Ind. J. Pharm. Edu. Res., 2023; 57(3s):s742-s748.
https://www.ijper.org

Original Article

Multicomponent Synthesis, Characterization of Novel Pyrimidine Derivatives with Anti-cancer Potential

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ABSTRACT

Background: Search for the better anti-cancer agents become central part for many research teams as the current drugs in use suffer lack of specificity to the cancer targets. Design and development of target specific anti-cancer agents increase the potency and safety of the drugs. **Materials and Methods:** Current research intends to develop a novel series of the pyrimidine derivatives owing to the anti-cancer potential of the pyrimidine scaffold. In a multicomponent reaction approach, 4,5-disubstituted pyrimidines (4) were synthesized from three component coupling reaction of substituted enamine (1), an orthoester (triethoxy methane) (2) and ammonium acetate (3). 4,5-disubstituted pyrimidines (4) were oxidized to corresponding aldehyde derivative (5) via Stephen aldehyde synthetic process. Then the pyrimidine aldehyde coupled with various aromatic amines to produce final pyrimidine imine derivatives (6a-6j). By using IR, ^{1}H-NMR, and mass spectral studies, all the prepared derivatives were characterized and subjected to anti-cancer activity evaluation by MTT Assay. Four cancer cells (A 549 (lung), B16F10 (mouse skin melanoma), SiHA (cervical), MCF-7 (breast), and one normal fibroblast (L929)] were employed to study the anti-cancer potential of the synthesized pyrimidine derivatives. **Results:** The synthesized compounds produced in moderate to good yields with proposed scheme of synthesis. All the synthesized derivatives displayed noticeable cytotoxicity against the tested cancer cell lines. **Conclusion:** All of the evaluated cell lines were susceptible to the potential cytotoxicity of the newly synthesized novel pyrimidine derivative. These brand-new pyrimidine compounds may be transformed into potent anti-cancer lead molecules.

Keywords: Multicomponent synthesis, Pyrimidines, Anti-cancer, MTT Assay.

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Received: 25-09-2022;
Revised: 30-01-2023;
Accepted: 23-06-2023.

INTRODUCTION

In 2018, it is appraised that there were 18,1 million new cases of cancer and that 9,6 million individuals perished as a consequence of the cancer.[1] Despite considerable advances and sophisticated technical advancements in cancer treatment techniques across the world, cancer remains to be a prevalent disease that presents a threat to human health.[2,3] Chemotherapy is recognized as an important realistic technique because of its relative efficacy in compared to other therapies, etc. This is one of the principal strategies used in the battle against cancer.[4,5] Despite the efficacy of several anti-cancer chemotherapeutic medicines in the treatment of different forms of cancer, the long-term sequelae and side-effects of anti-cancer therapies continue to be a major cause of concern for both patients and physicians. This is because anti-cancer medications might promote cancer in other places of the body. Current efforts to combat anti-cancer drug-induced adverse effects are successful in the majority of circumstances. These include surgical operations and the effects of radiation. Nevertheless, these measures do not even come close to addressing the possible long-term consequences.[6] In order to address and overcome this challenge, innovative anti-cancer medicines that target cancer targets, have an improved safety profile, and are effective are necessary. In order to address and overcome this challenge, innovative anti-cancer medicines that target cancer targets, have an improved safety profile, and are effective are necessary.

Research institutions of all shades and the pharmaceutical sector are hard at work generating innovative anti-cancer drugs that are target-specific, effective against cancer cells, and have the ability to induce selective action. In addition, the obvious similarities between normal and malignant cells, as well as the variety of tumors, are the fundamental challenges preventing the development of decisive chemotherapeutic agents.[7] As a consequence, the medical and pharmaceutical industries are continually involved in a process involving the research and development of novel anti-cancer medications.

DOI: 10.5530/ijper.57.3s.84

The pyrimidine nucleus is emerging as a lead nucleus for the treatment of cancer among the significant nitrogen-containing heterocyclic scaffolds. Imatinib, nilotinib, Dasatinib, and Pazopanib are the four anti-cancer drugs that comprise the pyrimidine nucleus (Figure 1). The US FDA has approved the combination of gefitinib and erlotinib for the treatment of NSCLC (non-small cell lung cancer).[8] These two drugs are new quinazolines that restrict epidermal growth factor receptor autophosphorylation and EGF-stimulated signal transduction in cancer cells, hence suppressing Epidermal Growth Factor Receptor Tyrosine Kinase (EGFR-TK).[9,10] The phosphorylation of EGFR in response to EGF is responsible for both of these results. The EGFR, is one of the targets of tyrosine kinase inhibitors that has been the focus of the most in-depth study.[11,12] Tyrosine kinases are important enzymes that play crucial roles in cell survival, metastasis, differentiation, and proliferation. Cancers such as breast, prostate, ovarian, lung, and brain are caused by the unregulated activation of these enzymes and the increased production of EGRF that results from mutations that occur throughout the expression process.[13] Due to the essential role that the EGRF-TK pathway plays in the development of a broad range of cancers, a significant number of research organizations are focusing on the development of novel anti-cancer medications that specifically inhibit this pathway.

Due to the fact that pyrimidine derivatives are the key lead compounds that block the EGRF-TK pathway directly, efforts were undertaken to generate new organic chemicals having a pyrimidine ring structure at their core. The cytotoxicity of these compounds was then evaluated *in vitro* against one normal human cell line and four distinct cancer cell lines.

MATERIALS AND METHODS

In this study, we employed unpurified synthetic compounds and solvents acquired from outside sources like Sigma-Aldrich. In order to see the reactions, we used Merck's precoated aluminium TLC plates of silica gel 60 F_{254} and saw the spots using a UV chamber and iodine vapours. Remi electronic melting point equipment was used to measure melting temperatures.

The Agilent FTIR was employed to capture infrared spectra using the KBr pellet technique. The BRUKER DRX - 500 MHz was used to record the ^{1}H NMR. All chemical shifts (δ) are expressed in terms of ppm relative to the internal standard Tetra Methyl Silane (TMS). The various kinds of splitting are indicated by the letters s

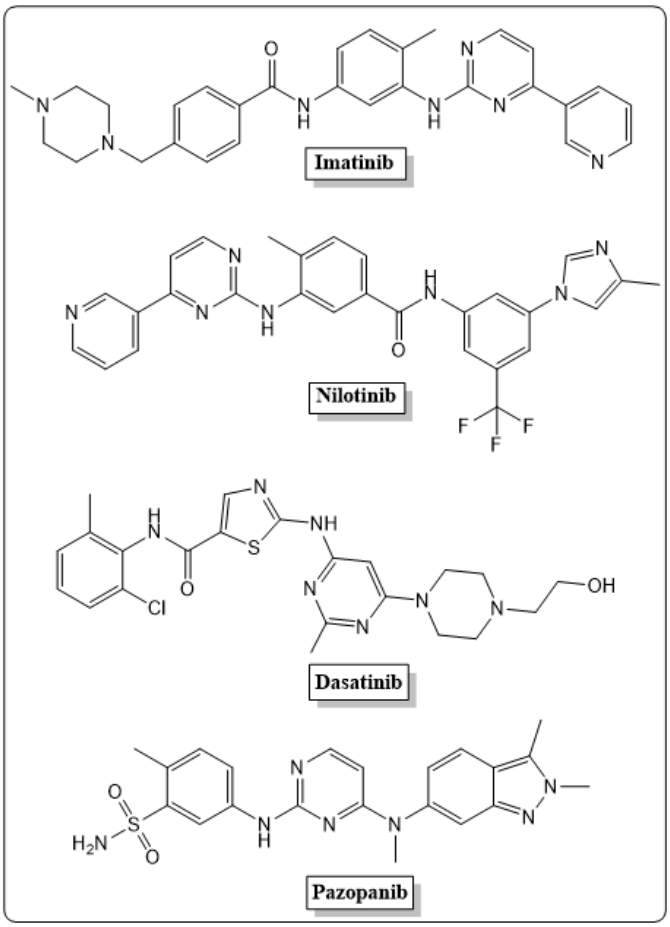

Figure 1: Major anti-cancer drugs containing pyrimidine scaffold.

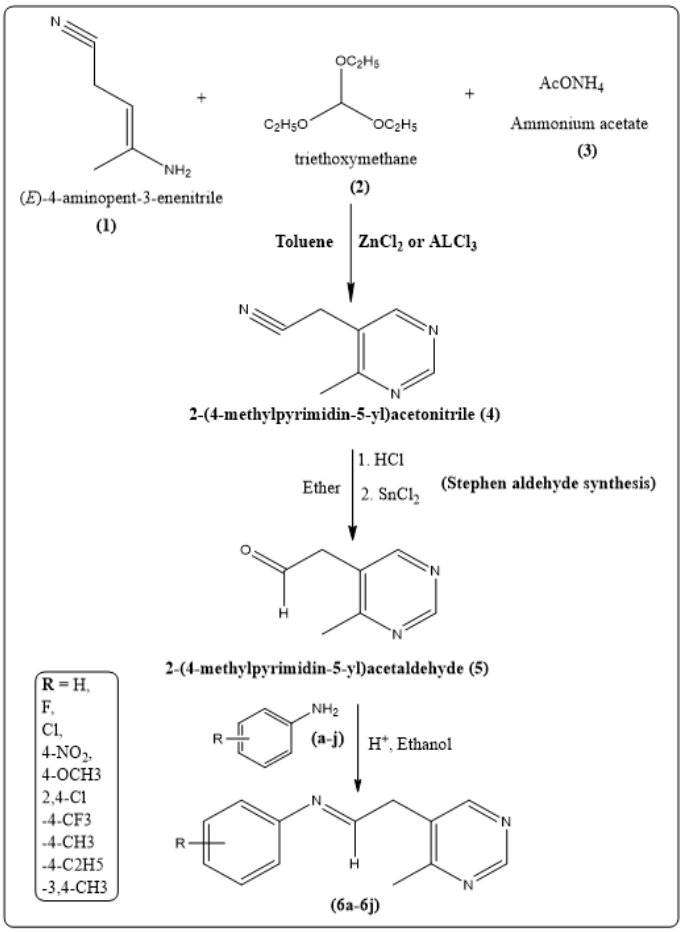

Figure 2: Scheme of synthesis of Pyrimidine-imine derivatives.

(singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). The BRUKER ESI-IT MS was used to record the MASS.

General procedure for the synthesis of Pyrimidine imine derivatives (6a-6j)

The scheme of synthesis for the title compounds is depicted in Figure 2.

Procedure for multicomponent synthesis of di-substituted pyrimidine (2-(4-methylpyrimidin-5-yl) acetonitrile) (4)

The reaction conducted by mixing the substituted enamine (1 equiv, 10mmol) with ortho ester (3 equiv, 30mmol) and ammonium acetate (3 equiv, 20mmol) in benzene or toluene at 70-80°C under reflux conditions using the Lewis acid zinc chloride or aluminium chloride as catalyst.[14-17]

TLC under UV light was used to continually monitor the reaction. After completion of reaction basic work up procedure with sodium bicarbonate was applied to get the crude product of pyrimidine. Isolated the intermediate product from the column chromatography by ethyl acetate: hexane (1:9 v/v) mobile phase system and purified by recrystallization in ethanol.

Procedure for Conversion of nitrile to aldehyde (2-(4-methylpyrimidin-5-yl) acetaldehyde) by Stephen aldehyde synthesis

4,5-Di substituted Pyrimidine (4) (1 equiv, 10mmol) that containing the nitrile group was dissolved in dry ether. To this the $SnCl_2$ solution in ether (1 equiv, 10mmol), that was previously saturated with HCl was added. After reaction completion the reaction mixture was quenched with cold water and washed with sodium bicarbonate solution followed by brine. Later the crude product extracted with three equal volumes of dry ether then purified from column chromatography to produce the aldehyde (5).[18]

Condensation of aromatic amines with the aldehyde (5)

Resulted from aldehyde (1 equiv, 5mmol) from the second step reaction was dissolved in ethanol and different aromatic amines (1 equiv, 5mmol) (a-j) were reacted with the aldehyde in the acidic conditions to produce the final derivatives of pyrimidines (6a-6j) that are linked to various aromatic amines through Imine bond. TLC under UV light was used to continually monitor the reaction. After completion of reaction, basic work up procedure with sodium bicarbonate was applied to get the crude product of pyrimidine. Isolated final the product from the column chromatography by ethyl acetate: hexane (1:9 v/v) mobile phase system and purified by recrystallization in ethanol.

Anti-cancer activity

From the American Type Culture Collection (ATCC), four cancer cell lines such as B16F10 (mouse skin melanoma), SiHA (cervical), MCF-7 (breast), and A 549 (lung) and one normal fibroblast (L929) were acquired.

Except for HT-29, all the cells were grown in DMEM medium (Gibco, Life Technologies), which was supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin, and 10% FBS (Gibco, Life Technologies) in a humid incubator with 5% CO_2 at 37°C. DMSO, phosphate-saline buffer (pH 7.4), paraformaldehyde, and MTT (chemically 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were acquired from Sigma-Aldrich India. The Carmichael *et al.,* 1987 standard procedure was employed to perform the MTT assay.[19]

RESULTS

Chemistry

Structural and physical data of the titled compounds was detailed below in the Table 1 and the pyrimidine-imine derivatives general structure was illustrated in Figure 3.

Table 1: Molecular formula, melting point and yield of compounds 6a-6o.

Comp. No	R	Mol. Form.	MP in °C	% Yield
6a	4-H	$C_{13}H_{13}N_3$	109-111	86
6b	4-F	$C_{13}H_{12}FN_3$	99-100	82
6c	4-Cl	$C_{13}H_{12}ClN_3$	103-104	80
6d	4-NO_2	$C_{13}H_{12}N_4O_2$	114-115	77
6e	4-OCH_3	$C_{14}H_{15}N_3O$	124-125	80
6f	2,4-Cl	$C_{13}H_{11}Cl_2N_3$	118-119	74
6g	-4-CF_3	$C_{14}H_{12}F_3N_3$	129-130	79
6h	-4-CH_3	$C_{14}H_{15}N_3$	121-122	74
6i	-4-C_2H_5	$C_{15}H_{17}N_3$	133-134	70
6j	-3,4-CH_3	$C_{15}H_{17}N_3$	127-128	76

Table 2: Anti-proliferative activity (IC_{50} values) of compounds 6a-6j.

Sample No.	MCF-7	SiHA	A 549	B16F10	L929
6a	22.9±3.36	14.25±1.14	21.62±2.18	20.39±1.88	42.6±1.3
6b	24.2±4.46	15.25±2.25	26.35±2.58	22.94±4.30	38.91±1.8
6c	>100	>100	66.12±1.10	69.31±2.1	>100
6d	17.19±1.14	18.53±2.25	39.63±2.11	>100	>100
6e	24.8±1.22	19.88±0.14	>100	19.12±1.62	44.6±1.31
6f	11.08±1.34	13.84±1.22	17.75±1.33	21.35±1.34	37.68±6.2
6g	8.12±1.33	9.34±1.04	14.92±2.32	21.85±1.26	41.36±1.74
6h	7.42±2.12	7.95±1.31	13.01±1.12	21.01±1.75	>100
6i	6.21±1.26	6.94±1.03	6.23±1.18	10.11±1.85	>100
6j	22.8±1.32	20.68±0.14	24.72±1.18	28.11±1.48	42.6±1.4
Doxorubicin	1.9±0.98	1.64±0.95	0.98±1.78	1.4±1.22	1.06±1.17

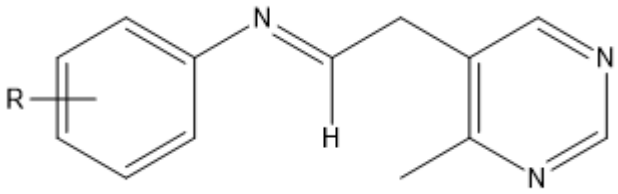

Figure 3: General structure of pyrimidine imine derivatives.

Spectral Data of Pyrimidine imine derivatives

"(E)-2-(4-methylpyrimidin-5-yl)-N-phenylethan-1-imine (6a): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1607.5 (C=N), 1646.5 (C=O), 3286.0 (N-H), 3065.5 (=C–H), 1286.0 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, *J* = 1.5 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.07 (t, *J* = 9.1 Hz, 1H), 7.37 – 7.30 (m, 2H), 7.16 – 7.07 (m, 3H), 4.19 (dd, *J* = 9.1, 1.0 Hz, 2H), 2.35 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{13}H_{13}N_3$ ([M + H]$^+$): 211.27, found 212.20.

(E)-N-(4-fluorophenyl)-2-(4-methylpyrimidin-5-yl)ethan-1-imine (6b): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1602.5 (C=N), 1641.8 (C=O), 3275.3 (N-H), 3059.7 (=C–H); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.28 – 7.18 (m, 4H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.35 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{13}H_{12}FN_3$ ([M + H]$^+$): 229.26, found 230.25.

(E)-2-(4-methylpyrimidin-5-yl)-N-phenylethan-1-imine (6c): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1600.8 (C=N), 1642.1 (C=O), 3275.9 (N-H), 3055.5 (=C–H), 1276.0 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.36 (q, J = 0.9 Hz, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.19 – 7.13 (m, 2H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.30 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{13}H_{12}ClN_3$ ([M + H]$^+$): 245.7, found 246.6.

(E)-2-(4-methylpyrimidin-5-yl)-N-(4-nitrophenyl)ethan-1-imine (6d): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1601.5 (C=N), 1643.5 (C=O), 3287.4 (N-H), 3060.5 (=C–H), 1282.2 (C-N), 675.5 (C-Cl); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.36 (q, J = 0.9 Hz, 1H), 8.21 – 8.15 (m, 2H), 8.07 (t, J = 9.1 Hz, 1H), 7.29 – 7.23 (m, 2H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.38 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{13}H_{12}N_4O_2$ ([M + H]$^+$): 256.27, found 257.15.

(E)-N-(4-methoxyphenyl)-2-(4-methylpyrimidin-5-yl)ethan-1-imine (6e): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1604.5 (C=N), 1643.5 (C=O), 3281.7 (N-H), 3064.4 (=C–H), 1281.0 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.36 (dt, J = 1.5, 0.9 Hz, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.20 – 7.14 (m, 2H), 6.95 – 6.89 (m, 2H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 3.78 (s, 3H), 2.33 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{14}H_{15}N_3O$ ([M + H]$^+$): 193.2, found 194.15.

(E)-N-(2,5-dichlorophenyl)-2-(4-methylpyrimidin-5-yl) ethan-1-imine (6f): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1608.3 (C=N), 1646.7 (C=O), 3279.5 (N-H), 3055.6 (=C–H), 1288.8 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.18 (t, J = 9.1 Hz, 1H), 7.46 (d, J = 6.7 Hz, 1H), 7.33 (d, J = 2.0 Hz, 1H), 7.18 (dd, J = 6.7, 1.8 Hz, 1H), 4.30 (dd, J = 8.9, 0.9 Hz, 2H), 2.33 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{13}H_{11}Cl_2N_3$ ([M + H]$^+$): 280.15, found 281.05.

(E)-2-(4-methylpyrimidin-5-yl)-N-(4-(trifluoromethyl)phenyl) ethan-1-imine (6g): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1605.5 (C=N), 1644.5 (C=O), 3283.0 (N-H), 3062.5 (=C–H), 1286.0 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.69 (dq, J = 10.8, 1.4 Hz, 2H), 7.30 – 7.24 (m, 2H), 4.28 (dd, J = 9.0, 1.0 Hz, 2H), 2.40 (s, 3H). ESI-MS: m/z Anal. Calcd. For $C_{14}H_{12}F_3N_3$ ([M + H]$^+$): 279.27, found 280.15.

(E)-2-(4-methylpyrimidin-5-yl)-N-(p-tolyl)ethan-1-imine (6h): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1600.5 (C=N), 1645.5 (C=O), 3275.5 (N-H), 3058.9 (=C–H), 1285.5 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.36 (q, J = 0.9 Hz, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.19 – 7.14 (m, 2H), 7.06 – 7.01 (m, 2H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.33 (s, 3H), 2.24 (d, J =

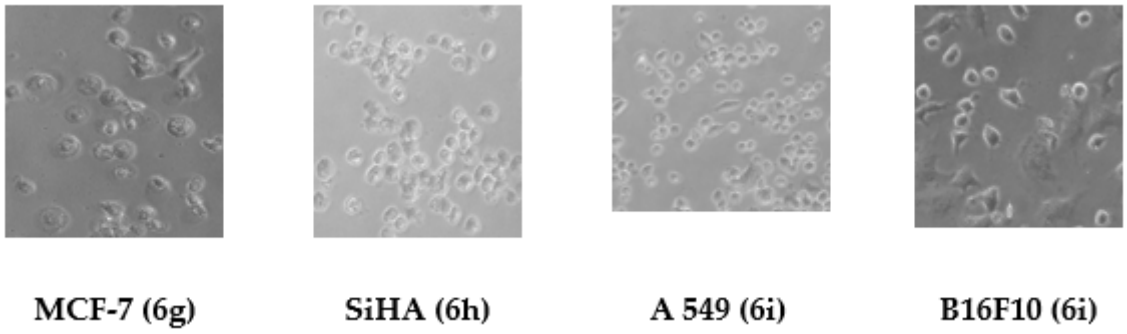

Figure 4: Cell lines images at IC_{50} concentration of potent compounds in the MTT assay.

1.2 Hz, 3H). ESI-MS: *m/z* Anal. Calcd. For $C_{14}H_{15}N_3$ ([M + H]$^+$): 225.30, found 226.25

(E)-N-(4-ethylphenyl)-2-(4-methylpyrimidin-5-yl)ethan-1-imine (6i): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1610.1 (C=N), 1639.8 (C=O), 3288.5 (N-H), 3058.5 (=C–H), 1280.3 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.07 (t, J = 9.1 Hz, 1H), 7.18 (dq, J = 8.1, 1.3 Hz, 2H), 7.09 – 7.03 (m, 2H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.72 – 2.63 (m, 2H), 2.33 (s, 3H), 1.20 (t, J = 7.3 Hz, 3H). ESI-MS: m/z Anal. Calcd. For $C_{15}H_{17}N_3$ ([M + H]$^+$): 239.32, found 240.2.

(E)-N-(3,4-dimethylphenyl)-2-(4-methylpyrimidin-5-yl) ethan-1-imine (6j): Light yellowish solid, IR (KBr): ν_{max} in cm^{-1}: 1602.5 (C=N), 1641.5 (C=O), 3281.2 (N-H), 3061.8 (=C–H), 1286.0 (C-N); ^{1}H NMR: (500 MHz, DMSO-d_6) δ 8.82 (d, J = 1.5 Hz, 1H), 8.36 (dt, J = 1.5, 0.9 Hz, 1H), 8.12 (t, J = 9.1 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.94 (dd, J = 7.9, 2.2 Hz, 1H), 4.19 (dd, J = 9.1, 1.0 Hz, 2H), 2.33 (s, 3H), 2.29 – 2.22 (m, 6H). ESI-MS: m/z Anal. Calcd. For $C_{15}H_{17}N_3$ ([M + H]$^+$): 239.32, found 240.2."

Anti-cancer evaluation

In this investigation, the antiproliferative effect of the pyrimidine-imine analogues was investigated using the anti-cancer medicine Doxorubicin as the standard. All the analogues 6a–6j were examined for their *in vitro* anti-cancer efficacy against one normal fibroblast cell and four cancer cell lines: B16F10 (mouse skin melanoma), A549 (lung), SiHA (cervical), and MCF-7 (breast). The dosage that decreases cell growth to 50%, i.e., IC_{50} (in μM), was used to quantify the cytotoxic effects of the tested analogues. The tested analogues demonstrated good to moderate anti-proliferative properties against the tested cell lines, according to the MTT assay findings mentioned in Table 2 and the assay results.

According to this study, analogue 6i exhibited potent cytotoxicity against the four cancer cell lines utilized in the assay, such as B16F10 (IC_{50} 10.11±1.85μM), A-549 (IC_{50} 6.23±1.18 μM), SiHA (IC_{50} 6.94 ±1.03 μM), and MCF-7 (IC_{50} 6.21±1.26 μM). Compound 6h displayed potent activity against the cancer cell lines A 549 (IC_{50} 13.01±1.12 μM), SiHA (IC_{50} 7.95±1.31 μM), and MCF-7 (IC_{50} 7.42±2.12 μM) and 6g showed potent anti-cancer activity against A 549 (IC_{50} 14.92±2.32 μM), SiHA (IC_{50} 9.34±1.04 M), and MCF-7 (IC_{50} 8.12±1.33 μM), cancer cell lines. The images of cell lines of potent compounds found in the cytotoxicity assay were displayed in Figure 4.

DISCUSSION

Table 1 lists the competitive yields of the generated compounds 6a-6j. The findings from infrared, mass spectrometry, and proton nuclear magnetic resonance (IR, MS, and ^{1}H-NMR) correlated well with the proposed structures of the newly synthesised chemicals. C-H aromatic peaks of the pyrimidine ring were identified with chemical shift (δ) values between 8.0 and 8.85.

According to the results, compounds with an alkyl group substitution on the phenyl ring of the pyrimidine-imine were more promising than those with electronegative and unsubstituted groups. Among the phenyl substituted compounds, the molecules with methyl, ethyl substitution on the phenyl rings (6h, 6i) disclosed highest activity and within these compounds the ethyl group substituted compound 6i is the most potent of all derivatives. From the results it is apparent that the planar geometry at 4th position (phenyl ring) and electron donating substitutions (methyl, ethyl) on the planar ring system is playing an essential role in the activity of the compounds. Most pyrimidine derivatives have been shown to inhibit tyrosine kinase-related pathways, indicating that perhaps this is their mechanism of action.

Shameem *et al.*, (2023) screened pyrimidine carboxamide hybrids for their *in vitro* anti-cancer activity against six cancer cell lines, such as B16F10, SiHA, MCF-7, HT 29, DU 145, and A 549, as well as one normal human L929 cell line, employing the MTT assay, and compared them with the reference drug doxorubicin. They reported that the methoxy derivative 5l outperformed among all the prepared compounds. The IC_{50} value of 5l against SiHA, B16F10, A549, and MCF-7 cell lines were found to be 15.2±1.5, 6.22±3.1, and 10.01±1.12 and 6.44±2.12 μM respectively. The IC_{50} values of 5l against MCF-7 are in good agreement with our reported IC_{50} values (6.21 and 7.42 μM, respectively) for compounds 6i and 6h. In contrast to this, the IC_{50} values of 5l against A-549 and B16 F10 were superior to our reported IC_{50}

values for compounds 6i, 6h, and 6g. However, against SiHA, our reported compounds (6i, 6h, and 6g) displayed significant anti-cancer activity than molecule 5l, with IC_{50} values of 6.94, 7.95, and 9.34 μM respectively.[20]

Ahmed and his coworkers (2020) prepared a set of nitrogen heterocyclic compounds containing pyrimidine moieties. Against a normal fibroblast (WI-38) cell and HepG2 and MCF7 cancer cell lines, the prepared pyrimidines were examined for *in vitro* anti-proliferative efficacy, and compared to that of doxorubicin. Spiroderivative 16 outperformed all other examined substances in terms of anti-cancer potential, with IC_{50} values of 6.7, 10.76, and 7.36 μM. Our synthesized compounds 6i, 6h, and 6g exhibited the highest anti-cancer activity against MCF-7 cell lines than molecule 16, with IC_{50} values of 6.21, 7.42, and 8.12 μM respectively.[21]

CONCLUSION

We describe the effective synthesis of a novel series of aromatic amine-linked pyrimidine-imine derivatives utilising a $ZnCl_2$-catalyzed multicomponent synthetic method. One normal human cell line and four distinct cancer cell lines were used to assess the efficacy of the synthesised compounds to kill cancer cells. In contrast to the gold reference medication doxorubicin, the majority of the compounds demonstrated moderate to promising cytotoxicity. The most potent derivative was 6i, which was followed by 6h, and 6g. To determine the precise mechanism of action of the prepared novel pyrimidine-imine derivatives, further investigations are required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance; **IC_{50}:** Half-maximal inhibitory concentration; **MHz:** Mega Hertz; **IR:** Infra Red; **ESI-MS:** Electro Spray Ionization Mass.

SUMMARY

As the currently available medications lack specificity for the cancer targets, the search for improved anti-cancer medicines has taken centre stage for many research teams. The effectiveness and safety of the medications are increased by the design and development of target-specific anti-cancer agents. Current study aims to develop a novel series of the pyrimidine derivatives owing to the anti-cancer potential of the pyrimidine scaffold. In a multicomponent reaction approach, 4,5-disubstituted pyrimidines (4) were synthesized from three component coupling reaction of substituted enamine (1), an orthoester (triethoxy methane) (2) and ammonium acetate (3). 4,5-disubstituted pyrimidines (4) were oxidized to corresponding aldehyde derivative (5) via Stephen aldehyde synthetic process. Then the pyrimidine aldehyde coupled with various aromatic amines to produce final pyrimidine imine derivatives (6a-6j). All the synthesized derivatives were characterized by IR, ^{1}H-NMR and Mass spectral studies and subjected to anti-cancer activity evaluation by MTT Assay. Four cancer cells (A 549 (lung), MCF-7 (breast), SiHA (cervical), B16F10 (mouse skin melanoma) and one normal fibroblast (L929)) were employed to study the anti-cancer potential of the synthesized pyrimidine derivatives. The synthesized compounds produced in moderate to good yields with proposed scheme of synthesis. All the synthesized derivatives displayed noticeable cytotoxicity against the tested cancer cell lines.

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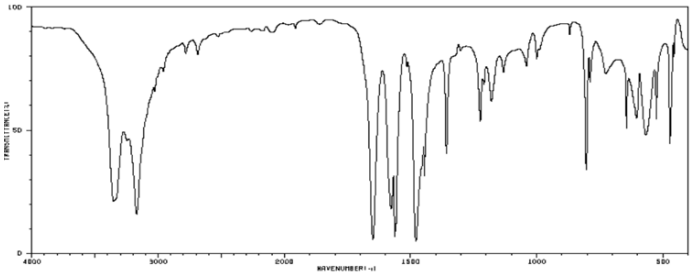
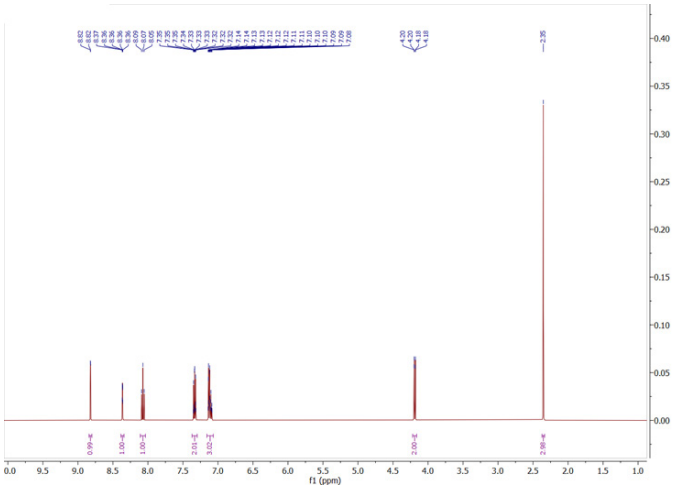
Cite this article: Devidi S, Manickam MS, Suresh R. Multicomponent Synthesis, Characterization of Novel Pyrimidine Derivatives with Anti-cancer Potential. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s742-s748.

Supplementary Data



6a IR Spectrum



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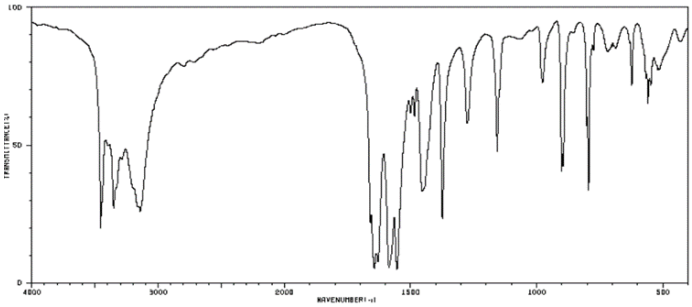

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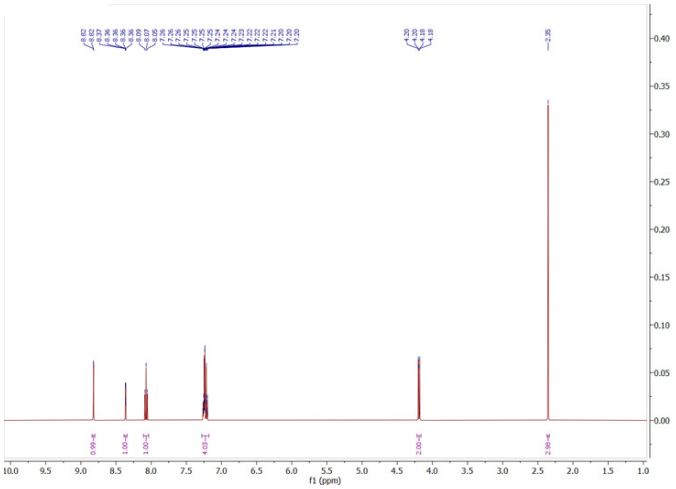

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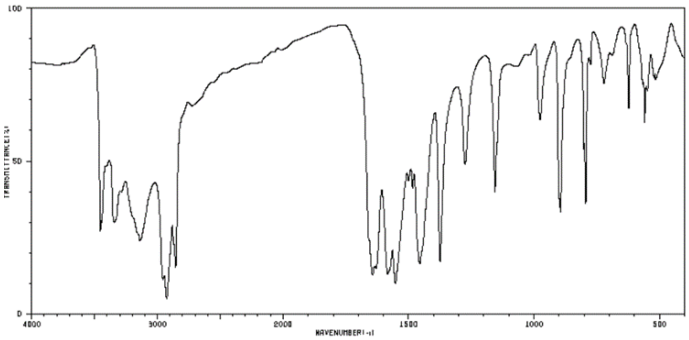
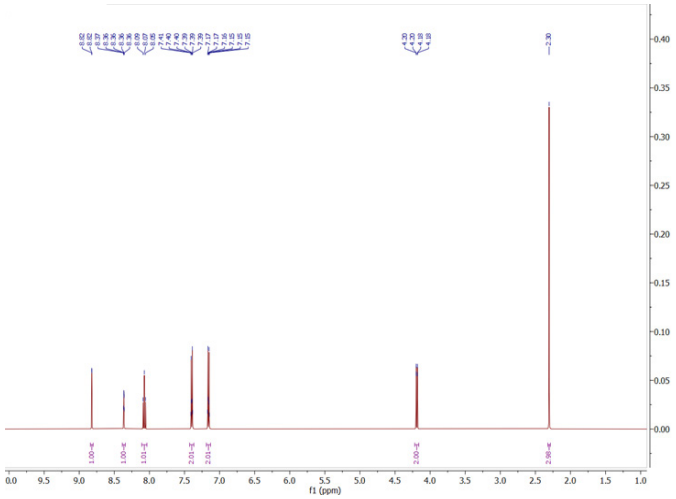
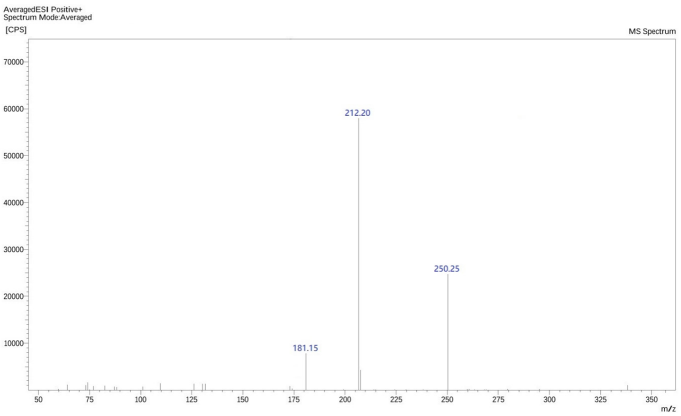


6c IR Spectrum



6c HNMR Spectrum

MASS REPORT



6a Mass Spectrum

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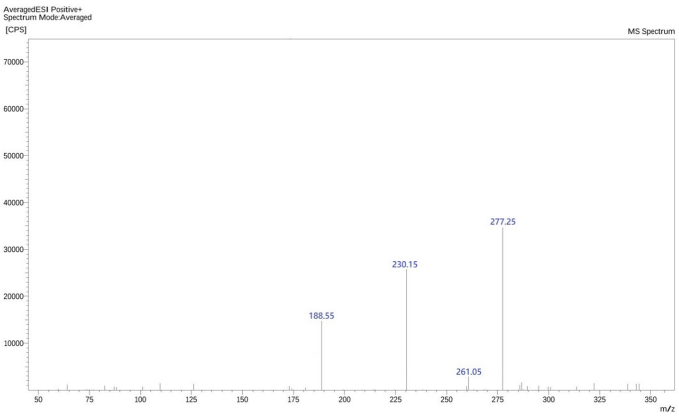

6b Mass Spectrum

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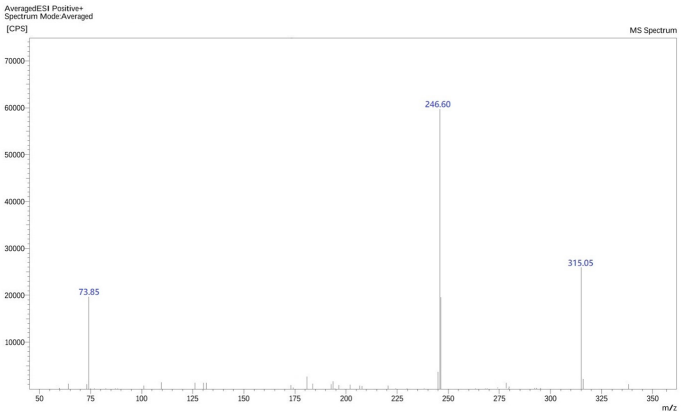

6c Mass Spectrum