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Research Article

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Synthesis and Biological Evaluation of Imidazopyrmidine-Propenone Conjugates as Potent Tubulin Polymerization Inhibitors

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ABSTRACT

A library of imidazopyrimidine-propenone conjugates (8a-8g) were synthesized and evaluated for their antitumor activity against three human cancer cell lines namely prostate (DU-145), lung (A549) and breast (MCF-7) cancer. These conjugates showed good to moderate activity against the tested cell lines. Among them two conjugates (8c and 8d) showed significant antiproliferative activity against human lung cancer cell line (A549) with an IC50 values of 1.236 µM and 1.327 µM respectively. Flow cytometric analysis revealed that these conjugates 8c and 8d arrest the cell cycle at the G2/M phase and induce cell death by apoptosis. The tubulin polymerization assay showed that these compounds 8c and 8d effectively inhibited the microtubule assembly in human lung cancer cells (A549). The molecular modeling studies showed that the compound 8c interacts and binds efficiently with the tubulin protein at the colchicine site. Overall, the present investigation demonstrated that the synthesized imidazopyrmidine-propenone conjugates are promising tubulin inhibitors.

Keywords: Imidazopyrimidine-propenone; Anticancer activity; Tubulin polymerization; Molecular modeling

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INTRODUCTION

Cancer is a dreadful disease marked by uncontrolled proliferation of abnormal cells which invades to other organs of body leading to morbidity and mortality. Cancer is one of the leading causes of death around the globe where nearly 8.5 million people die of cancer in 2015. Microtubule is a heterodimeric protein made of two sub units that is α-tubulin and β-tubulin. They are long, filamentous, tube shaped protein polymers that are essential in all eukaryotic cells. Microtubule perform various funtions in cell such as development and maintenance of cell shape, cell signalling, transport of vesicles and other components throughout the cells apart from cell division (mitosis) [1,2]. Microtubules plays a prominent role in building up of mitotic spindle during mitosis which makes them an promising target for anticancer drugs. Microtubules and their dynamics with multiple tubulin-binding sites are the targets of a chemically diverse group of antimitotic drugs that have been used with great success in the treatment of cancer. Here are some of the microtubule targeting agents shown in (Figure 1). Imidazopyrimidine is a fused bicyclic heterocylce with three nitrogen that represent an important class of privileged scaffold [3-6]. imidazopyrimidine scaffold displays a broad profile of biological activity, tumour suppression being important among them [7]. Many attempts to discover new drugs with novel technologies have fallen short of producing the desired results. Henceforth, privileged structure guided scaffold re- modification is one of the primary strategy to identify structurally new chemo types by modifying either the central core of the scaffold or by modifying the side-chain of existing active compounds [8]. Thus imidazopyrimidine scafflod provide an immense scope to utilize undescribed bioactivities by making use of existing motifs with well established synthetic protocols of imidazopyrimidine.

Aryl-aminopropenone [9] represents a new class of compound reported recently with potent antimitotic property via distrubtion on tubulin dynamics and induction of apoptosis in tumours cells. In our efforts to discover newer conjugates we designed and synthesized a series of imidazopyrimidine-propenone by introducing amino propenone chain to central imidazopyrimidine core scaffold and the synthesized conjugates were tested for their antiproliferative effect in a set of cancer cell lines. The two most active molecules in the series (8c and 8d) were further investigated for their tubulin inhibition. The results of our investigations along this direction are presented in this work.

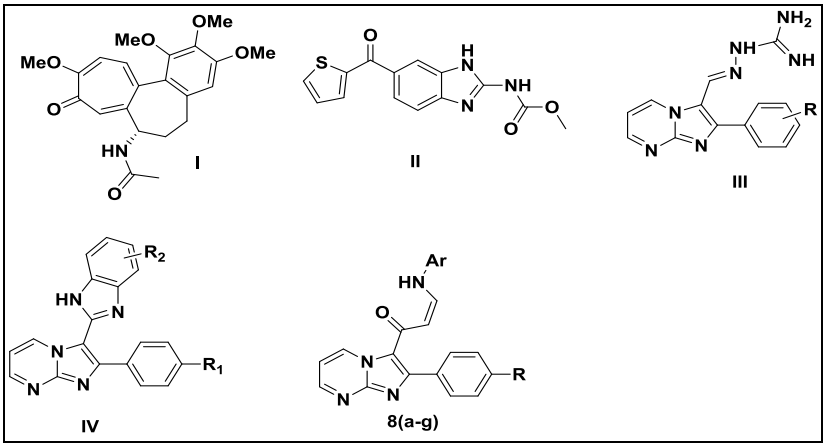

Figure 1: Chemical structures of microtubule targeting agents. Colchicine (I), nocodazole (II), imidazopyrimidine guanylhydrazones (III) imidazopyrimidine-benzimidazoles (IV) and imidazopyrimidine-propenone conjugates (8a-g)

EXPERIMENTAL SECTION

Chemistry

All chemicals and reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Reactions were monitored by TLC performed on silica gel coated glass plates containing 60 GF254 and visualized by UV light or iodine staining. Column chromatography was performed with Merck 60–120 mesh silica gel. NMR spectra were recorded on Bruker UXNMR/ XWIN-NMR (300 MHz) or Inova Varian-VXR-unity (400 or 500 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from an internal TMS standard. Data are reported as follows: chemical shift (ppm) (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). ESI spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS–MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus and are uncorrected.

Synthesis of 2-(aryl)imidazo[1,2-a]pyrimidine (4a-b)

2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidine:

2-Bromo-1-(4-methoxyphenyl) ethanone (1a, 6.026 g, 26 mmol) and 2-aminopyrimidine (2, 2.46 g, 26 mmol) were dissolved in acetone and the reaction mixture was refluxed for 4–5 h. The resulting salt (3a) was collected by filtration, washed with acetone, dissolved in 3 N HCl (200 mL) and refluxed again for 1 h. Before complete cooling, the solution was cautiously basified by drop wise addition of 15% aq. NH_4OH to pH 8. The resulting base was collected by filtration and crystallized from EtOH to afford compound 2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidine (4a) as a white solid (5 g, 85% yield); Mp: 187-189ºC; 1H (ppm): 8.54 (d, J = 7.3 Hz, 1H), 8.46 (m, 1H), 7.92 (d, J = 6.2 Hz, δNMR ($CDCl_3$, 300 MHz) 2H), 7.82 (d, J = 4.1 Hz, 1H), 6.94 (d, J = 6.2 Hz, 2H), 6.85 (d, J = 4.1 Hz, 1H), 3.85 (s, 3H); MS (ESI): m/z 226 (M+1)+.

2-phenylimidazo[1,2-a]pyrimidine (**4b**):

Compound 4b was prepared according to the method described for compound 4a, employing 2-bromo-1-(phenyl)ethanone (1b, 5.026 g, 25 mmol) and 2-aminopyrimidine (2, 2.36 g, 25 mmol) to obtain the pure product 4b as a white solid (4.5 g, 90% yield); Mp: 144–46ºC; 1H (ppm): 8.51-8.50 (m, 1H), 8.42 (d, J = 6.1 Hz, 1H), 8.01 (d, J = 7.4 δNMR (CDCl3, 300 MHz) Hz, 2H), 7.81-7.80 (m, 1H), 7.44 (t, J = 7.2 Hz, 14.7 Hz, 2H), 7.35 (d, J = 7.17 Hz, 1H), 6.85- 6.82 (m, 1H); MS (ESI): m/z 196 [M+1]+.

Synthesis of 2-(aryl)imidazo[1,2-a]pyrimidine -3-carbaldehyde (5a-b)

2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidine-3-carbaldehyde (**5a**):
Vilsmeier reagent was prepared by addition of $POCl_3$ (10 mL, 111 mmol) to a stirred solution of DMF (8.6 mL, 111 mmol) in $CHCl_3$ (10 mL) at 0–5°C. To this reagent, 2-phenylimidazo[1,2-a]pyrimidine 4a (5 g, 22 mmol) in chloroform (20 mL) was added while maintaining cold conditions. After complete addition, the reaction mixture was stirred at room temperature for 3 h and at reflux conditions for 10–12 h. After completion of the reaction, as indicated on TLC, chloroform was removed under reduced pressure and the resulting oily liquid was poured onto ice. The aldehyde 5a was collected by filtration and crystallised from EtOH (5 mL) to obtain the pure product 5a as a white solid (5 g, 90% yield); Mp: 178-180ºC; 1H NMR ($CDCl_3$, 300 MHz) 10.13 (s, 1H), 9.88 (dd, J = 1.8 Hz, 6.7 Hz, 1H), 8.82-8.81 (m, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 2.3 Hz, 6.7 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 3.89 (s, 3H); MS (ESI): m/z 254 (M+1)+.

2-Phenylimidazo[1,2-a]pyrimidine-3-carbaldehyde (5b):
Compound 5b was prepared according to the method described above for 5a, employing 2-(phenyl)imidazo[1,2-a]pyrimidine (4b, 4.5 g, 23 mmol) to obtain the pure product 5b as a white solid (4.3 g, 83% yield); Mp: 155–158ºC; 1H NMR ($CDCl_3$, 300 MHz) 10.19 (s,1H), 9.89 (dd, J = 1.9 Hz, 8.7 Hz, 1H), 8.86-8.84 (m, 1H), 7.93-7.90 (m, 2H), 7.56-7.54 (m, 3H), 7.20 (dd, J = 2.5 Hz, 8.7 Hz, 1H); MS (ESI): m/z 224 [M+1]+.

Synthesis of 1-(2-(aryl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-ol (6a-b)

1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-ol (**6a**):
Compound 6a was obtained by stirring solution of 2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidine-3-carbaldehyde (5a, 5 g, 19.8 mmol) with ethynyl magnesium bromide solution (60 mL, 30.0 mmol) (0.5 M) in tetrahydrofuran at 0°C and then stirred at room temperature for 4-5 h. After completion of reaction saturated aqueous ammonium chloride solution (5-10 mL) was added, THF removed under vacuum, followed by addition of ethyl acetate. The organic layer was extracted and washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain pure compound 6a as brown solid (4g, 72% yield); M.p: 184–186°C; ^{1}H NMR ($CDCl_3$+DMSO, 300 MHz): δ 8.63 (d, *J* = 6.9 Hz, 1H), 7.63-7.52 (m, 2H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.08-7.00 (m, 1H), 6.91 (d, *J* = 6.3 Hz, 1H), 6.35 (s, 1H), 5.97 (s, 1H), 3.87 (s, 3H), 2.91 (s, 1H); MS (ESI): m/z 280 $[M+H]^+$.

1-(2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-ol (6b):
Compound 6b was obtained using the above described method, employing 2 (phenyl)imidazo[1,2-a]pyrimidine -3-carbaldehyde (5b, 4.3 g, 19.3 mmol) to the ethynyl magnesium bromide solution (58.1 mL, 29 mmol) to obtain pure product as brown solid (3 g, 62.7% yield); M.p: 171–173°C; 1H NMR (300 MHz, CDCl3+DMSO) δ 8.69 (d, *J* = 6.9 Hz, 1H), 7.70-7.67 (m, 2H), 7.60 – 7.53 (m, 1H), 7.45 – 7.39 (m, 2H), 7.32 (t, *J* = 8.5 Hz, 1H), 6.89 (t, *J* = 6.6 Hz, 1H), 6.02 (s, 1H), 2.89 (s, 1H); MS (ESI): m/z 250 $[M+H]^+$.

Synthesis of 1-(2-(aryl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7a-b)

1-(2-(4methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (**7a**):
A solution of 2-iodoxy benzoic acid (IBX) (6.02 g, 21.5 mmol) and dimethyl sulfoxide (DMSO) was stirred for 10 min at room temperature until homogeneous solution. A solution of 1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-3-yl)prop-2-yn-1-ol (6a, 4 g, 14.3 mmol) in dimethyl sulfoxide was added slowly and stirred for 2 h. After completion of reaction, ice water was added to reaction mixture and the mixture was stirred for another 10 min. To this mixture ethyl acetate was added and filtered through celite. The organic layer was separated and washed sequentially with water, saturated Na_2CO_3 solution and brine; dried over anhydrous Na_2SO_4 and evaporated in vacuum. It was recrystallized from methanol to obtain the pure compound 7a (2.8 g, 70% yield); M.p: 145–147°C;

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.92-9.85 (m, 1H), 8.83 (dt, *J* = 4.5, 2.3 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.81 – 7.72 (m, 1H), 7.23-7.19,(m, 1H), 7.07 (d, *J* = 8.8 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 3.90 (s, 3H), 2.69 (s, 1H).MS (ESI): 278 $[M+H]^+$.

1-(2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7b):
Compound 7b was obtained using the method described for 7a by adding 1-(2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-ol (6b, 3 g, 12 mmol) to the 2- iodoxy benzoic acid (5.08 g, 18 mmol) in DMSO solution (20 mL) resulting compound obtain as pale yellow solid (2.3 g, 77% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.92-9.85 (m, 1H), 8.83-8.79 (m, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.79 – 7.74 (m, 1H), 7.23-7.19,(m, 1H), 7.07-6.93 (m,3H), 2.65 (s, 1H). MS (ESI): 248 $[M+H]^+$.

Synthesis of (Z)-3-(arylamino)-1-(2-arylimidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one (8a-g)

(Z)-3-((3,5-dimethoxyphenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one (8a):
(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7a, 100 mg, 0.361 mmol) was added to stirred solution of 3,5-dimethoxyaniline (55.23 mg, 0.361 mmol) in ethanol (5 mL) and stirred at 25°C for 4 h. The progress of the reaction was monitored by TLC (Hexane/EtOAc=6:4). After completion of reaction (TLC), the yellow color solid, that precipitated on addition of water, was filtered and washed with ethanol to give the titled compound in good yield (115 mg). M.p: 167-169°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.82 (d, *J* = 12.3 Hz, 1H), 9.92 (dd, *J* = 6.9,2.0 Hz, 1H), 8.78-8.69 (m, 1H), 7.75 (d, *J* = 8.6 Hz, 2H),7.12- 7.01 (m, 3H), 6.84 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 5.49 (d, *J* = 7.8 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.79, 160.09, 152.07, 149.98, 145.86, 144.60, 136.16, 131.82, 126.23, 114.01, 112.16, 109.89, 107.84, 101.39, 97.19, 56.27, 56.02, 55.36; ESI-MS: m/z 431 $[M+H]^+$.

(Z)-3-((2-fluorophenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one (8b):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7a, 100 mg, 0.361 mmol) and 2-fluoroaniline (40.07 mg, 0.361 mmol) in ethanol (5 mL) as yellow color solid in good yield (103). M.p: 224–226°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (d, *J* = 12.0 Hz, 1H), 9.94 (dd, *J* = 6.9, 2.0 Hz, 1H), 8.69 (dd, *J* = 4.1, 2.0 Hz, 1H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.17 – 6.99 (m, 6H), 6.93-6.88 (m, 1H), 6.78 – 6.73 (m, 1H), 5.55 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 3H); 13C NMR (125 MHz, $CDCl_3$) δ 183.41, 163.36, 152.24, 152.38, 151.29, 148.16, 142.50, 136.40, 131.93, 128.88, 126.60, 124.91, 123.41, 118.99, 116.28, 114.96, 113.87, 110.11, 99.08, 55.49; ESI-MS: m/z 389 $[M+H]^+$.

(Z)-3-((4-bromophenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one (8c):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7a, 100 mg, 0.362 mmol) and 4-bromoaniline (62.26 mg, 0.362 mmol) in ethanol (5 mL) as yellow color solid in good yield (115 mg). M.p 243–245°C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO) δ 11.71 (d, *J* = 12.6 Hz, 1H), 9.87-9.77 (m, 1H), 8.70 (d, *J* = 5.1 Hz, 2H), 8.19 (s, 1H), 7.41 (dd, *J* = 24.8, 8.6 Hz, 3H), 7.11 – 7.03 (m, 3H), 6.96 (d, *J* = 8.5 Hz, 2H), 5.44 (d, *J* = 7.9 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.33, 181.25, 153.54, 148.15, 142.26, 139.69, 135.41, 134.42, 132.12, 129.46, 128.41, 118.34, 117.65, 116.37, 110.31, 109.11, 98.55, 55.7; ESI-MS: m/z 449 $[M+H]^+$.

(Z)-3-((4-fluorophenyl)amino)-1-(2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one (8d):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(4-methoxyphenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7a, 100 mg, 0.361 mmol) and 4-fluoroaniline (40.07 mg, 0.361 mmol) in ethanol (5 mL) as yellow color solid in good yield (108). M.p: 185–187°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.86 (d, *J* = 12.0 Hz, 1H), 10.01 (dd, *J* = 6.9, 2.0 Hz, 1H), 8.69 (dd, *J* = 4.1, 2.0 Hz, 1H), 7.78 – 7.70 (m, 3H), 7.22 (dd, *J* = 12.2, 8.1 Hz, 1H), 7.17 – 7.09 (m, 3H), 7.05 – 7.00 (m, 3H), 5.61 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 3H); 13C NMR (125 MHz, $CDCl_3$) δ 183.41, 160.63, 153.24, 152.38, 151.29, 149.16, 142.50, 136.40, 131.93, 128.88, 126.60, 124.91, 123.41, 118.99, 116.08, 114.97, 113.85, 110.01, 99.00, 55.39; m/z 389 $[M+H]^+$.

(Z)-1-(2-phenylimidazo[1,2-a]pyrimidin-3-yl)-3-((3,4,5-trimethoxyphenyl)amino)prop-2-en-1-one (8e):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7b, 100 mg, 0.404 mmol) and 3,4,5-trimethoxyaniline

(73.92 mg, 0.404 mmol) in ethanol (5 mL) as yellow color solid in good yield (126 mg). M.p: 199–201°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.80 (d, *J* = 12.3 Hz, 1H), 10.00 – 9.91 (m, 1H), 8.69 (dd, *J* = 4.0, 2.0 Hz, 1H), 7.79 (dd, *J* = 7.1, 2.2 Hz, 2H), 7.47 (dd, *J* = 4.9, 1.9 Hz, 3H), 7.17 (dd, *J* = 12.3, 8.0 Hz, 1H), 7.05 (dd, *J* = 6.9, 4.2 Hz, 1H), 6.27 (s, 2H), 5.46 (d, *J* = 7.9 Hz, 1H), 3.86 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.89, 154.19, 152.15, 144.09, 136.30, 134.42, 130.42, 128.81, 110.06, 97.58, 93.91, 61.07, 56.20; ESI-MS: m/z 431 $[M+H]^+$.

(Z)-3-((4-chlorophenyl)amino)-1-(2-phenylimidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one(8f):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7b, 100 mg, 0.404 mmol) and 4-chloroaniline (51.83 mg, 0.404 mmol) in ethanol (5 mL) as yellow color solid in good yield (128 mg,). M.p: 191–193°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.79 (d, *J* = 12.1 Hz, 1H), 10.01 - 9.93 (m,1H), 8.71 (dd, *J* = 4.1, 2.0 Hz, 1H), 7.78 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.50 – 7.46 (m, 3H), 7.31 – 7.26 (m, 2H), 7.15 (dd, *J* = 12.3, 8.0 Hz, 1H), 7.06 (dd, *J* = 6.9, 4.2 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 5.48 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.89, 153.84, 148.56, 144.27, 139.33, 138.39, 137.45, 135.33, 130.45, 128.40, 119.88, 117.29, 110.65, 98.90, 95.19; ESI-MS: m/z 375 $[M+H]^+$.

(Z)-3-((4-bromophenyl)amino)-1-(2-phenylimidazo[1,2-a]pyrimidin-3-yl)prop-2-en-1-one(8g):
The titled compound was prepared using the method described for compound 8a, by addition of (2-(phenyl)imidazo[1,2-a]pyrimidin-3-yl)prop-2-yn-1-one (7b, 100 mg, 0.404 mmol) and 4-chloroaniline (69.63 mg, 0.404 mmol) in ethanol (5 mL) as yellow color solid in good yield (131 mg). M.p: 206–208°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.78 (d, *J* = 12.2 Hz, 1H), 9.97-9.91 (m, 1H), 8.71 (dd, *J* = 4.1, 2.1 Hz, 1H), 7.80 – 7.76 (m, 2H), 7.50 – 7.46 (m, 3H), 7.45 – 7.42 (m, 2H), 7.15 (dd, *J* = 12.3, 8.1 Hz, 1H), 7.06 (dd, *J* = 6.9, 4.2 Hz, 1H), 6.93 (d, *J*=8.0, 2H), 5.49 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.23, 180.25, 154.54, 149.15, 143.26, 139.29, 136.31, 134.32, 132.72, 130.46, 130.03, 129.37, 129.03, 128.41, 119.30, 117.65, 116.07, 111.41, 110.19, 98.35; ESI-MS: m/z 419 $[M+H]^+$.

BIOLOGY

Anti Cancer Activity

The anticancer activity of the compounds was determined using MTT assay [10]. 1×10^4 cells/well were seeded in 100 µL DMEM supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37°C in a CO_2 incubator. After 24 h of incubation, all the synthesized compounds were added to the respective wells and incubated for 48 h. After 48 h of drug treatment, 10 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. The supernatant from each well was carefully removed, formazan crystals were dissolved in 100 µL of DMSO and absorbance at 570 nm wavelength was recorded.

Cell Cycle Analysis

A549 cancer cells were seeded in 6 well plates and treated with conjugates 8c and 8d at concentration of 1 µM for 48 h. After the treatment, both the floating as well as trypsinised adherent cells were collected, washed with phospahate buffer saline and fixed with 70% ethanol. After fixation the cells were washed with PBS and stained with 50 µg/mL propidium iodide in hypotonic lysis buffer (0.1% sodium citrate, 0.1% Triton X-100) containing DNase free RNase-A for about 20 min. Stained cells were analyzed then using fluorescence-activated cell sorter caliber (Becton Dickinson) [11].

Inhibition of Tubulin Polymerization

A fluorescence based *in vitro* tubulin polymerization assay was performed according to the manufacturer's protocol (BK011, Cytoskeleton, Inc.) [12]. Briefly, the reaction mixture in a total volume of 10 µL contained PEM buffer, GTP (1 µM) in the presence or absence of test compounds (final concentration of 3 µM). Tubulin polymerization was followed by a time dependent increase in the fluorescence due to the incorporation of a fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured by using a Varioscan multimode plate reader (Thermo Scientific Inc.). Nocodazole was used as the positive control in each assay. The reaction mixture for these experiments include: tubulin (3 mg ml^{-1}) in PEM buffer, GTP (1 mM), in the presence or absence of test compounds at 3µM concentration. Polymerization was monitored by increase in the fluorescence as mentioned above at 37°C.

Molecular Docking Procedure

The optimization of all the geometries is carried out in Gaussian 09 using PM3 semi-empirical method. The crystal structures of PDB (3E22) were obtained from the RSCB protein data bank. Docking studies were performed using AutoDock 4.2 software [13]. The Analysis of intermolecular interactions has been performed using Pymol, v. 0.99 [14].

RESULTS AND DISCUSSIONS

Chemistry

Synthesis of the imidazopyrmidine-propenones (8a-g) is shown in Scheme 1. To obtain 2-arylimidazopyrimidine (4a-b), equimolar mixtures of substituted 2-bromoacetophenones and 2-aminopyrmidine were refluxed for 4-5h followed by 2N HCl under reflux conditions. The intermediates, imidazopyrimidine aldehydes (5a–b) were prepared by means of Vilsmeier-Hack reaction on the corresponding 2-arylimidazopyrmidine (4a–b). These aldehydes (5a-b) were further treated with ethynyl magnesium bromide in THF to obtain the intermediates (6a-b) followed by oxidation with IBX in DMSO providing the corresponding precursors (7a-b). Subsequently, the desired compounds (8a-g) were prepared by reaction of corresponding precursors (7a-b) with arylamines in ethanol. The list of substitutions along with their corresponding yields and respective melting points are listed in Table 1.

Scheme 1: Synthesis of imidazopyrimidine-propenone

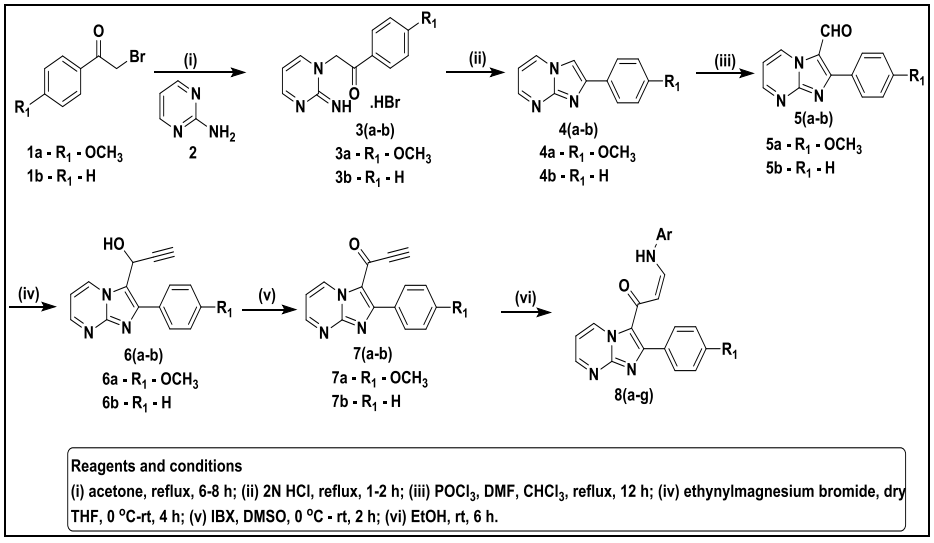

Table 1: Structures of compounds 8(a–g) and their yields

Compound	Ar	R1	Yield %
8a	3,5-dimethoxyphenyl	OCH3	74%
8b	2-flourophenyl	OCH3	73%
8c	4-bromophenyl	OCH3	71%
8d	4-flourophenyl	OCH3	77%
8e	3,4,5-trimethoxyphenyl	H	71%
8f	4-chlorophenyl	H	66%
8g	4-bromophenyl	H	62%

Cytotoxic Activity

In order to explore the structure activity relationship of imidazopyrimidine-propenone conjugates (8a-8g), the scaffold consist of 3 rings as depicted in the Figure 2. for convenience the rings are labelled A, B and C. For SAR study Changes were made at 4th position of rings B and C respectively whereas ring A was remains unchanged. The cytotoxicity of the synthesized derivatives was evaluated against three human cancer cell lines namely prostate (DU-145), lung (A549) and breast (MCF-7) cancer cells by employing MTT[15] assay using Nocodazole as reference drug. The results are summarised in Table 2. The IC_{50} values of these conjugates shows considerable cytotoxic activity ranging from 1.236 µM – 18.32 µM. Particularly conjugate 8c, having an OCH_3 group at ring B and bromine at ring

C, posses significant activity against human lung cancer and prostrate cancer cells with IC_{50} values of 1.236 µM and 1.483 µM respectively. Another conjugate 8d, having OCH_3 atom at 4th position of ring B and flourine atom at 4th position on ring C, displayed IC_{50} value 1.327 µM and 1.541 µM respectively. An outlook on the substituents on ring B shows that the potency is maximum when the ring is substituted with electron donating substituent (methoxy) and decreases when no subsititution is present at the ring.

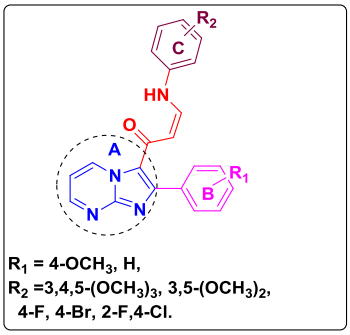

Figure 2: Structure activity relationship of imidazopyrmidine-propenone conjugates

Table 2: IC_{50} (µM) values[a] for compounds 8(a-g) on selected human cancer cell lines.

Compound	DU-145b	A549c	MCF-7d
8a	5.212	4.482	4.836
8b	12.15	9.635	18.32
8c	1.732	1.236	1.483
8d	2.133	1.327	1.541
8e	7.234	3.251	9.2
8f	9.78	7.713	9.553
8g	5.281	2.532	7.923
Nocodazole	1.291	1.589	1.091

Note: 50% inhibitory concentration after 48 h of drug treatment; b Human prostate cancer; c Human lung cancer; d Human breast cancer

Effect on Cell Cycle Arrest

In order to investigate the mode of action underlying the antiproliferative activity of these potential conjugates (8c and 8d), the cell cycle analysis in lung cancer cell line was analyzed by using the flow cytometry technique. In this present experiment, lung cancer cells were treated with compounds 8c and 8d at 1 µM concentration for 48 h. The data obtained clearly suggested that these compounds show G2/M cell cycle arrest in comparison to the untreated control. These compounds (8c and 8d) showed 40.2% and 42.1% of cell accumulated in G2/M phase at 1 µM concentration respectively as shown in Figure 3 and Table 3 whereas in control (untreated cells) 5.6% of G2/M phase arrest was observed. The effect of these conjugates on cell cycle progressions correlated well with their cytotoxic activity.

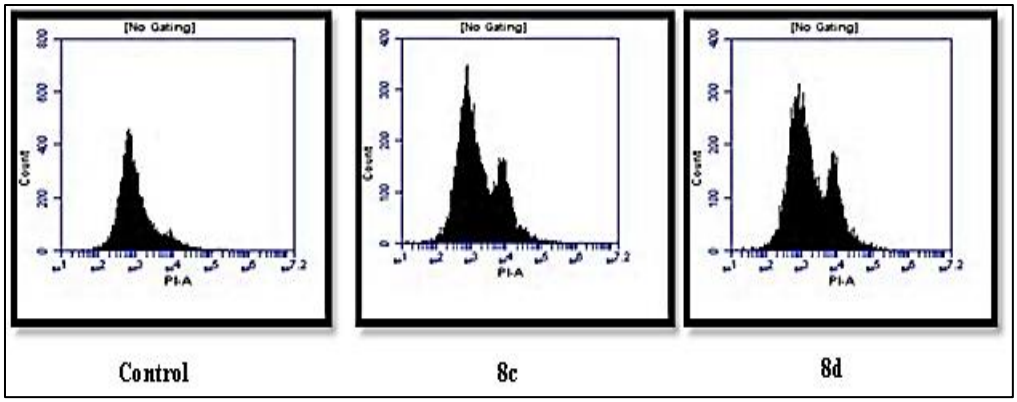

Figure 3: Antimitotic effects of compounds 8c and 8d by fluorescence-activated cell sorting (FACS) analysis. Generation of cell-cycle arrest at G2/M by compounds 8c and 8d in Lung cancer cells

Table 3: Distribution of cells at G0/G1, S phase and G2/M phase of cell cycle

Compound	% of cells in Sub-G1phase	% of cells in G1phase	% of cells in S phase	% of cells in G2/M phase
Control	3.2	79.5	11.7	5.6
8c	2.9	47.7	9.2	40.2
8d	2.8	46.5	8.6	42.1

Effect on Tubulin Polymerization

To elucidate whether the antiproliferative activities of these conjugates 8c and 8d were related to the interaction with tubulin, we studied their effects on the tubulin polymerization in a cell-free system. As tubulin subunits heterodimerize and self-assemble to form microtubules in a time dependent manner, we have investigated the progression of tubulin polymerization by monitoring the increase in fluorescence emission at 420 nm (excitation wavelength is 360 nm) in a 384 well plate for 30 min at 37°C with and without the compounds at 3 µM concentration. Conjugates 8c and 8d inhibited tubulin polymerization by 52.04%, 56.11% respectively, compared to the control (Figure 4). Tubulin polymerization inhibition was also observed in the case of the standard like nocodazole (50.75%).

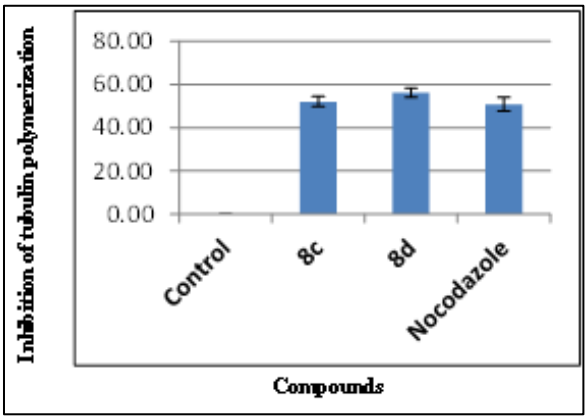

Figure 4: Tubulin polymerization assay was carried out in a reaction mixture that contained PEM buffer and GTP (1 mM) in the presence or absence of test compounds (8c and 8d) at 3 µM concentration. The reaction was firstly initiated by the addition of GTP to all the wells. Tubulin polymerization was monitored by the increase in fluorescence at 420 nm (excitation wavelength is 360 nm) was measured for 1h at 1 min interval in a multimode plate reader (Tecan) at 37°C

Docking Studies

To check the interactions of the compound 8c in the colchicine binding site of tubulin. The tubulin- colchicine binding site protein was taken from RCSB PDB (ID 3UT5). All natural substrates, ions and water molecules were

removed as part of protein preparation. AutoDock 4.2 was used to add Kollman and Gasteiger charges. A grid box encapsulating the colchicine site was generated-the grid spacing was 1.0 Å and the box size was 25 Å in each coordinate. The protein was kept rigid and compound 8c was flexible during the course of docking. The maximum number of binding modes was set to 10. The conformation with the lowest binding energy was used and assumed to be the best docked. Docking results were visualised using Pymol, v. 0.99. Various interactions were observed between the conjugate 8c and the protein. Conventional hydrogen bonds were formed with the carbonyl oxygen and βLys254 and also with αAsn101. αGln11 formed hydrogen bond with the π bond nature of the carbon. Hydrophobic interactions were observed between βAla250 and phenyl ring bearing bromo- substitution. αGlu183 shows a π-π interaction with imidazopyrimidine ring. Hydrophobic bond between αSer178 and nitrogen of the imidazole ring was observed. αAla180 exhibited π bonding with the methoxy- substituted phenyl ring as shown in Figure 5.

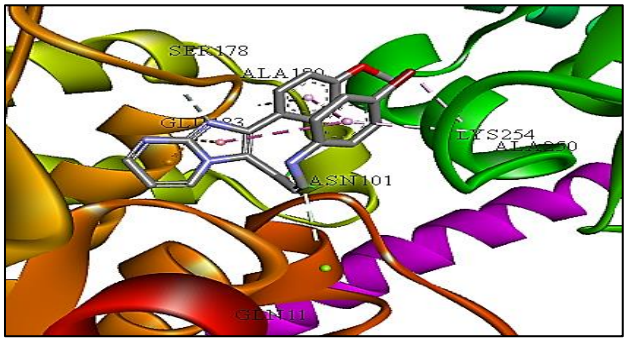

Figure 5: Binding interactions of compound 8c in the colchicine binding site

CONCLUSION

In the conclusion, we synthesized conjugates of imidazopyrmidine-aminopropenone (8a-8g) and evaluated for their antiproliferative activity against three human cancer cell lines namely prostate (DU-145), lung (A549) and breast (MCF-7) cancer. Among them, compounds 8c and 8d showed significant antiproliferative activity ($IC_{50,}$ 1.236 µM and 1.327 µM respectively) against human lung cancer cell line (A549). Moreover, preliminary studies showed that they arrest cells in the G2/M phase of the cell cycle. The tubulin polymerization assay showed the level of tubulin inhibition of both the compounds is similar to that of standard nocodazole. The docking experiment displayed the binding mode for the interaction of compound 8c with the colchicine binding site. These results suggest that these conjugates have the potential to be developed as strong tubulin polymerization inhibitors for the treatment of cancer.

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