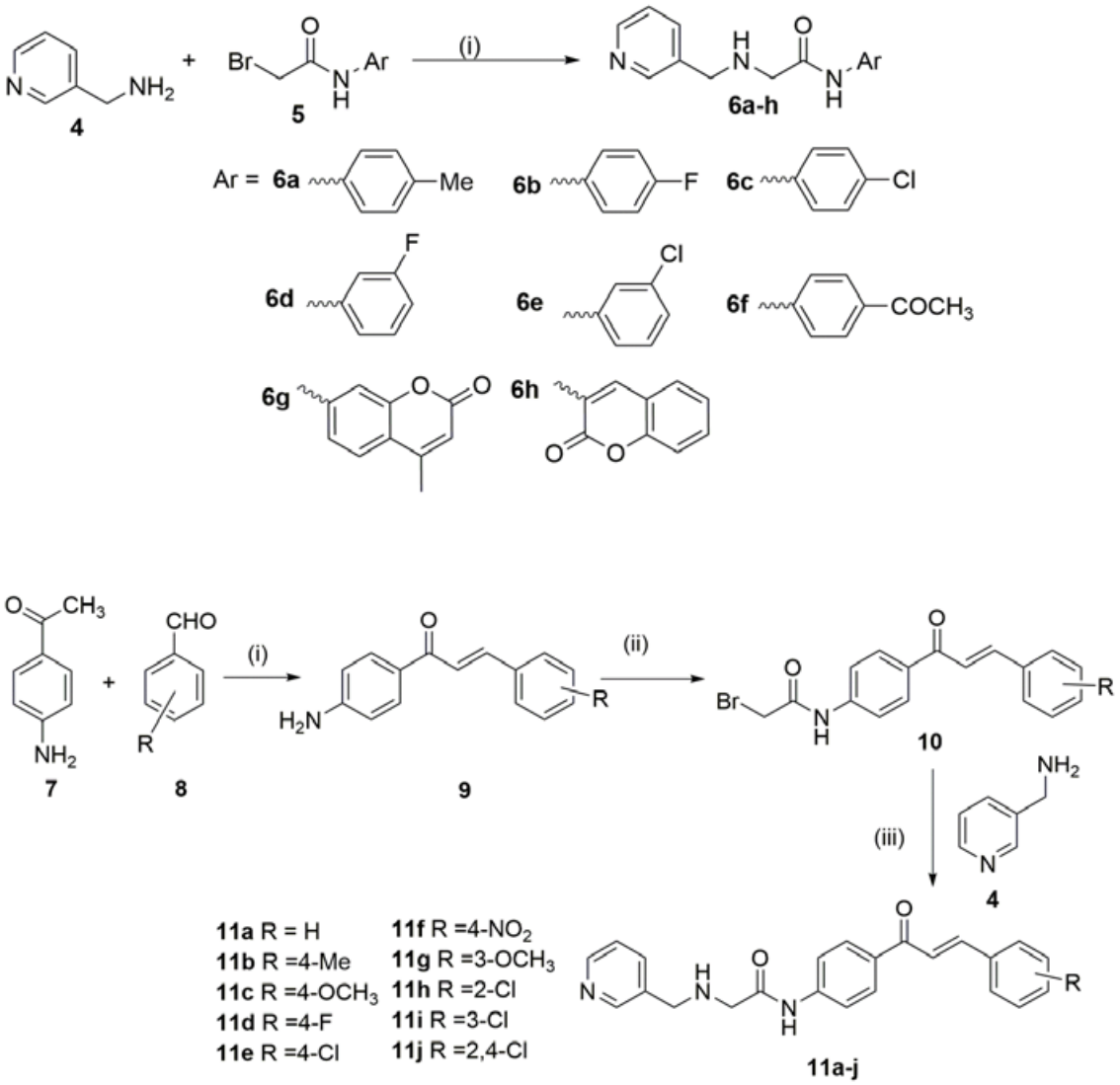


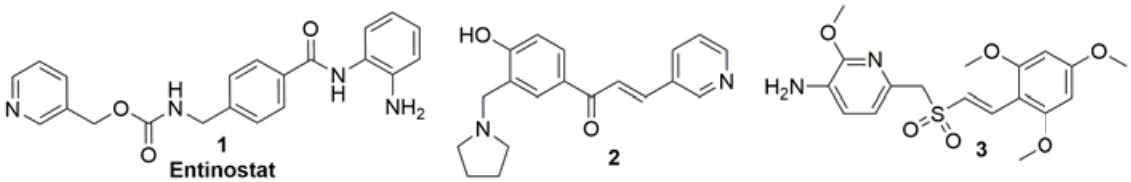
Compound no	Ar	IC_{50} in µM[a]	
		A549	MCF-7
11a		6.18 ± 0.11	53.27 ± 3.56
11b		132.00 ± 9.86	90.61 ± 5.96
11c		0.269 ± 0.0089	n/a
11d		16.04 ± 3.43	n/a
11e		5.14 ± 1.07	n/a
11f		28.19 ± 1.19	n/a
11g		32.42 ± 2.08	0.174 ± 0.0076
11h		46.89 ± 6.08	71.72 ± 5.32
11i		0.245 ± 0.011	0.0067 ± 0.0002
11j		62.26 ± 5.9	92.21 ± 7.21
Fluorouracil		11.13 ±0.083	45.04 ±1.02

[a]IC_{50} values were were determined based on MTT assay using GraphPad Prism software. NA = Not active

Table 3: K_b and K_{SV} values for compound **11g** and **11i**.

Compound No	λ_{max} nm	UV based assay K_b (M^{-1})	Emission λ_{max} nm	Fluorescence assay K_{SV} (M^{-1})
11g	325	2.12×10^4	610	5.21×10^3
11i	325	1.06×10^4	608	5.85×10^3

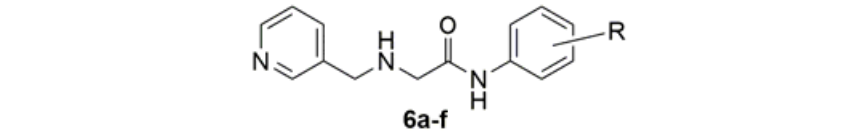




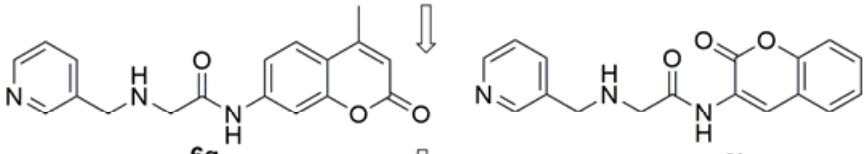
1
Entinostat

2

3

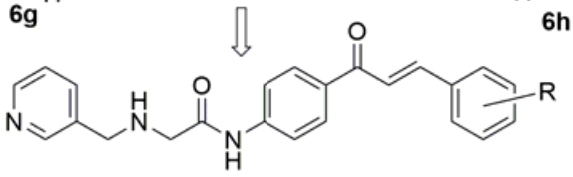


6a-f



6g

6h



11a-j