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Synthesis and Molecular Docking of Pyrazoline

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ABSTRACT
Pyrazoline is a nitrogen-containing heterocyclic compound with five members and various biological activities. It exists in three chemical forms, with 2-pyrazoline being the most researched due to its greater stability. There are different methods for synthesis the of pyrazoline.Nitrogen heterocycles can be synthesized from alkyl dihalides and primary amines by hydrazine in a single step using microwave irradiation.Condensation of ketones, aldehydes, and hydrazine monohydrochloride forms pyrazoline intermediates, which can be oxidized with bromine to produce pyrazoles in good yields. Alternatively, DMSO and oxygen can be used to produce 3,5-disubstituted or 3,4,5-trisubstituted pyrazoles.Molecular docking is a crucial technique in drug discovery. AutoDock 4.2 is the most widely used program. In one study, compounds were successfully docked against the 3POZ crystal structure. TAK-285 is an innovative small molecule that inhibits EGFR and targets HER2. It has antiproliferative and in vivo cytotoxic activity against cancer cells

Keywords: Pyrazoline, heterocyclic compound, microwave irradiation, condensation, pyrazoline intermediates, molecular docking,3POZ crystal structure, TAK -285, inhibits EFGR, HER 2, antiproliferative.

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I. INTRODUCTION

Pyrazoline is a type of nitrogen-containing heterocyclic compound with five members that possess various biological activities. It is a dihydro derivative of pyrazole and comprises two adjacent N-atoms (nitrogen) and one endocyclic double bond in the ring. Pyrazoline exists in three different chemical forms: 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline, with 2-pyrazoline being the most researched due to its greater stability compared to the other two forms.

Properties of pyrazoline
1. Molecular formula: C3H6N2[
2. Melting point: 66-70 C
3. Acidity Pka: 14,0
4. Boiling point: 186-188C
5. Molar mass: 68.08 mol
6. Physical state: colorless solid

Materials and Methods

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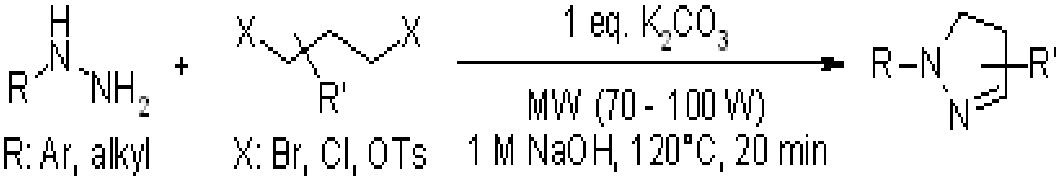
Synthesis of pyrazoline

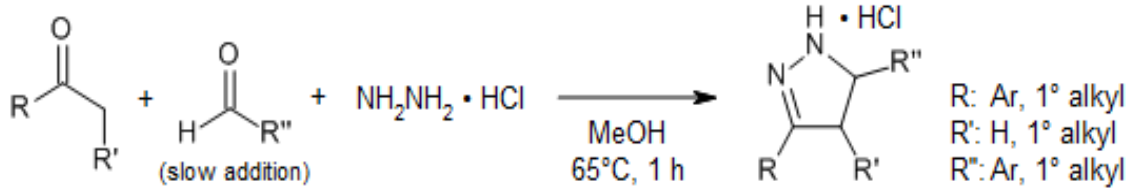
Method 1

nitrogen-containing heterocycles from alkyl dihalides and primary amines and hydrazine can be accomplished in a single step under microwave irradiation. This efficient process occurs via a simple cyclocondensation reaction in an alkaline aqueous medium

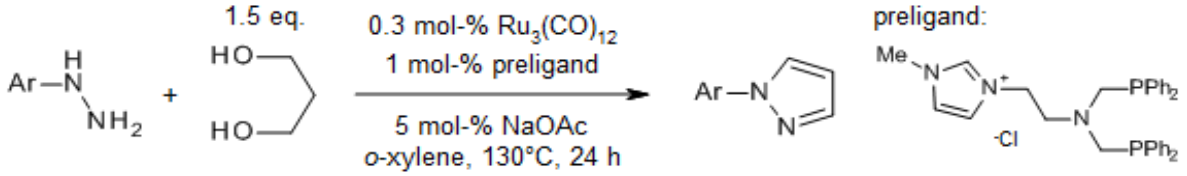


Method 2

Onepot reaction involves the condensation of ketones, aldehydes, and hydrazine monohydrochloride, which readily forms pyrazoline intermediates under mild conditions. These intermediates can then be oxidized using bromine to produce a wide variety of pyrazoles in excellent yields. Alternatively, a gentler oxidation protocol employs DMSO and oxygen to afford3,5-disubstituted or 3,4,5-trisubstituted pyrazoles.



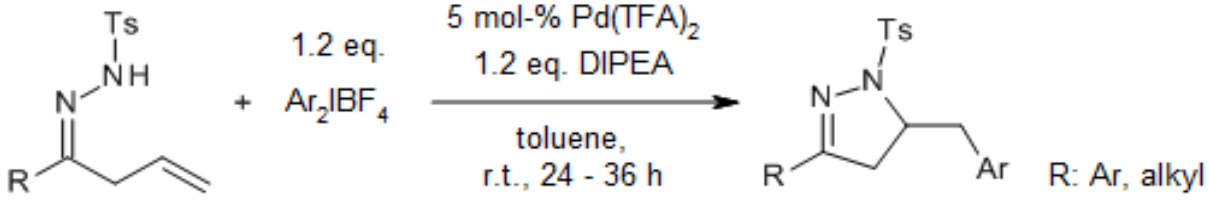
Method3



Low amounts of a combination of Ru3(CO)12 and an NHC-diphosphine ligand catalyze acceptor dehydrogenative coupling reactions of 1,3-diols with arylhydrazines to provide pyrazoles and 2-pyrazolines in good yields. The reactions offer high selectivity and a wide range of substrate scope.

Method 4

A palladium-catalyzed aminoacylation of unactivated alkenes in β,γ-unsaturated hydrazones provides dihydropyrazoles via simultaneous formation of C(sp3)-N and C(sp3)-C(sp2) bonds under mild conditions, without requiring ligands, yielding diverse substitutions.



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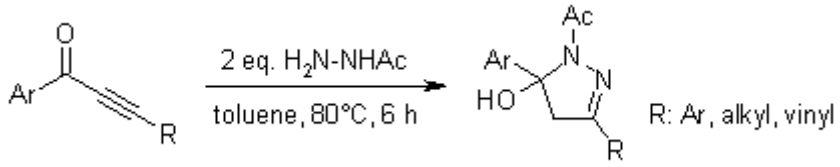
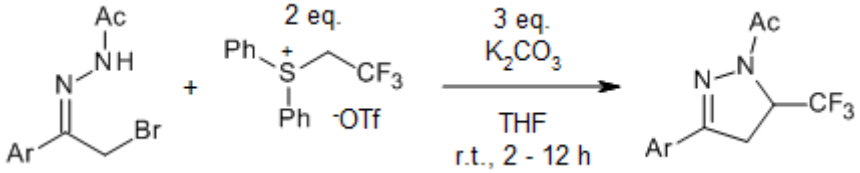
Method 5



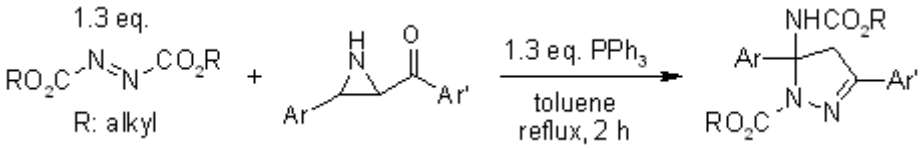
Numerous 1-acyl-5-hydroxy-4,5-dihydro-1H-pyrazoles have been synthesized with high yields from their corresponding 2-alkyn-1-ones. The resulting dihydropyrazoles undergo dehydration and iodination in the presence of NaCl and Li2CO3 at room temperature, producing 1-acyl-4-iodo-1H-pyrazoles.

Method 6



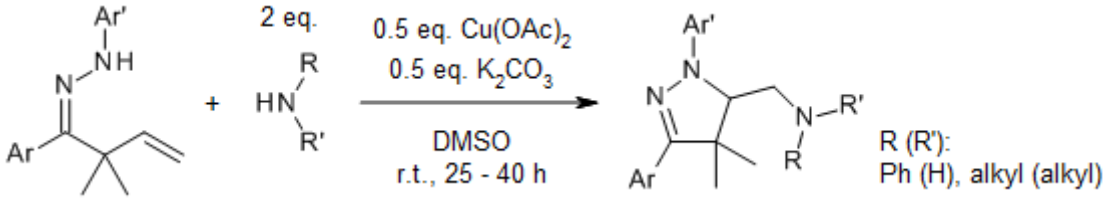
In situ formed 1,2-diaza-1,3-dienes were employed in formal [4 + 1]-annulation reactions along with fluorinated sulfur ylides, resulting in the formation of 5-(trifluoromethyl)pyrazolines with good yields.

Method 7



A new, effective, and universal domino reaction of 2-acylaziridines with Huisgen zwitterions has been discovered to produce 2-pyrazolines. A possible mechanism for the domino sequence has been proposed.

Method 8



A convenient copper-catalyzed intra-/intermolecular diamination of β,γ-unsaturated hydrazones with simple amines enables efficient access to various nitrogen-containing pyrazolines under mild reaction conditions.

MOLECULAR DOCKING OF PYRAZOLINE

Molecular docking is a crucial technique in drug discovery that helps predict the orientation, interaction, and binding energies of ligands in their targeted binding sites. Currently, AutoDock 4.2 is considered the most widely used molecular docking program due to its high accuracy and versatility, as demonstrated in various studies. In one such study, compounds containing chalcone, pyrazoline, and pyrimidine moieties were successfully docked against the 3POZ crystal structure, and the results are detailed in Table 1. TAK-285 is an innovative

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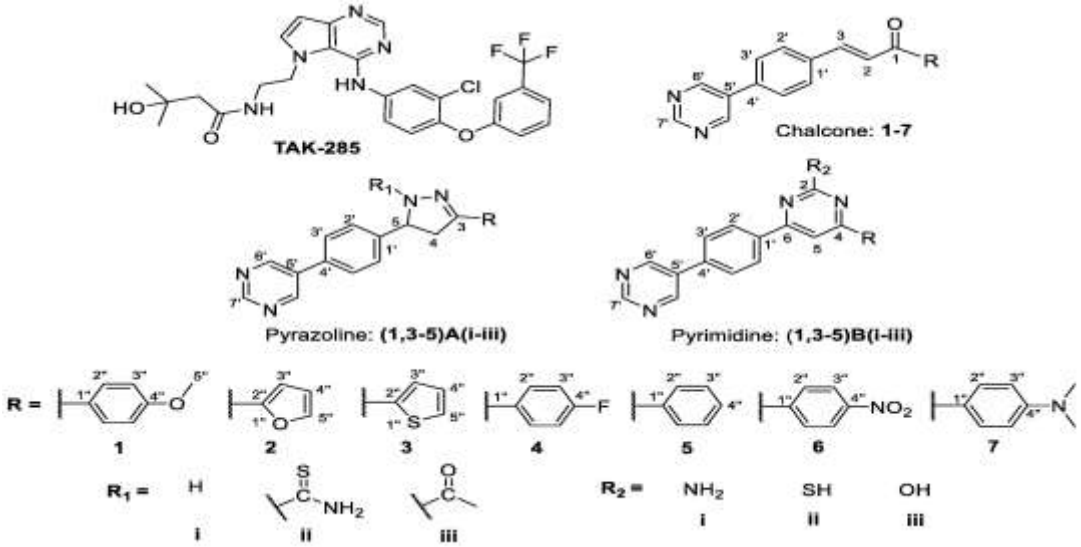
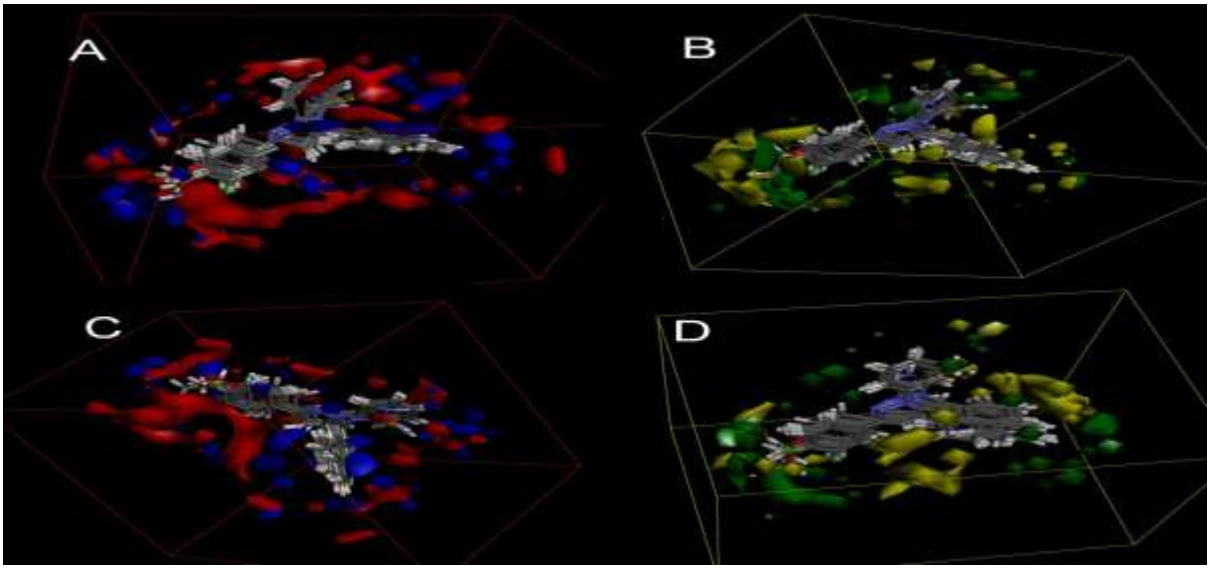
investigative small molecule that inhibits EGFR and specifically targets HER2, similar to lapatinib. It has been found to have antiproliferative activity in vitro and in vivo cytotoxic activity against cancer cells and was used as a control ligand.



In this study, we conducted an in-depth analysis of the structures of Plate Number 1 and several new chalcone, pyrazoline, and pyrimidine derivatives. Our findings revealed that the docking score results for the chalcone were almost identical to those of Plate Number 1, except for compounds 2 and 7, where the extra chains did not affect the binding affinity inside the pocket. We excluded compound 6 from further investigation due to its environmental toxicity, carcinogenicity, and mutagenicity. We observed that most of the proposed pyrazoline and pyrimidine derivatives showed excellent binding energies, ranging from 9.71 to 7.32 kcal/mol. Compound 5Bii had the lowest binding energy of 9.71 kcal/mol, while 5Aii had the highest binding energy of 7.32 kcal/mol.



Our analysis of the docked compounds' intermolecular interactions is presented in the Supplementary. We found that Figure 1 shows how TAK-285 binds within the adenosine triphosphate (ATP) binding pocket of the catalytic tyrosine kinase domain, competing with ATP. Previous

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studies have shown that a hydrogen bond with the ATP binding site of EGFR (Plate Number 2 and/or Plate Number 3) helps to inhibit its activity, as seen in all the proposed compounds except 7, 5Aii, and 3Bii. Plate Number 4 formed conventional hydrogen bonds with Plate Number 1, which matched compounds 3Aii, 5Aiii, 1B(i-iii), 4B(i-ii), and 4B(ii-iii) through hydrogen bond interaction. However, amino acid residue Plate Number 3 performed a hydrophobic interaction with Plate Number 1 but formed conventional hydrogen bonds with compounds 1Aiii, 3Aiii, 4Aii, 4Aiii, 5Aiii, and 4Biii.

Based on the comprehensive analysis of the binding energy of all the designed compounds, we decided to synthesize compounds 1, 3, 4, and 5 of chalcone derivatives and some heterocyclic derivatives of pyrazoline (Ai-iii) and pyrimidine (Bi-iii). These compounds showed good binding energies and strong interactions in the targeted active site. Our findings provide valuable insights into the design of new chalcone, pyrazoline, and pyrimidine derivatives with improved binding affinity and selectivity towards the ATP binding site of EGFR, which may have potential therapeutic applications in cancer treatment.

Table 1 The lowest binding energies from AutoDock 4.2 and interacting amino acids for the pyrazoline and pyrimidine derivatives.

Compound	Binding energy (Kcal/mol)	Interacting amino acids	Compound	Binding energy (Kcal/mol)	Interacting amino acids
TAK-285	−10.15	MET 793, ARG 776, LEU 777, THR790, ARG 841, THR 854,			
Chalcone					
1	−10.91	MET 793, LYS 745	**5**	−10.31	MET 793
2	−8.27	MET 793	**6**	−9.63	MET 793
3	−10.76	MET 793	**7**	−8.23	LEU 777
4	−10.19	MET 793, LEU 777			
Pyrazoline					
1Ai	-0.8.49	MET 766, MET 793	**4Ai**	−7.81	MET 793
1Aii	-0.8.16	MET 793, ASP 855	**4Aii**	−8.82	LYS 745, MET 793
1Aiii	−9.16	LYS 745, MET 793, ASP 855	**4Aiii**	−8.80	LYS 745, MET 793, ASP 855
3Ai	−8.26	MET 793, ASP 855	**5Ai**	−7.66	MET 793
3Aii	−8.80	MET 793, ASN 842, ASP 855	**5Aii**	−7.32	ASP 855
3Aiii	−8.92	LYS 745, LEU 788, MET 793	**5Aiii**	−8.92	LYS 745, MET 793, ASN 842
Pyrimidine					
1Bi	-0.9.32	MET 793, ASN 842, ASP 855	**4Bi**	−9.41	MET 793, ASN 842, ASP 855
1Bii	-0.9.22	MET 793, ASN 842, ASP 855	**4Bii**	−9.43	MET 793, ASN 842, ASP 855
1Biii	−8.96	MET 793, ASN 842, ASP 855	**4Biii**	−8.40	LYS 745, MET 793, ASP 855
3Bi	−9.23	MET 793, THR 854, ASP 855	**5Bi**	−7.82	MET 793, ASP 855
3Bii	−7.41	LEU 788, ARG 841	**5Bii**	−9.71	MET 793, ASN 842, ASP 855
3Biii	−9.42	MET 793, THR 854, ASP 855	**5Biii**	−9.15	MET 793, ASN 842, ASP 855

Chemistry

In this study, a series of pyrazoline and pyrimidine derivatives were synthesized by cyclization reactions of chalcone compounds 1, 3-5. The synthesized compounds were confirmed using analytical techniques such as FT-IR, NMR spectrometry, and CHN elemental analysis. The IR spectra revealed the presence of diagnostic bands, including absorbance bands of C sp2-H str, C sp3-H of the asymmetrical and symmetrical str, C = N str, C = C str, and C-N str at appropriate frequencies.

The 1 H NMR spectra provided diagnostic tools for positional elucidation of the protons. The characteristic signals were identified based on the chemical shifts and intensity patterns. In all pyrazoline derivatives, two protons of Ha and Hb at C-4 of the pyrazoline ring were observed and appeared as two doublets of doublets. A proton at C-5 of the pyrazoline ring also appeared as a doublet of doublets due to the vicinal coupling with two non-equivalent geminal protons of C-4 carbon. Furthermore, the proton at C-5 was observed to be more downfield than those of Ha and Hb because of its proximity to a benzene ring.

The coupling constants for two non-equivalent geminal protons become smaller when the HCH angle becomes larger. In this study, small changes in bond angles resulting from stereochemical changes influenced the geminal

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coupling constant. A singlet centered at pH 7.24 and pH 7.83 was consistent with the formation of a pyrimidine ring in 2,4,6-trisubstituted pyrimidine derivatives of Bi-iii. Both the pyrazoline and pyrimidine derivatives exhibited aromatic protons in the expected region.

In all the compounds, a singlet was assigned to H60 and H70 of the terminal pyrimidine ring. Signals for NH (Ai), NH2 (Aii, Bi), SH (Bii), acetyl-CH3 (Aiii), and OH (Bi) protons resonated as a singlet at appropriate frequencies. The aromatic protons were observed within the expected region along with the integral values. Overall, these observations provide valuable insights into the chemical properties of the synthesized pyrazoline and pyrimidine derivatives

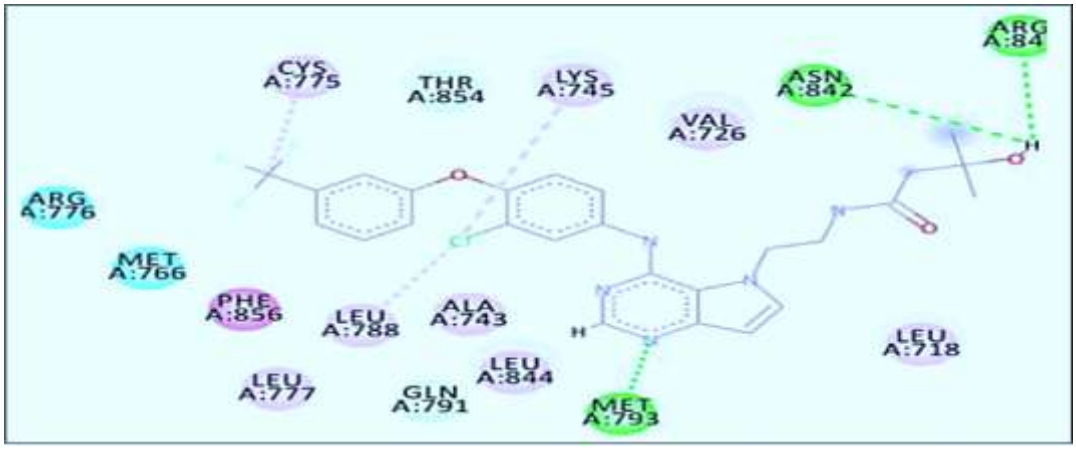
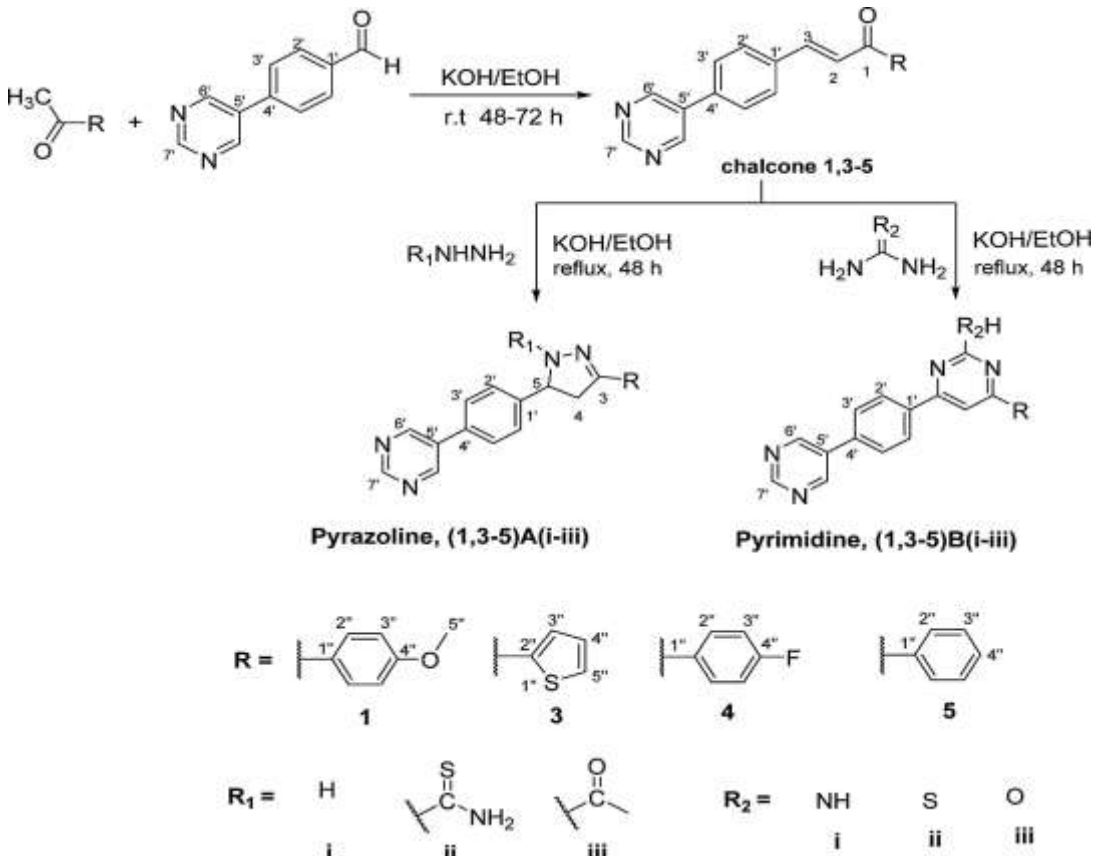


Fig. 1 2D intermolecular interactions between docked (TAK285) and 3POZ protein. Green and pink colored amino acids represent their contribution to hydrogen bonds and hydrophobic interactions



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Scheme 1. Synthesis pathway of chalcone, pyrazoline, and pyrimidine derivatives

The derivatives Ai-iii and Bi-iii have been analyzed using 13C and 1H NMR spectroscopy to determine their structure. The aromatic protons were observed as doublets in the NMR spectra, and the signals of the pyrazoline and pyrimidine rings were identified. The absence of trans-alkene carbons was confirmed, which further supports the proposed structures of the compounds.

The compound 1Aiii was found to have aliphatic carbons at DC 21.9, 55.4, 42.3, and 59.5 for the acetyl-CH3, methoxy-C50', C-4 and C-5 of pyrazoline, respectively. The carbonyl carbon was observed at DC 168.8, and the aromatic carbons appeared in the range of DC 161.4–114.2. These results were confirmed by analyzing the compound using DEPT-90 and DEPT-135 NMR spectra, which showed the presence of all methine (CH), methylene (CH2), methyl (CH3), and quaternary carbons (C) in the molecule.

Similarly, compound 1Bii was found to have an aliphatic carbon at DC 55.6 for the methoxy-C50' and C-5 for the 2,4,6-trisubstituted pyrimidine ring at DC 101.9. The aromatic carbons appeared in the range of DC 114.1–189.5. These results were also confirmed using DEPT-90 and DEPT-135 NMR spectra, which showed the presence of all methine (CH), methylene (CH2), methyl (CH3), and quaternary carbons (C) in the molecule.

To further confirm the structures of the compounds, 2D-NMR correlation using 1H–1H COSY and 1H–13C HSQC spectra were used for unambiguous assignment. These spectra showed the correlations between the protons and carbons and confirmed the assigned structures of pyrazoline 1Aiii and pyrimidine 1Bii.

In pyrazoline derivative 1Aiii, a cross-peak was observed between both protons H4a and H4b at dH 3.09 and dH 3.71 with the methylene carbon C4 (CH2) at dC 42.3. Similarly, in pyrimidine derivative 1Bii, a cross-peak was observed between proton H5 at dH 7.59 with methine carbon C5 (CH) at DC 101.9. These cross-peaks provided further confirmation of the structures of the compounds.

All the carbons were determined and represented in Supplementary Figure S2, which shows the NMR spectra and the assigned structures of the compounds.

Invitro assay

Recombinant assay of EFGR kinase

To validate the results of molecular docking showing the capability of compounds 4Aiii and 5Bii to bind and inhibit EGFR kinase, a luminescence assay was performed using the ADP-GloTM kinase assay at two different concentrations of 50 and 0.19 µM. The findings indicate that both compounds significantly inhibited the recombinant kinase when incubated with the inhibitors. Compound 4Aiii demonstrated an inhibition rate of 83% and 82% for both concentrations, respectively. Similarly, compound 5Bii showed an inhibition rate of 89% at 50 µM concentration. However, its inhibition rate decreased to 72% at 0.19 µM concentration, which is still considered significant inhibitory activity (as shown in Fig. 2). Moreover, the findings are consistent with the molecular docking results where both compounds showed a similar binding affinity and number of interactions with the surrounding amino acids located in the EGFR binding pocket. This suggests the high potential of pyrazoline and pyrimidine derivatives to be further evaluated for their cytotoxic activity.

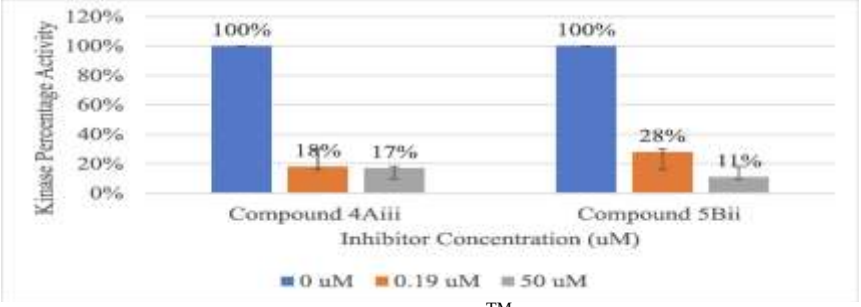

Fig. 2. Recombinant kinase activity measured using ADP-Glo^{TM} for compounds **4Aiii** and **5Bii** at three different concentrations (0, 0.19 and 50 µM).

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Cytotoxicity assay

The study aimed to assess the cytotoxicity potential of newly synthesized chalcone, pyrazoline, and pyrimidine derivatives against human breast cancer cell lines (MCF7 and MDA-MB-231) and a non-cancerous breast cell line (MCF-10A) with tamoxifen as the reference drug. The researchers calculated the IC50 and SI values, which indicate the concentration required for 50% inhibition of cell viability and the selectivity index, respectively.

The results indicated that pyrazoline derivatives showed better anti-cancer activity compared to the pyrimidine-derived compounds. Furthermore, the chalcone derivatives attached to the pyrimidine ring showed different cytotoxic activity based on the attached substituent. Compounds with C6H5- and thiophene ring exhibited higher cytotoxicity against MCF-7 compared to C6H4-OMe and C6H4F.

All chalcone compounds exhibited moderate cytotoxicity against the MDA-MB-231 cell line, with compound 5 exhibiting the highest cytotoxic selectivity index compared to tamoxifen. However, compounds 1, 3, and 4 exhibited lower cytotoxic selectivity against MCF-7, which can lead to increased cytotoxicity towards healthy cells.

Among the pyrazoline derivatives, 5Ai showed the lowest IC50 value, indicating excellent cytotoxic activity, and high cytotoxic selectivity index towards MCF-7, avoiding massive toxicity of normal cells (MCF-10A). The researchers suggest that the presence of 1H and the 3-phenyl group within the pyrazole ring may enhance the activity of 5Ai. Another pyrazoline, 4Ai, showed moderate cytotoxic activity but exhibited 1H and 3-(p-fluorophenyl).++--++++++++++++++++++

Compound 3Bi showed superior cytotoxic activity against the MCF-7 cell line compared to the other tested pyrimidine derivatives and tamoxifen. The researchers attribute this superior activity to the presence of thiophene-2-yl over position no. 4 of the pyrimidine ring. On the other hand, pyrazoline 4Aii showed only moderate cytotoxic activity against MCF-7.

Overall, the study suggests that the newly synthesized pyrazoline and pyrimidine derivatives have promising cytotoxicity potentials against human breast cancer cell lines. Further research is necessary to elucidate the underlying mechanisms of these compounds and their potential for clinical use.

Table 2 Cytotoxic effects of chalcones, pyrazoline and pyrimidine derivatives against two breast cancer cell lines (MCF-7 and MDA-MB-231) and non-cancerous cell lines (MCF-10A).

Compounds	IC_{50} (72 h) (μM)			Selective Index	
	MCF-7	MDA-MB-231	MCF-10A	MCF-7	MDA-MB-231
1	31.2 ± 2.17	100 ± 0.01	6.33 ± 0.58	0.2	0.06
3	9.55 ± 0.22	100 ± 0.01	4.73 ± 0.49	0.5	0.05
4	100 ± 0.01	100 ± 0.01	6.86 ± 1.68	0.07	0.07
5	9 ± 0.94	100 ± 0.01	32.9 ± 1.15	3.66	0.33
1Ai	100 ± 0.01	64.56 ± 7.8	100 ± 0.01	1	1.55
1Aii	53.7 ± 8.9	100 ± 0.01	100 ± 0.01	1.86	1
1Aiii	66.8 3 ± 1.8	51.2 9 ± 6.75	100 ± 0.01	1.5	1.95
3Ai	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
3Aii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
3Aiii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
4Ai	40.27 ± 1.08	100 ± 0.01	100 ± 0.01	2.48	1
4Aii	38.01 ± 2.96	100 ± 0.01	100 ± 0.01	2.63	1
4Aiii	58.88 ± 2.63	100 ± 0.01	100 ± 0.01	1.7	1
5Ai	26.3 ± 0.61	83.17 ± 0.97	100 ± 0.01	3.8	1.2
5Aii	67.61 ± 1.15	93.32 ± 2.15	100 ± 0.01	1.48	1.07
5Aiii	50.12 ± 4.13	69.18 ± 2.46	100 ± 0.01	2	1.45
1Bi	60.25 ± 7.25	100 ± 0.01	100 ± 0.01	1.66	1
1Bii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
1Biii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
3Bi	5.5 ± 0.07	100 ± 0.01	100 ± 0.01	18.18	1
3Bii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
3Biii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
4Bi	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
4Bii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
4Biii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
5Bi	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
5Bii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
5Biii	100 ± 0.01	100 ± 0.01	100 ± 0.01	1	1
Tamoxifen	26.95 ± 3.01	23.36 ± 3.84	–	–	0.3

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II. CONCLUSION

The study analyzed chalcone, pyrazoline, and pyrimidine derivatives as potential EGFR inhibitors. Selected compounds were synthesized and analyzed using advanced spectroscopic techniques. In vitro testing showed promising inhibitory activity against the EGFR kinase enzyme. Molecular docking revealed strong binding affinities towards EGFR kinase. Pyrazoline derivatives showed cytotoxic activity against breast cancer cell lines, with 5Ai being the best hormonal breast cancer candidate, and 1Aiii the most potent against non-hormonal breast cancer. One pyrimidine derivative showed excellent hormonal breast cancer activity, while others were active against non-hormonal breast cancer. Further studies and modifications are recommended to improve efficacy.

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