

Novel Imidazole-Linked 3,4-Dihydropyrimidine-2(1*H*)-thione Derivatives: Synthesis, Optimization, Computational Study and Cytotoxic Activity Against Breast Cancer

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Running Title: Imidazole-Linked DHPMs: Synthesis and Cytotoxicity

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ABSTRACT

Background: Breast cancer remains one of the leading causes of cancer-related mortality worldwide, necessitating the development of new chemotherapeutic agents with improved efficacy and safety. In this context, molecular hybridization of biologically active heterocycles such as imidazole and dihydropyrimidine offers a promising strategy for anticancer drug discovery. The present study aimed to design, synthesize, characterize, and evaluate a novel series of imidazole-linked 3,4-dihydropyrimidine-2(1*H*)-thione hybrids as potential anticancer agents against the MCF-7 breast cancer cell line.

Methods: The target compounds (**4a-4j**) were synthesized through an optimized one-pot Biginelli reaction using acetic acid as a green catalyst under mild conditions. Structural elucidation of the synthesized derivatives was carried out using FT-IR, ¹H NMR, ¹³C NMR, mass spectrometry, and elemental analysis. The synthesized compounds were screened for cytotoxic activity against MCF-7 cells using the MTT assay. Molecular docking studies were performed against EGFR tyrosine kinase (PDB ID: 1M17), followed by DFT calculations and in silico ADME/drug-likeness evaluation.

Results: Among the synthesized derivatives, compounds **4c** and **4g** exhibited the most potent cytotoxic activity with IC₅₀ values of 8.3 ± 0.60 μM and 14.8 ± 0.20 μM, respectively.

Molecular docking revealed strong binding interactions with EGFR, showing binding affinities of -18.7 kcal/mol for **4c** and -16.2 kcal/mol for **4g** through hydrogen bonding, π - π stacking, and hydrophobic interactions. DFT analysis indicated favorable electronic properties with low HOMO-LUMO energy gaps, supporting enhanced molecular reactivity. ADME profiling confirmed acceptable drug-likeness, high gastrointestinal absorption, and compliance with Lipinski's rule of five.

Conclusion: The present study demonstrates that imidazole-linked dihydropyrimidine-2(1*H*)-thione hybrids represent promising anticancer scaffolds. Particularly, compounds **4c** and **4g** emerged as potential lead molecules for further optimization and preclinical investigation targeting EGFR-mediated breast cancer therapy.

Keywords: 3,4-Dihydropyrimidine-2(1*H*)-thione, Breast cancer, Computational study, Imidazole, *in silico* study, MCF-7 cell line.

Introduction

Breast cancer is one of the most prevalent malignancies affecting women worldwide, accounting for approximately 2.3 million new cases and nearly 685,000 deaths annually, making it a leading cause of cancer-related mortality among women.¹ It primarily originates from epithelial cells of the breast tissue and exhibits diverse molecular subtypes, including estrogen receptor-positive (ER⁺), progesterone receptor-positive (PR⁺), and HER2-enriched forms, each requiring distinct therapeutic strategies. Current treatment options, such as surgery, radiotherapy, hormone therapy, and chemotherapy, have significantly improved patient survival; however, major limitations persist, including the emergence of multidrug resistance, non-selective cytotoxicity, severe side effects, and poor bioavailability of existing drugs.²⁻³ These challenges have accelerated the search for novel chemotherapeutic agents with enhanced potency, selectivity, and reduced toxicity. In recent years, heterocyclic compounds have emerged as versatile scaffolds in anticancer drug discovery due to their structural diversity and ability to interact effectively with biological targets.⁴ Among various pharmacologically active scaffolds, nitrogen-rich scaffolds such as imidazole and 3,4-dihydropyrimidine derivatives have emerged as promising frameworks owing to their broad spectrum of therapeutic potentials.⁵ Understanding their electronic characteristics through global reactivity parameters, such as electronegativity, hardness, and electrophilicity, can provide valuable insights into molecular stability, reactivity, and potential biological interactions, thereby supporting the rational design of potent anticancer agents.⁶

Combining multiple pharmacophores via a covalent link is the tactic known as molecular hybridization. Hybrid compounds have the potential to increase potency, interact with numerous targets (multifunctionally), mitigate adverse effects, or influence various aspects of a single disease.⁷⁻⁸ When considering the pharmaceutical industry's financial costs and the minimization of adverse effects caused by undesired drug interactions in drug combinations, this strategy has become increasingly paradigmatic. Due to the hybridization technique of different pharmacophores, including hybrid molecules based on natural substances, there are several examples of highly bioactive compounds in the literature.⁹

Within the medicinal chemistry domain, heterocyclic scaffolds such as imidazole and pyrimidine occupy a prominent position in anticancer drug discovery due to their broad biological activity, structural diversity, and synthetic flexibility.¹⁰ Imidazole, an important nitrogen-containing heterocycle found in biomolecules such as histidine and histamine, contributes significantly to enzyme inhibition, hydrogen bonding, and metal ion coordination, thereby enhancing ligand–receptor interactions. Several clinically important drugs containing the imidazole pharmacophore, including Ketoconazole, Dacarbazine, Temozolomide, Tipifarnib, and Ponatinib, have demonstrated remarkable anticancer potential through kinase inhibition, DNA alkylation, and apoptosis induction mechanisms.¹¹⁻¹³ Similarly, pyrimidine derivatives represent one of the most successful classes of heterocycles in medicinal chemistry because of their close structural resemblance to nucleic acid bases. Anticancer agents such as 5-Fluorouracil, Capecitabine, Gemcitabine, and Pemetrexed exert their therapeutic effects by disrupting DNA synthesis and folate metabolism pathways.¹⁴⁻¹⁷ Recent literature has increasingly focused on molecular hybridization strategies combining two or more bioactive pharmacophores into a single molecular framework to improve potency, selectivity, and pharmacokinetic behavior.¹⁸ In particular, imidazole–pyrimidine hybrid systems have attracted considerable attention owing to their ability to simultaneously modulate multiple biological targets, including EGFR, VEGFR, CDKs, and topoisomerases.¹⁹⁻²⁰ Several recently reported imidazole-linked pyrimidine derivatives exhibited promising cytotoxic activity against MCF-7 breast cancer cells; however, many of these compounds required multistep synthesis, expensive catalysts, or harsh reaction conditions.²¹⁻²² In contrast, the present work describes a facile and environmentally benign acetic acid-mediated Biginelli strategy for the synthesis of novel imidazole-linked 3,4-dihydropyrimidine-2(1*H*)-thione hybrids with excellent yields and significant anticancer potential. Furthermore, the integration of cytotoxicity evaluation, molecular docking, DFT analysis, and ADME profiling provides a comprehensive structure-

activity relationship framework, highlighting the significance and novelty of the designed hybrids as promising EGFR-targeted anticancer leads.

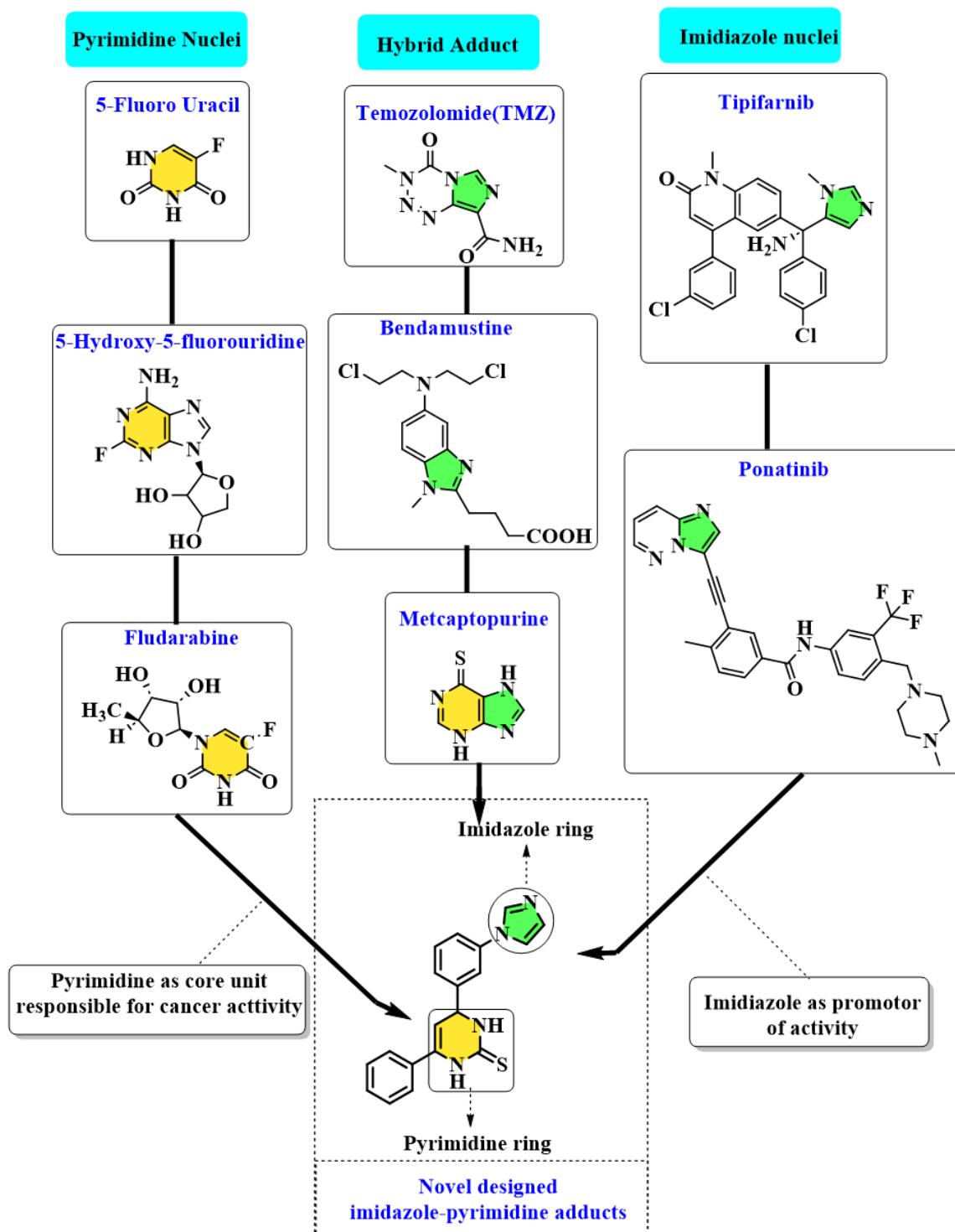


Figure 1. Examples of imidazole and pyrimidine-based anticancer drugs and a rational design strategy.

In light of these findings, we propose the construction of hybrid molecules combining imidazole and dihydropyrimidine pharmacophores in an effort to discover compounds with cytotoxic properties. To achieve this, the hybrid framework was designed by incorporating an imidazole moiety. The aryl ring connection between the two pharmacophores was achieved using the Biginelli procedure.

Materials and Methods

Materials

Except for ethyl acetate and hexanes, which were refined by straightforward distillation, all of the reagents were bought from commercial sources and utilized without additional purification. Thin-layer chromatography (TLC) was used to track the reactions using plates containing silica gel (Merck 60GF245), and the spots were visible when exposed to ultraviolet light. Bruker (^1H at 400 MHz and ^{13}C at 100 MHz in $\text{DMSO}-d_6$ as the solvent) was used to record the NMR spectra. Tetramethylsilane (TMS) was utilized as an internal standard, and the chemical shifts (δ) are reported in ppm units downfield from TMS. The coupling constants (J), which relate to peak multiplicities, are given in Hz. Bruker Alpha-P equipment was used to gather Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectra. Using a non-calibrated thermometer, the melting points were measured on a Buchi Melting Point M-565 apparatus.

Chemistry

Procedure for the synthesis 4-(3-(1H-imidazol-1-yl)phenyl)-6-(substituted phenyl)-3,4-dihydropyrimidine-2(1H)-thione (4a-4j).

A solution of 3-(1H-imidazol-1-yl)benzaldehyde (**1**, 1.0 mmol) and thiourea (**2**, 1.1 mmol) in ethanol (10 ml) was stirred at ambient temperature for 30 minutes. Subsequently, glacial acetic acid (0.5 vol, pH: $\sim$ 4.0) and various phenacyl bromide derivatives (**3a–3j**, 1.0 mmol) were added to the reaction mixture. The reaction mixture was heated at 70 °C for 12 hours, and the completion of the reaction was confirmed by TLC (mobile phase: 1. Hexane: ethyl acetate, 7:3; 2. Pure ethyl acetate). After the reaction was completed, the reaction mixture was poured into crushed ice. The product was filtered, washed with brine solution, and recrystallized from ethyl acetate to give a pure compound. The purity of the synthesized compounds was confirmed by ^1H NMR analysis.

Analytical data

4-(3-(1H-imidazol-1-yl)phenyl)-6-(p-tolyl)-3,4-dihydropyrimidine-2(1H)-thione (4a).

This compound was obtained as a white colour solid, Yield: 82%; MP 164-166 °C. IR (cm⁻¹): 3309 (N-H), 3101, 2909 (C-H), 1680 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.17 (s, 1H, -NH), 7.64 (s, 1H, -NH), 7.57-7.49 (m, 2H, Ar-H), 7.37 (s, 1H, Ar-H), 7.24-7.23 (d, 1H, J = 4.4 Hz, Pyrimidine ring-CH), 7.17-7.15 (d, 2H, J = 8.8 Hz, Ar-H), 7.09 (s, 1H, Ar-H), 6.99-6.97 (d, 3H, J = 8.8 Hz, Ar-H), 5.78 (s, 1H, -Chiral), 2.25 (s, 3H, -CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 167.4, 145.0, 142.8, 140.5, 135.1, 132.0, 131.4, 129.9, 129.9, 128.9, 128.7, 127.8, 127.2, 125.7, 124.7, 123.3, 123.1, 120.7, 117.3, 64.8; MS (*m/z*): [M]⁺ 347.1; Elemental analysis of C₂₀H₁₈N₄S: Calculated= C, 69.34; H, 5.24; N, 16.17; and Experimental= C, 69.33; H, 5.27; N, 16.15.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (4b).

Light pink colour solid, Yield: 74%; MP 122-124 °C. IR (cm⁻¹): 3309 (N-H), 3101, 2929 (C-H), 1680 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.18 (s, 1H, -NH), 7.64-7.55 (m, 1H, -NH), 7.36 (s, 1H, Ar-H), 7.25-7.19 (m, 1H, Pyrimidine ring-CH), 7.09 (s, 4H, Ar-H), 6.96 (s, 3H, Ar-H), 6.96 (s, 3H, Ar-H), 6.75-6.73 (d, 3H, J = 8.8 Hz, Ar-H), 5.73 (s, 1H, -Chiral), 3.70 (s, 3H, -OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 166.6, 158.9, 147.0, 146.8, 130.7, 129.6, 128.0, 126.2, 122.0, 120.0, 113.9, 64.4, 55.4; MS (*m/z*): [M]⁺ 362.9; Elemental analysis of C₂₀H₁₈N₄OS: Calculated= C, 66.28; H, 5.01; N, 15.46; O, 4.41; and Experimental= C, 66.27; H, 5.02; N, 15.44; O, 4.43.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-(trifluoromethyl)phenyl)-3,4-dihydropyrimidine-2(1H)-thione (4c).

Dark brown colour solid, Yield: 85%; MP 180-182 °C. IR (cm⁻¹): 3302 (N-H), 3085, 2909 (C-H), 1685 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.26 (s, 1H, -NH), 7.81-7.70 (m, 1H, -NH), 7.59-7.34 (m, 6H, Ar-H & Pyrimidine ring-CH), 7.15 (s, 6H, Ar-H), 5.81 (s, 1H, -Chiral); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 167.2, 145.9, 145.7, 139.2, 137.6, 130.8, 130.1, 128.9, 128.2, 127.9, 126.1, 125.9, 125.4, 124.7, 123.2, 120.3, 120.1, 118.6, 65.3; MS (*m/z*): [M]⁺ 401.1; Elemental analysis of C₂₀H₁₅F₃N₄S: Calculated= C, 59.99; H, 3.78; N, 13.99; and Experimental= C, 59.98; H, 3.81; N, 13.97.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-bromophenyl)-3,4-dihydropyrimidine-2(1H)-thione (4d).

Off-white colour solid, Yield: 78%; MP 164-166 °C. IR (cm⁻¹): 3317 (N-H), 3126, 2936 (C-H), 1689 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.21 (s, 1H, -NH), 7.68 (s, 1H, -NH), 7.59-7.51 (m, 2H, Ar-H), 7.47 (s, 1H, Ar-H), 7.39-7.37 (d, 2H, J = 8.7 Hz, Ar-H), 7.30-7.28

(d, 1H, J = 8.8 Hz, Pyrimidine ring-CH), 7.20-7.18 (d, 4H, J = 8.8 Hz, Ar-H), 7.10-7.06 (q, 2H, J = 16.1 Hz, Ar-H), 5.74 (s, 1H, -Chiral); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 166.9, 146.3, 145.8, 136.0, 134.5, 131.5, 130.8, 130.4, 130.2, 126.0, 123.7, 121.1, 120.2, 119.9, 118.5, 65.0; MS (m/z): $[\text{M}]^+$ 411.1, 413.1; Elemental analysis of $\text{C}_{19}\text{H}_{15}\text{BrN}_4\text{S}$: Calculated= C, 55.48; H, 3.68; N, 13.62; and Experimental= C, 55.47; H, 3.70; N, 13.61.

4-(6-(3-(1H-imidazol-1-yl)phenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl) benzonitrile (4e).

Yellow colour solid, Yield: 80%; MP 190-192 °C. IR (cm^{-1}): 3296 (N-H), 3078, 2915 (C-H), 1685 ($>\text{C}=\text{S}$); ^1H NMR (400 MHz, DMSO- d_6) δ : 8.59 (s, 1H, -NH), 7.84 (s, 1H, -NH), 7.67-5.57 (m, 5H, Ar-H), 7.43-7.31 (m, 1H, Pyrimidine ring-CH), 7.14 (s, 6H, Ar-H), 5.83 (s, 1H, -Chiral); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 168.7, 147.5, 145.1, 144.0, 140.1, 135.2, 129.7, 128.8, 127.5, 126.3, 125.8, 121.8, 120.9, 119.2, 117.9, 64.8; MS (m/z): $[\text{M}]^+$ 358.1; Elemental analysis of $\text{C}_{20}\text{H}_{15}\text{N}_5\text{S}$: Calculated= C, 67.21; H, 4.23; N, 19.59; and Experimental= C, 67.23; H, 4.20; N, 19.60.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-(methylthio)phenyl)-3,4-dihydropyrimidine-2(1H)-thione (4f).

White colour solid floppy, Yield: 90%; MP 178-180 °C. IR (cm^{-1}): 3304 (N-H), 3101, 2909 (C-H), 1681 ($>\text{C}=\text{S}$); ^1H NMR (400 MHz, DMSO- d_6) δ : 9.25 (s, 1H, -NH), 8.17 (s, 1H, -NH), 7.78-7.58 (m, 1H, Ar-H), 7.45-7.44 (d, 1H, J = 4.4 Hz, Pyrimidine ring-CH), 7.31-7.18 (m, 9H, Ar-H), 7.08-7.06 (d, 1H, J = 8.8 Hz, Ar-H), 5.77 (s, 1H, -Chiral); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 167.1, 146.5, 145.7, 138.3, 136.1, 135.5, 131.0, 128.8, 128.2, 125.7, 122.8, 122.1, 121.6, 121.0, 118.6, 65.6, 14.8; MS (m/z): $[\text{M}]^+$ 379.1; Elemental analysis of $\text{C}_{20}\text{H}_{18}\text{N}_4\text{S}_2$: Calculated= C, 63.46; H, 4.79; N, 14.80; and Experimental= C, 63.44; H, 4.80; N, 14.81.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-nitrophenyl)-3,4-dihydropyrimidine-2(1H)-thione (4g).

Light pink colour solid amorphous, Yield: 87%; MP 174-176 °C. IR (cm^{-1}): 3302 (N-H), 3117, 2949 (C-H), 1689 ($>\text{C}=\text{S}$); ^1H NMR (400 MHz, DMSO- d_6) δ : 8.79 (s, 1H, -NH), 8.01-7.93 (m, 5H, Ar-H & -NH), 7.71-7.41 (m, 5H, Ar-H & Pyrimidine ring-CH), 7.22 (s, 3H, Ar-H), 5.97 (s, 1H, -Chiral); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 167.2, 146.5, 145.6, 141.5, 137.0, 135.8, 131.0, 129.4, 129.2, 127.1, 126.9, 126.1, 123.8, 123.6, 121.2, 120.8, 119.6, 64.7; MS (m/z): $[\text{M}]^+$ 378.5; Elemental analysis of $\text{C}_{19}\text{H}_{15}\text{N}_5\text{O}_2\text{S}$: Calculated= C, 60.47; H, 4.01; N, 18.56; O, 8.48; and Experimental= C, 60.45; H, 4.03; N, 18.55; O, 8.49.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione (4h).

White colour solid, Yield: 69%; MP 188-190 °C. IR (cm⁻¹): 3309 (N-H), 3101, 2909 (C-H), 1691 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.24 (s, 1H, -NH), 7.70 (s, 1H, -NH), 7.59-7.43 (m, 9H, Ar-H), 7.34-7.32 (d, 1H, J = 8.7 Hz, Pyrimidine ring-CH), 7.15-7.11 (t, 2H, J = 16.1 Hz, Ar-H), 5.81 (s, 1H, -Chiral); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 167.2, 145.7, 139.1, 137.6, 136.1, 130.8, 130.3, 128.8, 126.1, 125.4, 124.7, 120.3, 120.1, 118.6, 65.3; MS (*m/z*): [M]⁺ 367.4; Elemental analysis of C₁₉H₁₅ClN₄S: Calculated= C, 62.20; H, 4.12; N, 15.27; and Experimental= C, 62.21; H, 4.12; N, 15.26.

4-(3-(1H-imidazol-1-yl)phenyl)-6-phenyl-3,4-dihydropyrimidine-2(1H)-thione (4i).

Dark brown colour solid, Yield: 71%; MP 172-174 °C. IR (cm⁻¹): 3317 (N-H), 3126, 2936 (C-H), 1689 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.49 (s, 1H, -NH), 7.96-7.94 (s, 1H, J = 8.8 Hz, -NH), 7.80-7.76 (t, 1H, J = 16.1 Hz, Pyrimidine ring-CH), 7.71-7.69 (d, 2H, J = 8.8 Hz, Ar-H), 7.60-7.58 (d, 4H, J = 8.7 Hz, Ar-H), 7.46-7.44 (d, 3H, J = 8.8 Hz, Ar-H), 7.18-7.16 (d, 2H, J = 8.8 Hz, Ar-H), 5.51 (s, 1H, -Chiral); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 168.5, 147.6, 145.1, 144.1, 140.2, 135.3, 129.8, 128.8, 127.6, 125.9, 121.8, 120.9, 118.8, 63.9; MS (*m/z*): [M]⁺ 333.7; Elemental analysis of C₁₉H₁₆N₄S: Calculated= C, 68.65; H, 4.85; N, 16.85; and Experimental= C, 68.63; H, 4.86; N, 16.86.

4-(3-(1H-imidazol-1-yl)phenyl)-6-(4-hydroxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (4j).

Light yellow colour solid, Yield: 65%; MP 170-172 °C. IR (cm⁻¹): 3309 (N-H), 3101, 2909 (C-H), 1676 (>C=S); ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.23 (s, 1H, -NH), 7.98-7.91 (m, 1H, -NH), 7.71-7.70 (d, 4H, J = 4.4 Hz, Ar-H), 7.50-7.41 (m, 3H, Ar-H), 7.38-7.35 (m, 2H, Ar-H), 7.12-7.08 (m, 2H, Ar-H), 6.97-6.95 (d, 1H, J = 8.8 Hz, Pyrimidine ring-CH), 5.76 (s, 1H, -Chiral), 5.40 (s, 1H, -OH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 168.0, 147.5, 145.1, 144.1, 140.2, 135.3, 129.7, 128.8, 127.5, 126.3, 125.8, 121.8, 120.9, 118.3, 66.3; MS (*m/z*): [M]⁺ 349.2; Elemental analysis of C₁₉H₁₆N₄OS: Calculated= C, 65.50; H, 4.63; N, 16.08; O, 4.59; and Experimental= C, 65.51; H, 4.62; N, 16.07; O, 4.60.

Biological Studies: Cytotoxicity study

To determine the cytotoxic potential of the synthesized compounds against the MCF-7 breast cancer cell line, the MTT assay was performed using cells procured from NCCS Pune and evaluated at Aakar Biotech, Lucknow. The cells were cultured in DMEM supplemented with

10% Fetal Bovine Serum (FBS) and 1% Penicillin–Streptomycin solution under a humidified atmosphere containing 5% CO₂ at 37°C. Approximately 10,000 cells per well were seeded into a 96-well plate and incubated for 24 h. The test compounds were initially dissolved in DMSO and subsequently diluted with serum-free medium to obtain the required concentrations, ensuring that the final DMSO concentration remained non-toxic to the cells. DMSO-treated control wells were also included and showed no significant cytotoxicity under the experimental conditions. After 24 h treatment, MTT solution (5 mg/mL) was added and incubated for an additional 2 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was recorded at 540 nm using an ELISA plate reader (iMark, Bio-Rad, USA). All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). IC₅₀ values were calculated using GraphPad Prism 6 software.²³⁻²⁴

Molecular Docking Protocol

The AutoDock v4.2 virtual screening program was used in the execution of the molecular docking studies. The protein EGFR was selected as a macromolecule, and compounds were considered ligand molecules. The cubical grid box size (X × Y × Z) for EGFR was set at 92.16 × 63.20 × 50.15 Å°. The optimal binding orientation was determined by selecting the pose with the lowest free binding energy (measured in kcal/mol). The interaction between the protein receptor's amino acid residues and ligand molecules was examined utilizing Discovery Studio Visualizer v21.1.0. To validate the docking protocol's accuracy, the compounds were subjected to re-docking (RMSD 0.2703 Å) with the same proteins employed in the study.²⁵

DFT Studies

DFT studies aim to offer comprehensive geometric information for a broad spectrum of systems. In this work, the DFT approach with the B3LYP-6-31G (d, p) basis set was used to conduct the theoretical calculations. In order to run crucial chemical calculations, such as creating HOMO and LUMO surfaces, molecular electrostatic potential surfaces, and global reactivity parameters, the required molecules' input files were organized and prepared using the GaussView 06 program.²⁶ The geometrical parameters were calculated using the formula reported previously.²⁷

ADME and Drug Likeness

It is widely acknowledged that understanding the pharmacokinetic (PK) characteristics of potential drug candidates, particularly their ADME, plays a critical role in the stages of drug discovery and development. Drug likeness analysis was performed using SwissADME. The

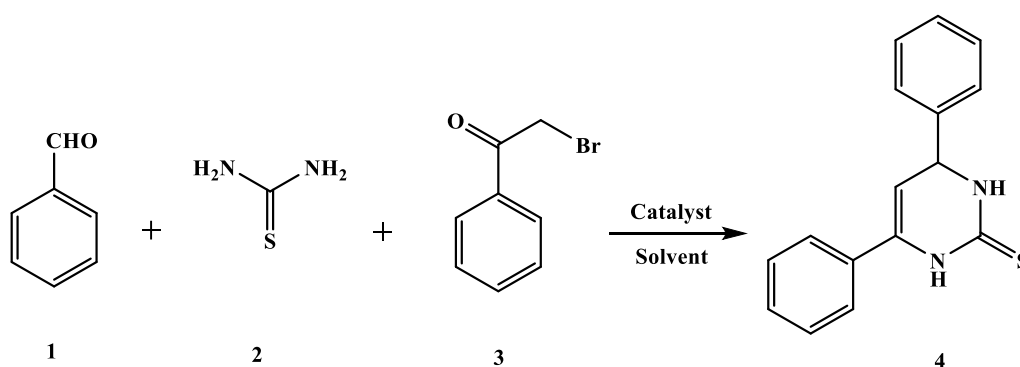
drug-likeness of the chosen hit compounds from the molecular docking analysis was evaluated by applying Lipinski's Rule of Five, a standard for favorable pharmaceutical properties, as well as Jorgensen's Rule of 3. Following the rule of good medicine, the criteria include a molecular weight below 500 g/mol, lipophilicity less than 5, and adherence to having no more than 5 hydrogen bond donors and no more than 10 hydrogen bond acceptors for competitor molecules.²⁸⁻²⁹ The ProTox-III server was used to analyze the toxicity class of the compounds.³⁰

Results and Discussion

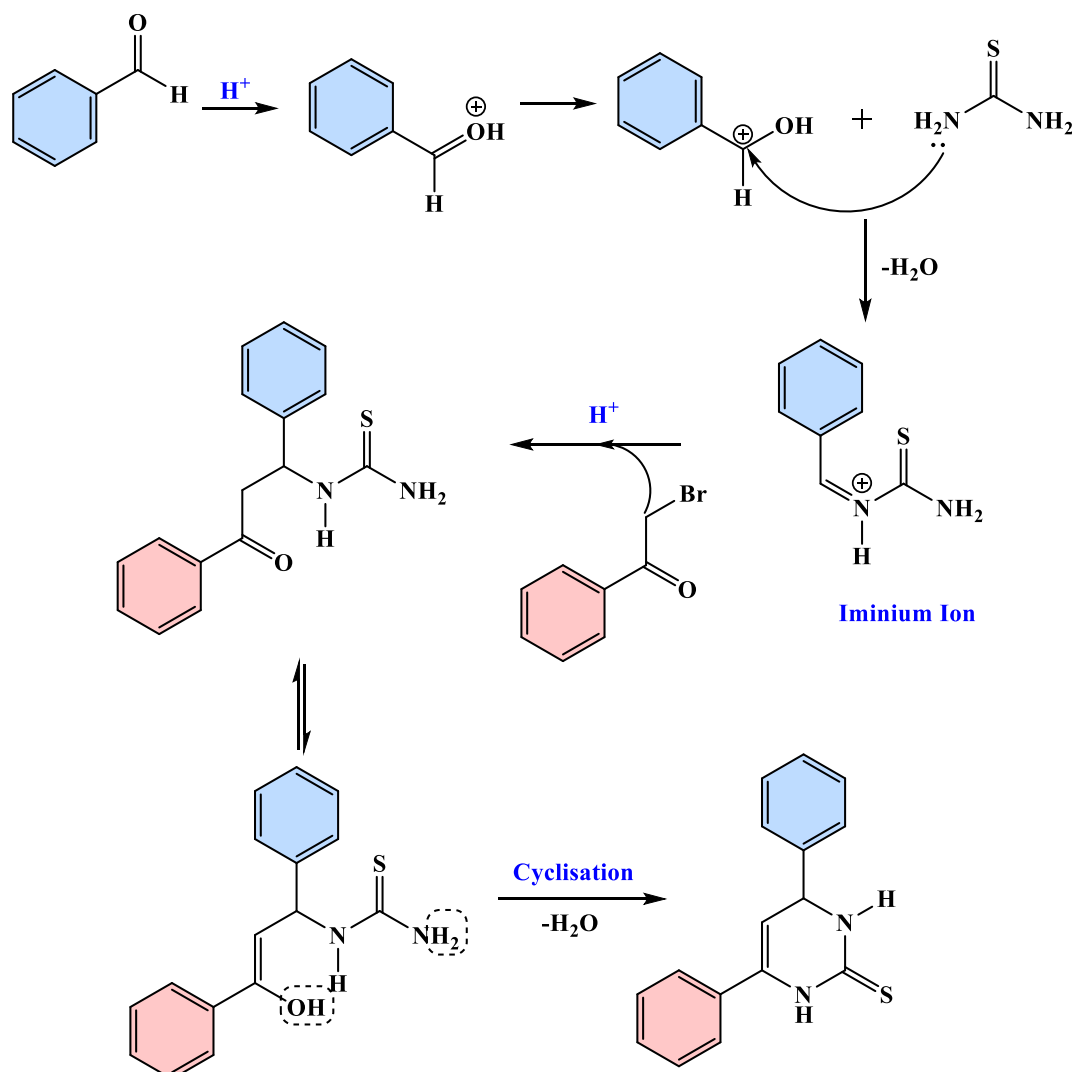
Synthesis and Optimizations

Pyrimidine–imidazole bearing novel hybrids were synthesized via a one-step *in situ* reaction to achieve greater efficiency, structural diversity, and synthetic simplicity (**Schemes 1** and **2**). This approach enables the rapid formation of complex molecular frameworks under mild conditions, minimizing the need for isolation of products and reducing reaction time and waste. The *in situ* strategy also promotes better yields and improved atom economy, making it highly attractive for generating biologically relevant heterocyclic scaffolds. By integrating both pyrimidine and imidazole moieties, known for their significant pharmacological potential, into a single hybrid system, the present work aims to explore enhanced bioactivity and synergistic effects in a streamlined synthetic process.

The Biginelli reaction, a multicomponent condensation of aldehyde, thiourea, and a carbonyl compound, is a powerful method for synthesizing dihydropyrimidine-2(1*H*)-thione or thiazine-type derivatives.³¹ Utilizing the same type of multicomponent reactions, initially to set a standard reaction condition and to forward optimized synthetic process for the library of compounds, benzaldehyde (**1**), thiourea (**2**) and phenacyl bromide (**3**) reacted by employing various reaction conditions concerning to the catalyst, temperature and solvents (**Scheme 1**) which proceeded through an iminium ion intermediate followed by cyclization (**Scheme 2**).



Scheme 1. Model reaction to optimize the reaction conditions and prediction of designed adducts (4).



Scheme 2. Generalized acid-catalyzed mechanism for the synthesis of pyrimidine adduct.

To establish optimal conditions for the synthesis of compound 4 via the Biginelli reaction, a series of reaction parameters was screened, and the results are summarized in **Table 1**. It was deliberately tested with representatives from major catalyst families: Brønsted acids, Lewis's acids, ionic liquids, organocatalysts, and inorganic iodine, considering the reported catalysts in the Biginelli reaction. The reaction was initially attempted under catalyst-free conditions using different solvents at room temperature. In methanol and ethanol (**Entries 1 and 2**), only trace amounts of the desired product were obtained, indicating that the reaction does not proceed efficiently in the absence of a catalyst. A trial in DMF across a temperature range from room temperature to 110°C (**Entry 3**) failed to yield any product, likely due to the aprotic nature of the solvent, which is not conducive to proton transfer processes required in the Biginelli

reaction. However, when the reaction was conducted in ethanol at 70°C (**Entry 4**), a modest 35% yield was obtained even without a catalyst, suggesting that elevated temperature does promote the condensation to some extent, though not sufficiently.

Table 1. Systematic optimization of reaction conditions. Representative catalysts from different categories (Brønsted acids, Lewis acids, ionic liquids, organocatalysts, and inorganic catalysts) were screened along with variations in temperature and solvent to identify optimal conditions for the model reaction.

Entries	Catalyst	Temperature (°C)	Solvent	Yield (%)
1.	-	RT	Me-OH	Trace
2.	-	RT	Et-OH	Trace
3.	-	RT - 110	DMF	No reaction
4.	-	70	Et-OH	35
5.	Con. HCl ^[a]	70	Et-OH	77
6.	Citric acid ^[a]	70	Et-OH	48
7.	Acetic acid ^[a]	70	Et-OH	91
8.	FeCl ₃ ^[b]	RT – 70	Et-OH	35
9.	p-TsOH ^[c]	70	Et-OH	55
10.	[Et ₃ NH][HSO ₄] ^[d]	RT – 70	Et-OH	< 20
11.	L-Proline ^[e]	70	Et-OH	< 20
12.	Iodine ^[f]	70	Et-OH	< 20

[a] Acid catalyst. [b] Lewis's acid catalyst. [c] Brønsted acid catalyst. [d] Ionic liquids. [e] Organocatalyst. [f] Inorganic/miscellaneous catalyst.

To improve the efficiency of the transformation, various acid catalysts were examined under similar conditions. Among the acids tested, concentrated hydrochloric acid (**Entry 5**) yielded 77%, reflecting its strong proton-donating capacity that aids in imine and enamine intermediate formation. Citric acid (**Entry 6**), a weaker organic acid, provided only 48% yield, possibly due to limited catalytic activity or lower solubility in the reaction medium. Remarkably, acetic acid (**Entry 7**) produced the best result with a high yield of 91%, highlighting its effectiveness as a mild, environmentally benign, and readily available catalyst for this transformation.

The balanced acidity of acetic acid seems to facilitate the key steps in the Biginelli mechanism: imine formation, enolization, and cyclocondensation, without over-stabilizing intermediates or

causing side reactions. Further catalyst screening included Lewis and Brønsted acid catalysts such as FeCl₃ and *p*-toluenesulfonic acid (*p*-TsOH). Ferric chloride (**Entry 8**) delivered only a 35% yield, which may be attributed to the formation of stable complexes with thiourea or the carbonyl substrate, thereby impeding the reaction progress. *p*-TsOH (**Entry 9**), a strong organic sulfonic acid, gave a better yield of 55%, though it still fell short compared to acetic acid. The moderate performance of these acids suggests that while they provide sufficient acidity, factors such as solubility, steric bulk, and compatibility with reaction intermediates may limit their catalytic efficiency in this system. Alternative catalytic approaches were also explored, including ionic liquids, organocatalysts, and inorganic reagents. The ionic liquid [Et₃NH][HSO₄] (**Entry 10**) did not give a recorded yield, which could be due to low solubility or phase incompatibility under the tested conditions. Similarly, L-proline (**Entry 11**), a commonly used organocatalyst, and iodine (**Entry 12**), an inorganic promoter, failed to provide significant product formation (yield < 20%). These results imply that such catalysts do not effectively mediate the multistep process required in the Biginelli mechanism, which relies heavily on efficient proton transfer and carbonyl activation.

In summary, the reaction optimization highlights the importance of catalyst selection and temperature control for efficient Biginelli-type synthesis of compound **4**. Among all tested systems, acetic acid at 70 °C in ethanol emerged as the optimal condition, delivering a high yield of 91%. These findings not only provide a reliable protocol for synthesizing compound **4** and expanding the designed library, **4a-4j** (**Figure 2**), but also demonstrate a green and practical approach suitable for broader application in heterocyclic compound synthesis.

Following successful optimization, the model Biginelli reaction was initiated by stirring a mixture of 3-(1*H*-imidazol-1-yl)benzaldehyde (1.0 mmol) and thiourea (1.1 mmol) at ambient temperature for 30 minutes. To facilitate the condensation under mild acidic conditions, glacial acetic acid was employed to maintain the pH in the range of 4.0–4.5. Subsequently, phenacyl bromide (1.0 mmol) was introduced, and the reaction mixture was heated at 70 °C. After 9-12 hours of stirring, the desired products, 4-(3-(1*H*-imidazol-1-yl)phenyl)-6-(substituted phenyl)-3,4-dihydropyrimidine-2(1*H*)-thione (**4a-4j**), were isolated in excellent yields exceeding 80% (**Scheme 3**). Notably, the crude products crystallized directly from the reaction mixture, eliminating the need for further purification steps. The identity and purity of the synthesized compounds were confirmed through comprehensive spectral analyses, including ¹H NMR, ¹³C NMR, mass spectrometry, and IR spectroscopy.

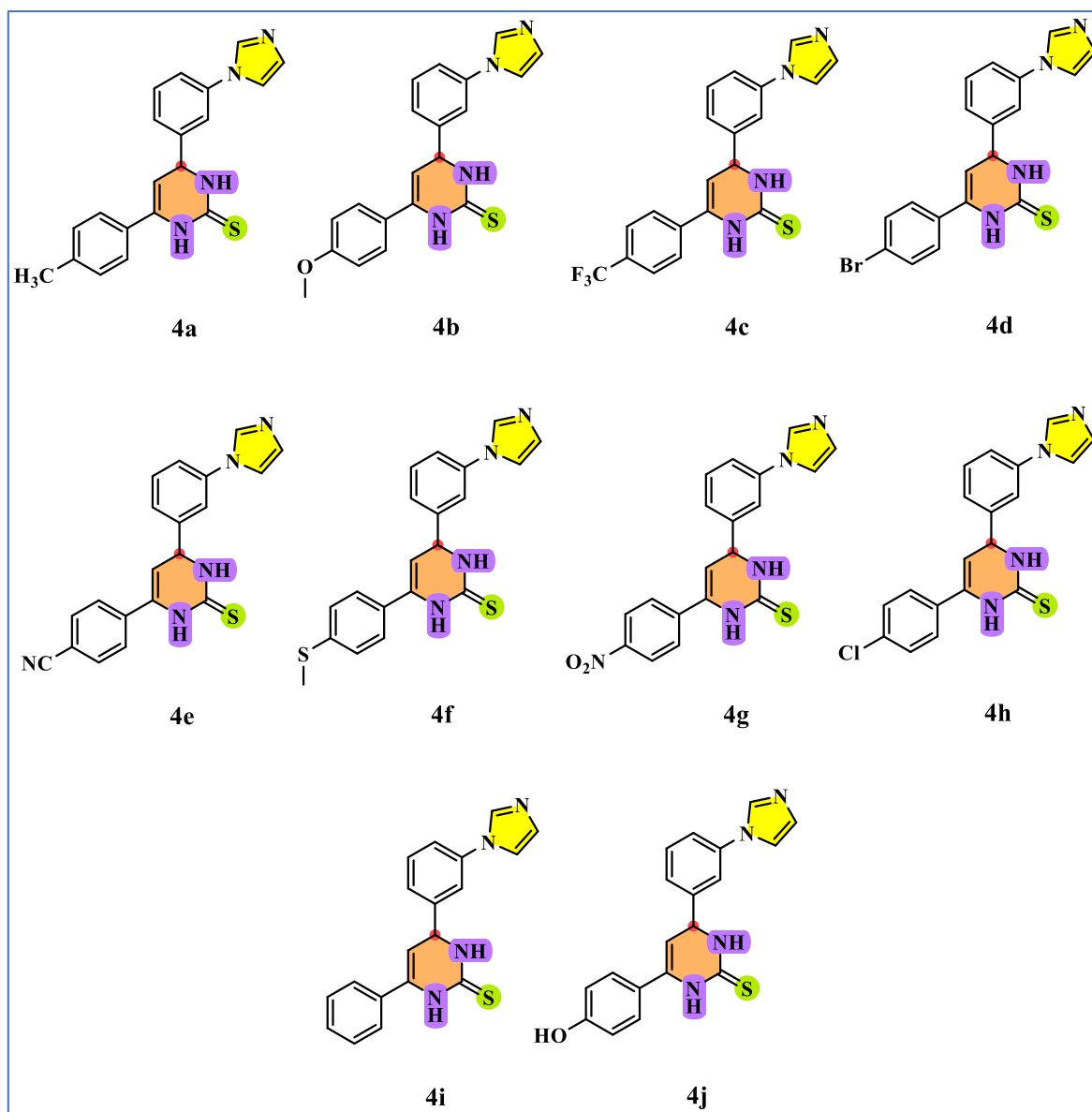
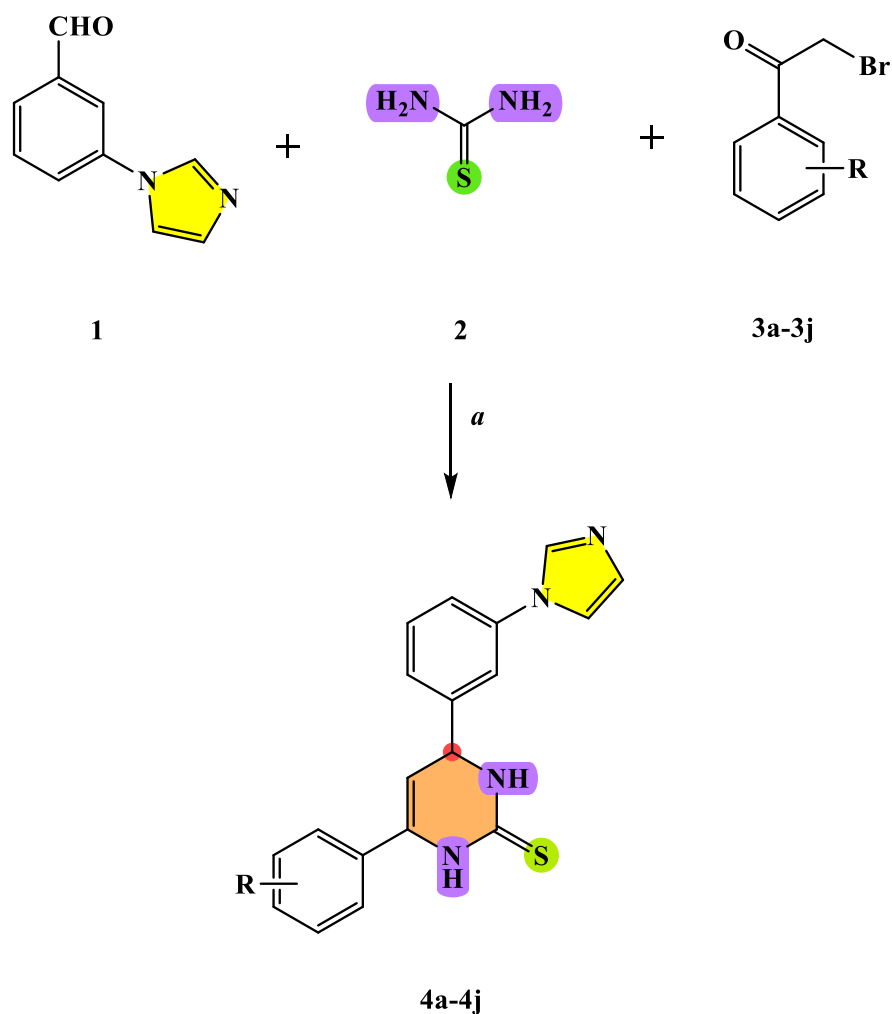


Figure 2. Chemical structure representation of newly synthesized compounds (4a-4j).



Reaction and Conditions: a) Acetic acid, Et-OH, 70°C, 12 h.

Scheme 3. Synthetic pathway for the novel imidazole-pyrimidine bearing framework (4a-4j).

Anticancer Activity (MTT Assay)

The synthesized imidazole-linked 3,4-dihydropyrimidine-2(1*H*)-thione hybrids (**4a-4j**) were evaluated for their *in vitro* cytotoxicity against the human breast cancer MCF-7 cell line using the MTT assay.

Table 2. Cytotoxicity of the synthesized compounds (**4a-4j**) against the MCF-7 breast cancer cell line.

Comp. ID	Substituents (-R)	IC ₅₀ (μM)	Binding affinity (kcal/mol)
4a	4-Me	37.2 ± 0.90	-13.9
4b	4-OMe	17.5 ± 1.10	-15.3
4c	4-CF ₃	8.3 ± 0.60	-18.7

4d	4-Br	26.4 ± 2.09	-14.8
4e	4-CN	40.2 ± 2.01	-13.2
4f	4-SMe	19.1 ± 0.28	-15.2
4g	4-NO ₂	14.8 ± 0.20	-16.2
4h	4-Cl	47.6 ± 1.20	-14.9
4i	-H	>100	-14.2
4j	4-OH	41.4 ± 0.45	-14.8
Sunitinib	-	5.00 ± 0.11	-7.5

The results, as presented in **Table 2**, reveal a structure–activity relationship (SAR) that highlights the critical influence of various para-substituents (-R) on the phenyl ring attached to the dihydropyrimidine-2(1H)-thione core. Visual confirmation of the cytotoxic effect is shown in Fig. 3, which illustrates the morphological changes in MCF-7 cells after treatment. Compared to the control group (**Figure 3i**), treated cells (**Figure 3ii–3xi**) exhibited characteristic signs of cytotoxicity, such as cellular shrinkage, membrane disruption, and decreased cell density.

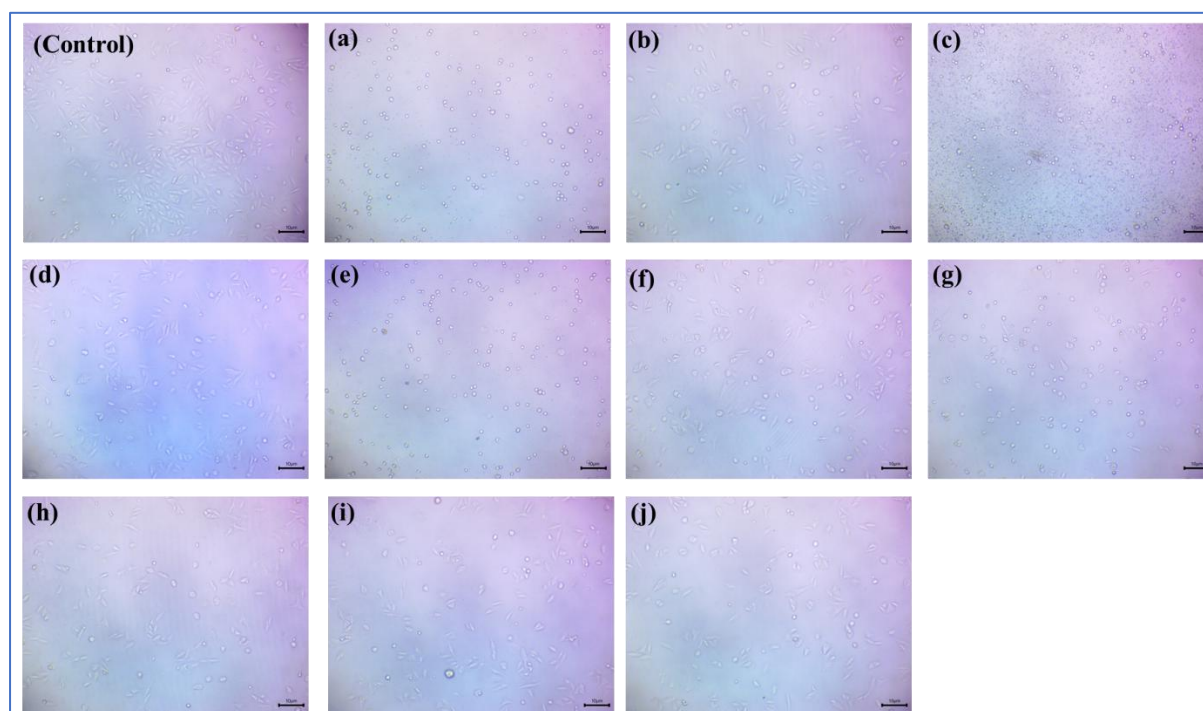


Figure 3. Cytotoxicity on MCF-7 cells (i) Control (before treatment), (ii) After treatment with **4a** (iii) After treatment with hybrid **4b** (iv) After treatment with hybrid **4c** (v) After treatment with hybrid **4d** (vi) After treatment with hybrid **4e** (vii) After treatment with hybrid **4f** (viii)

After treatment with hybrid **4g** (ix) After treatment with hybrid **4h** (x) After treatment with hybrid **4i** (xi) After treatment with hybrid **4j**.

Among the tested derivatives, compound **4c**, bearing a 4-trifluoromethyl (4-CF₃) substituent, exhibited the most potent cytotoxic activity with an IC₅₀ of 8.3 ± 0.60 μM, which was significantly more active compared to other analogues. Notably, this compound also exhibited the highest binding affinity (-18.7 kcal/mol) in the molecular docking study, suggesting a strong interaction with the active site of the target protein, which likely contributes to its enhanced anticancer activity. Other promising candidates include compound **4g** (4-NO₂) and **4b** (4-OMe) with IC₅₀ values of 14.8 ± 0.20 μM and 17.5 ± 1.10 μM, respectively. Their relatively strong binding affinities (-16.2 and -15.3 kcal/mol) also correlate well with their cytotoxic potential. These results indicate that electron-withdrawing groups (like CF₃ and NO₂) or electron-donating groups (such as OMe and SMe) at the *p*-position can enhance activity. In contrast, compound **4i**, the unsubstituted analogue (-H), displayed weak cytotoxicity (IC₅₀ > 100 μM), suggesting that substitution at the *p*- position is essential for biological activity. Additionally, compounds such as **4e** (4-CN) and **4j** (4-OH) showed only moderate inhibition, with IC₅₀ values of 40.2 ± 2.01 μM and 41.4 ± 0.45 μM, respectively. When compared to the standard anticancer agent Sunitinib (IC₅₀ = 5.00 ± 0.11 μM), several compounds showed significant, though lesser, cytotoxic effects, yet their binding affinities were markedly better.

The cytotoxicity data revealed a meaningful structure-activity relationship (SAR), indicating that the nature of the para-substituent on the phenyl ring of the dihydropyrimidine-2(1*H*)-thione scaffold strongly influences anticancer activity. Among the synthesized derivatives, compounds containing strong electron-withdrawing substituents showed comparatively higher activity than unsubstituted or weakly substituted analogues. In particular, compound **4c** bearing a 4-trifluoromethyl (-CF₃) group exhibited the highest potency (IC₅₀ = 8.3 ± 0.60 μM), followed by compound **4g** containing a 4-nitro (-NO₂) substituent (IC₅₀ = 14.8 ± 0.20 μM). The improved activity of these derivatives may be associated with the electron-deficient nature of these groups, which can enhance molecular interactions within the EGFR binding pocket. Additionally, the increased lipophilic character of these substituents may favour better membrane permeability and stronger hydrophobic interactions with the receptor.

Compounds carrying electron-donating groups such as **4b** (4-OMe) and **4f** (4-SMe) also demonstrated promising activity, although slightly lower than the strongly electron-withdrawing analogues. The methoxy and methylthio groups may contribute to stabilization of

ligand–protein interactions through π – π stacking and hydrophobic contacts. However, excessive electron donation could reduce the overall electrophilic nature of the scaffold, which may explain their moderate activity relative to compounds **4c** and **4g**. The halogen-substituted derivatives **4d** (4-Br) and **4h** (4-Cl) displayed moderate cytotoxicity. This behavior may arise from a balance between increased lipophilicity and steric effects, where the bulky halogen atoms could partially interfere with optimal accommodation inside the active site. On the other hand, compounds containing more polar substituents such as **4e** (4-CN) and **4j** (4-OH) showed comparatively weaker activity, possibly due to reduced hydrophobic interactions and altered cellular permeability. Interestingly, the unsubstituted derivative **4i** was found to be the least active compound ($IC_{50} > 100 \mu M$), suggesting that substitution at the para-position is important for achieving significant anticancer activity in this series. Conclusively, the SAR trend clearly indicates that both electronic and steric factors play a crucial role in determining biological activity. The observed cytotoxicity pattern also correlated well with the molecular docking results, where compounds **4c** and **4g** exhibited the strongest binding affinities toward EGFR. These findings provide useful insight for further structural optimization of imidazole–dihydropyrimidine hybrids as potential anticancer agents (**Figure 4**).

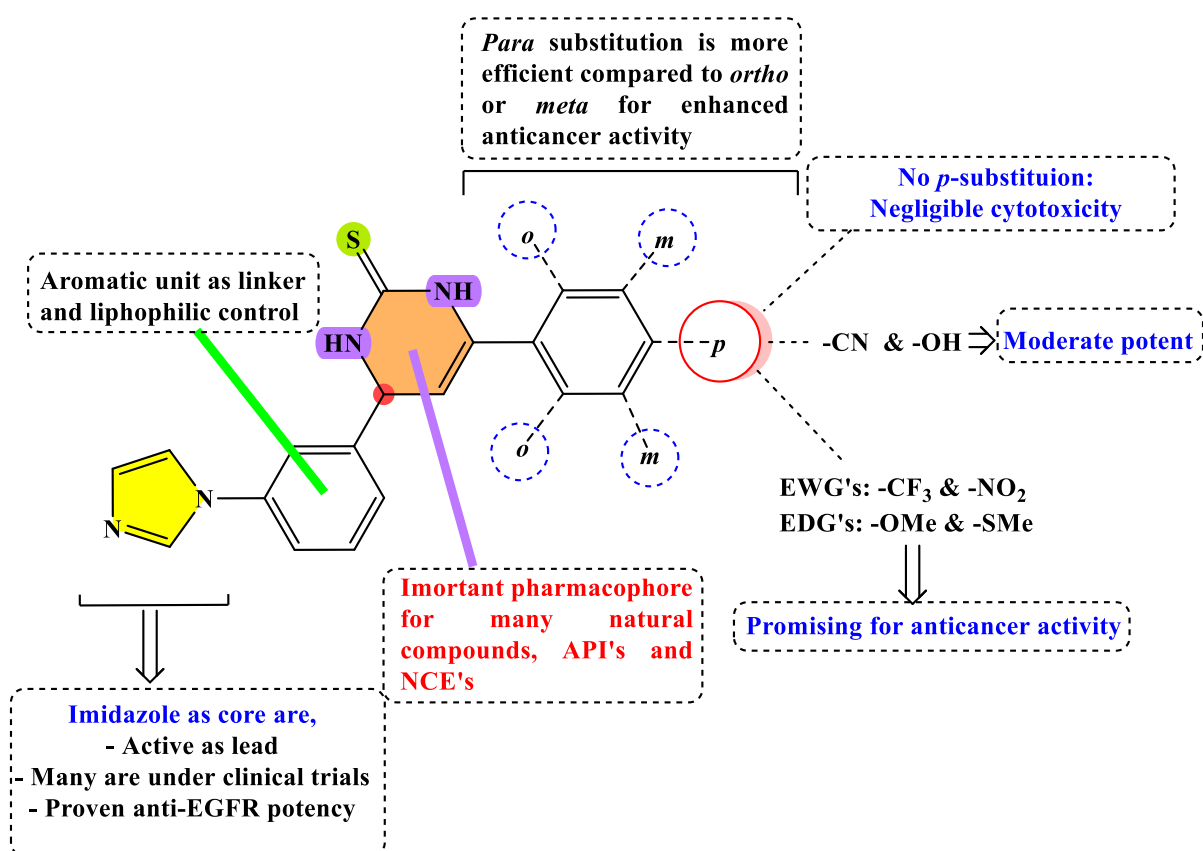


Figure 4. SAR analysis of synthesized compounds.

Molecular Docking Study

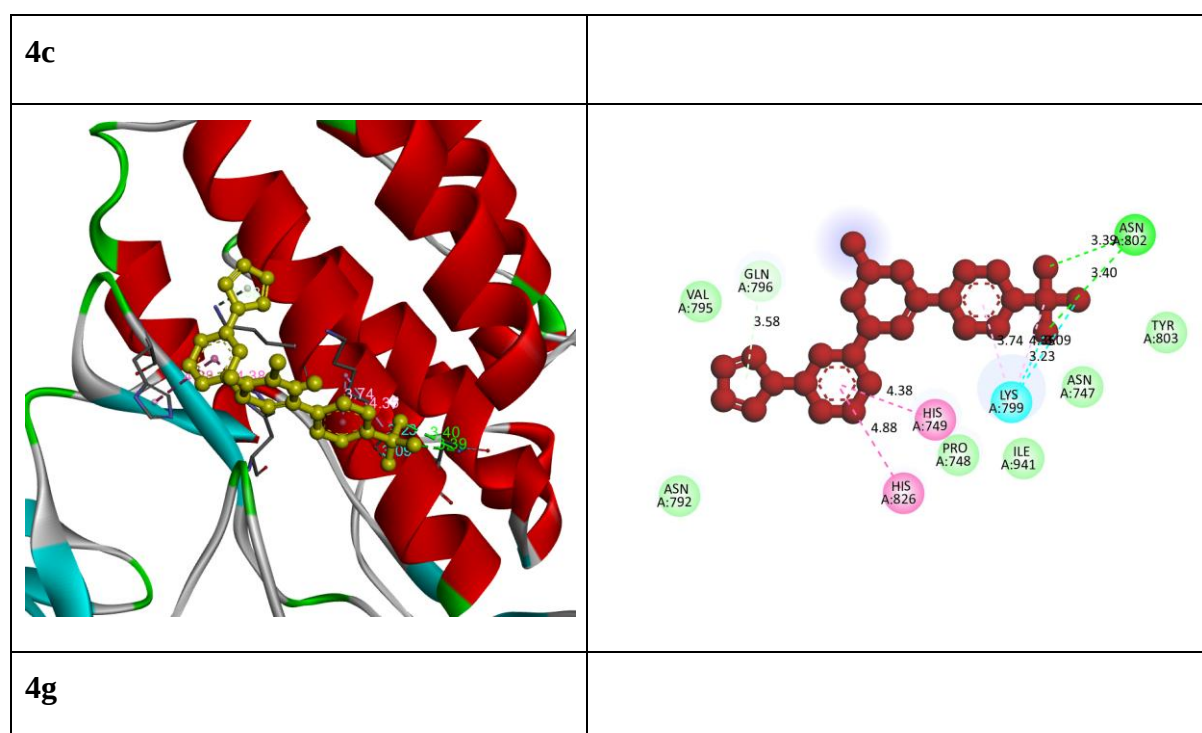
To elucidate the binding affinity and interaction profile of the synthesized compounds (**4a-4j**), molecular docking simulations were performed against the crystal structure of the Epidermal Growth Factor Receptor tyrosine kinase (PDB ID: 1M17), which plays a critical role in breast cancer progression. Sunitinib, a clinically approved tyrosine kinase inhibitor, served as the reference ligand. The binding affinities of the synthesized ligands are summarized in **Table 3**. All compounds exhibited stronger binding than sunitinib, whose docking score was -7.5 kcal/mol. Sunitinib shows higher activity than the synthesized compounds but has a lower docking score. This mismatch between the reference compound's higher experimental activity and its initial docking score was aligned with the previous reports.³²⁻³³ Notably, compound **4c** demonstrated the strongest binding affinity (-18.7 kcal/mol), followed by **4g** (-16.2 kcal/mol), **4b** (-15.3 kcal/mol), and **4f** (-15.2 kcal/mol). These values reflect a superior binding capability compared to the standard drug, suggesting enhanced stability of the ligand-receptor complexes.

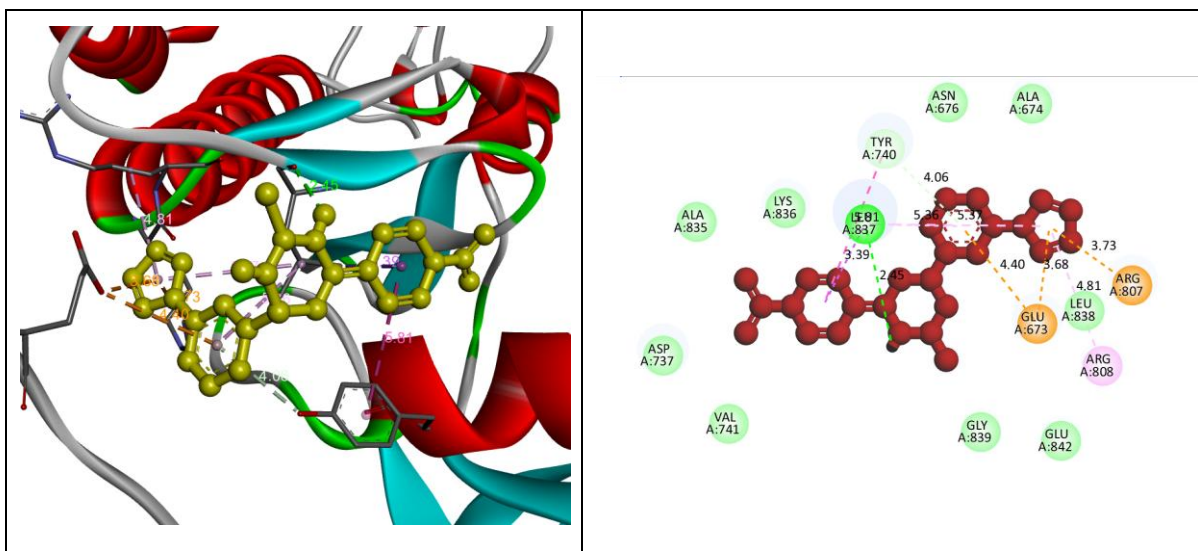
Table 3. Docking score of the synthesized compounds.

Comp. ID	Binding affinity (kcal/mol)
4a	-13.9
4b	-15.3
4c	-18.7
4d	-14.8
4e	-13.2
4f	-15.2
4g	-16.2
4h	-14.9
4i	-14.2
4j	-14.8
Sunitinib	-7.5

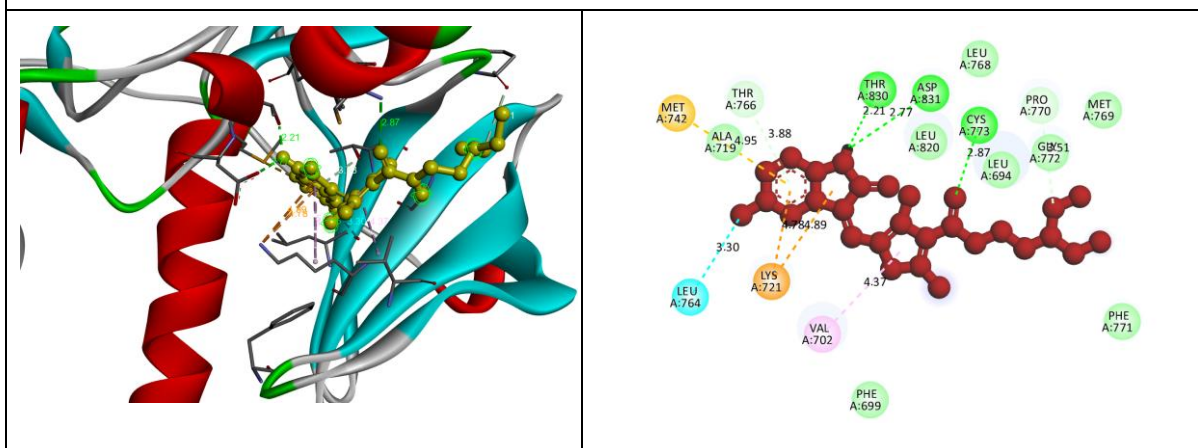
Among the synthesized derivatives, compounds **4c** and **4g** exhibited the most favorable molecular interactions within the ATP-binding site of the EGFR tyrosine kinase domain (PDB ID: 1M17), correlating with their high docking scores of -18.7 and -16.2 kcal/mol, respectively. Compound **4c** was found to form three conventional hydrogen bonds with ASN-747, LYS-799, and ASN-802, which likely play a crucial role in anchoring the ligand within the active site.

Additionally, strong π - π stacking interactions were observed between the aromatic rings of **4c** and the imidazole side chains of HIS-749 and HIS-826, enhancing electronic complementarity. π -alkyl interactions with PRO-748 and ILE-941, along with van der Waals contacts involving GLN-796, VAL-795, and TYR-803, contributed further to the binding stabilization (**Figure 5**). These diverse interactions collectively suggest a robust and specific binding orientation for compound **4c**. Similarly, compound **4g** exhibited a well-packed conformation inside the binding pocket. It engaged in hydrogen bonding with LYS-837 and TYR-740, while also forming a π -cation interaction with ARG-807, which is a favorable electrostatic attraction between the ligand's aromatic system and the positively charged residue. Furthermore, π - π stacking and π -alkyl interactions were established with GLU-673, LEU-838, and ARG-808, suggesting a strong hydrophobic component to the binding. The presence of multiple van der Waals contacts with surrounding residues such as ALA-835, GLY-839, and GLU-842 further reinforced the ligand's fit within the active site cavity (**Figure 5**). Together, these interactions highlight the structural and electronic complementarity of compounds **4c** and **4g** with the kinase active site, supporting their potential as effective tyrosine kinase inhibitors for breast cancer therapy.





Sunitinib



Common docking interactions with Sunitinib (Stick) and compounds **4c** and **4g** (Ball and stick)

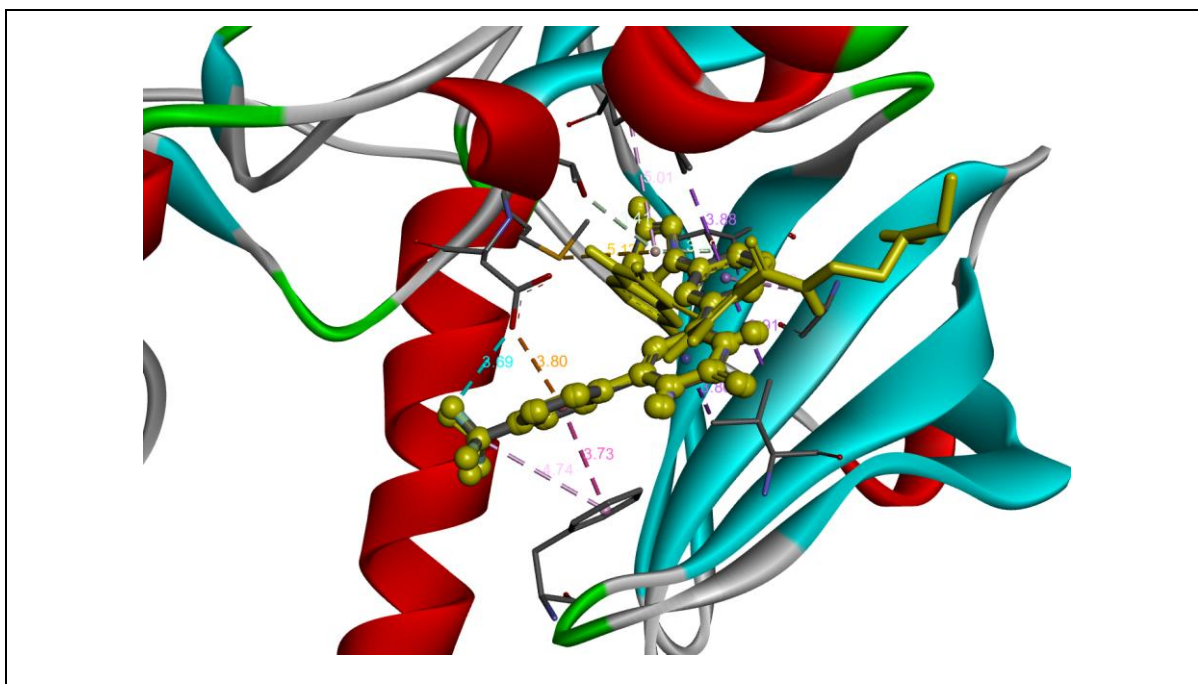


Figure 5. 3D and 2D binding poses of compound **4c**, **4g**, and Sunitinib in the active site of the EGFR domain (PDB ID: 1M17), with a common binding pose with Sunitinib and compounds **4c** and **4g**.

Density Functional Theory (DFT) Analysis of Lead Compounds

To complement the molecular docking results and gain deeper insights into the electronic behavior of the lead molecules, DFT calculations were performed on compounds **4c** and **4g** using the B3LYP/6-31G level of theory. The analysis focused on frontier molecular orbitals (HOMO and LUMO) and the derivation of global reactivity descriptors to assess chemical stability and bioactivity. Compound **4c** exhibited a HOMO energy of -0.1865 eV and a LUMO energy of -0.1444 eV, resulting in a narrow energy gap (ΔE) of 0.0421 eV (**Figure 6**).

4c

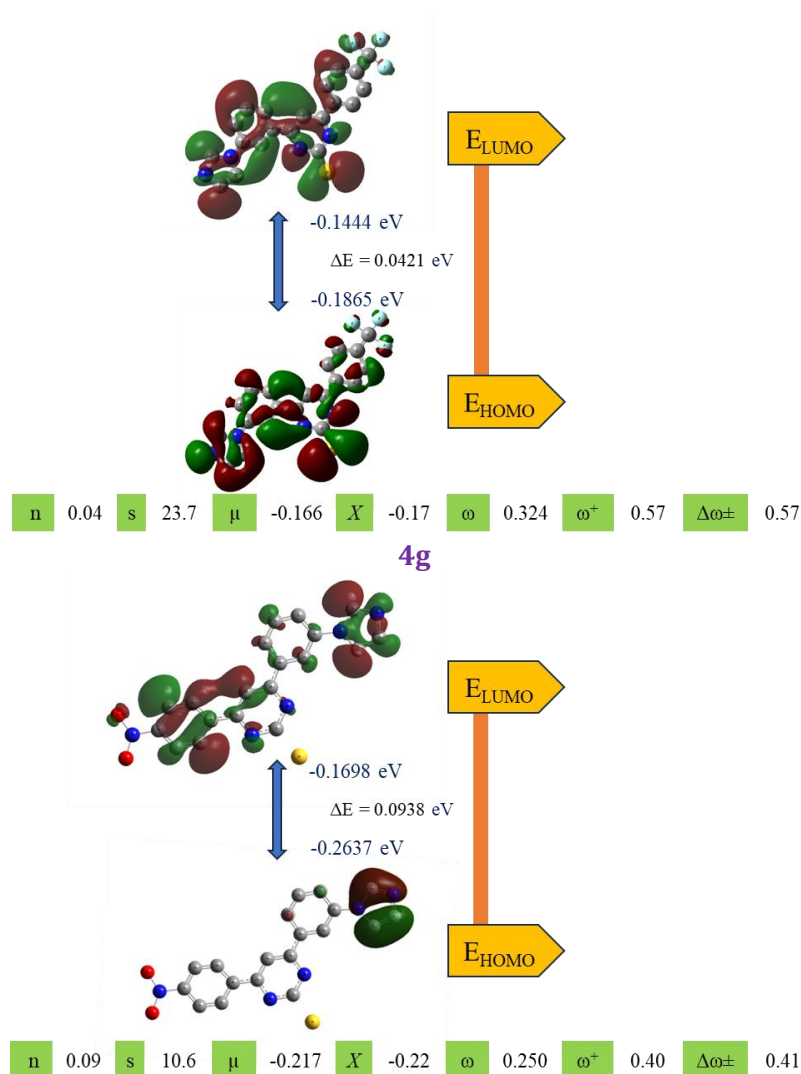


Figure 6. Frontier molecular orbitals (FMOs) and global reactivity parameters of compounds **4c** and **4g**.

This small gap indicates a high degree of chemical softness and enhanced reactivity, which are beneficial for molecular recognition in biological systems. In contrast, compound **4g** presented a HOMO energy of -0.2637 eV and a LUMO energy of -0.1698 eV , with a slightly larger energy gap of 0.0939 eV (**Figure 6**). Although **4g** is somewhat less reactive than **4c**, it still displays a favorable electronic profile for interaction with biological targets. In terms of HOMO–LUMO electron density distributions, compound **4c**'s HOMO was primarily localized on the fused aromatic ring system and nitrogen-containing heterocycles, indicating potential nucleophilic interaction sites, while its LUMO extended over the carbonyl and aryl moieties, suggesting electrophilic susceptibility. Compound **4g** similarly showed HOMO localization on the electron-rich phenyl and amide groups and LUMO delocalization on aromatic substituents, enabling dual reactivity. The global reactivity descriptors further reinforced these findings.

Compound **4c** exhibited a softness (*S*) of 7.57, chemical potential (μ) of -0.166 eV, electronegativity (χ) of 0.166 eV, and electrophilicity index (ω) of 8.17, indicative of high reactivity and balanced nucleophilic/electrophilic behavior. Compound **4g**, while showing slightly lower softness (10.6) and chemical potential (-0.217 eV), also maintained a reasonable electronegativity (0.22 eV) and is predicted to engage favorably in biological interactions. Overall, the DFT analysis of both compounds underscores their electronic compatibility and binding potential, corroborating their strong docking scores. Particularly, the lower HOMO–LUMO gap and higher electrophilicity of compound **4c** highlight its suitability as a lead scaffold for further optimization as a breast cancer therapeutic candidate. Compound **4g** also remains promising due to its complementary reactivity and stable electronic configuration.

ADME and Pharmacokinetic Profiling

To comprehensively evaluate the pharmacokinetic and drug-likeness profiles of the synthesized compounds (**4a–4j**), an *in silico* ADME analysis was conducted using SwissADME. Key parameters such as molecular weight, number of hydrogen bond donors and acceptors, rotatable bonds, bioavailability score, rule-based violations (Lipinski, Ghose, Veber, etc.), PAINS and Brenk alerts, and synthetic accessibility were analyzed alongside radar plots and BOILED-Egg diagrams. All compounds exhibited favorable profiles for key drug-likeness criteria. Importantly, none of the molecules violated Lipinski's Rule of Five, and all passed Ghose, Veber, Egan, and Muegge filters, indicating good oral bioavailability potential and acceptable physicochemical properties for drug development (**Figure 7**). The calculated bioavailability score for all compounds was consistent at 0.55, which is considered satisfactory for oral drugs. Structural evaluations revealed molecular weights ranging from 346.45 to 411.32 Da, with 3-4 rotatable bonds and 1-4 hydrogen bond acceptors, aligning well with desirable drug-like features. The synthetic accessibility scores ranged between 3.67 and 3.85, suggesting moderate ease of synthesis. No PAINS (pan-assay interference compounds) alerts were detected, indicating low risk of non-specific assay interference. However, most compounds showed one Brenk alert, typically associated with reactive or less favorable substructures, which may require minor structural optimization.

The bioavailability radar plots revealed that all compounds occupied a desirable physicochemical space, with balanced lipophilicity, polarity, molecular size, and solubility. These visual tools confirmed that the compounds were well-positioned within the drug-like envelope, with minimal deviation in flexibility or saturation. The BOILED-Egg model further

supported the ADME findings by predicting high gastrointestinal absorption (HIA) for all compounds (**Figure 8**). Compounds **4c** and **4g**, which had already demonstrated excellent binding affinities and electronic characteristics in molecular docking and DFT analyses, also showed promising ADME properties. Compound **4c** exhibited optimal oral absorption and balanced lipophilicity. Similarly, compound **4g** displayed strong oral bioavailability and favourable synthetic accessibility, reinforcing its potential as a viable lead molecule for further preclinical development in breast cancer therapy. All the synthesized compounds belong to toxicity class 3. The BOILED-Egg model, based on their physicochemical characteristics, predicted that the compounds **4c** and **4g** are located near the yolk region, suggesting a probability of crossing the blood–brain barrier (BBB). Although the SwissADME, BOILED-Egg, and ProTox-based analyses provide useful preliminary insight into the drug-likeness and pharmacokinetic behaviour of the synthesized compounds, these predictions should be interpreted with caution. Such *in silico* tools are valuable for early-stage prioritization, but they do not substitute for experimental ADMET evaluation.

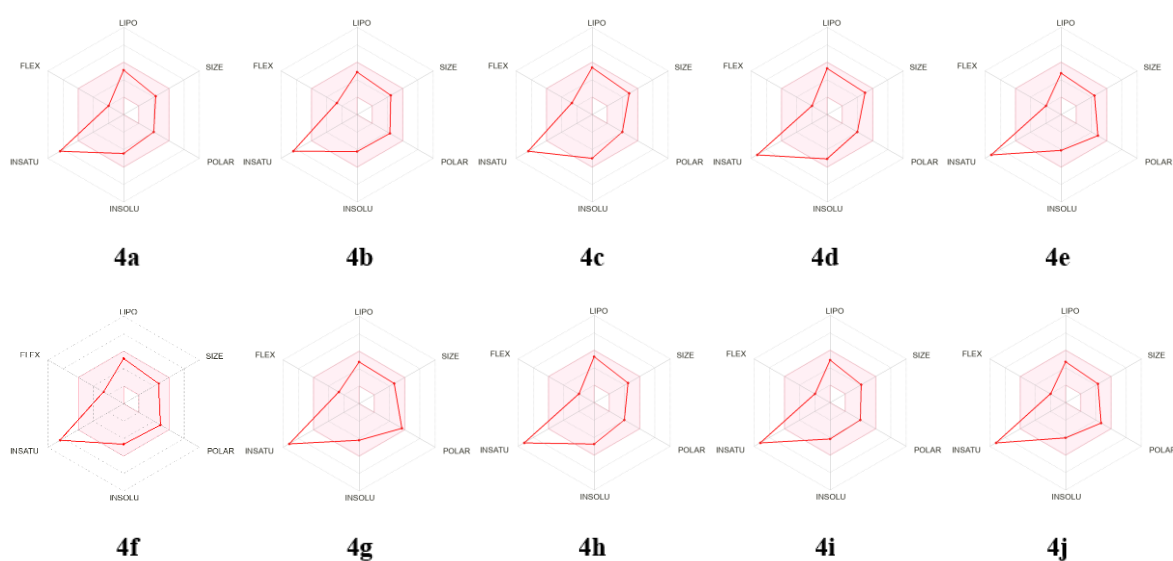


Figure 7. Bioavailability radar plots of compounds **4a-4j**.

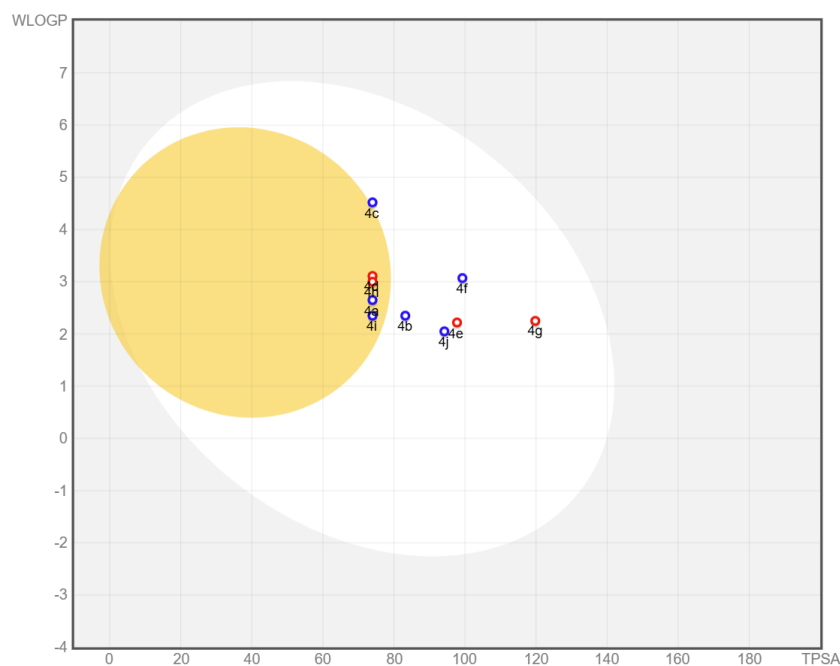


Figure 8. BOILED-Egg plot for gastrointestinal absorption and BBB penetration of compounds **4a-4j**.

Conclusion

In conclusion, the present work has successfully designed, synthesized, and evaluated a novel series of imidazole-linked 3,4-dihydropyrimidine-2(1*H*)-thione hybrids as potential anticancer agents. The synthesis was achieved via a green, one-pot Biginelli protocol with excellent yields and structural diversity. Among the synthesized compounds, **4c** and **4g** emerged as lead candidates, exhibiting significant cytotoxicity against MCF-7 cells, superior EGFR binding affinity, and favorable electronic characteristics as evidenced by molecular docking and DFT studies. Furthermore, both compounds demonstrated promising pharmacokinetic properties with no Lipinski or PAINS violations. The results suggest that compounds **4c** and **4g** represent promising scaffolds for further development in cancer biology.

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Graphical Abstract

