INTRODUCTION

One of the most important roles of medicinal chemistry is in finding new bioactive molecules, their structure elucidation, therapeutic behaviour and action mechanism [1]. Further new threats due to the emergence and diffusion of multi drug resistant bacteria have again emphasized the need for the emergence of new bioactive molecules [2,3]. Studies suggest that heteroatoms like nitrogen, sulphur, oxygen constitute more than 85% of biological active moieties [4]. Nitrogen containing heterocycles are of great importance as they are a part of pharmaceutical products as anticancer, antidepressive, antifungal and antibacterial agents and are an essential constituent of several biologically important molecules like vitamins, nucleic acids and dyes [5,6].

Among the five membered nitrogen containing heterocyclic compounds, pyrazole and imidazole motifs have shown promising biological activities in the field of medicinal and pharmaceutical chemistry [7,8]. Pyrazole moieties possess excellent anticancer [9], antibacterial [10], anti-inflammatory [11], antifungal [12] and analgesic activities [13], etc. Studies also show that the pyrazoles along with other heterocyclic compounds act as potent anticancer agents targeting EGFR tyrosine kinase and FabH [14-17].

Also, imidazole is known to exhibit excellent pharmacokinetic properties and is also present in various natural products like purines, nucleic acids and histamines [8]. Imidazole displays excellent anticancer activity [18] and therefore provides new areas in the designing of new anticancer drugs along with several new pharmacophores. Finally, from the molecular design point of view, combining two such promising pharmacophores into one single unit using one pot multicomponent reaction (MCR) was considered in the given study. The excellent biological activities of both pyrazole and imidazole were taken into consideration while carrying out the current study. In the present work, we have tried to synthesize a new antimicrobial moiety by combining pyrazole and imidazole derivatives and did the biological evaluation for any significant changes and carried out DFT studies and molecular docking for the same.

Herein, the synthesis, biological evaluation, DFT studies and molecular docking of novel 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile and its derivatives using one pot MCR approach is reported.
**EXPERIMENTAL**

All the chemicals were purchased commercially and used as such. Solvents like DMF and ethanol used were of analytical grade. Potassium carbonate and pyridine were used as catalyst in the synthesis route. TLC was carried out to closely monitor all the steps. Elemental analysis (% C, H, N and O) was done with the help of CHN/S/O elemental analyzer 2400 Series II, Perkin-Elmer. A mass spectrum for all the synthesized compounds was done using LCMS spectrometer was involved in $^1$H NMR and $^{13}$C NMR spectra in DMSO-$d_6$, with solvent peak as internal standard. IR Spectra was also investigated for the synthesized compounds with the help of Perkin Elmer Spectrum-GX spectrophotometer.

**Synthesis of 3-methyl-1-phenyl-1H-pyrazole-5-one (3a-d):**

The starting compound 3-methyl-1-phenyl-1H-pyrazole-5-one (3a-d) was synthesized by refluxing an equimolar mixture of 3-methyl-1-phenyl-5-chloro-1H-pyrazole-4-carbaldehyde with imidazole. The crude product was filtered, washed with water to remove all acidic precipitates were filtered and washed with water to remove all acidic.

**Synthesis of 3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (4a-d):**

3-Methyl-1-phenyl-1H-pyrazole-5-one (3a-d) was then converted to 3-methyl-1-phenyl-5-chloro-1H-pyrazole-4-carbaldehyde (4a-d) using the VMH reaction where equimolar amount of POCl$_3$ (0.4 mol) was added dropwise to 3-methyl-1-phenyl-5-chloro-1H-pyrazole-4-carbaldehyde (4a-d), the reaction mixture was cooled to room temperature and then kept in an ice-bath. To a mixture ether and 13C NMR spectra in DMSO-$d_6$ with solvent peak as internal standard. IR Spectra was also investigated for the synthesized compounds with the help of Perkin Elmer Spectrum-GX spectrophotometer.

**Synthesis of 3-methyl-1-phenyl-1H-pyrazole-5-carboxaldehyde (6a-d):**

The aldehyde compound 3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (6a-d) was synthesized by refluxing an equimolar mixture of 3-methyl-1-phenyl-5-chloro-1H-pyrazole-4-carbaldehyde (4a-d) with imidazole (5) for 2 h at 85 °C in presence of anhydrous K$_2$CO$_3$ as a base catalyst and DMF as a solvent. To isolate 5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (6a-d), the reaction mixture was cooled to room temperature, poured onto ice water and stirred constantly until the precipitate formed. It was a nucleophilic substitution of the chloro group by imidazole. The crude product was filtered, washed with water and recrystallized with ethanol with an overall yield of 67-70%.

**Synthesis of target molecule 4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9a-h):** Target molecules 9a-h were synthesized using the one pot multi-component reaction approach. For this calculated amount of the reaction mixture consisting of 5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (6a-d), 2-amino acetophenine (7) and 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazole-3-one (8a-b) was refluxed for 3-4 h using ethanol as a solvent and pyridine in catalytic amount was added to the round bottom flask. After the reaction was completed, the reaction mixture was brought to room temperature when the crude product separated out and was washed finally with ethanol to obtain the pure form (Scheme-I).

4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9a): Yield: 85%. IR (KBr, $\nu_{\text{max}}$, cm$^{-1}$): 3320 and 3075 (asym. and sym. str. -NH$_2$), 2140 (C≡N str.). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm: 1.82, 1.89 (s, 3H, 2×CH$_3$), 4.76 (s, 1H, H4), 8.41 (s, 2H, NH$_2$), 6.73-7.90 (m, 13H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm: 13.82, 14.10 (CH$_3$), 26.42 (C4), 57.56 (C-CN), 97.93, 108.18, 114.35, 118.30, 119.56, 120.24, 121.00, 125.31, 130.13, 131.01, 132.45, 132.97, 133.56, 137.34, 145.25, 146.15, 147.74, 155.21, 160.83 (Ar-C). MS (m/z): 474.1 (M$^+$). Anal. calcd. (found) % for C$_{28}$H$_{24}$N$_{8}$O: C, 66.65 (66.69); H, 4.79 (4.75); N, 23.61 (23.47).

4-(5-(1H-imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazole-4-yl)-6-amino-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9b): Yield: 82%. IR (KBr, $\nu_{\text{max}}$, cm$^{-1}$): 3345 and 3090 (asym. and sym. str. -NH$_2$), 2115 (C≡N str.). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm: 1.92, 1.98, 2.41 (s, 3H, 3×CH$_3$), 4.64 (s, 1H, H4), 8.38 (2H, NH$_2$), 6.78-7.72 (m, 12H, Ar-H). $^{13}$C NMR spectrum (400 MHz, DMSO-$d_6$) $\delta$ ppm: 13.71, 14.15, 21.18 (CH$_3$), 26.27 (C4), 57.10 (C-CN), 96.98, 109.11, 115.50, 118.82, 119.00, 120.54, 121.77, 126.43, 130.07, 130.67, 132.12, 132.92, 135.38, 137.08, 145.43, 145.99, 147.14, 154.25, 160.34 (Ar-C). MS (m/z): 488.2 (M$^+$). Anal. calcd. (found) % for C$_{25}$H$_{26}$N$_{8}$O: C, 68.84 (68.71); H, 4.95 (4.77); O, 3.27 (3.45); N, 22.94 (23.07).
(s, 2H, NH2), 6.90-7.81 (m, 12H, Ar-H). 13C NMR (400 MHz, DMSO-$d_6$) δ ppm: 13.87, 14.09 (CH3), 26.48 (C4), 57.51 (C-CN), 97.00, 108.45, 115.12, 118.37, 119.10, 120.55, 121.56, 125.74, 130.27, 131.00, 132.60, 133.16, 135.74, 137.40, 145.45, 146.67, 147.40, 155.30, 160.23 (Ar-C). MS (m/z): 508.1 (M+).

Anal. calcd. (found) % for C27H21N8OCl (m.w. 508.97): C, 63.72 (68.84); H, 4.16 (4.23); Cl, 6.97 (6.72); O, 3.14 (3.24); N, 22.02 (21.97).

4-(5-(1H-Imidazol-1-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1-(p-tolyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9e): Yield: 83%. IR (KBr, νmax, cm$^{-1}$): 3320 and 3085 (asym. and sym. -NH2), 2145 (-C≡N str.). 1H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.84, 1.92, 2.40 (s, 3H, 3×CH3), 4.76 (s, 1H, H4), 8.42 (s, 2H, NH2), 6.82-7.78 (m, 12H, Ar-H). 13C NMR (400 MHz, DMSO-$d_6$) δ ppm: 13.86, 14.18, 21.24 (CH3), 26.28 (C4), 57.44 (C-CN), 97.32, 109.56, 115.11, 118.56, 119.20, 120.43, 121.57, 126.35, 130.64, 131.14, 132.78, 133.46, 136.00, 137.45 145.72, 146.34, 147.65, 154.90, 160.38 (Ar-C). MS (m/z): 488.2 (M$^+$). Anal. calcd. (found) % for C28H24N8O (m.w. 488.56): C, 68.84 (69.02); H, 4.95 (5.12); O, 3.27 (3.39); N, 22.94 (22.73).

4-(5-(1H-Imidazol-1-yl)-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-6-amino-3-methyl-1-(p-tolyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9f): Yield: 78%. IR (KBr, νmax, cm$^{-1}$): 3340 and 3075 (asym. and sym. -NH2), 2130 (-C≡N str.). 1H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.90, 1.96, 2.38, 2.46 (s, 3H, 4×CH3), 4.72 (s, 1H, H4), 8.50 (s, 2H, NH2), 6.88-7.80 (m, 11H, Ar-H). 13C NMR (400 MHz, DMSO-$d_6$) δ ppm: 13.82, 14.11, 21.14, 21.22 (CH3), 26.21 (C4), 57.43 (C-CN), 97.12, 109.35, 115.21, 118.45, 119.45, 120.00, 121.70, 126.34, 130.45, 130.98, 132.60, 133.12, 135.88, 137.75 145.60, 146.12, 147.78, 154.45, 160.65 (Ar-C). MS (m/z): 502.2 (M$^+$). Anal. calcd. (found) % for C29H26N8O (m.w. 502.58): C, 69.31 (69.20); H, 5.21 (4.97); O, 3.18 (3.35); N, 22.30 (22.48).

4-(5-(1H-Imidazol-1-yl)-1-(4-methoxyphenyl)-3-methyl-1H-pyrazol-4-yl)-6-amino-3-methyl-1-(p-tolyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9g): Yield: 82%. IR (KBr, νmax, cm$^{-1}$): 3345 and 3015 (asym. and sym. -NH2), 2130 (-C≡N str.).

Scheme-I: Synthesis of compounds 9a-h (Reagents and conditions: (i) DMF, POCl$_3$, reflux; (ii) DMF, K$_2$CO$_3$, reflux; (iii) EtOH, piperidine, reflux)

4-(5-(1H-Imidazol-1-yl)-3-methyl-1-(p-tolyl)-1H-pyrazol-4-yl)-6-amino-3-methyl-1-(p-tolyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9e): Yield: 83%. IR (KBr, νmax, cm$^{-1}$): 3340 and 3075 (asym. and sym. -NH$_2$), 2130 (-C≡N str.). 1H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.90, 1.96, 2.38, 2.46 (s, 3H, 4×CH$_3$), 4.72 (s, 1H, H4), 8.50 (s, 2H, NH$_2$), 6.88-7.80 (m, 11H, Ar-H). 13C NMR (400 MHz, DMSO-$d_6$) δ ppm: 13.82, 14.11, 21.14, 21.22 (CH$_3$), 26.21 (C4), 57.43 (C-CN), 97.12, 109.35, 115.21, 118.45, 119.45, 120.00, 121.70, 126.34, 130.45, 130.98, 132.60, 133.12, 135.88, 137.75 145.60, 146.12, 147.78, 154.45, 160.65 (Ar-C). MS (m/z): 502.2 (M$^+$). Anal. calcd. (found) % for C$_{29}$H$_{26}$N$_8$O (m.w. 502.58): C, 69.31 (69.20); H, 5.21 (4.97); O, 3.18 (3.35); N, 22.30 (22.48).

4-(5-(1H-Imidazol-1-yl)-1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-6-amino-3-methyl-1-(p-tolyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (9g): Yield: 82%. IR (KBr, νmax, cm$^{-1}$): 3345 and 3015 (asym. and sym. -NH$_2$), 2130 (-C≡N str.).
Synthesis, Biological Evaluation, DFT Studies and Molecular Docking of Pyrazole-5-carbonitrile and its Derivatives

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Two pyrazole rings were noted as twist angles between two substituted phenyl pyrazole rings and other two planes were passed through the molecule in order to understand the influence of substituents at R1 and R2 positions. The angle between two planes of the particular pharmacophore and their potency to guide the SAR, the interaction of the synthesized compounds with EGFR (PDB code: 1M17) was examined by docking the protein and ligand molecules into the active pocket of the protein. The protein structure was corrected for any structural error using homology model preparation and protonation of the molecule with partial charge into MOE software. The prepared ligand molecules were then docked at the ligand site with induced fit refinement.

With FabH: Similarly, to get the understanding of the behaviour of the particular pharmacophore and their potency to guide the SAR, molecular docking of compounds and E. coli FabH was performed and examined on the binding model based on E. coli FabH-CoA complex structure (PDB code: 1HJN).

RESULTS AND DISCUSSION

The IR spectra was carried out for all the eight synthesized compounds 9a-h in order to confirm the type of functional groups present in them. The symmetric and asymmetric stretching bands of the primary amine (-NHR) were observed in the region of 3090-3015 and 3350-3320 cm⁻¹ for compounds 9a-h. The stretching bands for cyano (–CN) group in compounds 9a-h appeared in the range of 2160-2115 cm⁻¹. A C=Cl stretching was observed for compounds 9d and 9h in the 718 cm⁻¹ region.

¹H NMR was carried out to confirm the structure of all the eight synthesized compounds 9a-h. A singlet for three protons each in two different methyl groups was observed in the region of δ 1.80-1.98 ppm for all the compounds 7a-h whereas in compounds 9b, 9e, 9g and 9h a singlet for third methyl group as R1 substitution and in compound 9f a singlet for fourth methyl group as R2 substitution with three protons appeared in the region of δ 2.38-2.42 ppm. Three protons as R3 substitution of methoxy group in compounds 9c and 9g resonated in the region of δ 3.72-3.75 ppm. An aromatic chiral proton for all the synthesized compounds 9a-h was observed in the region of δ 6.73-7.90 ppm. A singlet for two protons of the amino group in all the synthesized compounds 9a-h was observed in the range of δ 6.38-6.58 ppm.

All the synthesized compounds (9a-h) were analyzed for their ¹³C NMR absorption spectra. The two methyl groups present on the pyrazole ring and pyrano-pyrazole ring of all synthesized compounds 9a-h showed signals in the range of δ 21.14-22.10 ppm. Active methylene (C4) displayed a single line in the range of δ 26.15-25.54 ppm for compounds 9a-h. The presence of a nitrite carbon atom in all eight compounds 9a-h was confirmed by a single peak at δ 57.10-58.04 ppm. A methoxy group substitution at R1 in compounds 9e and 9g was confirmed by a single line in the range of δ 54.05-54.25 ppm. A multiplet of aromatic carbon atoms for compounds 9a-h was confirmed in the range of δ 97-160.83 ppm.

Biological evaluation

Antiproliferation and EGFR inhibitory activity: All the synthesized eight compounds having two phenyl pyrazole
groups with various substitution matrix were tested for EGFR inhibitory activity as well as antiproliferation activity against known lung cancer cell line A549 known as adenocarcinoma human alveolar basal epithelial cell line and liver cancer cell line HepG2. In case of EGFR inhibition, the mode of action to inhibit tyrosine kinase is well known, by stopping the transferring of signal between the two EGFR molecules. When the synthesized molecules were tested for these activities, it was found that compound 9g showed most potent activity amongst the synthesized compounds with IC50 of 0.11 ± 0.02 µM, while compounds 9d and 9a showed compared good activity with IC50 of 0.18 ± 0.06 µM and IC50 of 0.21 ± 0.04 µM, respectively. In case of antiproliferative activity against A549 and Hep G2 compound 9d showed most potent activity with IC50 of 1.21 ± 0.05 µM and 1.96 ± 0.03 µM, respectively, whereas compound 9e showed potency against the A549 with IC50 of 1.52 ± 0.11 µM and compound 9d showed potency against the Hep G2 with IC50 of 2.84 ± 0.11 µM, although among all the synthesized compounds only compound 9d showed the nearest potency compared to the standard erlotinib.

E. coli FabH inhibitory activity: The E. coli FabH inhibitory activity of the synthesized eight compounds 9a-h were examined and their result as a concentration in micro mole is represented in Table-1. Among eight compounds, three of them showed a fair inhibitory activity. Compound 9h showed the most potent inhibitory activity with the IC50 of 2.6 µM, while compound 9a showed inhibitory action at the IC50 of 3.2 µM and compound 9g showed inhibitory action at the IC50 of 5.4 µM. While the other derivatives 9b, 9c, 9d, 9e and 9f showed the inhibitory action at the IC50 of 14.2 µM, 12.7 µM, 6.3 µM, 10.2 µM and 15.4 µM, respectively.

Computational studies

Density functional theory: It has been generally accepted that materials will be more reactive and less kinetically stable the smaller the molecular orbital energy gap ∆E [20,21]. However, recent research has shown that this conventional relationship is not always followed [22]. The theoretical values of the energy gap of molecular orbital obtained from DFT study is represented in Table-2. The molecular surface plot of all the prepared molecules is represented in Fig. 1. It can be observed that in molecules 9a and 9g the HOMO density is spread over the center of the molecule indicating easy mobility of electron around the center enhancing inter molecular charge transfer while LUMO is delocalized over the whole R1 phenyl pyrazole fraction of the molecule, in molecule 9b and 9c, the HOMO

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<th>E. coli FabH IC50 (µM)</th>
<th>Hemolysis LC30a (mg/mL)</th>
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<td>3.936</td>
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<td>2.980</td>
<td>2.390</td>
<td>2.640</td>
<td>2.204</td>
</tr>
<tr>
<td>ΔN_{occ} = ω/η</td>
<td>3.113</td>
<td>1.652</td>
<td>1.500</td>
<td>2.235</td>
<td>1.722</td>
<td>2.369</td>
<td>1.411</td>
<td>2.231</td>
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density is localized over R₁ pyrazole ring and LUMO is delocalized over the R₁ phenyl pyrazole fraction of molecule, in 9d molecule HOMO density is localized over R₁ phenyl ring and LUMO is localized over R₁ phenyl ring, this localization of density over phenyl ring indicating favourable atomic center within phenyl rings for possible nucleophilic attack. In molecules 9e, 9f and 9h the HOMO density is delocalized over the R₁ phenyl pyrazole fragment of the molecule and LUMO is localized over on R₂ phenyl ring. Other parameters based on the molecular orbital energy level gap are the softness, hardness, global softness, absolute softness, chemical potential, global electrophilicity, electronegativity and additional electronic charge are reported in Table-2 with the molecular energy, twist angles between planes and dipole moment. It is clear that the correlation between molecule softness and reactivity does not hold, hence this kind of information on its own is insufficient to account for molecule reactivity.

**Molecular docking study:** From the inhibition concentration data of EGFR kinase inhibition and antiproliferative activities it was observed that among all the prepared compounds, compound 9g exhibited greater potency against all three cancer cell lines, which are EGFR, A549 and HepG2 with the IC₅₀ of 0.11 ± 0.02, 1.21 ± 0.05 and 1.96 ± 0.03, respectively. Compound 9h showed least activity against the EGFR with IC₅₀ of 4.03 ± 0.26, while compound 9c showed the least activity against A549 cell line and HepG2 IC₅₀ of 8.57 ± 0.31 and 9.26 ± 0.17, this can be attributed to the electron donating and electron withdrawing groups at the R₂ position as they are of opposite character and can affect the molecular orbital localization at the R₁ phenyl group as well as localization of occupied orbital density over the core. This change in substitution bring a change in the twist angle between the aromatic part of the molecule allowing the molecule to fit perfectly in the pocket of EFGR, which can be quantitatively seen in Table-2 and qualitatively observed in Fig. 2. A strong interaction of compound 9g with protein with residues CYS773, ARG817, ASP831 and LYS721, among which former three residues form two hydrogen or one hydrogen and one π hydrogen bond with the binding energy of -9.0233 kcal/mol. These interaction of compound 9g with protein residues are shown in Fig. 3.

The binding energy of all compounds are summarized in Table-3. It was found that compound 9d was bound into the active pocket of the EGFR with the minimum binding energy ΔGᵣ of -9.2430 kcal/mol, the 2D and 3D binding interaction of compound 9d is showed in Fig. 4. All the amino acid residues that had interacted with the ligand within the active radius of about 5 Å are labeled. Binding showed that compound 9d bound in the site of EGFR through hydrophobic interaction. The binding was stabilized by seven hydrogen bonds, among which one hydrogen bond formed between the chlorine at R₁ substitution and GLN767 with bond distance of 2.87 Å, second and third hydrogen bond between pyrazolidine nitrogen and LYS721 and pyrazolidine nitrogen and ASP831 with respective bond

<table>
<thead>
<tr>
<th>Table-3</th>
<th>BINDING ENERGIES OF COMPOUNDS 9a-h HAVING ERLOTINIB WITH EGFR AND MALONYL CoA WITH FabH</th>
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<tbody>
<tr>
<td>Compound</td>
<td>EGFR</td>
</tr>
<tr>
<td>9a</td>
<td>-7.8844</td>
</tr>
<tr>
<td>9b</td>
<td>-7.2793</td>
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<tr>
<td>9c</td>
<td>-7.3714</td>
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<tr>
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<td>9f</td>
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<td>9g</td>
<td>-9.0233</td>
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<tr>
<td>9h</td>
<td>-7.6501</td>
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</table>
Fig. 2. Twist angle 1 of 9a-h

Fig. 3. 2D and 3D Binding model of compound 9g into the active pocket of EGFR

Fig. 4. 2D and 3D Binding model of compound 9d into the active pocket of EGFR
distance of 3.18 Å and 1.87 Å. Fourth was between the amine nitrogen and ARG817 with distance of 1.82 Å while the fifth and sixth bond was between the nitrogen of the cyanide with ARG817 and CYS773 with respective bond length of 3.62 Å and 3.75 Å and the seventh hydrogen bond was between the CYS773 and nitrogen of the imidazole with bond length of 3.65 Å. From this binding model, it could be concluded that these seven hydrogen bonds from the pyrazole, imidazole as well as cyanide and amine group with the pocket are responsible for the effective EGFR inhibitory of compound 9d.

From the inhibition concentration data of FabH E. coli for all the synthesized compounds, it was observed that among them compound 9h exhibited greater potency with the IC\textsubscript{50} concentration of 2.6 µM, while compound 9f showed the least potency against E. coli with the IC\textsubscript{50} concentration of 15.4 µM, from the docking score it can be seen that compound 9b bound with the least binding energy with the protein, but among the three binding sites in the docking results, two of them are of receptor exposure, while in the docking result of compound 9h, there is one strong π–π interaction between the R\textsubscript{1} aromatic ring and aromatic ring of residue TRP32 and two π–H bond with residue PHE213 and MET207 as can be seen in Fig. 5 and hence shows a greater activity despite comparative lower binding energy than compound 9b.

A catalytic triad tunnel composed of Cys-His-Asn is found in the active site of the FabH, which is found in several bacteria. This triad which works as catalysis, plays an important role in the controlling of chain elongation as well as substrate binding and hence the alkyl chain of the CoA is broken at the Cys residue of the triad of FabH, the interaction occurring between the Cys and substrate seems to playing a significant role in the binding of the substrate. From studied eight compounds, compound 9b was found to be bound strongly into the active pocket of the FabH with the binding energy Δ\textsubscript{G} of -8.0856 kcal/mol on primary analysis of docking results. Binding affinity score of all the prepared compounds is represented in

![Fig. 5. 2D and 3D Binding model of compound 9h into the active pocket of FabH](image_url)

![Fig. 6. 2D and 3D Binding model of compound 9b into the active pocket of FabH](image_url)
Table-3. The interactive binding model of compound 9b with the active site of FabH protein in 2D and 3D model is depicted in Fig. 6, respectively. Where it can be seen that in figures that among all three hydrogen bonds, one was formed between cyanide nitrogen and PHE213 with bond distance of 3.35 Å and the other two are strong receptor exposure with amine nitrogen and ARG249 and one between pyrazole nitrogen and ARG36. From this interaction and the binding score, it can be primarily concluded that the strong hydrogen bonding is responsible for the effective FabH inhibitory of compound 9b in docking results.

Conclusion

A series of molecule with phenyl pyrazole molecules as a pharmacophore with small substitutions and imidazole as well as primary amine and cyanide groups as potential binding sites is designed and synthesized. Among the prepared compounds, a small but significant number of biological results were obtained, leading us to the conclusion that using the same type of active groups, like phenyl pyrazole as in the present work, increases the probability of binding with greater affinity to the active pocket of the proteins. In current work, compound 9g was found to be the most active against EGFR, A549 and HepG2, while compound 9h was found to be most active against FabH E. coli, although other members of the synthesized series were also found to be active with comparatively higher inhibition concentration. In addition to the molecular docking, DFT study were also performed to evaluate the distance and angle between the active parts of the molecules as well as distribution of the charge density over the molecule affecting the binding of molecule in the active pocket with greater binding affinity. From this study and observation, it can be concluded that molecules with multiple pharmacophores can be used as template and can be further engineered for the target-based application, discovering more scope as well as any biological activity limitations.

ACKNOWLEDGEMENTS

The authors are thankful to Shri Maneklal M. Patel Institute of Sciences and Research, Kadi Sarva Vishwavidhyalaya for giving infrastructure and laboratory facility.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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