

Synthesis, cytotoxic evaluation, DFT analysis, molecular dynamics simulation and ADME studies of novel 1-(4-(substituted-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-yl)-4-methylpiperazine derivatives

Amol S. Kulal^{a,b}, Shavi Thakur^{a,*}, Ranjitsinh C. Dabhi^b, Vidhi A. Trivedi^c,
Ashish Patel^d, Anjali B. Thakkar^e, Parth Thakor^f, Bhimsen Rout^b, Tushar N. Patel^b,
Nandu S. Khairnar^b, Sumit Mittal^g

^a Shree Maneklal M. Patel Institute of Sciences & Research Sector 15/23, Gandhinagar, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

^b Piramal Pharma Limited, Plot No. 18, Pharmez, Matoda Village, Ahmedabad, Gujarat, India

^c Department of Chemistry, Shree Govind Guru University, Godhra, Gujarat, India

^d Department of Pharmaceutical Chemistry, Parul Institute of Pharmacy, Parul University, Vadodara, Gujarat, India

^e Department of Applied and Interdisciplinary Sciences, Sardar Patel University, Vallabhvidyanagar, Gujarat, India

^f Bapubhai Desaihai Patel Institute of Allied and Healthcare Sciences, Charotar University of Science and Technology, Changa, Gujarat, India

^g School of Advanced Sciences and Languages(SASL), VIT Bhopal University Kothri Kalan, Ashtha, near Indore Road, Bhopal, Madhya Pradesh, India, 466114

ARTICLE INFO

Keywords:

Lung cancer

A-549

MTT assay

Docking

Molecular dynamics

ADME

ABSTRACT

This study presents the rational design, synthesis, and pharmacological evaluation of a novel series of fifteen 1-(4-(substituted)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-yl)-4-methylpiperazine derivatives (**7a-7o**), developed by integrating a trifluoromethylated pyrazole core with a bioreducible arylpiperazine moiety to target non-small cell lung cancer (NSCLC). All compounds were structurally confirmed using advanced spectroscopic techniques, and *in vitro* cytotoxic activity was assessed against the A-549 cell line via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) assay. Among them, 3-methoxy-*N*-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (**7m**) and 4-isopropyl-*N*-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (**7o**) exhibited the most potent anti-proliferative effects ($IC_{50} \approx 69 \mu\text{g/mL}$). Molecular docking and molecular dynamics studies supported these findings, with compound **7m** showing a strong binding affinity (-8.114 kcal/mol) toward the target protein (PDB ID: 1M17) through a key hydrogen bond with CYS773 and extensive hydrophobic interactions, and lower root mean square deviation (RMSD) values, respectively. Absorption, distribution, metabolism, and excretion (ADME) predictions confirmed favorable pharmacokinetic characteristics, including compliance with Lipinski's criteria and 100% predicted oral absorption. Collectively, these results establish the trifluoromethylated pyrazole-piperazine scaffold as a promising lead framework for the development of next-generation NSCLC therapeutics. Density functional theory (DFT) computations were carried out to discover the electrical structure and molecular characteristics of the system. DFT achieves an efficient balance between computing cost and precision by representing the ground-state energy as a functional of electron density.

1. Introduction

Cancer remains a leading cause of mortality worldwide, with the emergence of drug resistance and the heterogeneity of tumor types presenting significant challenges to effective therapy [1–3]. Among the diverse chemotypes explored for anticancer potential, pyrazole-based scaffolds have garnered considerable attention due to their versatile

biological activities [4–6] particularly in kinase inhibition and anti-proliferative assays [7]. The incorporation of trifluoromethyl (-CF₃) substituents on the pyrazole ring has been shown to enhance pharmacokinetic properties such as lipophilicity, membrane permeability, and metabolic stability, rendering these moieties attractive in drug design [8–10].

Similarly, the piperazine nucleus is a privileged structure in

* Corresponding author.

E-mail addresses: kshavirana@gmail.com, shavithakur92@gmail.com (S. Thakur).

<https://doi.org/10.1016/j.molstruc.2026.146668>

Received 28 March 2026; Received in revised form 24 May 2026; Accepted 25 May 2026

Available online 25 May 2026

0022-2860/© 2026 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

medicinal chemistry [11,12], often used to improve water solubility and facilitate interactions with biological targets, as seen in clinically approved agents like Imatinib and Cinnarizine [13,14]. Hybrid molecules that integrate multiple bioactive fragments have emerged as promising anticancer strategies [15–17]. However, reports on frameworks (Fig. 1) that simultaneously feature a trifluoromethyl-substituted pyrazole core, a *p*-tolyl group at N1, and a 4-substituted arylpiperazine unit at C5 are limited, particularly in the context of NSCLC [18–21].

Given the high prevalence of NSCLC and the frequent involvement of EGFR and related kinase pathways in its pathogenesis, novel scaffolds targeting these mechanisms are urgently needed [22,23].

Herein, we report the design, synthesis, and characterization of a new class of 1-(4-(substituted)-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)-4-methylpiperazine derivatives. The synthesized analogs were evaluated for their cytotoxic potential against the A-549 human lung adenocarcinoma cell line, alongside molecular docking studies to assess binding affinity and *in silico* ADME profiling to predict drug-likeness.

2. Experimental

2.1. Materials and methods

Commercial reagent grade chemicals and solvents were procured from Merck, Sigma Aldrich chemical Co., SD fine and other chemical vendors, and were utilized without any further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Spectrochem and SD fine limited INDIA, respectively. The compound was purified by using reverse-phase Combi-Flash chromatography, monitored with UV light detection at 220 nm and 254 nm. Eluents are used (0.1% Formic acid in water: ACN). Several compounds were purified by normal phase Combi-Flash chromatography as well as column chromatography by using glass column with the help of silica gel 60-120 mesh (SDFCL, Baroda, India). The quantity of silica gel used was 50–100 times the product weight charged on the glass column, eluents are used ethyl acetate (EtOAc): *n*-hexane and DCM: Methanol. Thin layer chromatography (TLC) and Column chromatography was run on the silica gel coated aluminium sheets (Kieselgel 60 F₂₅₄, Merck, made in Germany) and silica gel (Flash silica 40-60 µm, Jin Chem, INDIA). Due to all the synthesized compounds containing aromatic rings, they were visualized and detected on TLC plates with UV lights (short wave, long wave or both). ¹H NMR, ¹⁹F NMR and ¹³C NMR spectra were recorded on a Bruker AMX 250 or 400 (400 MHz, FT for ¹H NMR and 100 MHz for ¹³C NMR and 376.94 MHz for ¹⁹F NMR and chemical shifts were calibrated according to TMS (locking the Residual and moisture peak). Chemical shifts (δ) were recorded in ppm and coupling constants (*J*) in hertz (Hz). DMSO-*d*₆ and CDCl₃ were solvents used for to run NMR spectral analysis. Melting point of all compounds were determined using the open capillary tubes method on an electrothermal device (HTLP Digital Melting Point Apparatus), and the results were uncorrected. HPLC analyses were performed using two Shimadzu LC-10AT pumps gradients-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu Class VP program. (HPLC methods either 54, 59 or 64) In case of LC-MS, a sample volume of 10 µL was injected in a Waters X-Terra^R.

2.2. Synthesis

2.2.1. Synthesis of 1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-5-ol (2)

p-Tolylhydrazine (1) (35g, 286mmol, 1eq.) and ethyl-4,4,4-trifluoro-3-oxobutanoate (52.65g, 286mmol, 1eq.) were dissolved in glacial acetic acid (350mL, 10V) at room temperature under nitrogen atmosphere and the reaction mixture was stirred at 120°C for 2 hours. Progress of reaction was monitored by TLC. After completion the reaction mixture was diluted with ice-water and basified with saturated solution of NaHCO₃ and extracted with EtOAc. The organic layer was

washed with brine, dried over sodium sulfate and concentrated under vacuum to afford the crude product which was purified by column chromatography using silica gel (mesh size 60-120) in gradient 5-35% EtOAc in *n*-hexane to get pure compound (2) as a pale white solid.

2.2.2. Synthesis of 5-chloro-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (3)

The POCl₃ (203.84g, 1329mmol, 7eq.) was slowly added into a *N,N*-dimethylformamide (416.61g, 5700mmol, 30eq.) at 0°C under nitrogen atmosphere and stirred reaction mixture at same temperature for 10 mins, followed by 1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-5-ol (2) (46g, 190mmol, 1eq.) was added, reaction mixture was stirred at 120°C for 4 hours. After completion of reaction, the reaction mixture was diluted with ice cold water and basified with saturated solution of NaHCO₃ and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum to afford the crude product which was purified by column chromatography using silica gel (230-400 mesh) in gradient 20-50% EtOAc in *n*-hexane to get pure compound (3) as a pale white solid.

2.2.3. Synthesis of 5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (5)

5-Chloro-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazole-4-carbaldehyde (3) (38g, 131mmol, 1eq.) and *N*-methylpiperazine (4) (14.42g, 144mmol, 1.1eq.) were dissolved in *N,N*-dimethylformamide (380mL, 10V) under nitrogen atmosphere followed by K₂CO₃ (36.20g, 262mmol, 2eq.) was added at 25°C and reaction mixture was stirred at 120°C for 4 hours. After completion the reaction mixture was diluted with ice cold water and extracted with EtOAc (3 times). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum to afford the crude product which was purified by column chromatography using silica gel (230-400 mesh) in gradient 20-60% EtOAc in *n*-hexane to get compound (5) as a pale yellow solid.

2.2.4. General method-A for the synthesis of compounds (7a-7o)

An oven dried pressure glass seal tube was charged with compound 5 (1eq.) and Aromatic amines (1.2eq.) were dissolved in ethanol (10V). The reaction mixture was cooled to 0°C under nitrogen atmosphere and added glacial acetic acid (1eq.) stirred for 45 mins followed by NaCNBH₃ (2eq.) was added, stirred reaction mixture at 25°C for 16 hours. Progress of reaction was checked by TLC. Upon completion the reaction, the mixture was diluted with ice cold-water and extracted with EtOAc (3 times). The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum to afford the crude product which was further purified by column chromatography using silica gel (320-400 mesh size) in gradient 40-90% EtOAc in *n*-hexane to obtain pure desired compounds (7a-7o) as mentioned in the Scheme 1. The synthetic route and chemical structures of compounds (7a-7o) are shown in Scheme 1.

The formation of the intermediate 2 occurs through multistep sequence mechanism as depicted in Scheme 2. In the initial stages (Steps A and B), the β-diketone (a) undergoes condensation with *p*-tolylhydrazine (1). The terminal, highly nucleophilic nitrogen atom of *p*-tolylhydrazine attacks the carbonyl carbon of the β-diketone, generating a carbinolamine intermediate (b). This intermediate promptly undergoes dehydration, eliminating a molecule of water to afford the acyclic hydrazone intermediate (c). Subsequently, in Step C, the reaction proceeds via intramolecular cyclization. Here, the nitrogen atom adjacent to the aryl group acts as a nucleophile, attacking the remaining electrophilic carbonyl carbon within the same molecule. This nucleophilic addition-elimination mechanism results in ring closure, forming a five-membered pyrazoline intermediate. Finally Step D and E, involves a series of proton transfer and tautomerization processes that drive the system toward its most stable form. Intramolecular proton shifts stabilize the cyclic intermediate, followed by a crucial tautomeric transformation that reorganizes the π-electron system to yield the compound

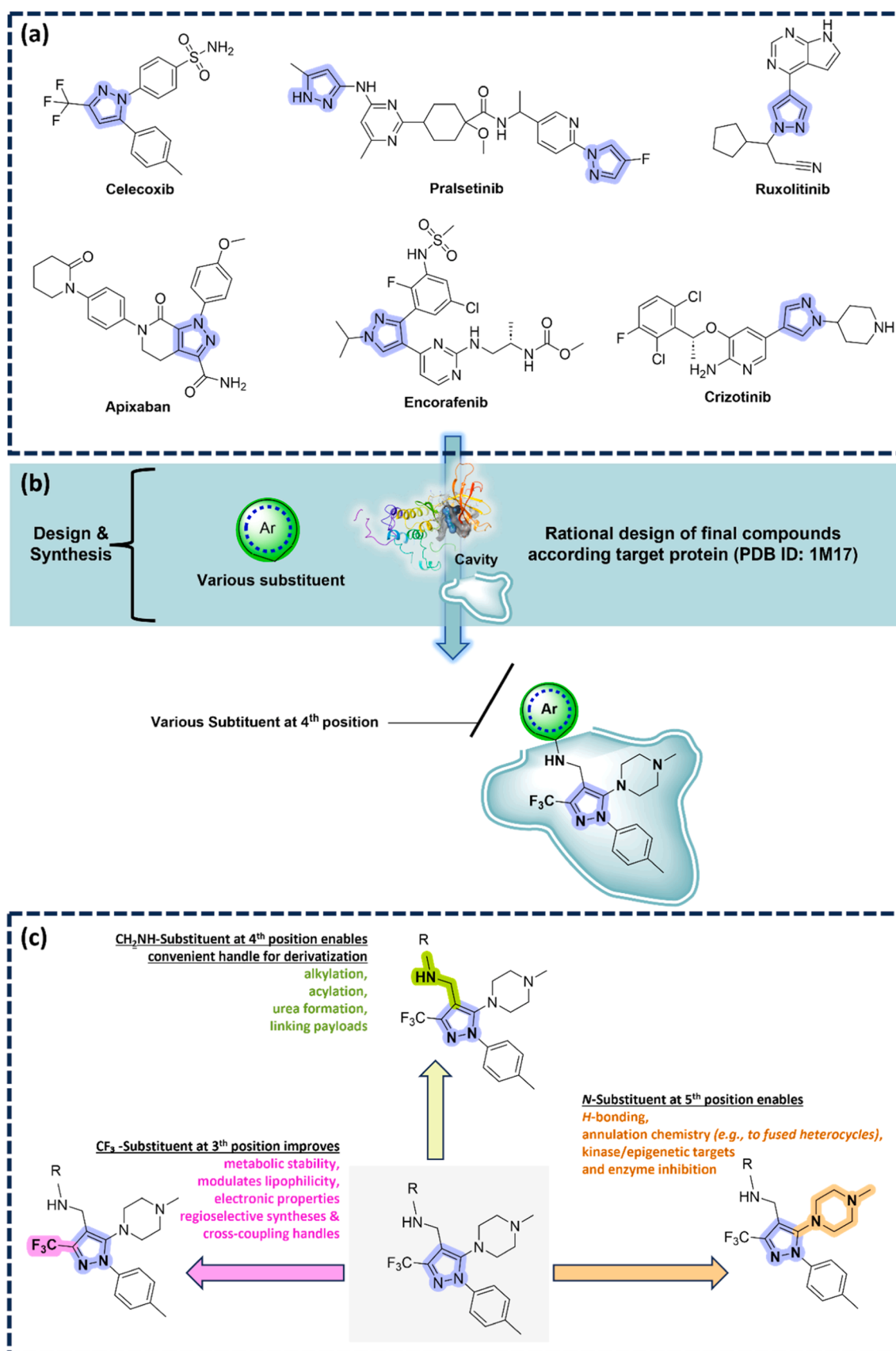


Fig. 1. (a) Chemical structures of pyrazole-based marketed drug molecules, (b) Rational design of synthesized target molecules, (c) Strategic substitution pattern enabling derivatization, metabolic stability, and target engagement.

2. The resulting product is thermodynamically favoured due to extensive conjugation within the pyrazole ring, accounting for its exclusive formation in the reaction.

The ^{13}C NMR, ^1H NMR, IR, LCMS and HPLC spectral data of representative compounds were found to be consistent with the proposed structure.

2.2.5. Synthesis of 1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-ol (2)

Pale white solid. Yield: 74%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.32 (s, 1H), 7.59 (d, J = 8 Hz, 2H), 7.31 (d, J = 8 Hz, 2H), 5.92 (s, 1H), 2.35 (s, 3H). ^{19}F NMR (377 MHz, $\text{DMSO}-d_6$) δ -61.36 (s, 3F). **MS** (m/z): 243.09 [$\text{M} + \text{H}$] $^+$ Exact mass 242.20. **LC-MS**: t_R 1.73 min; (m/z): 243.10 [$\text{M} + \text{H}$] $^+$ purity 99%; Exact Mass: 242.20. **HPLC**: t_R 3.99 min, purity 99%.

2.2.6. Synthesis of 5-chloro-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carbaldehyde (3)

Pale white solid. Yield: 70%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.94 (s, 1H) (aldehyde proton), 7.57 (d, J = 8 Hz, 2H), 7.45 (d, J = 8 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 182.27, 141.22, 140.94-140.04 (q, J_{CF} = 39 Hz), 135.53, 133.87, 130.47, 126.27, 124.60-116.55 (q, J_{CF} = 268.2 Hz), 20.23. **LC-MS**: t_R 1.93 min; (m/z): 289.2/291.1 [$\text{M} + \text{H}$] $^+$ purity 97%; Exact Mass: 288.65. **HPLC**: t_R 5.59 min, purity 99%.

2.2.7. Synthesis of 5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carbaldehyde (5)

Pale yellow solid. Yield: 44%. M.P.: 150-152°C. **FTIR** (KBr, cm^{-1}): 3035 (-C-H, aromatic), 2940 (-C-H, aliphatic), 2750 (-C-H, aldehyde), 1710 (-C=O, aldehyde), 1593 (-C=C- & -C=N-), 1330 (-C-F, trifluoromethyl group), 830 (-C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.94 (s, 1H), 7.50 (d, J = 8 Hz, 2H), 7.39 (d, J = 8 Hz, 2H), 3.11 (t, J = 4.8 Hz, 4H), 2.40 (s, 3H), 2.28 (t, J = 5.0 Hz, 4H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 181.45, 153.55, 140.67-139.54 (q, J_{CF} = 37.6 Hz), 139.029, 135.74, 129.82, 125.259, 124.67-116.63 (q, J_{CF} = 268 Hz), 110.84, 54.17, 50.025, 45.73, 20.71. ^{19}F NMR (377 MHz, $\text{DMSO}-d_6$) δ -61.36 (s, 3F). **MS** (m/z): 353.24 [$\text{M} + \text{H}$] $^+$ Exact Mass: 352.36. **LC-MS**: t_R 1.43 min; (m/z): 353.6/354.2 [$\text{M} + \text{H}$] $^+$ purity 98%; Exact Mass: 352.36. **HPLC**: t_R 3.99 min, purity 99%.

2.2.8. Synthesis of 4-methyl-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl) methyl) aniline (7a)

General method-A, White solid. Yield: 83%. M.P.: 104-106°C. **FTIR** (KBr, cm^{-1}): 3035 (-C-H, aromatic), 2940 (-C-H, aliphatic), 2750 (-C-H, aldehyde), 1710 (-C=O, aldehyde), 1593 (-C=C- & -C=N-), 1330 (-C-F, trifluoromethyl group), 830 (-C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.54-7.48 (m, 2H), 7.36 (d, J = 8.1 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2H), 6.58 (d, J = 8.2 Hz, 2H), 5.50 (d, J = 4.6 Hz, 1H), 4.07 (d, J = 4.6 Hz, 2H), 3.00 (t, J = 4.8 Hz, 4H), 2.39 (s, 3H), 2.22 (d, J = 5.5 Hz, 4H), 2.17 (s, 3H), 2.10 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 5.50 (t, J = 4.6 Hz, 1H) (exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 150.10, 146.74, 140.19-139.12 (q, J_{CF} = 35.4 Hz), 138.44, 136.64, 130.01, 129.75, 126.18-118.51 (q, J_{CF} = 268.54 Hz), 124.90, 112.64, 109.82, 55.08, 49.82, 46.25, 36.68, 21.14, 20.57. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ - 60.05 (s, 3F). **MS** (m/z): 444.44 [$\text{M} + \text{H}$] $^+$ Exact Mass: 443.52. **LC-MS**: t_R 1.65 min; (m/z): 444.30 [$\text{M} + \text{H}$] $^+$ purity 99%; Exact Mass: 443.52. **HPLC**: t_R 6.07 min, purity 99%.

2.2.9. Synthesis of 3-methyl-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (7b)

General method-A, Cream solid. Yield: 80%. M.P.: 123°C -125°C. **FTIR** (KBr, cm^{-1}): 3035 (-C-H, aromatic), 2945 (-C-H, aliphatic), 1595 (-C=C- & -C=N-), 1330 (-C-F, trifluoromethyl group), 830 (-C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.56-7.48 (m, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.03-6.95 (m, 1H), 6.47 (d, J = 6.7 Hz, 2H), 6.41 (d, J = 7.4 Hz, 1H), 5.65 (d, J = 4.5 Hz, 1H), 4.08 (d, J = 4.5 Hz, 2H), 3.01 (t, J = 4.8 Hz, 4H), 2.40 (s, 3H), 2.22 (d, J = 5.5 Hz, 4H), 2.20 (s, 3H),

2.10 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 5.65 (t, J = 4.5 Hz, 1H) (Exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 150.14, 148.94, 140.19-139.12 (q, J_{CF} = 35 Hz), 138.44, 136.64, 130.01, 129.18, 126.18-118.51 (q, J_{CF} = 268.54 Hz), 124.96, 117.49, 113.05, 109.94, 55.10, 49.86, 46.26, 36.46, 21.85, 21.14. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ - 60.06 (s, 3F). **MS** (m/z): 444.44 [$\text{M} + \text{H}$] $^+$ Exact Mass: 443.52. **LC-MS**: t_R 1.63 min; (m/z): 444.30 [$\text{M} + \text{H}$] $^+$ purity 99%; Exact Mass: 443.52. **HPLC**: t_R 6.18 min, purity 99%.

2.2.10. Synthesis of 4-fluoro-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (7c)

General method-A, Pale violet solid. Yield: 79%. M.P.: 114°C-116°C. **FTIR** (KBr, cm^{-1}): 3035 (-C-H, aromatic), 2945 (-C-H, aliphatic), 1590 (-C=C- & -C=N-), 1335 (-C-F, trifluoromethyl group), 1180 (C-F stretches), 830 (-C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.53-7.48 (m, 2H), 7.37 (d, J = 8.3 Hz, 2H), 6.98-6.92 (m, 2H), 6.71-6.63 (m, 2H), 5.70 (t, J = 4.6 Hz, 1H), 4.07 (d, J = 4.6 Hz, 2H), 3.01 (t, J = 4.8 Hz, 4H), 2.40 (s, 3H), 2.23 (d, J = 5.2 Hz, 4H), 2.11 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 5.70 (t, J = 4.6 Hz, 1H) (Exchanged), ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 156.13, 153.84, 150.14, 145.70, 140.19-139.12 (q, J_{CF} = 35 Hz), 138.44, 136.64, 130.01, 126.18-118.51 (q, J_{CF} = 268.54 Hz), 124.96, 120.82, 115.80, 113.29, 109.58, 55.08, 49.86, 46.24, 36.99, 21.14. ^{19}F NMR (376.65 MHz, $\text{DMSO}-d_6$) δ : -60.07 (s, 3F), -129.34 (s, 1F). **MS** (m/z): 448.42 [$\text{M} + \text{H}$] $^+$ Exact Mass: 447.48. **LC-MS**: t_R 1.59 min; (m/z): 448.30 [$\text{M} + \text{H}$] $^+$ purity 96%; Exact Mass: 447.48. **HPLC**: t_R 6.13 min, purity 94%.

2.2.11. Synthesis of 4-chloro-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (7d)

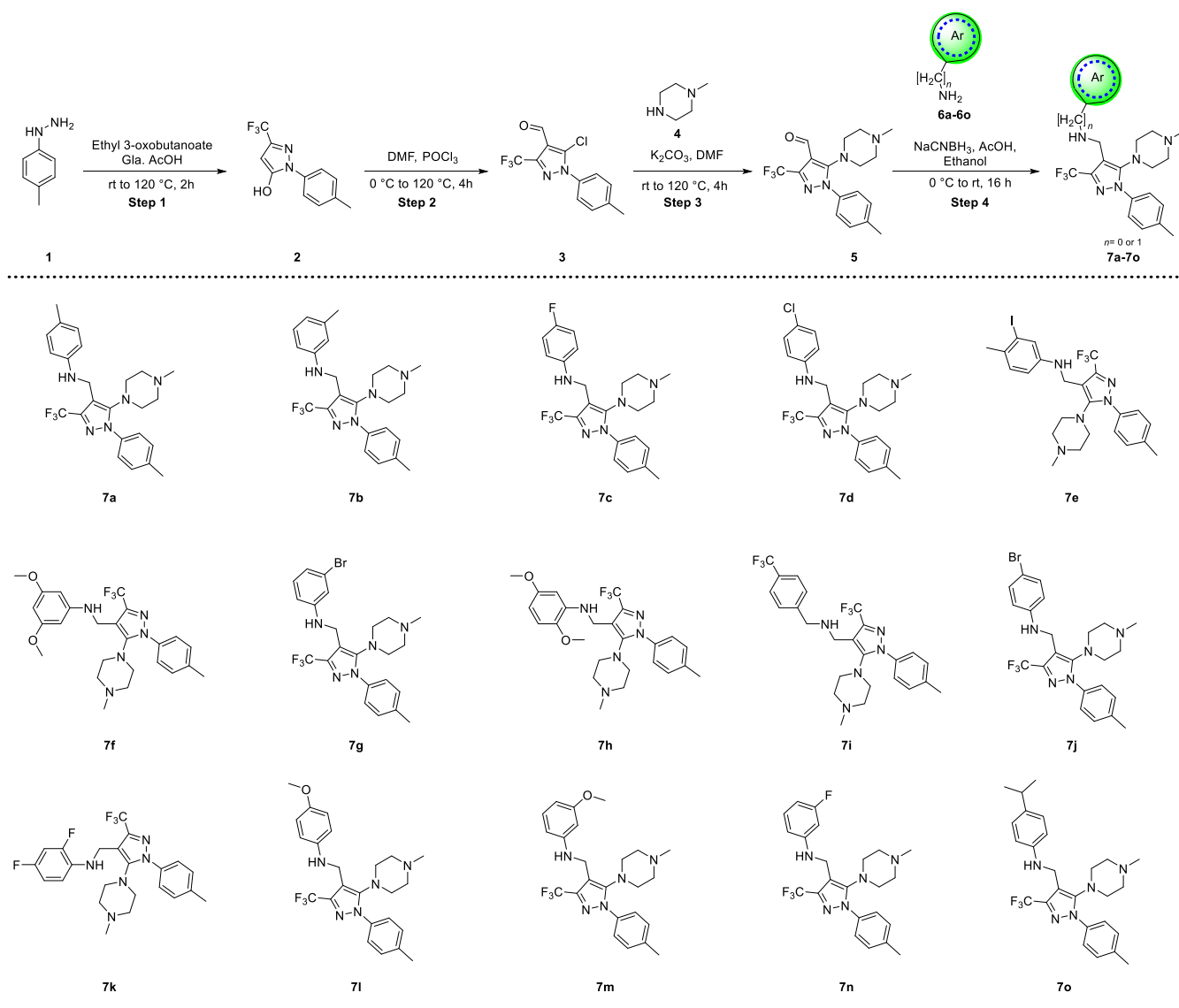
General method-A, White solid. Yield: 82%. M.P.: 140°C-142°C. **FTIR** (KBr, cm^{-1}): 3035 (-C-H, aromatic), 2940 (-C-H, aliphatic), 1590 (-C=C- & -C=N-), 1335 (-C-F, trifluoromethyl group), 700 (aromatic C-Cl, stretches), 830 (-C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.54-7.48 (m, 2H), 7.37 (d, J = 8.2 Hz, 2H), 7.16-7.09 (m, 2H), 6.71-6.63 (m, 2H), 6.01 (t, J = 4.5 Hz, 1H), 4.08 (d, J = 4.4 Hz, 2H), 2.99 (t, J = 4.8 Hz, 4H), 2.40 (s, 3H), 2.23 (t, J = 4.9 Hz, 4H), 2.10 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 6.01 (t, J = 4.5 Hz, 1H), (Exchanged), ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 150.15, 147.77, 140.19-139.12 (q, J_{CF} = 35 Hz), 138.54, 136.63, 130.04, 129.04, 124.99-119.80 (q, J_{CF} = 268.54 Hz), 123.47, 113.82, 109.28, 55.07, 49.88, 46.23, 36.48, 21.15. ^{19}F NMR (376.65 MHz, $\text{DMSO}-d_6$) δ : -60.09 (s, 3F). **MS** (m/z): 464.27 [$\text{M} + \text{H}$] $^+$ Exact Mass: 463.93. **LC-MS**: t_R 3.19 min; (m/z): 464.5 [$\text{M} + \text{H}$] $^+$ purity 99%; Exact Mass: 463.93. **HPLC**: t_R 4.88 min, purity 99%.

2.2.12. Synthesis of 3-iodo-4-methyl-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (7e)

General method-A, White solid. Yield: 75%. M.P.: 125°C-127°C. **FTIR** (KBr, cm^{-1}): 3035 C-H, aromatic), 2945 (-C-H, aliphatic), 1590 (-C=C- & -C=N-), 1330 (-C-F, trifluoromethyl group), 830 (-C-H, methyl *p*-substituted), 550 (aromatic C-Cl, stretches). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.50 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 7.9 Hz, 2H), 7.12 (s, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 5.83 (s, 1H), 4.06 (d, J = 4.5 Hz, 2H), 3.00 (t, J = 4.9 Hz, 4H), 2.40 (s, 3H), 2.30-2.19 (m, 7H), 2.11 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 5.83 (s, 1H) (Exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 150.14, 148.11, 140.19-139.12 (q, J_{CF} = 35 Hz), 138.53, 136.65, 130.31, 130.04, 127.68, 125.00, 123.47-119.80 (q, J_{CF} = 268.54 Hz), 112.84, 109.39, 102.31, 57.69, 55.111, 49.89, 46.26, 26.70, 21.15. ^{19}F NMR (376.65 MHz, $\text{DMSO}-d_6$) δ : -60.08 (s, 3F). **MS** (m/z): 570.29 [$\text{M} + \text{H}$] $^+$ Exact Mass: 569.41. **LC-MS**: t_R 1.71 min; (m/z): 570.3 [$\text{M} + \text{H}$] $^+$ purity 95%; Exact Mass: 569.27. **HPLC**: t_R 5.14 min, purity 96%.

2.2.13. Synthesis of 3,5-dimethoxy-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-4-yl)methyl)aniline (7f)

General method-A, Pale brown solid, Yield: 81%. M.P.: 145°C



-147°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1590 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1200 (aryl -OCH₃), 830 (C-H, methyl *p*-substituted). **¹H NMR** (400 MHz, DMSO-*d*₆) δ : 7.51 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 5.86 (d, *J* = 2.1 Hz, 2H), 5.81–5.74 (m, 2H), 4.08 (d, *J* = 4.3 Hz, 2H), 3.67 (s, 6H), 3.01 (t, *J* = 4.7 Hz, 4H), 2.40 (s, 3H), 2.24 (t, *J* = 4.6 Hz, 4H), 2.11 (s, 3H). **D₂O exchange**: **¹H NMR** (101 MHz, DMSO-*d*₆) δ : 5.77 (s, 1H) (Exchanged). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ : 161.63, 150.67, 150.13, 140.19–139.12 (q, *J*_{CF} = 35 Hz), 138.44, 136.63, 130.00, 124.99–119.80 (q, *J*_{CF} = 268.54 Hz), 124.91, 109.44, 91.36, 89.19, 55.20, 55.06, 49.82, 46.24, 36.44, 21.12. **¹⁹F NMR** (376.49 MHz, DMSO-*d*₆) δ : -60.04 (s, 3F). **MS (*m/z*)**: 490.41 [M + H]⁺ Exact Mass: 489.5/4491.3. **LC-MS**: *t*_R 1.55 min; (*m/z*): 490.3 [M + H]⁺ purity 98%; Exact Mass: 489.54. **HPLC**: *t*_R 4.58 min, purity 97%.

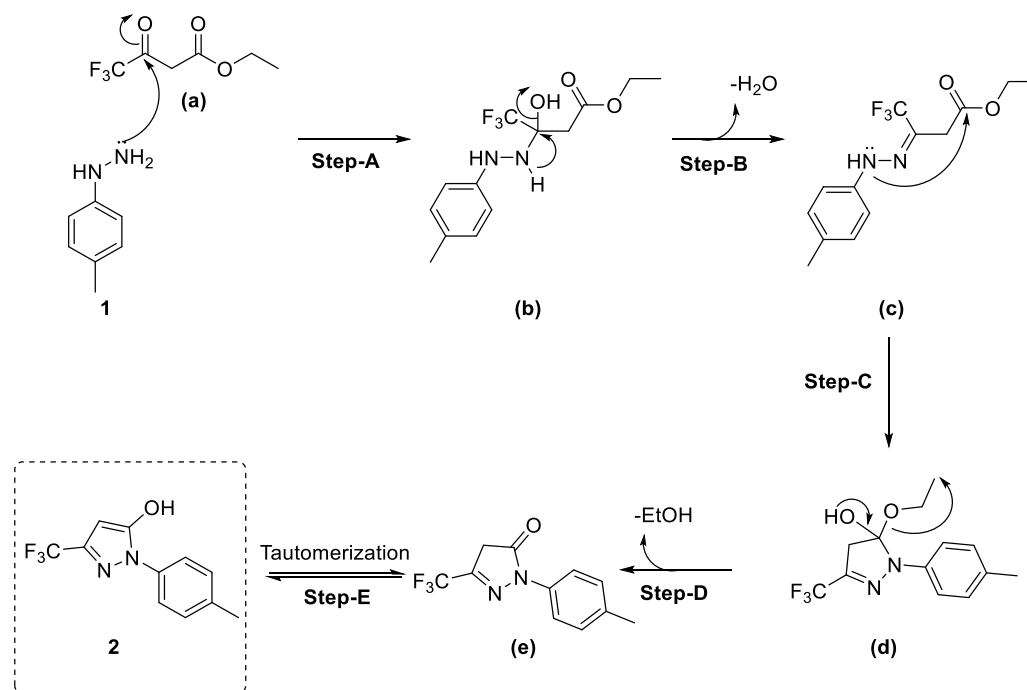
2.2.14. Synthesis of 3-bromo-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7g)

General method-A, Pale brown solid, Yield: 80%. M.P.: 123°C–125°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1590 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 830 (C-H, methyl *p*-substituted), 650 (Aryl -C-Br). **¹H NMR** (400 MHz, DMSO-*d*₆) δ : 7.50 (d, *J* = 8.4 Hz, 2H), 7.38 (s, 2H), 7.04 (t, *J* = 8.0 Hz, 1H), 6.81 (t, *J* = 2.0

Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.69–6.63 (m, 1H), 6.15 (d, *J* = 4.6 Hz, 1H), 4.10 (d, *J* = 4.3 Hz, 2H), 3.00 (t, *J* = 4.7 Hz, 4H), 2.40 (s, 3H), 2.25 (t, *J* = 5.5 Hz, 4H), 2.11 (s, 3H). **D₂O exchange**: **¹H NMR** (400 MHz, DMSO-*d*₆) δ : 6.15 (d, *J* = 4.6 Hz, 1H) (exchanged). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ : 150.40, 150.17, 140.19–139.12 (q, *J*_{CF} = 35 Hz), 138.58, 136.61, 131.18, 130.05, 125.00, 124.99–119.80 (q, *J*_{CF} = 268.54 Hz), 118.74, 114.38, 111.554, 109.085, 55.08, 49.90, 46.25, 36.19, 21.15. **¹⁹F NMR** (376.49 MHz, DMSO-*d*₆) δ : -60.13 (s, 3F). **MS (*m/z*)**: 508.38/510.4 [M + H]⁺ Exact Mass: 508.39. **LC-MS**: *t*_R 1.63 min; (*m/z*): 508.38/510.44 (fragment: 337.2/338.2) [M + H]⁺ purity 94%; Exact Mass: 508.39. **HPLC**: *t*_R 4.84 min, purity 94%.

2.2.15. Synthesis of 2,5-dimethoxy-N-((5-(4-methylpiperazin-1-yl)-1-(*p*-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7h)

General method-A, Pale brown solid. Yield: 84%. M.P.: 96°C–98°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1590 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1240 (C-OCH₃), 830 (C-H, methyl *p*-substituted). **¹H NMR** (400 MHz, DMSO-*d*₆) δ : 7.51 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 1H), 6.30 (d, *J* = 2.8 Hz, 1H), 6.16 (dd, *J* = 8.6, 2.8 Hz, 1H), 4.62 (t, *J* = 5.0 Hz, 1H), 4.16 (d, *J* = 5.0 Hz, 2H), 3.71 (s, 3H), 3.68 (s, 3H), 2.98 (t, *J* = 4.8 Hz, 4H), 2.40 (s, 3H), 2.25 (t, *J* = 5.5 Hz, 4H), 2.11 (s, 3H). **D₂O**



Scheme 2. Plausible mechanism for formation of compound 2.

exchange: ^1H NMR (101 MHz, $\text{DMSO}-d_6$) δ : 4.62 (t, $J = 5.0$ Hz, 1H) (exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 154.38, 149.58, 14.03, 140.19–139.12 (q, $J_{\text{CF}} = 35$ Hz), 138.13, 136.20, 129.44, 124.99–119.80 (q, $J_{\text{CF}} = 268.54$ Hz), 124.90, 110.50, 108.91, 98.95, 97.83, 55.98, 55.07, 55.57, 49.41, 36.04, 20.64. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ : -60.13 (s, 3F). **MS** (m/z): 490.42 $[\text{M} + \text{H}]^+$ Exact Mass: 489.54. **LC-MS:** t_{R} 1.54 min; (m/z): 490.3/491.3 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 489.54. **HPLC:** t_{R} 4.60 min, purity 99%.

2.2.16. Synthesis of 1-(5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)-N-(4-(trifluoromethyl)benzyl) methanamine (7i)

General method-A, Pale brown solid. Yield: 72%. M.P.: 85–88°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1400 (alkane, bending), 1330 (C-F, trifluoromethyl group), 1240 (C-OCH₃), 830 (C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.69 (d, $J = 7.9$ Hz, 2H), 7.60 (d, $J = 8.0$ Hz, 2H), 7.52–7.44 (m, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 3.85 (s, 2H), 3.63 (s, 2H), 3.06 (t, $J = 4.8$ Hz, 4H), 2.48 (s, 6H), 2.38 (t, $J = 5.5$ Hz, 4H), 1.91 (d, $J = 2.3$ Hz, 1H). **D₂O exchange:** ^1H NMR (101 MHz, $\text{DMSO}-d_6$) δ : (not to exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 171.96, 158.23, 157.93, 148.83, 145.09, 139.66–138.60 (q, $J_{\text{CF}} = 35$ Hz), 138.03, 136.12, 129.52, 128.73, 128.43–126.93 (q, $J_{\text{CF}} = 32$ Hz), 129.04, 124.99–119.80 (m, $J_{\text{CF}} = 268.54$ Hz), 110.62, 53.92, 52.08, 48.29, 44.43, 40.33, 21.00, 20.63. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ : -59.89 (s, 3F), -60.75 (s, 3F). **MS** (m/z): 512.38 $[\text{M} + \text{H}]^+$ Exact Mass: 511.52. **LC-MS:** t_{R} 2.91 min; (m/z): 512.4/ 513.5 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 511.52. **HPLC:** t_{R} 3.76 min, purity 97%.

2.2.17. Synthesis of 4-bromo-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7j)

General method-A, Pale brown solid. Yield: 78%. M.P.: 95°C–98°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 830 (C-H, methyl *p*-substituted), 650 (Aryl -C-Br). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.50 (d, $J = 8.1$ Hz, 2H), 7.37 (d, $J = 8.0$ Hz, 2H), 7.27–7.20 (m, 2H), 6.63 (d, $J = 8.6$ Hz, 2H), 6.03 (d, $J = 4.7$ Hz, 1H), 4.08 (d, $J = 4.4$ Hz, 2H), 2.99 (t, $J = 4.8$ Hz, 4H), 2.40 (s, 3H), 2.23 (d, $J = 5.6$ Hz, 4H), 2.10 (s, 3H).

D₂O exchange: ^1H NMR (101 MHz, $\text{DMSO}-d_6$) δ : 6.03 (d, $J = 4.6$ Hz, 1H) (Exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 149.64, 147.58, 139.66–138.60 (q, $J_{\text{CF}} = 35$ Hz), 138.03, 136.12, 131.33, 129.53, 128.43–126.93 (q, $J_{\text{CF}} = 32$ Hz), 113.89, 108.72, 106.66, 54.56, 49.38, 45.74, 235.87, 20.64. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ : -60.11 (s, 3F). **MS** (m/z): 510.35 $[\text{M} + \text{H}]^+$ Exact Mass: 508.39. **LC-MS:** t_{R} 1.69 min; (m/z): 508.38/510.44 (fragment: 337.2/338.2) $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 508.39. **HPLC:** t_{R} 6.46 min, purity 97%.

2.2.18. Synthesis of 2,4-difluoro-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7k)

General method-A, White solid. Yield: 84%. M.P.: 90°C–92°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 830 (C-H, methyl *p*-substituted), 800 (Aryl -C-F, 2,4-difluorine). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.49 (d, $J = 8.0$ Hz, 2H), 7.35 (d, $J = 7.9$ Hz, 2H), 7.09 (d, $J = 9.4$ Hz, 1H), 6.89 (d, $J = 9.0$ Hz, 1H), 6.83 (d, $J = 9.4$ Hz, 1H), 5.33 (s, 1H), 4.16 (d, $J = 4.7$ Hz, 2H), 2.99 (t, $J = 4.7$ Hz, 4H), 2.39 (s, 3H), 2.23 (d, $J = 5.6$ Hz, 4H), 2.12 (s, 3H). **D₂O exchange:** ^1H NMR (101 MHz, $\text{DMSO}-d_6$) δ : 5.33 (s, 1H) (exchanged). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ : 154.45–154.34 (d, $J_{\text{CF}} = 11.1$ Hz), 152.12–152.01 (d, $J_{\text{CF}} = 11$ Hz), 149.39, 148.96–148.84 (d, $J_{\text{CF}} = 11.6$ Hz), 39.66–138.60 (q, $J_{\text{CF}} = 35$ Hz), 138.07, 136.18, 133.26–133.19 (d, $J_{\text{CF}} = 10$ Hz), 129.50, 128.43–126.93 (q, $J_{\text{CF}} = 32$ Hz), 122.98, 112.23, 110.74–110.54 (d, $J_{\text{CF}} = 20$ Hz), 108.93, 103.69–103.19 (t, $J_{\text{CF}} = 27$ Hz), 54.39, 49.09, 45.33, 36.39, 28.94, 20.65. ^{19}F NMR (376.94 MHz, $\text{DMSO}-d_6$) δ : -60.14 (s, 3F), -126.61 (s, 1F), -129.18 (s, 1F). **MS** (m/z): 466.35/ 467.34 $[\text{M} + \text{H}]^+$ Exact Mass: 465.47. **LC-MS:** t_{R} 1.66 min; (m/z): 466.3 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 465.37. **HPLC:** t_{R} 4.70 min, purity 99%.

2.2.19. 4-Methoxy-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7l)

General method-A, White solid. Yield: 74%. M.P.: 115°C–117°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1240 (C-OCH₃), 830 (C-H, methyl *p*-substituted). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.51 (d, $J = 7.9$ Hz, 2H), 7.37 (d, $J = 8.0$ Hz, 2H), 6.75 (d, $J = 8.8$ Hz, 2H), 6.63 (d, $J = 8.9$ Hz, 2H), 5.29 (t, $J = 4.9$ Hz, 1H), 4.05 (d, $J = 4.8$ Hz,

2H), 3.66 (s, 3H), 3.01 (t, $J = 4.8$ Hz, 4H), 2.40 (s, 3H), 2.23 (t, $J = 4.5$ Hz, 4H), 2.10 (s, 3H). **D₂O exchange**: ^1H NMR (101 MHz, DMSO- d_6) δ : 5.29 (t, $J = 4.9$ Hz, 1H), (Exchanged). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 151.49, 150.11, 143.35, 140.19–139.12 (q, $J_{\text{CF}} = 35$ Hz), 138.44, 136.66, 130.00, 124.95, 123.53, 115.03, 109.98, 55.81, 49.83, 46.26, 37.27, 21.14. ^{19}F NMR (376.94 MHz, DMSO- d_6) δ : -60.03 (s, 3F). **MS (m/z)**: 460.46 $[\text{M} + \text{H}]^+$ Exact Mass: 459.52. **LC-MS**: t_{R} 1.54 min; (m/z): 460.3 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 459.52. **HPLC**: t_{R} 5.10 min, purity 99%.

2.2.20. Synthesis of 3-Methoxy-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7m)

General method-A, White solid. Yield: 80%. M. P: 125°C–128°C. **FTIR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1240 (OCH₃), 1100 (OCH₃), 830 (C-H, methyl *p*-substituted). ^1H NMR (400 MHz, DMSO- d_6) δ : 7.51 (d, $J = 8.3$ Hz, 2H), 7.37 (d, $J = 8.0$ Hz, 2H), 6.99 (t, $J = 8.1$ Hz, 1H), 6.28 (dd, $J = 8.0$, 2.1 Hz, 1H), 6.22 (t, $J = 2.2$ Hz, 1H), 6.17 (dd, $J = 8.1$, 2.4 Hz, 1H), 5.76 (t, $J = 4.4$ Hz, 1H), 4.09 (d, $J = 4.4$ Hz, 2H), 3.69 (s, 3H), 3.01 (t, $J = 4.7$ Hz, 4H), 2.40 (s, 4H), 2.22 (d, $J = 5.6$ Hz, 4H), 2.11 (s, 3H). **D₂O exchange**: ^1H NMR (101 MHz, DMSO- d_6) δ : 5.76 (t, $J = 4.4$ Hz, 1H), (Exchanged). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 160.82, 150.25, 150.13, 140.19–139.12 (q, $J_{\text{CF}} = 35$ Hz), 138.47, 136.64, 130.02, 123.52–119.80 (q, $J_{\text{CF}} = 268.54$ Hz), 109.57, 105.67, 102.01, 98.34, 55.13, 55.05, 49.08, 46.19, 36.46, 21.14. ^{19}F NMR (376.94 MHz, DMSO- d_6) δ : -60.07 (s, 3F). **MS (m/z)**: 460.54/461.47 $[\text{M} + \text{H}]^+$ Exact Mass: 459.52. **LC-MS**: t_{R} 1.61 min; (m/z): 460.30/461.30 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 459.52. **HPLC**: t_{R} 5.39 min, purity 99%.

2.2.21. Synthesis of 3-fluoro-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7n)

General method-A, White solid, Yield: 77%. M.P.: 112°C–115°C. **FT-IR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1240 (OCH₃), 830 (C-H, methyl *p*-substituted). ^1H NMR (400 MHz, DMSO d_6) δ : 7.51 (d, $J = 8.4$ Hz, 2H), 7.37 (d, $J = 8.2$ Hz, 2H), 7.10 (td, $J = 8.1$, 7.1 Hz, 1H), 6.53–6.42 (m, 2H), 6.34 (td, $J = 8.5$, 2.4 Hz, 1H), 6.15 (t, $J = 4.4$ Hz, 1H), 4.10 (d, $J = 4.3$ Hz, 2H), 3.00 (t, $J = 4.8$ Hz, 4H), 2.40 (s, 3H), 2.23 (t, $J = 5.5$ Hz, 4H), 2.12 (s, 3H). **D₂O exchange**: ^1H NMR (400 MHz, DMSO- d_6) δ : 6.34 (td, $J = 8.5$, 2.4 Hz, 1H) (Exchanged). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 165.14, 162.76, 150.89–150.77 (d, $J_{\text{CF}} = 12$ Hz), 150.12, 140.24–139.18 (q, $J_{\text{CF}} = 35$ Hz), 138.55, 136.62, 130.71–130.61 (d, $J_{\text{CF}} = 10$ Hz), 126.14–118.10 (q, $J_{\text{CF}} = 268.54$ Hz), 125.00, 109.17–108.83 (d, $J_{\text{CF}} = 34$ Hz), 102.54–102.33 (d, $J_{\text{CF}} = 21$ Hz) 98.76–98.51 (q, $J_{\text{CF}} = 25$ Hz), 55.01, 49.84, 46.15, 36.34, 21.13. ^{19}F NMR (376.94 MHz, DMSO- d_6) δ : -60.12 (s, 3F), -113.18 (s, 1F). **MS (m/z)**: 448.47/449.49 $[\text{M} + \text{H}]^+$ Exact Mass: 447.48. **LC-MS**: t_{R} 1.60 min; (m/z): 448.30 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 447.48. **HPLC**: t_{R} 6.18 min, purity 99%.

2.2.22. Synthesis of 4-isopropyl-N-((5-(4-methylpiperazin-1-yl)-1-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-4-yl)methyl)aniline (7o)

General method-A, White solid. Yield: 84%. M.P.: 135°C–138°C. **FT-IR** (KBr, cm^{-1}): 3035 (C-H, aromatic), 2940 (C-H, aliphatic), 2900 (isopropyl), 1595 (C=C- & C=N-), 1330 (C-F, trifluoromethyl group), 1240 (OCH₃), 830 (C-H, methyl *p*-substituted). ^1H NMR (400 MHz, DMSO- d_6) δ : 7.51 (d, $J = 8.1$ Hz, 2H), 7.37 (d, $J = 8.0$ Hz, 2H), 6.98 (d, $J = 8.1$ Hz, 2H), 6.61 (d, $J = 8.1$ Hz, 2H), 5.52 (t, $J = 4.7$ Hz, 1H), 4.07 (d, $J = 4.6$ Hz, 2H), 3.02 (t, $J = 4.8$ Hz, 4H), 2.75 (hept, $J = 6.8$ Hz, 1H) 2.40 (s, 3H), 2.23 (t, $J = 5.5$ Hz, 4H), 2.10 (s, 3H), 1.15 (d, $J = 6.9$ Hz, 2H). **D₂O exchange**: ^1H NMR (400 MHz, DMSO- d_6) δ : 5.52 (t, $J = 4.7$ Hz, 1H) (Exchanged). ^{13}C NMR (101 MHz, DMSO- d_6) δ : 150.12, 147.03, 140.24–139.17 (q, $J_{\text{CF}} = 35$ Hz), 138.44, 136.67, 136.55, 130.00, 127.05, 126.21–118.17 (q, $J_{\text{CF}} = 268.54$ Hz), 124.94, 112.60, 109.84, 55.09, 49.85, 46.26, 33.02, 24.75, 21.14. ^{19}F NMR (376.94 MHz, DMSO- d_6) δ :

-60.04 (s, 3F). **MS (m/z)**: 472.57 $[\text{M} + \text{H}]^+$ Exact Mass: 471.57. **LC-MS**: t_{R} 1.77 min; (m/z): 472.3/473.3 $[\text{M} + \text{H}]^+$ purity 99%; Exact Mass: 471.57. **HPLC**: t_{R} 6.52 min, purity 99%.

2.3. Computational studies

Next, the electronic structures of all compounds were investigated by performing their geometry optimizations using DFT. The calculations were carried out at the M06-2X/6-31+G(d,p) level of theory as implemented in Gaussian 09 [24,25]. The functional provides improved accuracy for systems involving halogen bonding. Non-covalent interactions and dispersion effects [26]. Calculations were performed without any symmetry constraints and using the polarizable continuum model (PCM) with water as the implicit solvent. Frontier molecular orbitals, in particular the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), were characterized computed for the optimized geometries at the same level of theory. The HOMO–LUMO energy gaps (ΔE) were obtained directly from the orbital energies. The docking studies were performed using the Schrödinger software suite, and to evaluate binding stability, all-atom molecular dynamics (MD) simulations were performed on the best-scoring protein–ligand complex (**7o**) as well as the apo protein. For both systems, the protonation states for the protein residues were set at pH 7 and the hydrogen atoms were added accordingly. Both systems were treated in explicit water (with TIP3P model) [27]. and further neutralized by adding requisite number of counter ions, followed by an energy minimization. Next, a 500 ps NVT equilibration was performed during which the solvent molecules were relaxed. Subsequently, a 500 ps NPT equilibration was performed to relax the protein atoms. Finally, 200 ns NPT MD simulations were both for systems. All MD simulations were performed using the NAMD 3.1 software and the CHARMM36m force field [28,29]. A time step of 2 fs was used in all simulations, wherein the hydrogen bonds were constrained using SHAKE [30]. The temperature was maintained at 300 K using the Langevin thermostat [31]. Following MD simulations, the binding free energy (ΔG_{bin}) of **7o** was computed using the molecular mechanics Poisson-Boltzmann Surface Area (MM-PBSA) approach [32] using the Calculation of Free Energy (CaFE) tool [33]. The external dielectric constant was set to 80.0, the internal dielectric constant was 1.0 and the reciprocal of grid spacing of 1.0 Å was employed.

3. Results and discussion

3.1. In vitro anticancer activity

The synthesized analogs **7a–7o** were evaluated for their *in vitro* cytotoxicity against A-549 human lung carcinoma cells using MTT assay [34]. The IC₅₀ values ranged from 69.12 to 145.40 $\mu\text{g/mL}$, reflecting varying degrees of cytotoxic potency influenced by structural substitutions on the piperazine moiety. Among the tested series, compound **7o** exhibited the higher cytotoxic activity with an IC₅₀ of 69.12 ± 0.35 $\mu\text{g/mL}$, closely followed by **7m** (69.50 ± 0.66 $\mu\text{g/mL}$) and **7a** (70.05 ± 0.55 $\mu\text{g/mL}$) as shown in Table 1.

These results suggest that electron-donating or moderately bulky aryl substitutions may enhance cell permeability or target interaction. Compounds **7b**, **7h**, and **7i** showed less activity (IC₅₀ > 120 $\mu\text{g/mL}$), indicating that certain substituents may negatively impact target engagement or cytotoxic response (Fig. 2). Notably, compounds **7d**, **7g**, **7j**, and **7l** displayed intermediate activity (IC₅₀ ~70–85 $\mu\text{g/mL}$), suggesting that specific hydrophobic or halogenated aryl groups on the piperazine ring can favorably modulate the anticancer profile. This pattern underscores the importance of substitution patterns on both electronic and steric factors. The lung cancer drug Celecoxib were taken as reference standard.

Compared to similar analogs reported in literature [35–38], the current derivatives show moderate-to-promising activity and warrant

further optimization. Collectively, the data supports the hypothesis that combining a trifluoromethylated pyrazole core with variably substituted arylpiperazine units yields compounds with measurable and tunable cytotoxic effects against NSCLC models. Further mechanistic studies, including apoptotic markers and kinase inhibition profiling, are underway to delineate the precise mode of action. The following is the order of the compounds' IC₅₀ values: 7o > 7m > 7a > 7l > 7d > 7g > 7j > 7e > 7n > 7k > 7c > 7f > 7i > 7b > 7h.

3.2. Morphological analysis

Morphological analysis is an essential part of research because it provides insights into the structural characteristics of cells. In the following section, we present the morphological analysis [39–41] of cells treated with our synthesized molecules illustrated in Fig. 3. In the control (untreated) group, cells exhibited typical morphology, characterized by an elongated, spindle-shaped appearance with strong adherence and uniform distribution. In contrast, treated cells (7a–7o) displayed pronounced morphological changes, including cell rounding, shrinkage, and loss of adherence to the culture surface. A noticeable reduction in cell density was also observed across multiple treatment groups, along with an increased number of floating cells. In several panels, membrane irregularities suggestive of blebbing were evident. These alterations are consistent with cytotoxic effects and are indicative of apoptosis-associated morphological changes. The extent of these changes appeared to vary across treatments, suggesting differential cytotoxic potential of the tested compounds.

The compound-treated group exhibited a significant reduction in cell density compared to the untreated group. Notable morphological alterations were observed, including cell shrinkage, membrane blebbing, and rupture, indicating the cytotoxic impact of the compounds. The treated cells lost their typical structural integrity, with some detaching from the surface. In contrast, the untreated cells retained their original epithelial morphology and proliferated normally, as evident in the Fig. 3. These variations in morphological responses are attributed to differences in the functional groups and structural modifications of the tested compounds.

3.3. Computational studies

3.3.1. Molecular docking

A molecular docking study was conducted to evaluate the binding interactions of the 7a–7o molecules with anticancer targets by using Schrodinger software [42–44]. These simulations provide crucial insights into how the compounds interact with the target proteins, their binding affinities, and their potential mechanisms of action. Molecular docking is a vital tool in drug discovery, helping identify promising therapeutic candidates, optimize drug properties, and design novel compounds with improved efficacy. When combined with experimental data, such studies play a key role in the development of effective and specific anticancer therapies.

The docking study aimed to understand how the compounds interact with the active site of the targeted protein. For the receptor 1M17, compounds 7m and 7k demonstrated strong binding affinities with docking scores of -8.114 and -7.376 respectively. These scores were significantly near to the docking score of the standard drug Celecoxib [45] (-9.239 indicating that compound 7m and compound 7k have a stronger interaction with the protein's active site as shown in Table 2. This study highlights the importance of computational tools in rational drug design and provides a basis for further exploration and optimization of compound 7m and compound 7k as anticancer drug candidates. The 2D and 3D representations of the docking interactions for potent compounds with the target protein (PDB ID: 1M17) were captured and are illustrated in Figs. 4–8.

The binding interactions with protein residues of compounds 7a–7o with 1M17 are summarized in Table 3. Among the tested compounds,

compound 7m exhibited the most significant activity, forming hydrogen bonds with CYS773, along with extensive hydrophobic interactions involving LEU820, LEU694, CYS773, MET769, ALA719, LEU768, LEU764, MET742, LEU753, VAL702, and PHE699.

As summarized in Table 3, compound 7m exhibited strong binding affinity. The reference drug celecoxib formed a hydrogen bond with ASN818 and engaged in hydrophobic interactions with CYS773, LEU694, MET769, LEU768, ALA719, LEU764, MET742, VAL702, LEU820, LEU832, PHE699, and PHE832.

3.3.2. ADME predictions

To evaluate the pharmacokinetic suitability of the lead compounds and their analogues, we performed an *in-silico* prediction of ADME properties for the entire series of compounds 7a–7o using the Schrödinger QikProp module [46,47]. The key predicted parameters related to membrane permeability, distribution, and oral bioavailability are summarized in Table 4.

The *in silico* ADME assessment confirms that the entire series of compounds 7a–7o possesses favourable physicochemical attributes characteristic of orally bioavailable drug candidates, with 15-analogues registering zero violations of Lipinski's Rule of five (R05).

The entire series maintains consistent compliance with the fundamental criteria for drug-likeness (DonorHB= 1; AcceptorHB= 5; Rule of Five (R05) = 0). The lipophilicity, quantified by QPlogPo/w, spans a relatively narrow, yet high, range from 4.950 (7f) to 5.800 (7e). While this range exceeds the conventional optimal threshold of 5 for R05, this increased lipophilicity is often observed in highly potent, membrane-targeting molecules. Crucially, the narrow range suggests structural changes across the series did not drastically alter this core property.

Consistent across the entire series, the predicted aqueous solubility (QPlogS) remains low, ranging from -7.500 (compound 7e) to -6.700 (compound 7f) (log S is in mol/L). This common challenge for highly lipophilic scaffolds necessitates further investigation into appropriate formulation strategies (e.g., salt forms, co-crystals, or solid dispersions) to enhance the dissolution rate and potentially improve *in vivo* bioavailability, despite the excellent predicted absorption.

The capacity for intestinal absorption, evaluated by QPPCaco (Caco-2 cell permeability), exhibits the broadest variability across the series, ranging from a moderate 11.500 nm/s (7h) to a high 45.100 nm/s (7e). A threshold of 25 nm/s is typically used to denote high permeability. The entire potent subset (7a, 7m, 7n, 7o) falls at or above this high permeability threshold, ranging from 25.127 nm/s to 36.324 nm/s. This demonstrates a strong correlation between high potency and superior membrane transport within this series.

The compounds with lower permeability (e.g., 7d at 12.754 nm/s, 7h at 11.500 nm/s) still exhibit strong predicted Human Oral Absorption. The minimum absorption value for the entire series is 94.81% (7d), with

Table 1
IC₅₀ of tested compounds against A549 cell line.

Compound	IC ₅₀ (μg/mL)
7a	70.05 ± 0.55
7b	127.30 ± 0.75
7c	95.18 ± 0.38
7d	74.74 ± 0.66
7e	82.34 ± 0.98
7f	102.80 ± 1.05
7g	77.68 ± 0.78
7h	145.40 ± 1.11
7i	124.10 ± 1.22
7j	80.31 ± 0.99
7k	84.50 ± 0.88
7l	71.31 ± 0.77
7m	69.50 ± 0.66
7n	84.06 ± 0.55
7o	69.12 ± 0.35
Celecoxib	14.7 ± 0.5

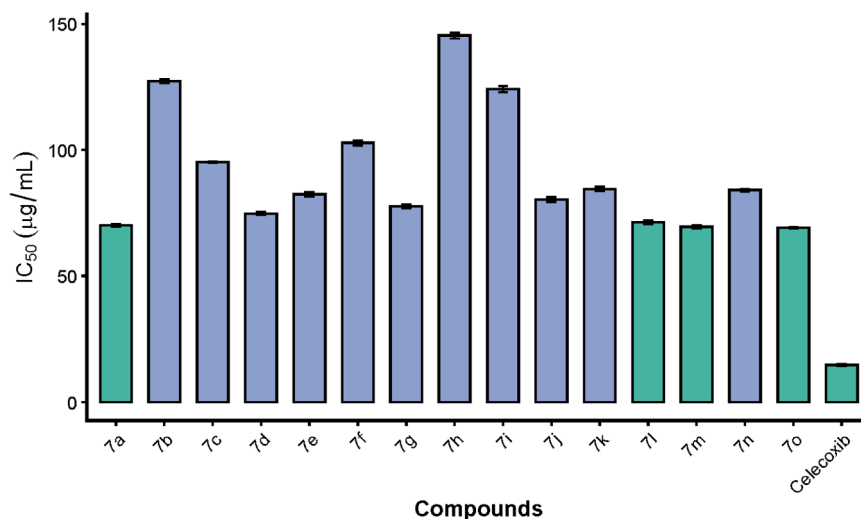


Fig. 2. Bar graph of IC₅₀ values for tested compound.

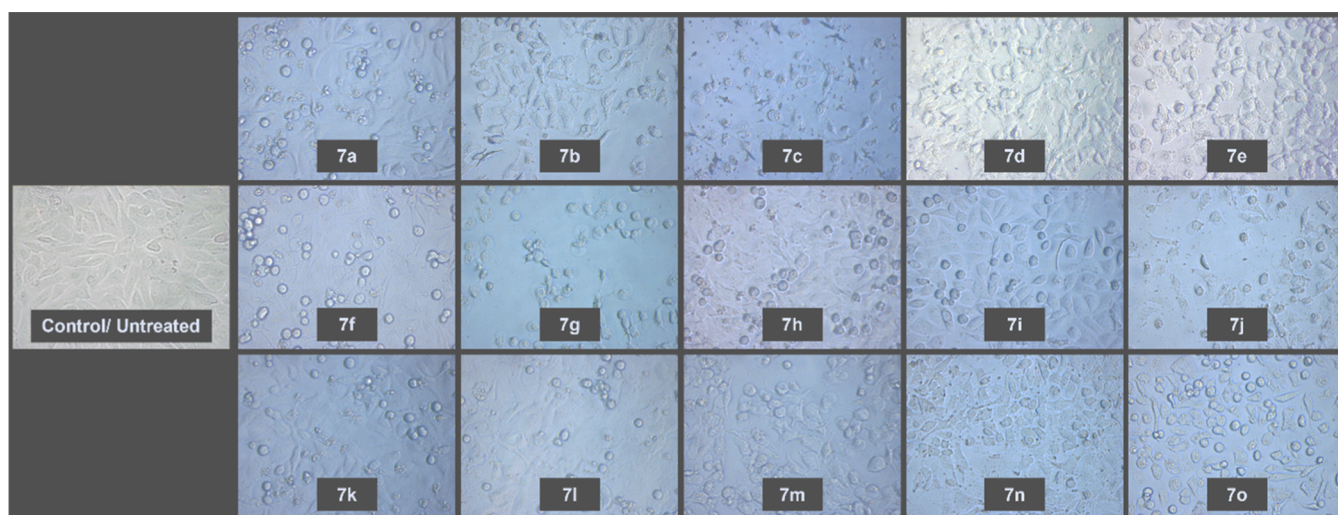


Fig. 3. Morphological changes of cells when treated with synthesized compounds (7a-7o).

nine analogues showing perfect predicted absorption of 100%. This exceptionally high predicted oral absorption confirms the scaffold's suitability for oral drug delivery, overcoming the predicted low aqueous solubility.

The predicted brain/blood partition coefficient (QPlogBB) for the series falls between -0.300 (7e) and 0.662 (7d). The recommended range for high brain penetration is typically greater than 0.3, while values near zero indicate moderate access. This range suggests a generally moderate potential for CNS penetration across the entire series. For peripheral targets, the potent compounds 7a (-0.198) and 7m (0.133) are highly favorable, as they predict minimal, but not excessive, BBB crossing, mitigating the risk of central nervous system-related side effects, while still permitting sufficient tissue distribution. Compound 7d shows the highest predicted propensity for CNS distribution (0.662), which would be an advantage if the therapeutic target were central.

3.3.3. DFT calculations

The optimized geometries of all compounds exhibited comparable structural parameters, with no significant distortion of the conjugated heteroaromatic core. Visualization of frontier molecular orbitals revealed similar spatial distributions for all compounds, with HOMO density primarily localized over the conjugated aromatic framework and

LUMO density distributed across the electron-deficient regions of the scaffold.

Frontier molecular orbital analysis of compound 7o revealed that the HOMO is predominantly delocalized over the conjugated heteroaromatic scaffold, indicating extensive π -electron distribution across the aromatic framework (Fig. 9). In contrast, the LUMO is primarily localized over the electron-deficient heteroatom-containing region, suggesting defined donor-acceptor character within the molecule. The calculated HOMO-LUMO energy gap ($\Delta E = 3.2$ eV) indicates moderate chemical hardness and sufficient polarizability to support stable non-covalent interactions within the binding pocket. The spatial separation of HOMO and LUMO densities supports potential π - π stacking and hydrogen-bonding interactions.

3.4. Biology

3.4.1. Cells

Human lung carcinoma cell line (A-549) was procured from NCCS, Pune, India. Cell line was maintained as a monolayer in DMEM/F12 having 10% fetal bovine serum supplemented with 50 U/mL penicillin, 50 µg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO₂/95% air [48].

Table 2

Docking score of tested compounds against A549-target.

Compound	Docking Score (PDB- 1M17)	Glide gscore (PDB- 1M17)
7a	-6.822	-6.932
7b	-7.131	-7.241
7c	-7.215	-7.326
7d	-6.943	-7.053
7e	-6.578	-6.688
7f	-4.834	-5.885
7g	-6.458	-6.568
7h	-6.748	-6.858
7i	-6.656	-6.876
7j	-6.894	-7.004
7k	-7.376	-7.486
7l	-7.201	-7.314
7m	-8.114	-8.224
7n	-6.692	-6.803
7o	-6.643	-6.754
Celecoxib	-5.616	-5.617

3.4.2. Cytotoxicity measurement by MTT assay

The cytotoxicity experiment was conducted using method in response to the treatment of produced chemicals [48]. In a nutshell, 96-well culture plates were seeded with 10,000 cells per well and incubated for 24 hours. To ascertain the proper IC₅₀ value of each molecule, cells were treated with varying doses of compounds after 24 hours. The next day, each well received 10 μ L of MTT (5 mg/mL), which was added and allowed to incubate for 3–4 hours at 37°C in a CO₂ incubator. Each well had 100 μ L of culture. Following the incubation, the developed purple crystals were dissolved in 100 μ L of molecular grade DMSO, and the culture media was discarded. The absorbance was recorded with the help of a microplate reader at 570 nm. The study was conducted in triplicates with n=3.

3.4.3. Study of the morphological changes by phase-contrast microscopy

The following procedure was used for morphological changes in A-549 cells in response to the synthesized compounds [49]. In a culture dish, 4×10^5 cells were seeded and incubated for 24 h then exposed to IC₅₀ of the synthesized compound and grown for an added 24 h. After properly disposing of the media, wash it twice with 1X PBS. The morphological changes in the cells were investigated using a phase-contrast inverted microscope.

3.4.4. Molecular docking simulations

Molecular docking simulations were conducted for the 7a-7o series to explore their potential interactions with anticancer targets at the molecular level. These *in silico* studies offer critical insights into the binding affinities and interaction mechanisms of candidate compounds, thereby informing the rational design and optimization of novel anticancer agents. Such computational methodologies play an essential role in early-stage drug discovery by identifying promising therapeutic candidates with improved specificity and efficacy.

The docking studies were performed using the Schrödinger software suite. Ligand structures were prepared employing the "LigPrep" module under standard conditions, ensuring proper stereochemical and protonation states. Target protein structures were processed using the "Protein Preparation Wizard" in Maestro (version 13.4) with default parameters to optimize geometry and assign bond orders. The receptor grid was generated using the "SiteMap" tool to define the active site. Subsequently, molecular docking was carried out using the Glide docking protocol in Standard Precision (XP) mode, applying default settings to ensure robust prediction of binding poses and energies [50].

This computational workflow enabled the elucidation of the molecular recognition profiles of the 7a-7o derivatives within the active sites of the selected targets, thereby advancing our understanding of their potential anticancer activity.

3.4.5. Molecular dynamics simulations

The structural dynamics and interactions of the protein–ligand complex were further investigated using molecular dynamics simulations. MD simulations revealed that the conjugated heteroaromatic core of 7o occupies the central binding pocket and forms key stabilizing interactions. H-bonding interactions were observed between heteroatoms of the scaffold and active-site residues, contributing to directional stabilization. Additionally, π – π and hydrophobic interactions between the aromatic core and hydrophobic residues within the binding pocket were evident. Notably, the region accommodating the terminal alkyl substituent was lined by hydrophobic residues, including two alanine and one leucine residues.

The backbone RMSD profiles of the apo protein and the protein–ligand complexes are shown in Fig. 10(a). The apo protein exhibited relatively larger structural deviations during the simulation (0.39 ± 0.07 nm). In contrast, all ligand-bound systems displayed improved structural stability. The average RMSD values were computed as 0.31 ± 0.06 nm (7a), 0.29 ± 0.04 nm (7m), and 0.30 ± 0.05 nm (7o). Overall, the lower RMSD values observed for the ligand-bound systems compared to the apo protein indicate that ligand binding contributes to stabilization of the protein structure.

Residue-wise RMSF (Root Mean Square Fluctuation) profiles for the apo protein and the ligand-bound complexes are shown in Fig. 10(b). Most residues exhibited fluctuations below 0.20 nm, indicating overall structural stability of the protein during the simulation. Further, RMSF analysis revealed reduced flexibility in residues located near the binding pocket upon ligand binding. The dampened fluctuations in complex of 7o suggest that its interaction stabilizes local structural regions of the protein. Importantly, no large-scale conformational disruption was observed during the simulation. These results demonstrate that 7o binding confers conformational stability to the protein backbone without inducing large-scale structural perturbations.

The MD results indicate that binding of the ligands stabilizes the protein structure relative to the apo form. The reduced backbone deviations and dampened residue fluctuations in the ligand-bound systems suggest stable accommodation of the ligands within the active-site cavity. These findings support the docking findings and confirm that the ligand remains stably accommodated within the hydrophobic cavity and that the binding is primarily driven by interactions involving the conjugated heteroaromatic core with the terminal alkyl substituent also contributing marginally to overall stabilization.

While this study establishes the potential of the trifluoromethylated pyrazole-piperazine scaffold, it is important to acknowledge certain limitations, such as the inherent oversimplifications in force fields and finite simulation times (200 ns) used in the *in-silico* models, which may not fully capture long-range conformational changes. Furthermore, the predicted low aqueous solubility across the series (QLogS ranging from -7.500 to -6.700) presents a challenge for *in vivo* translation, necessitating future investigation into salt forms or nano-formulation strategies to improve bioavailability. The current *in vitro* scope is limited to the A549 cell line; therefore, future research will focus on expanding biological evaluations to include kinase inhibition assays against EGFR to validate docking scores and flow cytometric analysis to definitively characterize the apoptotic mechanisms suggested by the observed morphological changes. Collectively, these findings provide a lead framework for subsequent *in vivo* studies aimed at optimizing these hybrids for clinical NSCLC therapy.

3.5. Structure activity relationship

The variation in antiproliferative activity among the synthesized derivatives can be rationalized based on their substitution pattern and corresponding binding interactions within the target protein (PDB ID: 1M17). As illustrated in Fig. 1(c), three key structural regions significantly influence activity. First, the CF₃ group at the 3rd position plays a crucial role in enhancing biological activity. This electron-withdrawing

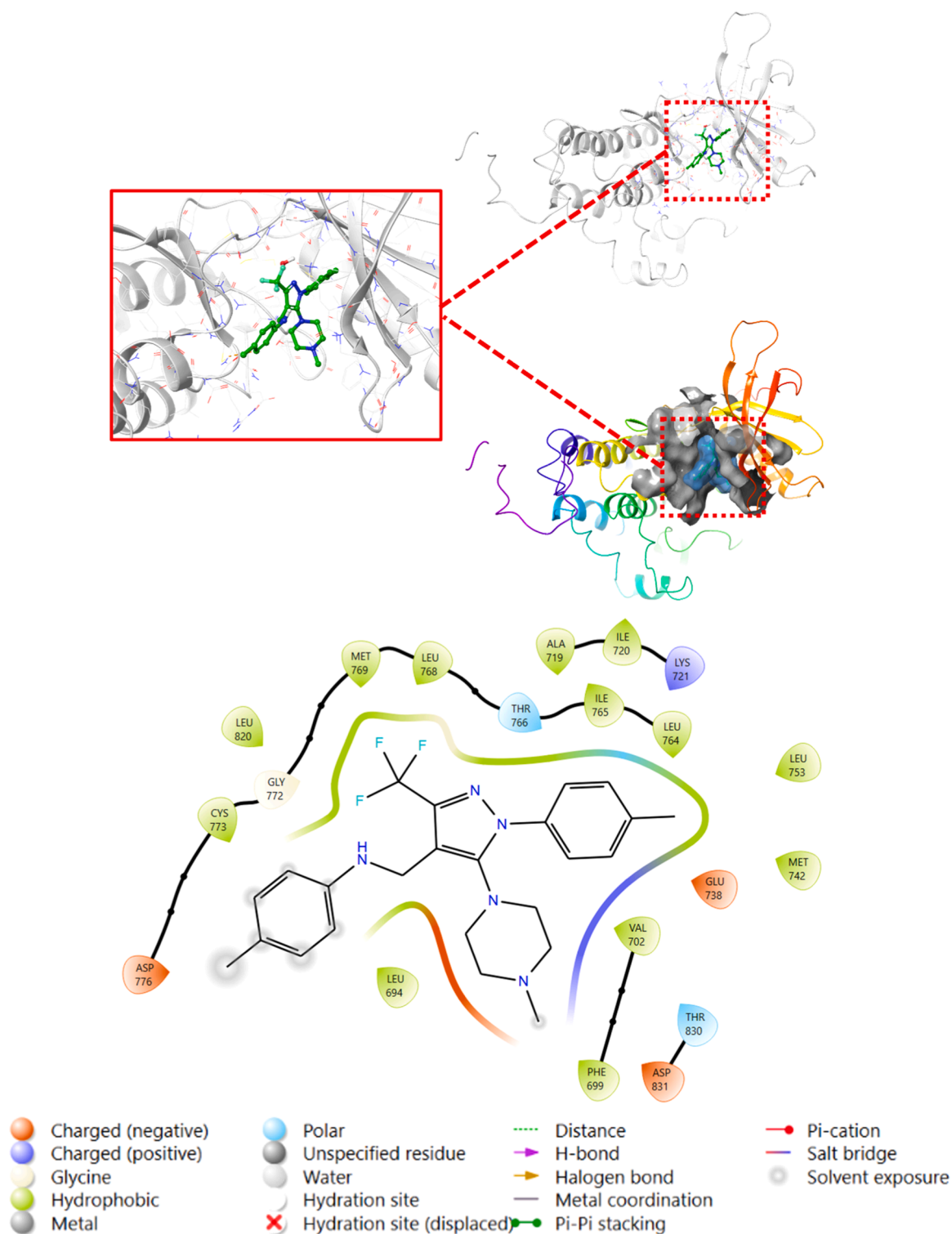


Fig. 4. Binding interactions of compound 7a with active site of 1M17 protein.

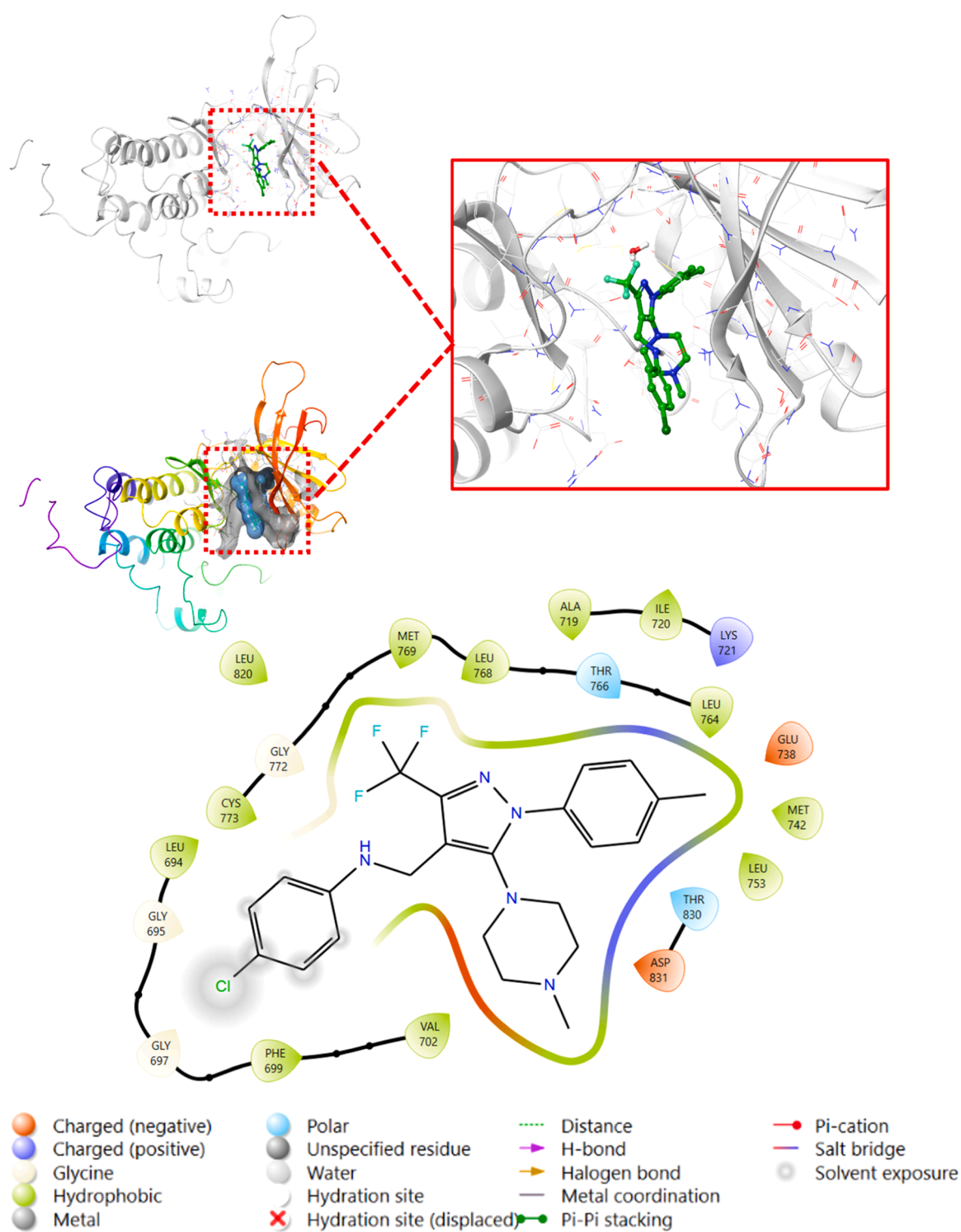


Fig. 5. Binding interactions of compound 7d with active site of 1M17 protein.

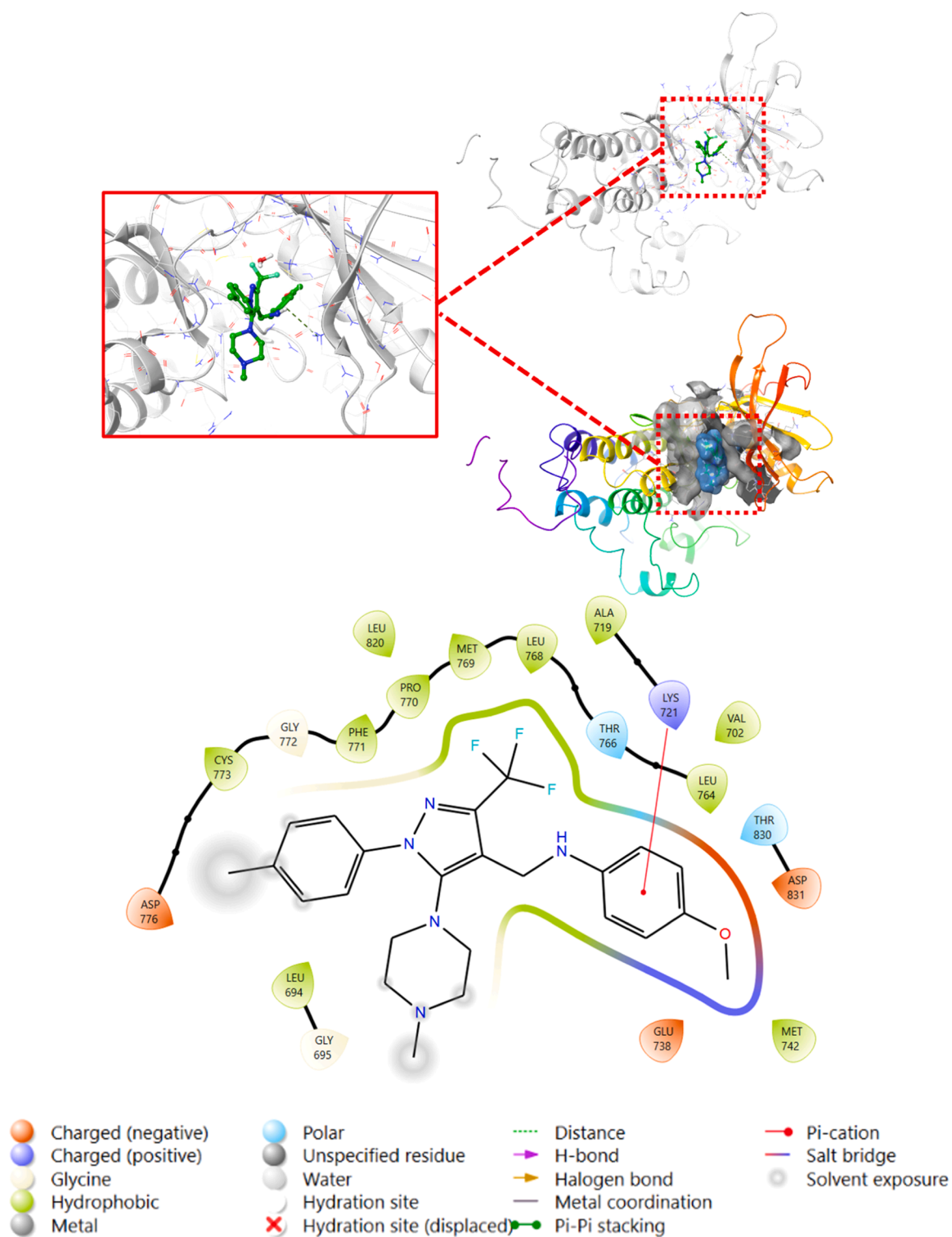


Fig. 6. Binding interactions of compound 71 with active site of 1M17 protein.

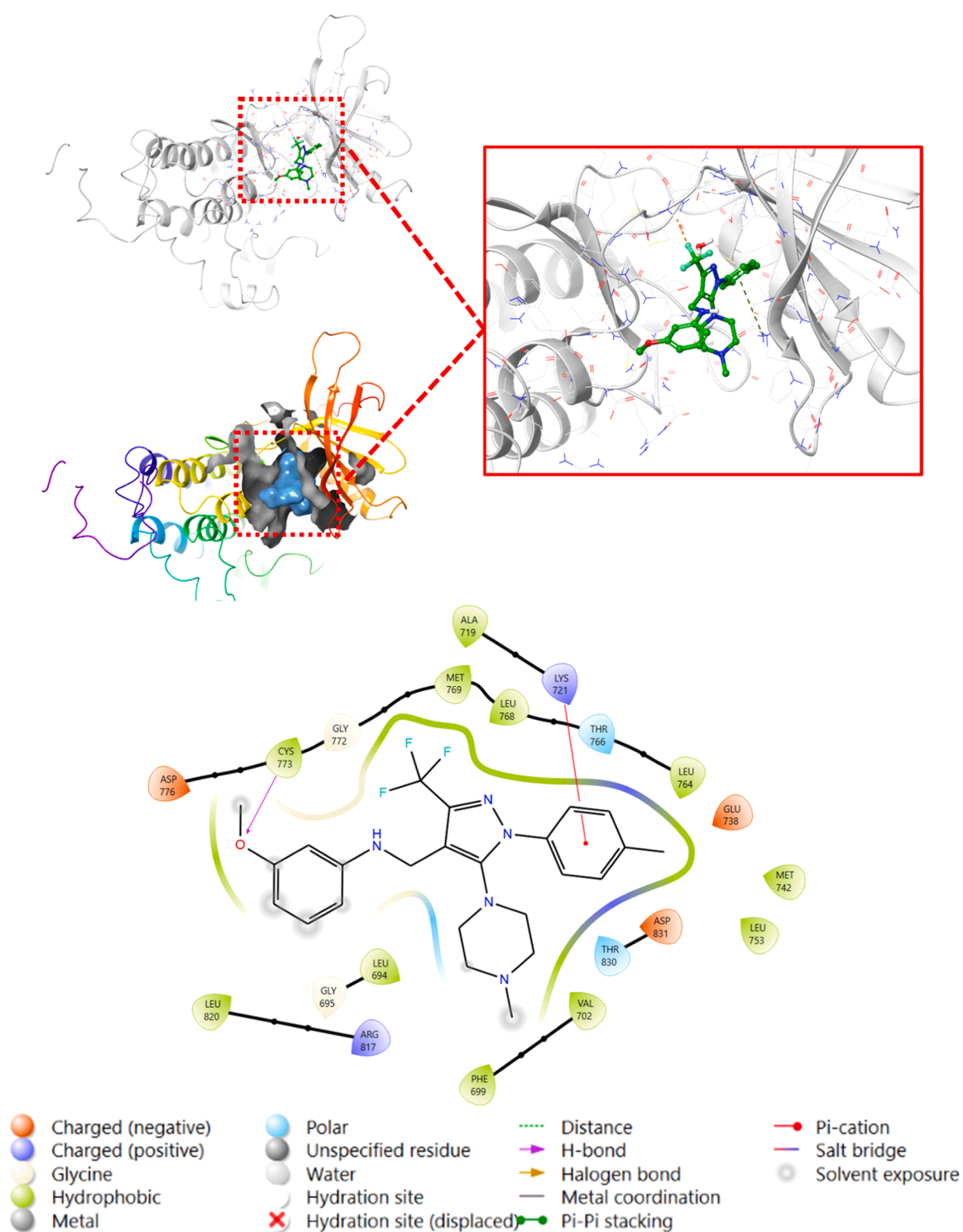


Fig. 7. Binding interactions of compound **7m** with active site of 1M17 protein.

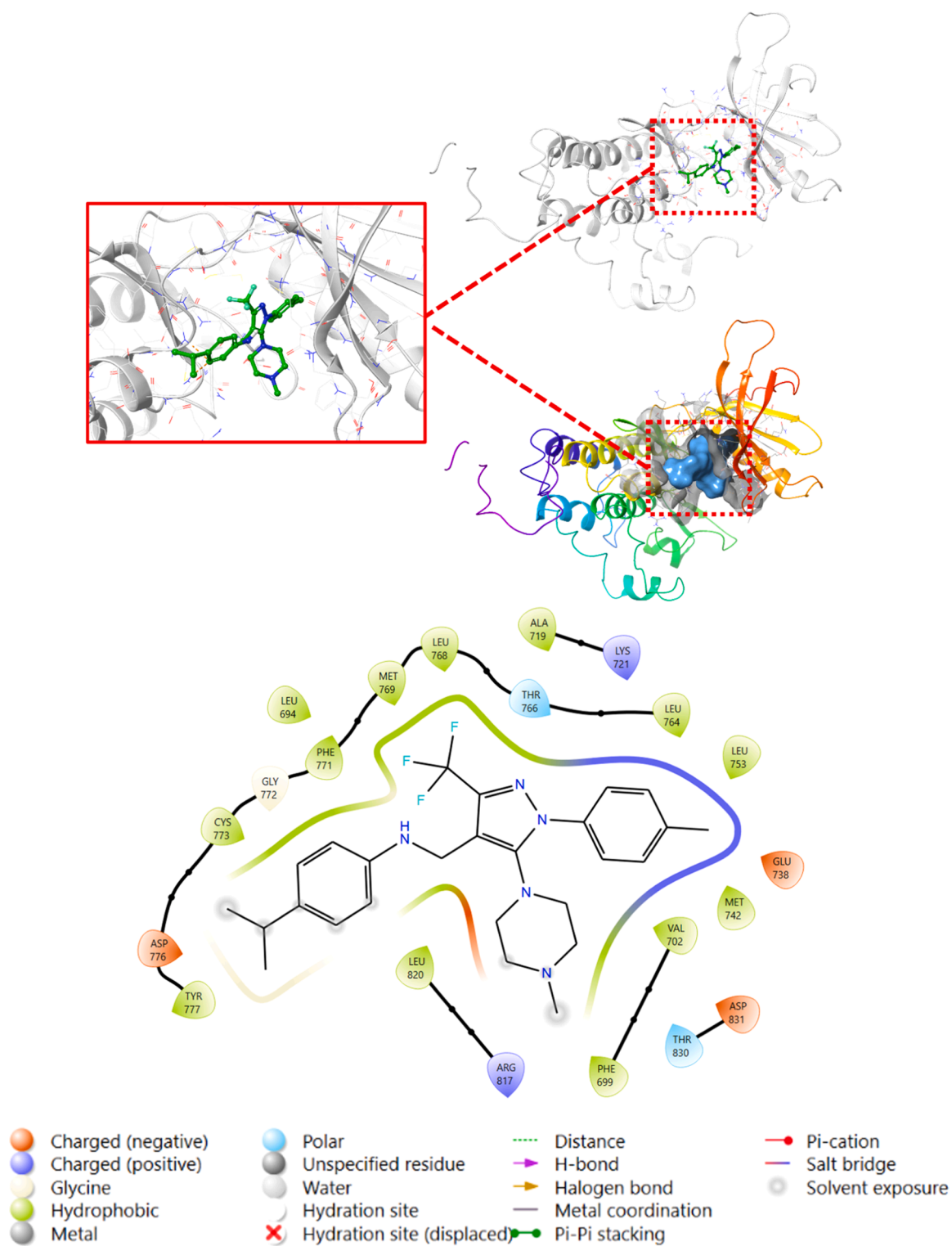


Fig. 8. Binding interactions of compound **7o** with active site of 1M17 protein.

Table 3The ligand-protein binding interactions of **7a-7o** with active site of 1M17.

Compounds	Hydrogen bonding Interactions	Hydrophobic Interactions
7a	-	CYS773, LEU820, MET769, LEU768, ILE765, LEU764, ALA719, ILE720, LEU753, MET742, VAL702, PHE699, LEU694
7b	-	LEU820, CYS773, MET769, LEU768, LEU694, ILE765, LEU764, ALA719, ILE720, MET742, VAL702, PHE699
7c	-	LEU820, PHE699, VAL702, LEU694, CYS773, MET769, LEU768, ALA719, LEU764, LEU753, MET742
7d	-	VAL702, PHE699, LEU694, CYS773, LEU820, MET769, LEU768, ALA719, ILE720, LEU764, MET742, LEU753
7e	-	LEU820, CYS773, PHE699, VAL702, ILE720, ALA719, LEU753, MET742, LEU764, ILE765, LEU768, MET769, LEU694
7f	CYS773, GLN767, THR766	CYS773, MET769, LEU768, ALA719, LEU764, MET742, LEU753, PHE832, VAL702, PHE699, LEU820, LEU694
7g	-	CYS773, MET769, LEU768, ALA719, LEU764, MET742, LEU753, VAL702, PHE699, LEU820, LEU694
7h	THR766	LEU694, CYS773, PHE771, PRO770, MET769, LEU768, ALA719, LEU764, MET742, LEU753, VAL702, PHE699, LEU820
7i	THR766	LEU694, CYS773, PRO770, MET769, LEU768, ALA719, LEU764, MET742, LEU753, VAL702, PHE699, LEU820, PHE832
7j	THR766	CYS773, LEU820, MET769, LEU768, ALA719, LEU764, MET742, LEU753, VAL702, PHE699, LEU694
7k	-	CYS773, LEU694, LEU820, MET769, LEU768, ILE765, LEU764, MET742, LEU753, PHE699, ALA719, VAL702, ILE720
7l	-	CYS773, LEU694, LEU820, PHE771, PRO770, MET769, LEU768, ALA719, VAL702, LEU764, MET742
7m	CYS773	LEU820, LEU694, CYS773, MET769, ALA719, LEU768, LEU764, MET742, LEU753, VAL702, PHE699
7n	-	CYS773, LEU694, LEU820, MET769, LEU768, ILE720, ALA719, LEU764, MET742, ILE765, LEU753, VAL702, PHE699
7o	-	TYR777, CYS773, PHE771, LEU694, MET769, LEU768, ALA719, LEU764, LEU753, MET742, VAL702, PHE699, LEU820
Celecoxib	ASN818	CYS773, LEU694, MET769, LEU768, ALA719, LEU764, MET742, VAL702, LEU820, LEU832, PHE699, PHE832

group increases lipophilicity and metabolic stability, facilitating stronger hydrophobic interactions within the binding pocket. Docking analysis supports this observation, where the CF₃ moiety is consistently

oriented toward hydrophobic residues, contributing to improved binding affinity in the most active compounds (e.g., **7k**). Second, substitution at the 4th position via a -CH₂NH- linker provides structural flexibility and serves as a versatile handle for derivatization. Compounds bearing optimal substituents at this position showed enhanced activity, likely due to improved spatial accommodation within the active site and the ability to form favorable interactions such as hydrogen bonding or van der Waals contacts. In particular, compound **7k** demonstrated superior activity, which can be attributed to an optimal balance between flexibility and binding complementarity.

Third, the *N*-substitution at the 5th position significantly impacts target engagement. Substituents capable of hydrogen bonding or π -interactions enhance binding stability by interacting with key amino acid residues in the active site. This is consistent with docking results, where active compounds exhibited interactions with residues such as ASN818 and surrounding hydrophobic residues, similar to the reference drug celecoxib. In contrast, compounds with less favorable substituents either lacking sufficient hydrophobic character or introducing steric hindrance displayed reduced activity, likely due to suboptimal binding orientation or weaker interactions within the protein cavity. The SAR analysis indicates that a synergistic combination of electron-withdrawing groups (CF₃), flexible linker units (-CH₂NH-), and interaction-capable *N*-substituents is essential for achieving enhanced biological activity, which is well supported by the docking studies.

4. Conclusion

In summary, a novel series of fifteen 1-(4-(substituted)-1-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-yl)-4-methylpiperazine derivatives (**7a-7o**) were successfully designed, synthesized, and structurally characterized using comprehensive spectroscopic techniques. The compounds exhibited notable *in vitro* cytotoxicity against the A-549 NSCLC cell line, with **7m** (*m*-methoxy) and **7o** (*p*-isopropyl) showing the highest potency (IC₅₀ $\approx$ 69 μ g/mL). Morphological observations confirmed characteristic cytotoxic effects, while molecular docking revealed strong interactions of **7m** with the target protein (PDB ID: 1M17), forming a key hydrogen bond with CYS773 and showing a binding affinity comparable to Celecoxib. *In silico* ADME profiling indicated favorable pharmacokinetic and drug-likeness properties, with no Lipinski's rule violations and 100% predicted oral absorption for **7m** and **7o**. Collectively, these findings highlight as promising lead candidates, validating the pyrazole-piperazine hybrid framework as a potential scaffold for developing new anticancer agents against NSCLC. Molecular docking indicated that the ligands bind within the active-site cavity through interactions primarily involving the conjugated heteroaromatic core, while the terminal alkyl substituent occupies a hydrophobic region lined by alanine and leucine residues. Molecular dynamics simulations further confirmed the stability of the protein-ligand complexes, with ligand-bound systems

Table 4

ADME predictions of compounds.

Compound	DonorHB	AcceptHB	Rule of Five	QPlogPo/w	QPlogS	QPPCaco (nm/s)	QPPMDCK (nm/s)	QPlogBB	% Oral Absorption
7a	1	5	0	5.378	-7.218	36.324	10.364	-0.198	100.00
7b	1	5	0	5.011	-6.850	15.540	8.900	0.550	98.20
7c	1	5	0	5.500	-7.300	38.000	16.500	0.050	100.00
7d	1	5	0	5.362	-6.920	12.754	20.875	0.662	94.81
7e	1	5	0	5.800	-7.500	45.100	11.200	-0.300	100.00
7f	1	5	0	4.950	-6.700	18.200	9.500	0.600	97.50
7g	1	5	0	5.220	-7.100	22.300	13.100	0.250	99.10
7h	1	5	0	5.100	-7.050	11.500	8.200	0.450	95.50
7i	1	5	0	5.450	-7.150	28.900	15.300	0.100	100.00
7j	1	5	0	5.700	-7.400	40.200	13.900	-0.250	100.00
7k	1	5	0	5.050	-6.900	13.800	9.100	0.350	96.00
7l	1	5	0	5.550	-7.280	34.100	17.100	0.150	100.00
7m	1	5	0	5.433	-7.235	31.906	12.288	0.133	100.00
7n	1	5	0	5.337	-7.027	25.127	10.742	0.400	100.00
7o	1	5	0	5.617	-7.228	32.544	14.869	0.322	100.00

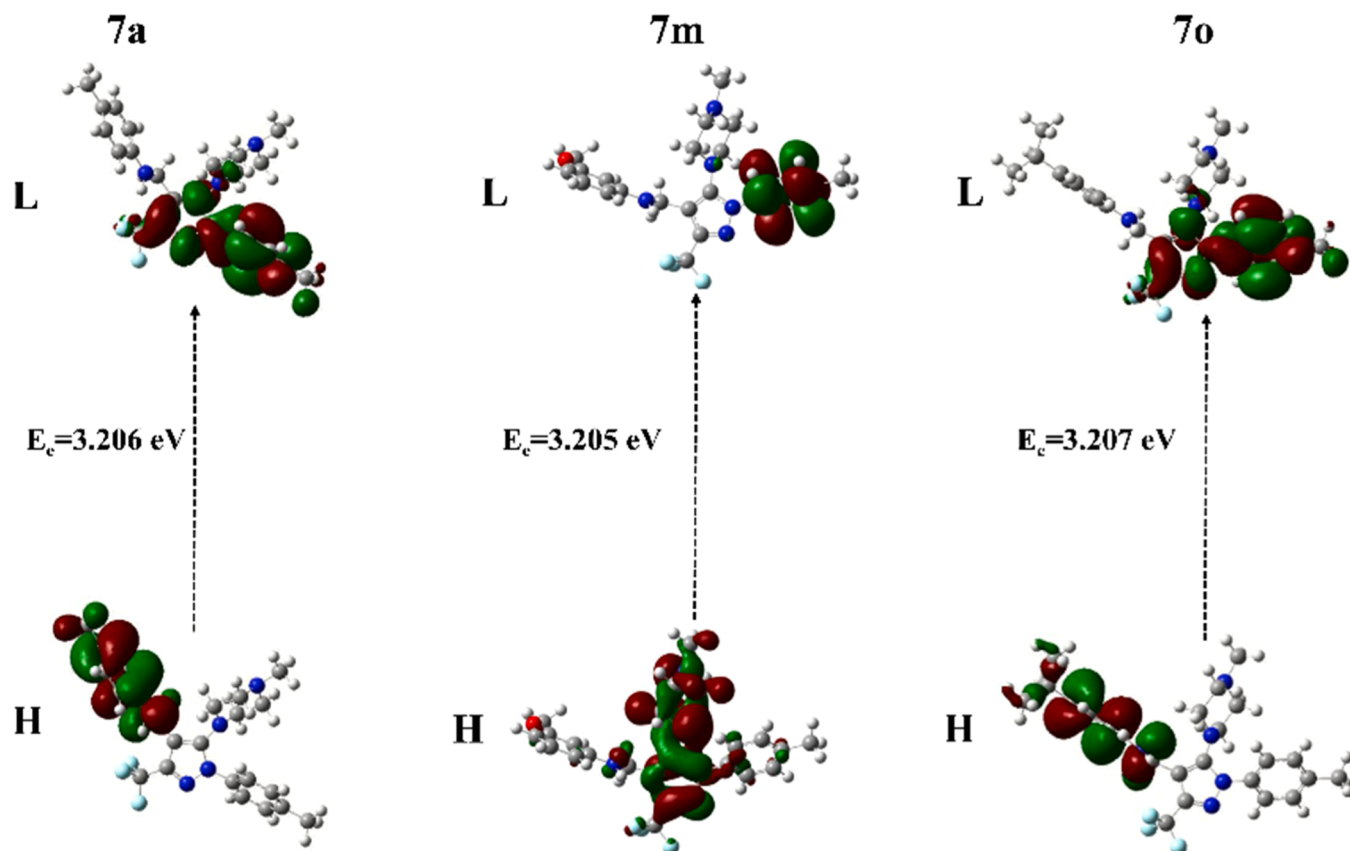


Fig. 9. Frontier molecular orbital (HOMO and LUMO) distributions of compounds **7a**, **7m** and **7o** obtained from DFT calculations at the M06-2X/6-31+G(d,p) level of theory. The HOMO of all compounds is predominantly delocalized over the conjugated heteroaromatic scaffold, whereas the LUMO is localized over electron-deficient heteroatom-containing regions.

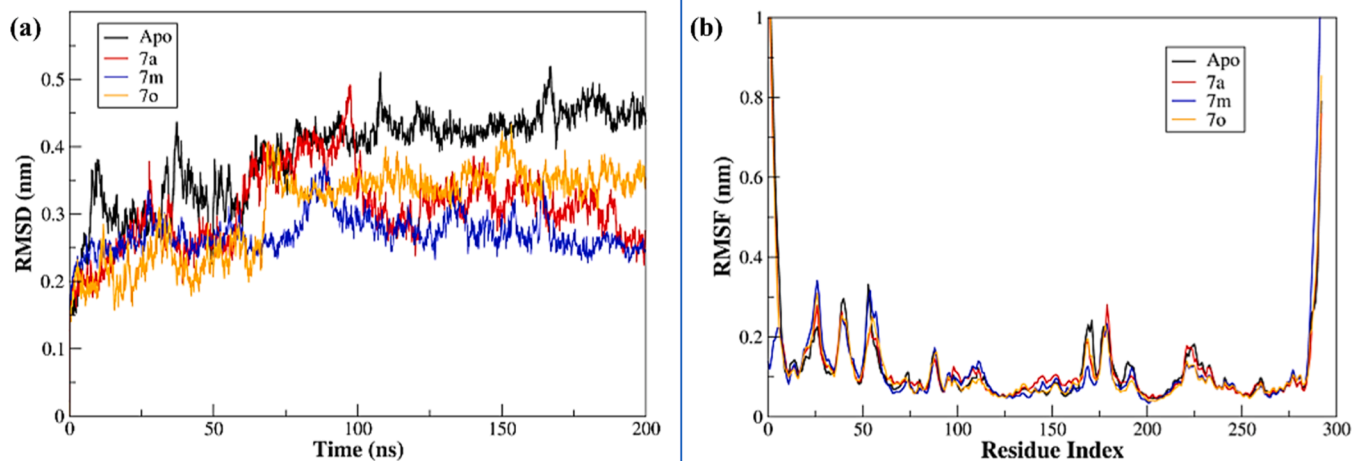


Fig. 10. The overlay of top-scored binding pose of all compounds against the target protein. (a) Backbone RMSD profiles demonstrating stabilization of the protein upon binding with compounds **7a**, **7m**, and **7o**, with the complex maintaining lower deviation relative to the apo form. (b) Residue-wise RMSF (Root Mean Square Fluctuation) analysis showing reduced fluctuations in the binding-site region upon ligand accommodation.

showing lower RMSD values and reduced residue fluctuations compared to the apo protein. The computational results support the experimental findings and suggest that the conserved heteroaromatic scaffold is the key contributor to binding stability.

CRediT authorship contribution statement

Amol S. Kulal: Writing – original draft, Methodology, Investigation, Conceptualization. **Shavi Thakur:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Ranjitsinh C. Dabhi:** Writing – original draft, Investigation, Conceptualization. **Vidhi A. Trivedi:** Visualization, Formal analysis.

Ashish Patel: Methodology, Investigation, Formal analysis. **Anjali B. Thakkar:** Investigation, Formal analysis. **Parth Thakor:** Investigation, Formal analysis. **Bhimsen Rout:** Visualization, Formal analysis. **Tushar N. Patel:** Visualization, Formal analysis. **Nandu S. Khairnar:** Visualization, Formal analysis. **Sumit Mittal:** Validation, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgment

The authors are thankful to Kadi Sarva Vishwavidhyalaya (MMPRC/2023-24/09) for providing the grant of minor research project titled “Spectroscopic, Dielectric studies and Biological Activity of liquid Crystalline derivatives with some new Heterocyclic Compounds.” and principal Dr. Keyur Shah, Shri M. M. Patel Institute of Sciences and Research, KSV, Gandhinagar, Gujarat, for providing research facilities. Authors are also thankful to Piramal Pharma Limited, Ahmedabad, Gujarat for providing support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.molstruc.2026.146668](https://doi.org/10.1016/j.molstruc.2026.146668).

Data availability

The data that has been used is confidential.

References

- [1] A. Kumar, K. Singh, K. Kumar, A. Singh, A. Tripathi, L. Tiwari, Drug resistance in cancer therapy: mechanisms, challenges and strategies, *Asian J. Nurs. Educ. Res.* 14 (2024) 95–100.
- [2] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, et al., Drug resistance in cancer: an overview, *Cancers* 6 (2014) 1769–1792.
- [3] H. Zahreddine, K.L. Borden, Mechanisms and insights into drug resistance in cancer, *Front. Pharmacol.* 4 (2013) 28.
- [4] R. Sharma, A. Mazumder Salahuddin, R. Kumar, A. Chauhan, M.J. Ahsan, et al., Pyrazoline derivatives: exploring the synthesis and development of new ligands for anti-cancer therapy, *Med. Chem.* 21 (2025) 938–955.
- [5] N. Maciejewska, M. Olszewski, J. Jurasz, M. Serocki, M. Dzierzynska, K. Cekala, et al., Novel chalcone-derived pyrazoles as potential therapeutic agents for the treatment of non-small cell lung cancer, *Sci. Rep.* 12 (2022) 3703.
- [6] Z. Xu, Y. Zhuang, Q. Chen, Current scenario of pyrazole hybrids with in vivo therapeutic potential against cancers, *Eur. J. Med. Chem.* 257 (2023) 115495.
- [7] E.S. Nossier, S.S. Abd El-Karim, N.M. Khalifa, A.S. El-Sayed, E.S. Hassan, S.M. El-Hallouty, Kinase inhibitory activities and molecular docking of a novel series of anticancer pyrazole derivatives, *Molecules* 23 (2018) 3074.
- [8] Y. Liu, Q. Tian, J. Ge, X. Wu, Z. Li, G. Cheng, Recent advances in the synthesis of trifluoromethyl-containing heterocyclic compounds via trifluoromethyl building blocks, *Org. Biomol. Chem.* 22 (2024) 6246–6276.
- [9] S. Schiesser, H. Chepliaka, J. Kollback, T. Quennesson, W. Czechtizky, R.J. Cox, N-Trifluoromethyl amines and azoles: An underexplored functional group in the medicinal chemist's toolbox, *J. Med. Chem.* 63 (2020) 13076–13089.
- [10] N.R. Emmadi, C. Bingi, S.S. Kotapalli, R. Ummanni, J.B. Nanubolu, K. Atmakur, Synthesis and evaluation of novel fluorinated pyrazolo-1, 2, 3-triazole hybrids as antimycobacterial agents, *Bioorg. Med. Chem. Lett.* 25 (2015) 2918–2922.
- [11] A. Mordini, G. Reginato, M. Calamante, L. Zani, Stereoselective synthesis of polysubstituted piperazines and oxopiperazines. Useful building blocks in medicinal chemistry, *Curr. Top. Med. Chem.* 14 (2014) 1308–1316.
- [12] A.K. Rath, R. Syed, H.S. Shin, R.V. Patel, Piperazine derivatives for therapeutic use: a patent review (2010–present), *Expert Opin. Ther. Pat.* 26 (2016) 777–797.
- [13] A.E. Evren, L. Yurttaş, B. Ekselli, G. Akalin-Ciftci, Novel tri-substituted thiazoles bearing piperazine ring: Synthesis and evaluation of their anticancer activity, *Lett. Drug Des. Discov.* 16 (2019) 547–555.
- [14] R. Gérardy, J.C. Monbaliu, Multistep continuous-flow processes for the preparation of heterocyclic active pharmaceutical ingredients. *Flow Chemistry for the Synthesis of Heterocycles*, 2018, pp. 1–2.
- [15] V. Ivasiv, C. Albertini, A.E. Gonçalves, M. Rossi, L. Bolognesi, Molecular hybridization as a tool for designing multitarget drug candidates for complex diseases, *Curr. Top. Med. Chem.* 19 (2019) 1694–1711.
- [16] Y. Luan, J. Li, J.A. Bernatchez, R. Li, Kinase and histone deacetylase hybrid inhibitors for cancer therapy, *J. Med. Chem.* 62 (2018) 3171–3183.
- [17] E.M. Domínguez-Martín, A.M. Díaz-Lanza, C.M. Faustino, Anticancer hybrid combinations: mechanisms of action, implications and future perspectives, *Curr. Pharm. Des.* 24 (2018) 4312–4333.
- [18] K. Świątek, G. Utecht-Jarzyńska, M. Jasiński, Exercise in 1-aryl-3-CF₃-1H-pyrazoles: regioselective synthesis of 4-/5-iodides and cross-coupling reactions, *RSC Adv* 15 (2025) 9225–9229.
- [19] M. Konwar, M. Saikia, R. Hazarika, D. Sarma, Nickel chloride catalyzed synthesis of pyrazoles and phthalazin-1 (2H)-ones from hydrazines at room temperature, *Tetrahedron Lett* 98 (2022) 153842.
- [20] G.K. Rastogi, M.L. Deb, P.K. Baruah, Copper-catalysed dehydrogenative self-coupling/cyclization of 5-aminopyrazoles: synthesis and photophysical study of pyridazines, *Chem. Commun.* 59 (2023) 9642–9645.
- [21] B. Maggino, D. Raffa, M.V. Raimondi, F. Plescia, M.L. Trincavelli, C. Martini, et al., Synthesis, benzodiazepine receptor binding and molecular modelling of isochromeno [4, 3-c] pyrazol-5 (1H)-one derivatives, *Eur. J. Med. Chem.* 54 (2012) 709–720.
- [22] V. Aran, J. Omerovic, Current approaches in NSCLC targeting K-RAS and EGFR, *Int. J. Mol. Sci.* 20 (2019) 5701.
- [23] Y. Xiao, P. Liu, J. Wei, X. Zhang, J. Guo, Y. Lin, Recent progress in targeted therapy for non-small cell lung cancer, *Front. Pharmacol.* 14 (2023) 1125547.
- [24] Y. Zhao, D.G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals, *Theor. Chem. Acc.* 120 (2008) 215–241.
- [25] H.S. Park, Y.K. Kang, Which DFT levels of theory are appropriate in predicting the prolyl cis–trans isomerization in solution? *New J. Chem.* 44 (2019) 17159–17173.
- [26] S. Kozuch, J.M.L. Martin, Halogen bonds: Benchmarks and theoretical analysis, *J. Chem. Theory Comput.* 9 (2013) 1918–1931.
- [27] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, Comparison of simple potential functions for simulating liquid water, *J. Chem. Phys.* 2 (1983) 926–935.
- [28] J.C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kale, K. Schulten, Scalable Molecular Dynamics with NAMD, *J. Comput. Chem.* 26 (2005) 1781–1802.
- [29] J. Huang, A.D.J. MacKerell, CHARMM36 All-Atom Additive Protein Force Field: Validation Based on Comparison to NMR Data, *J. Comput. Chem.* 34 (2013) 2135–2145.
- [30] H.C. Andersen, Rattle: A “Velocity” Version of the Shake Algorithm for Molecular Dynamics Calculations, *J. Comput. Phys.* 52 (1983) 24–34.
- [31] J.A. Izaguirre, D.P. Catarello, J.M. Wozniak, R.D. Skeel, Langevin stabilization of molecular dynamics, *J. Chem Phys* 114 (2001) 2090–2098.
- [32] P.A. Kollman, et al., Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models, *Acc Chem Res* 33 (2000) 889–897.
- [33] H. Liu, T.J. Hou, CaFE: a tool for binding affinity prediction using end-point free energy methods, *Bioinformatics* 32 (2016) 2216–2218.
- [34] P. Thakor, R.B. Subramanian, S.S. Thakkar, A. Ray, V.R. Thakkar, Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor Glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549), *Biomed. Pharmacother.* 92 (2017) 491–500.
- [35] K. Świątek, G. Utecht-Jarzyńska, M. Palusiak, J.A. Ma, M. Jasinski, One-pot synthesis of 1-Aryl-3-Trifluoromethylpyrazoles using nitrile imines and mercaptoacetaldehyde as a surrogate of acetylene, *Org. Lett.* 25 (2023) 4462–4467.
- [36] M. Konwar, P. Phukan, A.K. Chaliha, A.K. Buragohain, K. Damarla, D. Gogoi, et al., An Unexplored Lewis Acidic Catalytic System for Synthesis of Pyrazole and its Biaryl Derivatives with Antimicrobial Activities through Cycloaddition-Iodination-Suzuki Reaction, *ChemistrySelect* 4 (2019) 10236–10245.
- [37] X. Chen, J. She, Z.C. Shang, J. Wu, P. Zhang, Room-temperature synthesis of pyrazoles, diazepines, β -enaminones, and β -enamino esters using silica-supported sulfuric acid as a reusable catalyst under solvent-free conditions, *Synth. Commun.* 39 (2009) 947–957.
- [38] J. Rimland, A. Dunne, S.S. Hunjan, R. Sasse, I. Uings, D. Montanari, et al., The identification a novel, selective, non-steroidal, functional glucocorticoid receptor antagonist, *Bioorg. Med. Chem. Lett.* 20 (2010) 2340–2343.
- [39] A.B. Thakkar, R.B. Subramanian, V.R. Thakkar, P. Thakor, Hydromethanolic leaves extract of *Dalbergia sissoo* Roxb. Ex DC. Induces apoptosis in lung adenocarcinoma cells, *Process Biochem* 134 (2023) 250–261.
- [40] P.A. Halim, S.M. Sharkawi, M.B. Labib, Novel pyrazole-based COX-2 inhibitors as potential anticancer agents: Design, synthesis, cytotoxic effect against resistant cancer cells, cell cycle arrest, apoptosis induction and dual EGFR/Topo-1 inhibition, *Bioorg. Chem.* 131 (2023) 106273.
- [41] U. Pathy, M.A. Azzam, F. Mahdy, S. El-Maghraby, R.M. Allam, Synthesis and in vitro anticancer activity of some novel tetrahydroquinoline derivatives bearing pyrazol and hydrazide moiety, *J. Heterocycl. Chem.* 57 (2020) 2108–2120.
- [42] Z. Zhang, P. Cao, M. Fang, T. Zou, J. Han, Y. Duan, et al., Design, synthesis, and SAR study of novel 4, 5-dihydropyrazole-Thiazole derivatives with anti-inflammatory activities for the treatment of sepsis, *Eur. J. Med. Chem.* 225 (2021) 113743.
- [43] J.J. Sahayarayan, K.S. Rajan, R. Vidhyavathi, M. Nachiappan, D. Prabhu, S. Alfarraj, et al., In-silico protein-ligand docking studies against the estrogen protein of breast cancer using pharmacophore based virtual screening approaches, *Saudi J. Biol. Sci.* 28 (2021) 400–407.

- [44] A. Kumar (Ed.), Signal Analysis in Pharmacovigilance: Principles and Processes, CRC Press, 2024.
- [45] L. Chen, Y. He, H. Huang, H. Liao, W. Wei, Selective COX-2 inhibitor celecoxib combined with EGFR-TKI ZD1839 on non-small cell lung cancer cell lines: in vitro toxicity and mechanism study, *Med. Oncol.* 25 (2008) 161–171.
- [46] K.M. Honorio, T.L. Moda, A.D. Andricopulo, Pharmacokinetic properties and in silico ADME modeling in drug discovery, *Med. Chem.* 9 (2013) 163–176.
- [47] A.J. Lucas, J.L. Sproston, P. Barton, R.J. Riley, Estimating human ADME properties, pharmacokinetic parameters and likely clinical dose in drug discovery, *Expert Opin. Drug Discov.* 14 (2019) 1313–1327.
- [48] A.B. Thakkar, R.B. Subramanian, V.R. Thakkar, P. Thakor, Hydromethanolic leaves extract of *Dalbergia sissoo* Roxb. ex DC. induces apoptosis in lung adenocarcinoma cells, *Process Biochem* 134 (2023) 250–261.
- [49] K. Nagayama, R. Danev, Phase contrast electron microscopy: development of thin-film phase plates and biological applications, *Philos. Trans. R. Soc. B.* 363 (2008) 2153–2162.
- [50] G. Yalcin-Ozkat, Molecular modeling strategies of cancer multidrug resistance, *Drug Resist. Updat.* 59 (2021) 100789.