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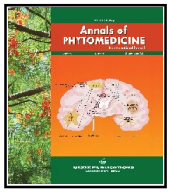

Original Article : Open Access

Evaluation of anticancer activity of novel pyrimidine aniline molecular hybrids: Synthesis and characterization

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Abstract

Current study aims to develop a novel series of the substituted pyrimidine derivatives having anticancer potential. A novel series of pyrimidine derivatives were synthesized through a feasible method. 4-(2-chloroethyl)-2,5,6-trimethylpyrimidine (3) was prepared by condensation of (E)-N-(but-2-en-2-yl) acetamide (1) and 3-chloropropanenitrile (2) in presence of trifluoromethanesulfonic anhydride and 2-chloropyridine under reflux. Amination of previously generated pyrimidine alkyl chloride (3) with aromatic amines in the presence of triethyl amine and DMF resulted in formation of final product (4a-4j). All the synthesized derivatives were characterized by ^{1}H NMR, ^{13}C NMR and MASS spectral methods and the MTT assay evaluated their anticancer activity for five cancer cell lines. All the synthesized compounds were screened for anticancer activity against five cancer cell lines such as human cervical cancer (HeLa), breast cancer (MCF7), ovarian cancer (PA-1), colorectal carcinoma (LoVo) and human dermal fibroblasts (NHDF) cell lines. All the compounds displayed decent cytotoxicity profile when compared with the standard drug doxorubicin. Among the synthesized compounds (4a to 4j) tested, four compounds, 4e, 4d, 4g and 4j have demonstrated excellent cytotoxicity against cancer cell lines.

1. Introduction

According to the WHO global cancer report 2020, over 10 million deaths reported from the cancer disease. Among these, deaths over 74% are contributed by breast, lung, colon, and prostate cancers (IARC). Despite advances in technology and cancer treatment worldwide, cancer remains a major threat to human health (Alafeefy *et al.,* 2014; Alafeefy *et al.,* 2014). Among the major strategies employed for the mitigation of cancer, chemotherapy is considered as key feasible method owing to its simplicity relative to other therapies (DeMartino and Boger, 2008; Curran, 2002). While many chemotherapy treatments have been successful in treating different types of cancers, the side effects and long-term effects of chemotherapy remain a concern for patients and doctors (Nurgali *et al.,* 2018). Therefore, target specific, widely accepted and effective cancer drugs are needed to address these challenges. Various research groups and the pharmaceutical industry are constantly striving to develop new anticancer drugs that specifically and efficiently target cancer cells with selective action. Moreover, the overwhelming similarity between normal cells and cancer cells and the diverse characteristics of tumours are major obstacles to the development of final chemotherapeutic agents (El-Messery *et al.,* 2016). Therefore, the discovery and development of new anticancer agents is an essential process that never ends in the pharmaceutical industry.

The pyrimidines chemistry has fascinating subject for researchers because of the presence of pyrimidine scaffold in biological systems such as cofactors, nucleic acids, various toxins, and other products (Rani *et al.,* 2016; Kumar *et al.,* 2015). Substituted pyrimidines, principally with 2,4,6-triamino or 2,4-diamino substitution are renowned pharmacophores in several structure-based drug design methods in medicinal chemistry. Their characteristic shape and hydrogen bonding ability makes this heterocyclic molecule appropriate for interaction with numerous biological targets (Choudhury *et al.,* 2008). Many recent reports displayed the potential of the pyrimidine-amine scaffolds against nasopharyngeal carcinoma, renal cell carcinoma (Zhang *et al.,* 2019), tyrosinase kinase inhibition (Mirmortazavi *et al.,* 2019), mGlu2 potentiation (Hu *et al.,* 2004), along with the major anticancer, antimalarial (Gangjee *et al.,* 1999; Rosowsky *et al.,* 1997; Phuangsawai *et al.,* 2016), antibacterial (Abdelgawad, 2019), antiHIV (Al-Masoudi *et al.,* 2014), and antihypertensive (Sekiya *et al.,* 1983). It has PAK1 inhibitory effect so, effective in neurodegenerative disorders (Xu *et al.,* 2013). Current study aims to develop a novel series of the substituted pyrimidine derivatives having anticancer potential.

2. Materials and Methods

All synthetic grade chemicals used for the study were purchased from Sigma Aldrich, Bangalore, India. The reaction was monitored by Merck-precoated aluminium TLC plates of silica gel 60 F254. The spots were visualized in the UV chamber and with iodine

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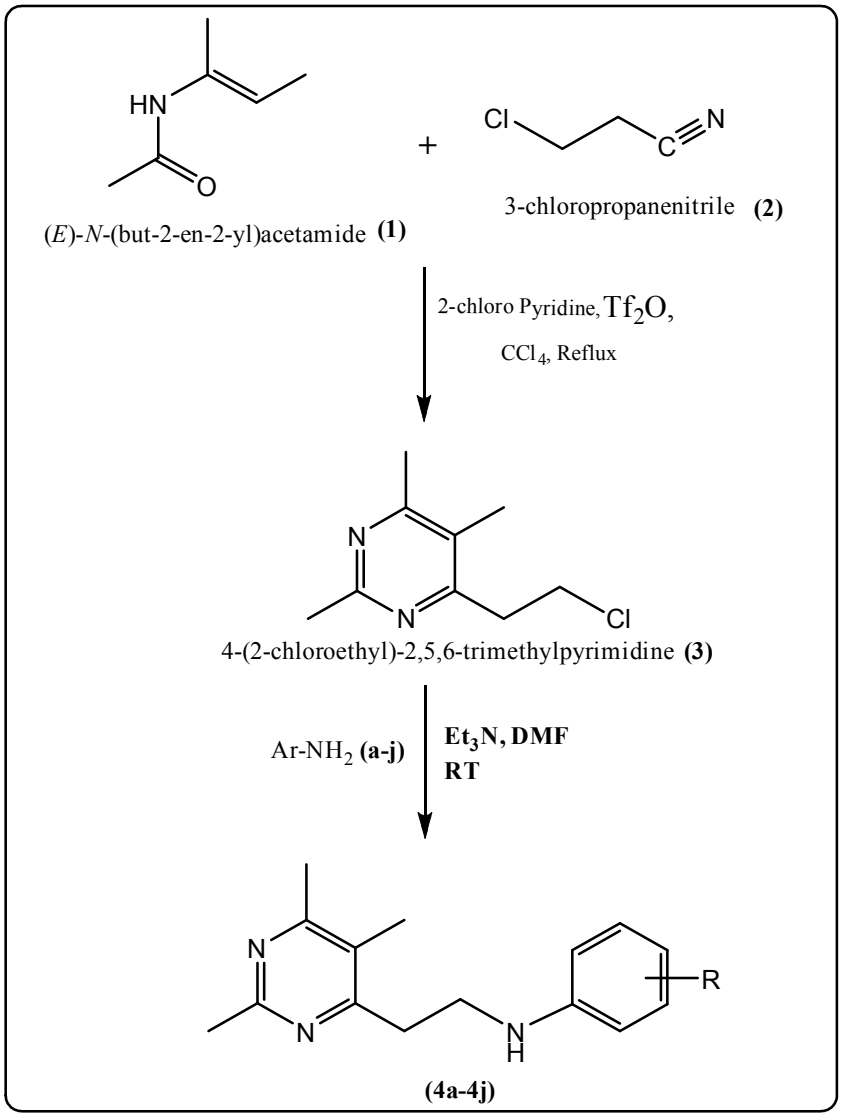
vapours. For the isolation and purification of the pure compounds column chromatography was used. Melting points of the compounds were determined by Remi electronic melting point apparatus. ^{1}H NMR recorded on BRUKER DRX - 500 MHz. Chemical shift values (δ) expressed in ppm with reference to internal standard TMS (tetramethyl silane). The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded on Agilent FTIR by the KBr pellet method. MASS recorded on BRUKER ESI-IT MS.

2.1 General procedure for the synthesis of substituted pyrimidines

The N-vinyl amides used as the starting materials for the two-step synthesis of the pyrimidine derivatives. This approach relies on electrophilic amide activation, employing the reagent combination of 2-chloropyridine (2-ClPyr) and trifluoromethanesulfonic anhydride (Tf_2O) (Sasada *et al.,* 2009; Stephen, 1925). Additionally, the pyrimidine moiety linked to different substituted anilines in an amination reaction with the previously generated pyrimidine alkyl chloride (Scheme 1) (Bhattacharyya *et al.,* 2014).

Scheme 1: Synthesis of substituted pyrimidines



2.1.1 Step 1: Procedure for the synthesis of 4-(2-chloroethyl)-2,5,6-trimethylpyrimidine (3)

Equimolar mixture of substituted N-vinyl amide (1) was combined with a substituted 3-chloropropanenitrile (2) in carbon tetrachloride, 0.5 equivalents of 2-chloropyridine and Tf_2O were added to reaction mixture and kept under reflux for 6 h (Sasada *et al.,* 2009). The reaction was constantly monitored by TLC under UV- light. After the reaction was finished, an acidic clean-up process using acetic acid was used to obtain the crude pyrimidine product (3). The final product was isolated from the column chromatography using 10% ethyl acetate and hexane mobile phase, and it was then purified by recrystallization in ethanol.

2.1.2 Step 2: Procedure for the synthesis of aniline linked pyrimidine derivatives (4a-4j)

4-(2-chloroethyl)-2,5,6-trimethylpyrimidine (3) and different substituted anilines (a-j) solubilized in DMF and the mixture was agitated at room temperature for 30 min employing triethylamine as a catalyst. After the reaction is complete, the mixture is treated with an acidic workup using dilute acetic acid, and the crude product is purified using column chromatography with 10% ethyl acetate and hexane mobile phase system to obtain the final products (4a-4j).

2.2 Anticancer activity

2.2.1 Cell culture

Human cervical cancer (HeLa), breast cancer (MCF7), ovarian cancer (PA-1), colorectal carcinoma (LoVo) and human dermal fibroblasts (NHDF) cell lines were purchased from American Type Culture Collection, Bengaluru, India. All the cells were cultured in DMEM media supplied with 1% L-glutamine, 1% streptomycin/penicillin, and 10% fetal bovine serum and incubated at 37°C in a five per cent carbon dioxide humidified atmosphere. The synthesized derivatives were solubilized in 0.01% dimethylsulfoxide (DMSO) and cells were treated with 0.1-50 µM of compounds (Desai *et al*., 2021).

2.2.2 MTT assay

The cytotoxic activity of synthesized compounds on cervical, breast, ovarian, colorectal carcinoma cancer and human dermal fibroblasts cell lines were assessed by MTT assay. The cells were treated with five different concentrations (1, 10, 25, 50, and 100 µM) of all synthesized compounds for 24 h in a CO_2 incubator. The cells were then treated for 4 h with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (5 mg/ml) and the reduced formazan crystals were solubilized in dimethylsulfoxide and the absorbance at 540 nm was recorded by a microplate reader. As control the untreated cells were used. Each trial was done in triplicate and the IC_{50} values were calculated by using following formula (Bhatti *et al.,* 2019)**.** The results data was statistically analyzed with Origin Pro 2022 software.

$$\% \text{ Cell viability} = [(A_t - A_0)/(A_c - A_0)] \times 100$$

where

Ac = Absorbance of cells treated with 0.2% DMSO.

At = Absorbance of cells treated with test compounds.

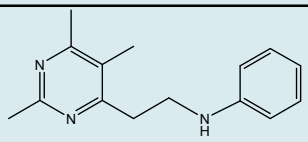
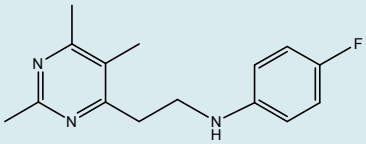
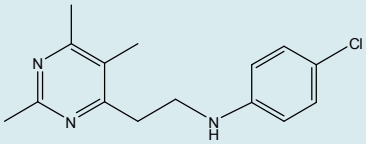
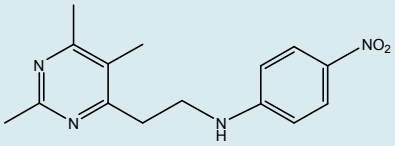
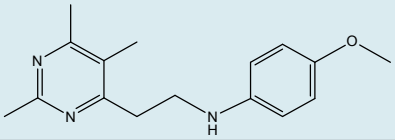
A0= Absorbance of blank.

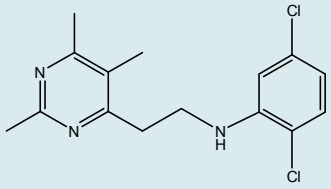
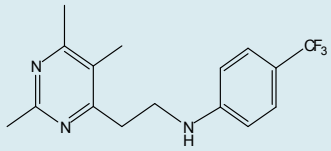
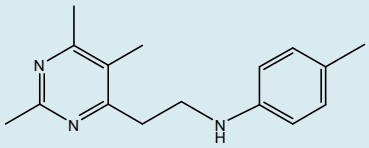
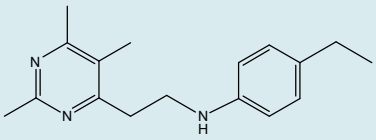
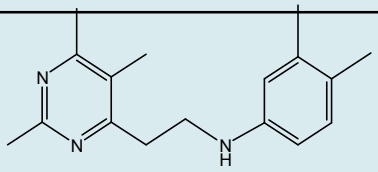
3. Results

3.1 Synthesis

The structure and physical data of the synthesized derivative was enumerated in Table 1.

Table 1: Synthesized pyrimidine derivatives physical characterization

Compound	Structure	Yield (%)	Melting point (^{0}C)
4a		69	202-203
4b		71	217-218
4c		71	211-212
4d		76	229-230
4e		62	194-195

4f		73	241-242
4g		79	256-257
4h		64	173-174
4i		59	189-190
4j		55	168-170

3.2 Spectral characteristics of the synthesized pyrimidine derivatives

N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4a)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.14 – 7.07 (m, 2H), 6.73 (m, J = 6.8, 1.0 Hz, 1H), 6.63 – 6.57 (m, 2H), 5.96 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.94 (t, J = 5.1 Hz, 2H), 2.37 (s, 3H), 2.18 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 119.9 (2C, s), 127.8 (1C, s), 128.2 (2C, s), 129.4 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{19}N_3$ ([M + H]$^+$): 241.34, found 242.15.

4-fluoro-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4b)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 6.97 – 6.89 (m, 2H), 6.80 – 6.74 (m, 2H), 5.74 (t, J = 5.5 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.90 (t, J = 5.0 Hz, 2H), 2.54 (s, 3H), 2.40 (s, 3H), 2.18 (s, 3H). **^{13}C NMR:** δ15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 120.5 (2C, s), 128.9 (2C, s), 129.4 (1C, s), 133.7 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{18}FN_3$ ([M + H]$^+$): 259.33, found 260.20.

4-chloro-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4c)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.14 – 7.08 (m, 2H), 6.65 – 6.59 (m, 2H), 5.71 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.98 (d, J = 5.1 Hz, 2H), 2.48 (s, 3H), 2.40 (s, 3H), 2.16 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 120.5 (2C, s), 128.9 (2C, s), 129.4 (1C, s), 133.7 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{18}ClN_3$ ([M + H]$^+$): 275.78, found 276.65.

4-nitro-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4d)

Light brown colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 8.03 – 7.97 (m, 2H), 6.84 – 6.78 (m, 2H), 6.29 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.97 (t, J = 5.1 Hz, 2H), 2.48 (s, 3H), 2.37 (s, 3H), 2.18 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 116.6 (2C, s), 125.0 (2C, s), 129.4 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{18}N_4O_2$ ([M + H]$^+$): 286.34, found 287.15.

4-methoxy-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4e)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 6.83

– 6.77 (m, 2H), 6.64 – 6.58 (m, 2H), 5.91 (t, J = 5.6 Hz, 1H), 3.78 (s, 3H), 3.48 (q, J = 5.2 Hz, 2H), 2.90 (t, J = 5.0 Hz, 2H), 2.54 (s, 3H), 2.38 (s, 3H), 2.16 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 56.0 (1C, s), 114.5 (2C, s), 120.5 (2C, s), 129.4 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 159.8 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{21}N_3O$ ([M + H]$^+$): 271.36, found 272.20.

2,5-dichloro-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4f)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.34 (d, J = 7.2 Hz, 1H), 7.03 – 6.95 (m, 2H), 6.85 (d, J = 2.2 Hz, 1H), 3.55 (q, J = 5.2 Hz, 2H), 2.92 (t, J = 5.1 Hz, 2H), 2.50 (s, 3H), 2.36 (s, 3H), 2.19 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 121.6 (1C, s), 121.8 (1C, s), 129.3-129.5 (2C, 129.3 (s), 129.4 (s)), 130.2 (1C, s), 132.3 (1C, s), 134.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{15}H_{17}Cl_2N_3$ ([M + H]$^+$): 310.22, found 311.15.

4-(trifluoromethyl)-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4g)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.54 (m, J = 7.5, 1.4 Hz, 2H), 6.77 – 6.71 (m, 2H), 5.82 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.97 (t, J = 5.0 Hz, 2H), 2.52 (s, 3H), 2.39 (s, 3H), 2.19 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 123.8 (1C, s), 126.5 (2C, s), 129.4 (1C, s), 130.3 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{18}F_3N_3$ ([M + H]$^+$): 309.34, found 310.15.

4-methyl-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4h)

Pale yellow colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.03 – 6.98 (m, 2H), 6.64 – 6.58 (m, 2H), 5.68 (t, J = 5.5 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.94 (d, J = 5.2 Hz, 2H), 2.48 (s, 3H), 2.35 (d, J = 17.4 Hz, 6H), 2.18 (s, 3H). **^{13}C NMR:** δ 15.0 (1C, s), 21.3 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 129.4 (1C, s), 129.6 (2C, s), 141.5 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{21}N_3$ ([M + H]$^+$): 255.37, found 256.25.

4-ethyl-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4i)

Off White colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 7.12 (m, J = 7.9, 0.9 Hz, 2H), 6.64 – 6.58 (m, 2H), 5.75 (t, J = 5.6 Hz, 1H), 3.48 (q, J = 5.2 Hz, 2H), 2.94 (t, J = 5.1 Hz, 2H), 2.63 (qt, J = 7.2, 1.0 Hz, 2H), 2.48 (s, 3H), 2.37 (s, 3H), 2.18 (s, 3H), 1.20 (t, J = 7.3 Hz, 3H). **^{13}C NMR:** δ 14.6 (1C, s), 15.0 (1C, s), 24.3 (1C, s), 25.8 (1C, s), 28.7 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (2C, s), 129.4 (1C, s), 129.9 (2C, s), 144.2 (1C, s), 148.4 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{23}N_3$ ([M + H]$^+$): 269.39, found 270.20.

3,4-dimethyl-N-(2-(2,5,6-trimethylpyrimidin-4-yl)ethyl)aniline (4j)

Off White colour solid; **^{1}H NMR:** (500 MHz, DMSO-d_6) δ 6.94 (m, J = 7.8, 1.1 Hz, 1H), 6.51 – 6.44 (m, 2H), 6.20 (t, J = 5.6 Hz, 1H), 3.50 (q, J = 5.1 Hz, 2H), 2.94 (t, J = 5.1 Hz, 2H), 2.48 (s, 3H), 2.37 (s, 3H), 2.23 – 2.16 (m, 9H). **^{13}C NMR:** δ 15.0 (1C, s), 19.9-20.1 (2C, 20.0 (s), 20.0 (s)), 24.3 (1C, s), 25.8 (1C, s), 32.3 (1C, s), 41.6 (1C, s), 117.9 (1C, s), 119.9 (1C, s), 129.4 (1C, s), 130.0 (1C, s), 130.9 (1C, s), 133.4 (1C, s), 148.5 (1C, s), 154.3 (1C, s), 163.0 (1C, s), 165.6 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{23}N_3$ ([M + H]$^+$): 269.39, found 270.25.

3.3 *In vitro* anticancer activity

The results of the MTT assay of all the synthesized compounds were given in Table 2.

Table 2: Anticancer results: IC_{50} values µM

Sample	Cervical cancer (HeLa)	Breast cancer (MCF7)	Ovarian cancer (PA-1)	Colorectal carcinoma (LoVo)	Human dermal fibroblasts (NHDF)
4a	16.78 ± 4.73	20.60 ± 0.51	16.81 ± 1.20	19.21 ± 0.69	46.65 ± 0.99
4b	14.23 ± 1.33	19.60 ± 0.68	18.01 ± 1.03	19.66 ± 1.01	51.07 ± 0.60
4c	12.51 ± 1.83	10.6 ± 1.01	15.70 ± 0.35	18.90 ± 1.49	44.25 ± 1.72
4d	6.23 ± 2.95	11.71 ± 1.02	15.31 ± 2.10	18.09 ± 3.32	54.96 ± 4.25
4e	8.64 ± 1.10	9.12 ± 0.45	6.64 ± 0.69	10.91 ± 1.20	52.56 ± 0.84
4f	24.70 ± 1.86	11.47 ± 1.05	34.94 ± 0.52	18.34 ± 0.67	43.74 ± 1.83
4g	19.59 ± 2.47	19.01 ±2.32	14.85 ± 0.97	13.78 ± 1.01	51.36 ± 1.26
4h	20.19 ± 0.90	21.05 ± 0.52	22.01 ± 0.51	20.09 ± 1.05	48.54 ± 3.74
4i	11.25 ± 0.51	22.63 ± 1.20	17.72 ± 0.52	18.99 ± 0.77	50.16 ± 0.69
4j	21.90 ± 0.99	26.78 ± 2.98	12.39 ± 4.25	11.47 ± 0.51	40.15 ± 0.65
Doxorubicin	0.98 ± 1.3	1.27 ± 1.27	1.23 ± 0.3	1.4 ± 0.44	1.02 ± 0.55

The results of cytotoxic activity of the synthesized compounds on cervical cancer (HeLa) cell line demonstrated that, amongst the titled compounds strong activity was recorded for 4d, 4e and 4i (IC_{50} = 6.23 ± 2.95, 8.64 ± 1.10 and 11.25 ± 0.51 µM, respectively). Regarding the results of the synthesized derivatives against breast cancer (MCF7) cell line, among all compounds, 4e and 4c (IC_{50} = 9.12 ± 0.45, 10.6 ± 1.01 µM, respectively), displayed good cytotoxic activity. The synthesized compounds against ovarian cancer (PA-1) cell lines 4e, 4g and 4j showed good cytotoxicity with IC_{50} 6.64 ± 0.69, 14.85 ± 0.97and 12.39 ± 4.25 µM, sequentially. Compound 4e was the most potent one having an IC_{50} of 10.91 ± 1.20 µM against colorectal carcinoma (LoVo) cell lines. That was followed by 4g (IC_{50} = 13.78 ± 1.01 µM) and 4j (IC_{50} =11.47 ± 0.51 µM) which exhibited cytotoxic activity. The cytotoxic activity of synthesized derivatives on human dermal fibroblasts (NHDF) showed that IC_{50} values of the compounds ranges from 40.15 ± 0.65 – 54.96 ± 4.25 µM, 4j being the highest and 4d being the least. Compound 4a (IC_{50} =16.81 ±1.20 µM) against ovarian cancer (PA-1), 4b (IC_{50} =14.23 ±1.33 µM) on cervical cancer (HeLa), **4d** (IC_{50} = 6.23 ± 2.95, 11.71 ± 1.02 µM) against these two cell lines displayed good activity. Compound **4e** showed good activity against four cell lines (cervical, breast, Ovarian, colorectal) and moderate activity against human dermal fibroblasts. Compound 4f (IC_{50} = 11.47 ± 1.05µM) against breast cancer (MCF7) cell lines showed good activity. Compound **4g** with IC_{50} values 14.85 ± 0.97, 13.78 ± 1.01 µM against ovarian and colorectal cancer cell lines exhibited good activity. Derivative 4h (IC_{50} = 20.09 ± 1.05 µM) on colorectal carcinoma, 4i (IC_{50} = 11.25 ± 0.51 µM) on cervical, and **4j** (IC_{50} = 12.39 ± 4.25, 11.47 ± 0.51 µM, respectively), on ovarian and colorectal cancer cell lines presented good activity.

4. Discussion

All the compounds were synthesized with moderate to good yields (55-79%). The spectral characterization of the synthesized compounds with ^{1}H NMR and ^{13}C NMR displayed the respective chemical shift values. Further the mass spectra of the compounds are in correspondence with the m/z values of the compounds.

The synthesized compounds (4a-j) were screened for *in vitro* cytotoxicity against cervical, breast, ovarian, colorectal cancer cell lines, and human dermal fibroblasts using MTT assay. All the compounds under investigation displayed moderate to potent cytotoxicity against the selected cancer cell lines relative to the standard doxorubicin. From the results, we can deduce that electron donating (**4j** (-CH_3) **4e** (4-OCH_3)), and electron withdrawing (**4g** (-CF_3), **4d** (4-NO_2)) groups showed good cytotoxic activity against all the cell lines.

The 4-NO_2 derivative **4d** was the most active against HeLa cell lines among all the tested derivatives, followed by the 4-OCH_3 derivative **4e** and the 4-OC_2H_5 derivative **4i**, with IC_{50} values 6.23 ± 2.95, 8.64 ± 1.10, and 11.25 ± 0.51 µM, respectively. The compound 4f (2,5-dichloro) with an IC_{50} of 24.70 ± 1.86 µM exhibited the least cytotoxic activity against HeLa cell lines. Weak electron-withdrawing group substituted 4f (2,5-diCl) showed the least activity while strong electron-withdrawing group substituted 4e showed good activity against HeLa cell lines. Moderate electron-donating groups, *i.e.,* compounds 4e (4-OCH_3) and 4i (4-OC_2H_5), also demonstrated good activity.

In the case of substances **4c** (4-Cl), **4f** (2,5-diCl), which contain one and two chlorine atoms, respectively, they were found to be active against MCF7 cell lines with IC_{50} = 10.6 ±1.01, 11.47 ±1.05 µM, respectively. In addition, the 4-nitro substituted compound **4d** and the 4-methoxy substituted compound **4e** also displayed good activity against MCF7 cell lines, with IC_{50} values of 11.71 ±1.02 and 9.12 ± 0.45 µM, respectively. The derivative 4j (3,4-dimethyl) showed the least activity against MCF cell lines (IC_{50} = 26.78 ± 2.98 µM). Strong and moderate electron-withdrawing group bearing compounds 4d (4-NO_2), 4c (4-Cl), and 4f (2,5-diCl), as well as moderate electron-donating group bearing compound 4e, demonstrated good activity, whereas weak electron-donating substituted compound 4j (3,4 dimethyl) demonstrated the least cytotoxicity.

The compound bearing a moderately electron-donating methoxy group at the 4 position, *i.e.*, **4e** (IC_{50} = 6.64 ± 0.69, 10.91 ± 1.20 µM), the strongly electron-withdrawing 4-trifluoromethyl group substituted compound **4g** (IC_{50} =14.85 ± 0.97, 13.78 ± 1.01 µM) and the weakly electron-donating 3,4 dimethyl group-bearing substance **4j** (IC_{50} =12.39 ± 4.25, 11.47 ± 0.51 µM) demonstrated good activity against PA-1 and LoVo cell lines. Compound 4h (4-methyl) showed the least activity against LoVo cell lines (IC_{50} =20.09 ±1.05 µM). The addition of another methyl group at the 3-position of 4h leads to increased activity, 4j (IC_{50} =11.47 ± 0.51 µM) against LoVo cell lines. Weak electron-withdrawing group containing compound 4f (2,5-diCl) showed the least activity (IC_{50} = 34.94 ± 0.52 µM) against PA-1 cell lines.

5. Conclusion

A novel series of pyrimidine aniline derivatives were synthesized through a feasible multicomponent synthetic route employing triethyl amine as a catalyst and this method provided a good yield for all the designed compounds. Synthesized compounds were characterized and evaluated for *in vitro* anticancer activity against five selected cancer cell lines. Relatively most of the compounds showed good to moderate cytotoxic activity comparative to the standard drug doxorubicin. The compound **4e** exhibited highest potency followed by **4d, 4g and 4j**. Further investigations are needed to establish the detailed mechanism of action of the developed novel pyrimidine derivatives.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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