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Evaluation of anticancer, antioxidant, and spectral data of newly prepared amino pyrimidine from 6-chloropyridine -3-carbaldehyde

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ABSTRACT
Chalcones were used as starting materials in the present research to create a new series of unique amino pyrimidine derivatives. Corresponding chalcones were synthesised from 6-chloropyridine-3-carbaldehyde. Synthesized compounds were assessed for their antioxidant and anticancer properties. Compounds 4b, 4e, and 4f demonstrated strong antioxidant capacity, whereas synthetic chemicals 4e and 4g had a GI50 of less than 10 µg/ml, according to the anticancer evaluation against human breast cancer line MCF-7. Novel generated pyrimidines (4a-4j) were analysed using GC-MS, ^{1}H and ^{13}C NMR, IR, as well as melting points. In silico ADME analysis of the synthetic substances (4a-4j) revealed that they are extremely effective for use as oral medicines.

Graphic Abstract:
Highlights
- **Synthesis of novel pyrimidines from chalcones.**
- **Evaluation of novel compounds for anticancer properties and antioxidant properties.**
- **Compounds 4e and 4g exhibited potential activity towards cell of humans strain for breast tumors MCF-7, whereas chemicals 4b, 4e, and 4g showed excellent antioxidant activity.**

Characterisation by NMR, GCMS, IR

INTRODUCTION:

Nature contains heterocyclic molecules, some of which are essential to biological functions [1]. The "aromatic organic heterocyclic chemical pyrimidine and its derivatives have long" been used in medicine[2]. Pyrimidine is a crucial heterocyclic compound with six members[3,4] that is a member of the alkaloid family[5]. "As vitamins, nucleic acid, purine, cytosine, uracil, thymine, folic acid, and coenzymes, it plays an important part in several biological processes[6–9]. Pyrimidine derivatives have also been reported to possess a wide range of biological properties" in the therapeutic industry, including anticancer[10–15], antihistamine[16], antinociceptive[17], antilipase[18], antifilarial[19], antiparasitic [20], antioxidant [21], antituberculosis [22], antihypertensive[23], antimicrobial[24–25], anticonvulsant[26], antiallergic [27], antipyretic [28], anti-inflammatory [29], antifungal [30], antiviral[31], antibacterial[32], antimalarial[33], antiHIV[34], antiulcer[35], insecticide [36], antineoplastic[37], antiepileptic[38], analgesic[39] and anthelmintic[40]. Sildenafil "(viagra), formycin A[41], sulfadiazine[42], pyrimethamine[43], roscovitine[44], indiplon, and zaleplon" are a few commercially available medications that are produced from pyrimidine.[45] etravirine [52], dinaciclib[46], ocinaplon [46], fluorastrol[47], 5-fluorouracil[48], ispinesib[49], volasertib[50], pictilisib, monastrol[51] and etravirine[52]

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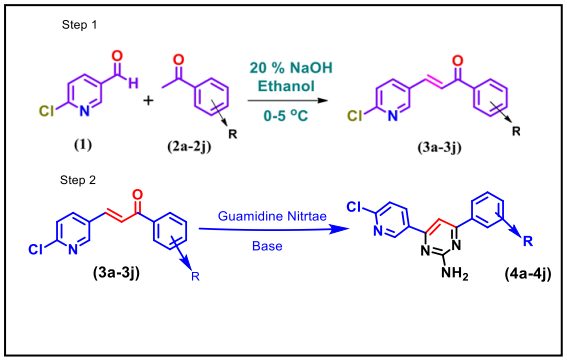
For instance, "thiourea reacts with chalcones in the presence of NaOH to form 4,6-disubstituted



pyrimidine2-yl thiol derivatives[53], guanidine hydrochloride condenses with chalcones in the presence of NaOH to form 2- amino-4,6-disubstituted pyrimidines[54], and substituted pyrimidin-2(1H)-ones have been synthesized from chalcones" [55] . These are the main methods used to synthesize pyrimidines from chalcones[56].

We have created a number of unique amino pyrimidine derivatives based on the aforementioned results and ongoing work. The anti-oxidant and anticancer properties of each of these compounds have been tested against human cancer cell lines.

MATERIALS AND METHODS:

Loba Chemical and TCI, respectively, provided all of the raw materials, which were used exactly as they were. Before being used, every solvent was meticulously distilled. "Thin layer chromatography (Merck, silica gel 60 F254) was used to" track the reactions. TLC was shown using a bright box. The product was described using FTIR, 1H, ^{13}C NMR, mass spectrometry. A Perkin Elmer FTIR spectrometer has been utilized to get infrared spectra from KBr pellets. Nuclear magnetic resonance (NMR) spectrum were acquired using a BRUKER 500MHz spectrophotometer, with TMS serving as a standard. The GCMS data were collected with a Shimadzu TQ 8040d instrument. Melting temperatures for every substance freshly produced chemical were determined by using an open capillary approach.

Experimental:

The primary approach for producing the molecules (4a-4j) [57] (scheme -I):

α, β-unsaturated ketones (3a-3j) were prepared as per reported procedure[58] and further reacted with guanidine nitrate as per reported procedure as follows:

50% aqueous KOH (2.2 mmoles) has been added dropwise to stirred solution of chalcone (one mmole) in 20 milliliters of ethanol after guanidine nitrate (1.5 mmol) has been charged at 30 degrees Celsius. After heating the reaction mixture to 80–85 degrees Celsius, it was agitated until the reaction has been finished. Thin layer chromatography has been utilised to monitor the reaction, and eluent has been a 70:30 mixture of hexane and ethyl acetate. Reaction gets complete after 5-6 hrs. Product was isolated by removal of solvent and further purification by column chromatography with an eluent of 80% hexane and 20% ethyl acetate. Pure product obtained in 60-77% yield as a white solid.

Scheme I: The synthesis pathway of the pyrimidine derivatives from chalcones.

RESULTS AND DISCUSSION:

Chemistry:

α, β-unsaturated ketones, also called as chalcone (3a-3j), were produced by reaction of 6-Chloropyridine-3-carbaldehyde (1) with various substituted acetophenones (2), as reported in the literature[58]. Chaconne compounds were reacted with guanidine nitrate /aq.KOH in a ethanol solution at reflux for 4-6 hours, until the reaction finished, which was recorded by thin layer chromatography. Pure amino pyrimidines were isolated by removal of solvents followed by column chromatography.

Spectroscopic tools like GCMS, ^{1}H NMR,^{13}C NMR, and FTIR have been utilised to verify structure of the newly formed pyrimidines Detail information on spectral data is provided in following chapter and chromatograms are provided in supplementary data file.

The ^{1}H NMR[59] supports the synthesis of amino pyrimidines, as predicted by a **singlet** in the δ 7.0 - 9.0 ppm region for the pyrimidine ring proton (integration of 1H). a **broad singlet** in the δ 4.0 - 7.0 ppm region for the amino group protons (integration of 2H), which disappear upon D2O exchange**, three signals** of three H in the δ 7.0 - 9.0 ppm region for the 6-chloropyridin-3-yl ring, and **signals** in the δ 6.5 - 8.0 ppm region for the phenyl ring protons, sowing splitting due to both proton-proton.

^{1}H NMR spectroscopy of every compound confirms the number of different types of protons in a molecule, and the integration of the signals directly correlates with the relative number of these protons, and it's consistent with the molecular formula. The ^{13}C NMR[60] showed all carbons are in agreement with structure of the compounds. Compound 4c showed Carbon **C2 (bearing -NH2) at** δ≈165.6 ppm, very electron-deficient due to two

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adjacent N atoms and the amine group, **C4 (bearing pyridyl) at δ 162.4 ppm a**ttached to N atom and the pyridyl group. Pyridine carbons observed at δ124.3, 137.2,148.4 ppm and Carbon connected to Cl at 153.2 due to deshielding effect of Cl group. Phenyl carbon attached to F atom observed at 163.5. Observed splitting resonance in carbon-connected fluorine atoms and carbons at ortho, meta, para positions.

Mass of produced compounds has been validated by GCMS. Mass spectra of bromine containing substance such as compound 4d exhibited a unique order of twin signals of almost equally strong split by distance of m/z 2 (i.e. 360 [M]+, 362 [M+2] + ions) .

Freshly synthesized compounds had their IR spectra[61] measured between 4000 and 400 cm-[1]. All compounds showed N-H Stretching of amine group typically shows two medium-intensity bands in region of ~3300-3500 cm^{-1}.Vibrations from C=N bonds within the pyrimidine and pyridine rings appears in the ~1500-1650 cm^{-1} region.

All the spectroscopic data are acceptable and reliable for the entire pyrazolines chemicals generated.

Scheme I: Synthetic method for Pyrimidines derivatives from 6-chloropyridine-based chalcones.

Physical and Spectroscopic descriptions of prepared pyrimidine Compounds (4a-4j)

4-(6-chloropyridin-3-yl)-6-(3,4-difluorophenyl) pyrimidin-2-amine (4a):
White color, "yield of 65%, MP: 138-140°C, ^{1}H NMR (DMSO) at 500MHz: δ 6.96 (s, 2H of NH_2 D2O exchangeable), 7.6 (q, 1H of Bz ring), 7.7 (d,1H of Bz ring) 7.89 (s, 1H, of Pyrimidine ring), 8.14 (m, 2H,, Ar-H), 8.3 (t, 1H", pyridine), 8.6 (dd, 1H, pyridine), 9.23(s, 1H,, Py-H);^{13}CNMR (CDCl3) at 125 MHz: δ;102.4,116.45/116.6,118.21/118.35,124.56/124.59, 124.6/124.64,124.8,132.57,135.03/135.05/135.07/1 35.1,138.4,149.1,150.4/150.5,152,4,162.3/162.4,16 4.3, Theoretical m/z of $C_{15}H_9ClF_2N_4$ is 318 and practical value found is 318 with single Cl pattern and also mol mass is even number, it indicated even number of Nitrogen atoms that is 4.

4-(6-chloropyridin-3-yl)-6-[4-(trifluoromethoxy) phenyl] pyrimidin-2-amine (4b):
Off "white color solid with Yield: 79%; MP: 141-143°C; ^{1}H NMR (DMSO) at 500MHz : δ 6.95 (s , 2H of NH_2, D2O exchangeable), 7.53 (d , 2H of Bz ring) , 7.72 (d ,1H of Pyridine ring) 7.88 (s, 1H,of Pyrimidine ring), 8.36 (m, 2H, Ar-H), 8.61(dd,1H,pyridine),9.23(s,1H,Py-H) ^{13}C NMR (125 MHz", DMSO): δ;102.7,119.5,121.43/121.54,124.8,129.6,132.67,13 6.63,138.4,149.1,150.5,152.4,162.2,164.4/164.43. Theoretical m/z of $C_{16}H_{10}ClF_3N_4O$:366.03, and practical value obtained is 366 with pattern of 1 Cl atom.

4-(6-chloropyridin-3-yl)-6-(4-fluorophenyl) pyrimidin-2-amine (4c):
whitish colour solid; melting point 106-108°C; yield 75%; ^{1}H NMR (CDCl3) at 500MHz : δ 5.13 (s , 2H of NH_2, D2O exchangeable), 7.10 (t , 2H of Bz ring) , "7.32 (s ,1H of Pyrimidine ring) 7.39 (d, 1H,of Pyridine ring), 8.0 (s, 2H, Bz ring), 8.27(d,1H,pyridine),8.97(s,1H,Py-H)^{13}C NMR (125 MHz, CDCl3)" δ 103.4,115.8/116,124.3,129.1/129.2,132.2,133.2/133 .3,137.2,148.4,153.2,162.4,163.5,163.5/165.5,165.6 . Theoretical m/z of $C_{15}H_{10}ClFN_4$: 300.05, found: 300.05 with single Cl pattern

4-(4-bromophenyl)-6-(6-chloropyridin-3-yl)-1,2-dihydropyrimidin-2-amine (4d):
Off white color solid with Yield: 72%; MP: 189-191°C; "^{1}H NMR (DMSO) at 500MHz : δ 6.94 (s , 2H of NH_2, D2O exchangeable), 7.7 (d , 1H of Pyridine ring) , 7.74 (d ,2H of Bz ring) 7.8 (s, 1H,of Pyrimidine ring), 8.2 (d, 2H, Bz ring), 8.6(d,1H,pyridine),9.22(s,1H,Py-H)"^{13}C NMR (125 MHz, DMSO):δ 102.42,124.83/124.88,129.55,132.12/132.68,136.6 2,138.41,149.11,152.39,162.23,164.42,164.61.

Theoretical m/z of $C_{15}H_{10}BrClN_4$: 359.96, and practical value obtained is 360 with pattern of 1Br and 1 Cl atom.

4-(3-bromophenyl)-6-(6-chloropyridin-3-yl)-1,2-dihydropyrimidin-2-amine (4e):
white solid with MP: 218-220°C; yield 73%; ^{1}H NMR (DMSO) at 500MHz: δ 6.98 (s, 2H of NH_2), 7.5 (t, 1H of Bz ring), 7.71 (q,2H of Bz ring) 7.9 (s, 1H, of Pyrimidine ring), 8.46 (s, 1H, Bz ring), 8.25(d,1H, pyridine), 8.62(d,1H, pyridine),9.24(s,1H, Py-H)^{13}C NMR (125 MHz, DMSO): δ 102.65,122.73,124.79,126.5,130.06,131.28,132.61, 133.8,138.44,139.74,149.17,152.44,162.34,164/164 .39.; Theoretical m/z of $C_{15}H_{10}BrClN_4$: 359.96, and practical value obtained is 360 with pattern of 1Br and 1 Cl atom.

6-(6-chloropyridin-3-yl)-4-(3-fluorophenyl)-1,2-dihydropyrimidin-2-amine (4f):
off white. yield of 79%; "MP :216-218°C; ^{1}H NMR (DMSO) at 500MHz : δ 6.96 (s , 2H of NH_2), 7.37 (t, 1H of Bz ring) , 7.6 (t ,1H of Bz ring) 7.7 (d, 1H,of Bz ring), 7.89 (s, 1H, pyrimidine

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ring), 8.05 (d,1H,Bz ring), 8.08(dd,1H,pyridine), 8.62(dd,1H,pyridine),9.24(s,1H,Py-H)^{13}C NMR (125 MHz, DMSO)" δ 102.7,114/114.19,117.85/118,123.56/123.58,124.83 ,131.13/131.19,132.63,138.43,139.97/140.03,149.1 4,152.43,162.06/162.31,163.99,164.33,164.35,164. 41.; Theoretical m/z of $C_{15}H_{10}ClFN_4$ is 300.05, found: 300 with single Cl pattern

4-(3-chlorophenyl)-6-(6-chloropyridin-3-yl)-1,2-dihydropyrimidin-2-amine (4g):
Solid light yellow; yield 74%; "melting point 214-216°C; ; ^{1}H NMR (DMSO) at 500MHz : δ 6.98 (s , 2H of NH_2), 7.6 (m, 2H of Bz ring) , 7.7 (d ,1H of pyridine ring) 7.9 (s, 1H,of pyrimidine ring), 8.2 (d, 1H, Bz ring), 8.32 (t,1H,Bz ring), 8.63(d,1H,pyridine),9.24(s,1H,Py-H)^{13}C NMR (125 MHz", $CDCl_3$)
δ
102.67,124.81,126.12,127.2,130.9/131,132.62,134. 18,138.44,139.55,149.16,152.44,162.35,164.16/164 .4.

Theoretical m/z of $C_{15}H_{10}Cl_2N_4$: 316.02 found: 316 with 2 Cl pattern
4-(6-chloropyridin-3-yl)-6-(2,4-dichlorophenyl) pyrimidin-2-amine (4h)
Solid light yellow; yield 76%; "melting point 211-213°C; ^{1}H NMR (DMSO) at 500MHz : δ 7.04 (s , 2H of NH_2), 7.47(s, 1H of Bz ring) , 7.67 (m ,1H of pyridine ring and 2H of Bz ring) 7.78 (s, 1H,of pyrimidine ring), 8.51(d,1H,pyridine),9.24(s,1H,Py-H)"
^{13}C NMR (125 MHz, DMSO)
δ
106.74,124.94,128.04,129.9,132.43,132.68/132.77, 134.97,137.06,138.38,148.99,152.49,161.45,164.27 ,165.73.
Theoretical m/z of $C_{15}H_9Cl_3N_4$: 349.98 found: 450 with 3 Cl pattern

6-(6-chloropyridin-3-yl)-4-(2,4-difluorophenyl)-1,2-dihydropyrimidin-2-amine (4i)
Pale yellow powder, "yield 70% with melting point 197-199°C, ^{1}H NMR (DMSO) at 500MHz: δ 7.01 (s, 2H of NH_2), 7.27(t, 1H of Bz ring), 7.42 (t,1H of Bz ring) 7.53 (s, 1H of pyrimidine ring), 7.68(d, 1H of pyridine ring),8.0(d, 1H of Bz ring),8.51(d,1H, pyridine),9.1(s,1H, Py-H)"
^{13}CNMR (CDCl3) at 125 MHz: δ;105.07,105.28/105.49,106.05/106.11,112.46/112. 49,112.63/112.66,122.86/122.92/122.95,124.92,132 .56,132.62/132.66,148.93,152.43,159.98/160.08,16 1.92,162.0/162.02/162.1,162.68/162.78,164.36/164 .67.
Theoretical m/z of $C_{15}H_9ClF_2N_4$: 318.04, found: 318 with single Cl atom.

6-(6-chloropyridin-3-yl)-4-(2,5-dichlorophenyl)-1,2-dihydropyrimidin-2-amine (4j):
whitish colour solid; melting point 216-218°C; yield 73%; ^{1}H NMR (DMSO) at 500MHz : δ 7.06 (s , 2H of NH_2), 7.5(t, 1H of pyrimidine ring) , 7.58 (m ,3H of Bz ring and 1H of pyridine ring)),8.51(d,1H,pyridine),9.1(s,1H,Py-H)
^{13}C NMR (125 MHz, $CDCl_3$) δ 106.67,124.94,130.36,130.94,130.96,132.2,132.37, 132.39,138.39,139.67,149.01,152.52,161.58,164.22 ,165.38. Theoretical m/z of $C_{15}H_9Cl_3N_4$: 349.98, found: 350 with three Cl pattern

Biological investigation:
Anticancer capabilities:
To ascertain the cancer-prevention qualities of medications, the Anti-tumor Medicines Medication Screening Service "(ACDSF) at ACTREC, Tata Memorial" Center, Kharghar, Navi Mumbai, carried out in vitro investigations. Anticancer efficacy of recently synthesized pyrimidines was assessed against MCF-7 cells by utilising Sulforhodamine B (SRB) assay[62-65.] The SRB evaluation procedure is provided in a separate file. Table 1 illustrates the exceptional anticancer activity of compounds 4e and 4g against MCF-7 breast cancer cell line.

Table 1 shows that compounds 4e and 4g had GI50 values of < 10µg/milliliters. A growth chart showing the percentage of control growth vs concentration (µg/ml) for female breast carcinoma cell types (MCF-7) is shown in **Figure 1**. The effects of the generated chemicals 4e and 4g, the positive medication used as a therapy agent, Adriamycin, and the negative control substance (DMSO) on a breast cancer cell line (MCF-7) are depicted in **Figure 2**.

Table 1: In vitro anticancer evaluation of produced pyrimidines against the MCF-7 cancer cell line

compound	**Drug concentrations (µg/mL)**	**Compound**	**Drug concentrations (µg/mL)**
	GI_{50}		**GI_{50}**
4a	>80	**4f**	>80
4b	>80	**4g**	**<10**
4c	>80	**4h**	>80
4d	>80	**4i**	>80
4e	**<10**	**4j**	**>80**
		ADR*	**<10**

***Adriamycin- a positive control drug**
"**GI50 = Concentration of drug causing 50% inhibition of cell growth**"

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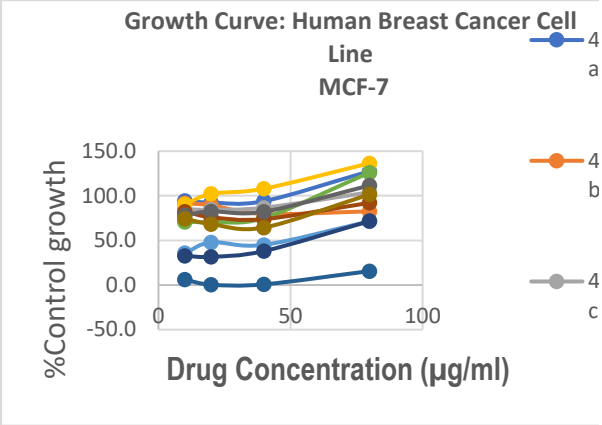

Figure 1: Progression curve for Malignancy of the woman's breast tumor cell line (MCF-7) % control growth vs dosage (µg/mL) of novel pyrimidines

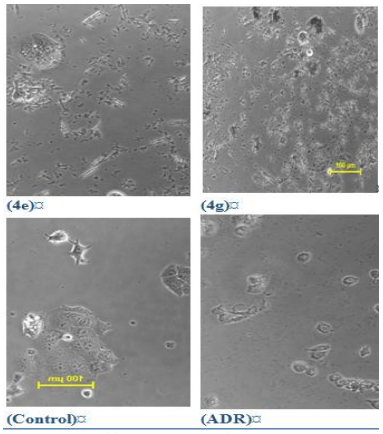

Figure 2: Effectiveness of created substances 4e, 4g, Negative influence. And favourable control drugs, Adriamycin, on breast tumor cell lines (MCF-7).

Antioxidant function:

According to the cited article, the DPPH radical scavenging assay has been used to assess the antioxidant ability of pyrazolines.[66] Every assessment was performed several times at 517 nm, and the degree of suppression was determined employing the standard deviation and mean. Results were evaluated using vitamin C and Vitamin E as positive controls. When examined with positive controls, the substances tested have comparatively high radical scavenging capacity. Chemicals **4b**, **4e**, and **4g** had the highest antioxidant properties between those examined. On the other hand, all the remaining substances demonstrated excellent antioxidant action. **Table 2** outlines the antioxidant investigation findings, whereas **Figure 3** graphically displays them.

Table 2: *In vitro* antioxidant power of produced amino pyrimidines (4a-4j)

Inhibition (%) *					**Antioxidant test IC_{50} (µg/mL)**
Compound	**Concentration (µg/mL)**				
	12.5	**25**	**50**	**100**	
4a	49.42±0.171	61.20±0.023	73.31±0.122	81.40±0.122	14.98±0.043
4b	52.19±0.039	64.35±0.027	77.21±0.105	86.31±0.034	**13.92±0.019**
4c	44.52±0.110	58.64±0.054	74.21±0.022	79.47±0.071	16.85±0.022
4d	50.67±0.103	62.19±0.034	72.75±0.027	78.28±0.020	14.49±0.016
4e	53.69±0.039	66.17±0.114	79.87±0.073	88.20±0.112	**13.43±0.007**
4f	52.62±0.052	63.38±0.043	77.48±0.121	87.19±0.109	14.04±0.031
4g	54.35±0.029	65.22±0.062	78.87±0.034	88.67±0.033	**13.43±0.005**
4h	50.70±0.043	62.57±0.071	74.58±0.036	84.46±0.022	14.46±0.051
4i	49.73±0.122	59.64±0.102	69.60±0.108	79.14±0.044	15.09±0.005
4j	50.32±0.104	61.68±0.024	76.48±0.084	83.90±0.073	14.82±0.058
Ascorbic acid	67.90±0.042	73.88±0.012	82.67±0.042	88.41±0.014	10.46±0.013
α-Tocopherol	56.82±0.025	67.66±0.141	77.14±0.033	86.91±0.042	12.56±0.012

* "Mean ± S.D. (n=3)"

*All "calculations related to IC_{50} are conducted with graphpad prism (http://www.graphpad.com/scientific-software/prism/)"

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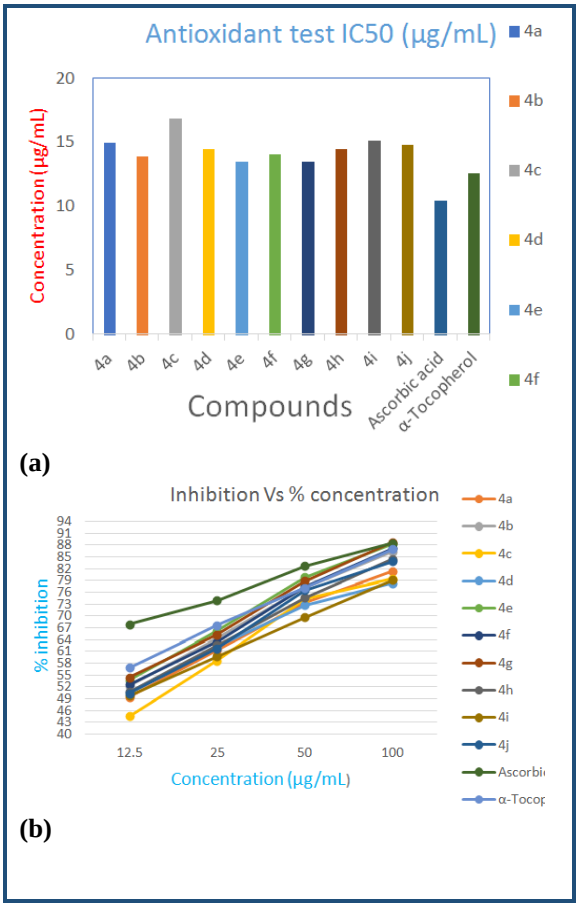

Figure 3 displays the antioxidant properties of generated pyrazolines, including resistance percentage versus dosage (µg/mL) and value of IC50 (µg/mL).

Predictions for physicochemistry, pharmacokinetics, and ADME:

The physical and chemical characteristics, pharmacokinetic, and characteristics of ADME were computed from the computational thinking investigations using the SwissADME internet appplication[67], and the findings are shown in **Table 4**. The expected studies indicated that all the generated pyrazolines have good physicochemical capabilities; yet, all the produced pyrazolines did not breach the five-point rule by Lipinski.

The generated pyrazolines had molecular mass ranging from 317.75-378.65 g/mol and TPSA figures ranging from 45.56 to 64.02 Å. The Absorption percentage (% Abs) is calculated applying formula (% Abs=109 - [0.345 × TPSA]). The % absorption of all produced pyrazolines ranged from 86.91 to 93.28%, indicating very strong bioavailability. The computational evaluation of all the generated pyrazolines revealed favourable pharmacokinetic features, indicating good bioavailability when consumed.

Table 4: Several in-silico physicochemical and pharmacokinetic features for maximum absorption after consumption of the generated pyrimidines(4a-4j).

Entry	MW[a]	H-bond acceptors[b]	H-bond donors[c]	MLogP[d]	Lipinski violations[e]	TPSA[f]	% ABS[g]	Rotatable bonds	Bioavailability score
Rule	**<500**	**≤10**	**≤5**	**≤4.15**	**0**	**<160**	**100%**	**≤10**	
4a	359.81	5	0	1.73	0	64.02	86.9131	5	0.55
4b	335.74	5	0	3.14	0	45.56	93.2818	3	0.55
4c	378.65	3	0	2.99	0	45.56	93.2818	3	0.55
4d	378.65	3	0	2.99	0	45.56	93.2818	3	0.55
4e	317.75	4	0	2.75	0	45.56	93.2818	3	0.55
4f	334.20	3	0	2.87	0	45.56	93.2818	3	0.55
4g	335.74	5	0	3.14	0	45.56	93.2818	3	0.55
4h	317.75	4	0	2.75	0	45.56	93.2818	3	0.55
4i	368.64	3	0	3.37	0	45.56	93.2818	3	0.55
4j	368.64	3	0	3.37	0	45.56	93.2818	3	0.55

Chemical "Weight b. Quantity of Hydrogen Bond Acceptors c. Amount of Hydrogen Bond Donors d. Estimated Lipophilicity (MLog Po/w) e. Deviation from Lipinski's Rule. Topological polar contact area g. % of absorption (% Abs = 109 - [0.345 × TPSA])".

Computational toxicology forecasting

In computational toxicity studies, were conducted using preADMET online program (http://preadmet.bmdrc.org/)[68]. Toxicological investigation indicated that all of produced pyrazolines are mutagenic. The pyrimidines 4b, 4c, 4d, 4e, 4g, and 4h were anticipated to be carcinogenic in rats, but none of the
Produced compounds are carcinogenic in mice. The projections also revealed that all of the generated pyrimidines provide a medium risk of cardiotoxicity **(Table 5).**

Table 5: Typical in-silico toxicity forecasts for generated Pyrazolines (4a-4j).

Sr. No.	Compounds code	Structure	Ames test	Carcino Mouse	Carcino Rat	hERG inhibition

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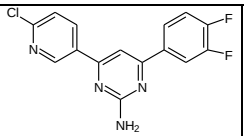
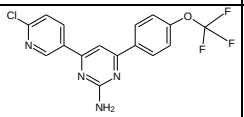
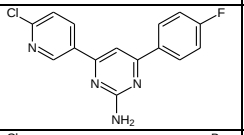
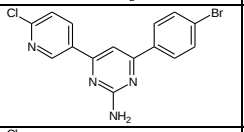
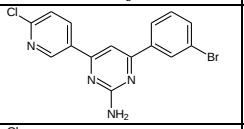
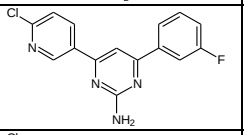
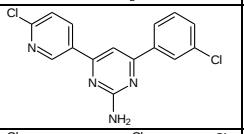
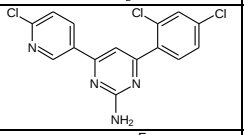
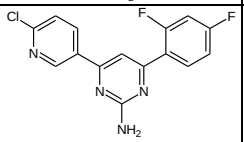
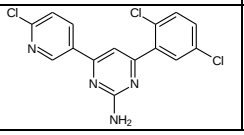
1	**4a**		Mutagen	Negative	Positive	Medium risk
2	**4b**		Mutagen	Negative	Negative	Medium risk
3	**4c**		Mutagen	Negative	Positive	Medium risk
4	**4d**		Mutagen	Negative	Negative	Medium risk
5	**4e**		Mutagen	Negative	Negative	Medium risk
6	**4f**		Mutagen	Negative	Positive	Medium risk
7	**4g**		Mutagen	Negative	Negative	Medium risk
8	**4h**		Mutagen	Negative	Negative	Medium risk
9	**4i**		Mutagen	Negative	Positive	Medium risk
10	**4j**		Mutagen	Negative	Negative	Medium risk

* Carcino: potential for cancer; hERG: human ether-a-gogo-related gene.

CONCLUSION:

In this research work, we employed 6-chloro-pyridine-based chalcones to create an entirely novel category of pyrimidine analogues. All generated pyrimidines were evaluated for antitumor properties in culture towards MCF-7 cell line. According to research, a couple of compounds are extremely successful against the female breast tumor line of cells, MCF-7. Substances 4e and 4g exhibit significant anticancer action, with GI_{50} values below 10 µg/mL. Chemicals 4b, 4e, and 4g demonstrate antioxidant action comparable to vitamin E and vitamin C, resulting in interesting options for future research. In summary, generated pyrimidines have the potential for further exploration as oral chemotherapy medication, as demonstrated using simulated physicochemical, pharmacokinetic parameters, and ADME analysis.

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Author Contributions:
Each author contributed to the design and conceptualization of the study. Both [Sharad Sankhe] and [Vilas Mukadam] prepared the materials, collected the data, and conducted the analysis. [Vilas Mukadam] wrote the original draft of the book, and all contributors provided feedback on earlier iterations. The final manuscript was read and approved by all writers.

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The authors attest that the information underlying the study's conclusions is accessible upon request.

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