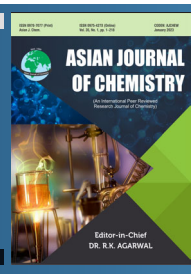
Asian Journal of Chemistry; Vol. 35, No. 4 (2023), 828-838

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.26894



Synthesis, Characterization and Molecular Modeling of Pyrazole-Quinoline Hybrids as New Class of Antibacterial, Antimicrobial, Anticancer Agents and DFT Study

RAJAT PATEL*, PUJA SHARMA, ROHIT R. KOSHTI, AKSHAY VYAS and CHETAN B. SANGANI

Shri Maneklal M. Patel Institute of Sciences & Research, Kadi Sarva Vishwavidyalaya, Gandhinagar-382024, India

*Corresponding author: E-mail: rajatmsc93@gmail.com

Received: 7 October 2022; *Accepted*: 21 January 2023; *Published online*: 30 March 2023; AJC-21178

A new series of pyrazole-quinoline hybrids were synthesized by a base-catalyzed cyclocondensation reaction through one-pot multi-component reaction, based on molecular hybridization techniques. All the compounds **10a-x** were examined for *in vitro* antibacterial and anticancer activities. Enzyme inhibitory activities were carried out against FabH and EGFR. From the studied compounds, most of the compounds showed effective antibacterial as well as anticancer activity against used strains and cancer cell lines, respectively. The most potent inhibitory activity was displayed by compound **10r** against EGFR and by compound **10i** against FabH. Spatial arrangement of the molecule and their HOMO-LUMO was studied and explained by DFT theory, to evaluate plane angle respective to the core and substitutions. Docking studies indicated that compound **10r** was bound to the active pocket of EGFR with hydrogen bond and π-H interaction with minimum binding energy and compound **10i** was bound to the active site of FabH with hydrogen bond and π-H interaction having minimum binding energy. Based on their substitutions, the hypothetical plane arising in the molecule and their twist angles were related with their activities against EGFR and FabH as well as antibacterial and anticancer activities.

Keywords: Pyrazole-quinoline hybrid, Molecular modeling, Enzyme inhibitory activity, One pot reaction, Anticancer activity.

INTRODUCTION

Since the occurrence of heterocyclic compounds from the nature and found major use in the pharma sector [1]. Foremost the nitrogen based heterocyclic compounds, due to their growing advantages in firstly the ease of synthesis with better biocompatibility and bioavailability, condensed drug resistance and fewer side effects proved to be a breakthrough step in the field of drug discovery [2]. Among the heterocyclic compounds with nitrogen, pyrazole and its analogs play a important role in medicinal chemistry field due to their exceptional pharmacological activities [3]. Pyrazoles, a five-membered heterocyclic compounds are especially helpful in the synthesis of organic compounds. Derivatives of the pyrazoles are considered to be a very important active scaffold pharmacologically that possesses all the types of pharmacological activities [4]. It is also known that in the presence of pyrazole nucleus in various pharmacological agents of the therapeutic classes as celecoxib, a potent anti-inflammatory, the antipsychotic CDPPB, the anti-obesity drug rimonabant, difenamizole, an analgesic, betazole, a H2-receptor agonist and the antidepressant agent fezolamide proved to be a potential agent with diversified applications [4,5]. Due to interesting pharmacological properties of the pyrazole molecules, they have grabbed the attention nowadays, the traces of the pyrazole and their derivatives can be found to be in a variety of well-established drugs [6-13]. Also, in the synthesis of new anticancer drug, pyrazole and its derivatives have emerged as a potential class of heterocyclic compounds due to their promising results for non-small cell lung cancer (A549) and liver cancer (HepG2) [14].

Nitrogen in a six-membered ring is also found to be significant in the pharma sector, which is generally known as quinoline. Quinoline and its analogs are found to have antibacterial and antifungal, anticancer, anti-inflammatory and antimalarial properties similar to the five-membered analogues pyrazole [15]. Similar to the nitrogen containing compounds, sulfur containing compounds also sows various biological activities and have their applications in the pharma sector [16-20]. Among the sulfur containing pharmacological agents, thioethers holds about 8.8% of the constituents [21]. In continuation of our

interest on the synthesis of potent pharmacological agents with various hybrid nucleus [22], 24 new analogues bearing pyrazole and quinoline nucleus with thioether group were synthesized to explore their potentiality as pharmacological agents.

EXPERIMENTAL

All the chemicals, catalyst and solvents used were of analytical grade and procured from the commerical sources. Nexus 870 FT-IR spectrophotometer was used to find out the FT-IR spectra (KBr). Bruker DPX300 model spectrometer in DMSO-d_6 was used for recording the ^{1}H NMR and ^{13}C NMR spectra and chemical shifts were reported in ppm (δ). The XT4 MP Apparatus (Taike Corp., China) was used to determine the melting points and are uncorrected. The ESI-MS spectra were recorded on a Mariner System 5304 mass spectrometer. TLC was performed on the glass backed silica gel sheets (Silica Gel 60 GF_{254}) and visualized in UV light (254 nm).

General procedure for the synthesis of 3-methyl-1-phenyl-1*H*-pyrazole-5-one (3a-b): An round bottom flask equimolar mixture of phenyl hydrazine (**1a-b**) and ethyl acetoacetate (**2**) was refluxed in a water bath at 70 ºC for 90 min using glacial acetic acid [23]. It was then brought to room temperature and then kept in an ice bath. Diethyl ether was added with constant stirring when 3-methyl-1-phenyl-1*H*-pyrazole-5-one (**3a-b**) separates out, which was filtered under vacuum and washed with ether to remove any impurities. The product was recrystallized using ethanol for recrystallization. TLC was done to ensure product formation.

General procedure for the synthesis of 3-methyl-1-phenyl-5-chloro-1*H*-pyrazole-4-carbaldehyde (4a-b): The Vilsmeier-Haack reaction was used to synthesize the starting material 3-methyl-1-phenyl-5-chloro-1*H*-pyrazole-4-carbaldehyde (**4a-b**). Phosphorous oxychloride (0.4 mol) was added to an ice-cold DMF (0.4 mol) dropwise with continuous stirring [24]. The addition procedure was designed in such a manner that it took nearly 30 min to complete. The stirring continued for 45 min further at 0 ºC. Then 3-methyl-1-phenyl-1*H*-pyrazole-5-one (0.08 mol) was added to the reaction mixture and allowed to cool to room temperature. The mixture was then refluxed for 5 h at 90 ºC, brought to room temperature and finally poured onto a mixture of crushed ice and water when the precipitates of 3-methyl-1-phenyl-5-chloro-1*H*-pyrazole-4-carbaldehyde (**4a-b**) separated out. The crude product was filtered, washed with water to remove any acidic impurities and then recrystallized using ethanol with an overall yield of 65-67%.

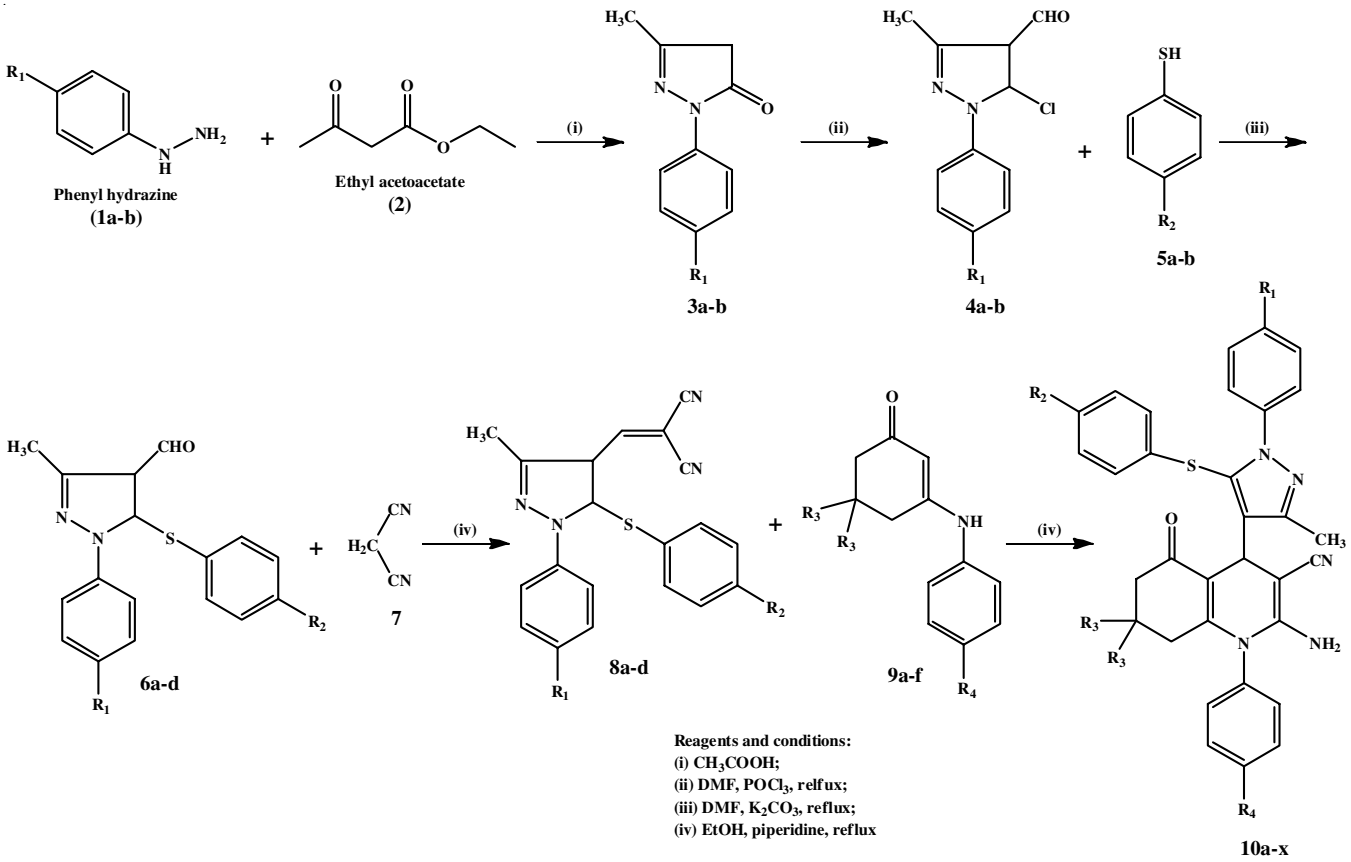
General procedure for the synthesis of 3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazole-4-carbaldehyde (6a-d): The nucleophilic substitution of chloro group with thiophenoxy group was carried out by refluxing a mixture of 3-methyl-1-phenyl-5-chloro-1*H*-pyrazole-4-carbaldehyde (**4a-b**) (5 mmol) with thiophenol (**5a-b**) (5 mmol) using anhydrous K_2CO_3 (10 mmol) as base and DMF (5 mL) as solvent. The mixture was refluxed at 85 ºC for almost 4 h and TLC was used to check the completion of the reaction [25]. After the completion of the reaction, the reaction mixture was cooled to room temperature and then poured onto chilled water with continuous stirring followed by neutralization with 1.5 N HCl when 3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazole-4-carbaldehyde (**6a-d**) separates out. The crude product was filtered, washed with water and recrystallized using ethanol with an overall yield of 68-69%.

General procedure for the synthesis of 2-amino-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10a-x): Target molecule (**10a-x**) was synthesized using one-pot MCR approach using 3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazole-4-carbaldehyde (**6a-d**) (5 mmol), malanonitrile (**7**) (5 mmol) and substituted 5-phenylamino cyclohexanone (**9a-d**) (5 mmol) in a round bottom flask containing a catalytic amount of pyridine and ethanol (10 mL) as solvent. The complete setup was refluxed for 3-4 h and the completion of the reaction was checked using TLC (ethyl acetate:hexane 1:1). Once the reaction was completed, the reaction mixture was brought to room temperature. After that, the solid product was filtered and recrystallized with ethanol (**Scheme-I**).

2-Amino-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10a): Yield 75%, IR (KBr, ν_{max}, cm^{-1}): 3483 and 3357 (asym. and sym. *str.* of -NH_2), 3012 (aromatic C-H *str.*), 2195 (-C≡N *str.*), 1675 (C=O *str.*), 1570 and 1460 (C=C *str.* of aromatic ring), 1198 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.70-2.64 (m, 6H, 3×C$\underline{H}_2$), 2.15 (s, 3H, C$\underline{H}_3$), 4.50 (s, 1H, C$\underline{H}$), 5.48 (s, 2H, N$\underline{H}_2$), 7.20-7.65 (m, 15H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 18.7, 21.4, 36.4 (3×CH_2), 31.8 (C4), 117.3 (C-CN), 57.6, 111.9, 153.3, 167.5 (C=C), 119.0, 119.9, 122.4, 122.8, 125.5, 126.2, 129.0, 129.3, 129.4, 129.5, 131.6, 138.6, 141.2, 141.8, 147.1 (Ar-C), 198.9 (C-C=O); Anal. calcd. (found) % for $C_{32}H_{27}N_5OS$ (529.7 g/mol): C, 72.56 (72.43); H, 5.14 (5.23); O, 3.02 (2.95); N, 13.22 (13.40); S, 6.05 (5.99); MS (*m/z*): 529.2 (M^+).

2-Amino-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10b): Yield 82%, IR (KBr, ν_{max}, cm^{-1}): 3471 and 3362 (asym. and sym. *str.* of -NH_2), 3021 (aromatic C-H *str.*), 2205 (-C≡N *str.*), 1693 (C=O *str.*), 1548 and 1464 (C=C *str.* of aromatic ring), 1209 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68-2.54 (m, 6H, 3×C$\underline{H}_2$), 1.86 (s, 3H, C$\underline{H}_3$), 2.24 (s, 3H, C$\underline{H}_3$), 4.65 (s, 1H, C$\underline{H}$), 5.59 (s, 2H, N$\underline{H}_2$), 6.75-7.43 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4 (CH_3), 21.7 (CH_3), 19.3, 21.9, 37.1 (3×CH_2), 31.0 (C4), 118.7 (C-CN), 57.9, 111.0, 152.7, 167.9 (C=C), 119.2, 120.4, 123.0, 124.8, 125.9, 128.0, 129.4, 129.9, 130.2, 131.6, 131.8, 138.3, 138.4, 141.8, 147.8 (Ar-C), 198.2 (C-C=O); Anal. calcd. (found) % for $C_{33}H_{29}N_5OS$ (543.7 g/mol): C, 72.90 (72.73); H, 5.38 (5.52); O, 2.94 (3.09); N, 12.88 (13.04); S, 5.90 (5.62). MS (*m/z*): 543.2 (M^+).

2-Amino-1-(4-methoxyphenyl)-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10c): Yield 79%, IR (KBr, ν_{max}, cm^{-1}): 3471 and 3362 (asym. and sym. *str.* of -NH_2), 3021 (aromatic C-H *str.*), 2225 (-C≡N *str.*), 1693 (C=O *str.*), 1548 and 1464 (C=C *str.* of aromatic ring), 1232 and 1034 (C-O-C asym & sym *str.* of -OCH_3), 1209 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72-2.58 (m, 6H, 3×C$\underline{H}_2$), 2.15 (s, 3H,



Scheme-I: Synthesis of compounds **10a-x**

C$\underline{H}_3$), 3.83 (s, 3H, OC$\underline{H}_3$), 4.52 (s, 1H, C$\underline{H}$), 5.66 (s, 2H, N$\underline{H}_2$), 6.90-7.51 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.1 (CH_3), 18.9, 21.2, 36.4 (3×CH_2), 31.4 (C4), 56.3 (OCH_3), 117.6 ($\underline{C}$-CN), 57.3, 111.3, 152.2, 167.3 (C=C), 119.4, 120.1, 122.9, 123.4, 124.7, 125.0, 128.3, 128.8, 129.5, 130.7, 131.6, 132.0, 138.9, 141.2, 147.4 (Ar-C), 197.8 ($\underline{C}$-C=O); Anal. calcd. (found) % for $C_{33}H_{29}N_5O_2S$ (559.7 g/mol): C, 70.82 (71.03), H, 5.22 (5.35), O, 5.72 (5.96), N, 12.51 (2.24), S, 5.73 (5.42); MS (*m/z*): 559.2 (M^+).

2-Amino-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10d): Yield 74%, IR (KBr, ν_{max}, cm^{-1}): 3467 and 3345 (asym. and sym. *str.* of -NH_2), 3046 (aromatic C-H *str.*), 2200 (-C≡N *str.*), 1680 (C=O *str.*), 1540 and 1472 (C=C *str.* of aromatic ring), 1195 (C-S-C *str.*), 1372 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, 3H, C$\underline{H}_3$), 0.93 (s, 3H, C$\underline{H}_3$), 1.82-2.66 (m, 4H, 2×C$\underline{H}_2$), 2.19 (s, 3H, C$\underline{H}_3$), 4.59 (s, 1H, C$\underline{H}$), 5.51 (s, 2H, N$\underline{H}_2$), 6.72-7.57 (m, 15H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5 (CH_3), 27.5 (CH_3), 31.8, 33.4 (2×C4), 43.9, 51.2 (2×CH_2), 117.4 ($\underline{C}$-CN), 57.1, 112.1, 153.4, 167.9 (C=C), 119.1, 119.7, 122.5, 122.9, 125.6, 126.4, 129.3, 129.5, 129.6, 129.7, 131.9, 138.6, 141.1, 141.9, 147.5 (Ar-C), 194.3 ($\underline{C}$-C=O); Anal. calcd. (found) % for $C_{34}H_{31}N_5OS$ (557.7 g/mol): C, 73.22 (73.13); H, 5.60 (5.47); O, 2.87 (2.96); N, 12.56 (12.70); S, 5.75 (5.74); MS (*m/z*): 557.2 (M^+).

2-Amino-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10e): Yield 76%, IR (KBr, ν_{max}, cm^{-1}): 3450 and 3358 (asym. and sym. *str.* of -NH_2), 3063 (aromatic C-H *str.*), 2185 (-C≡N *str.*), 1680 (C=O *str.*), 1544 and 1430 (C=C *str.* of aromatic ring), 1213 (C-S-C *str.*), 1350 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.89 (s, 3H, C$\underline{H}_3$), 0.95 (s, 3H, C$\underline{H}_3$), 1.87-2.45 (m, 4H, 2×C$\underline{H}_2$), 1.93 (s, 3H, C$\underline{H}_3$), 2.21 (s, 3H, C$\underline{H}_3$), 4.45 (s, 1H, C$\underline{H}$), 5.56 (s, 2H, N$\underline{H}_2$), 6.65-7.67 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 21.5 (CH_3), 27.9 (CH_3), 31.1, 33.9 (2×C4), 43.2, 51.7 (2×CH_2), 117.1 ($\underline{C}$-CN), 57.9, 111.6, 153.1, 167.3 (C=C), 118.9, 119.5, 122.6, 123.0, 125.7, 126.9, 129.1, 129.7, 129.8, 130.0, 132.2, 138.7, 141.4, 142.0, 147.9 (Ar-C), 194.8 ($\underline{C}$-C=O); Anal. calcd. (found) % for $C_{35}H_{33}N_5OS$ (571.7 g/mol): C, 73.53 (73.79); H, 5.82 (6.07); O, 2.80 (2.66); N, 12.25 (12.07); S, 5.41 (5.85); MS (*m/z*): 571.2 (M^+).

2-Amino-1-(4-methoxyphenyl)-7,7-dimethyl-4-(3-methyl-1-phenyl-5-(phenylthio)-1*H*-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10f): Yield 70%, IR (KBr, ν_{max}, cm^{-1}): 3443 and 3362 (asym. and sym. *str.* of -NH_2), 3045 (aromatic C-H *str.*), 2215 (-C≡N *str.*), 1689 (C=O *str.*), 1534 and 1435 (C=C *str.* of aromatic ring), 1223 and 1022 (C-O-C asym & sym *str.* of -OCH_3), 1190 (C-S-C *str.*), 1354 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92 (s, 3H, C$\underline{H}_3$), 0.96 (s, 3H, C$\underline{H}_3$), 1.80-2.56 (m, 4H, 2×C$\underline{H}_2$), 2.19 (s, 3H, C$\underline{H}_3$), 3.80 (s, 3H, OC$\underline{H}_3$), 4.56 (s, 1H, C$\underline{H}$), 5.52 (s, 2H, N$\underline{H}_2$), 6.70-7.61 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.5 (CH_3), 27.3 (CH_3), 31.3, 33.4 (2×C4), 43.8, 51.3 (2×CH_2), 55.6 (OCH_3),117.8 ($\underline{C}$-CN), 57.0, 111.2, 153.4,

167.6 (C=C), 118.1, 119.8, 122.4, 122.7, 125.3, 127.0, 129.2, 129.7, 129.8, 129.9, 132.7, 138.9, 141.5, 141.7, 148.0 (Ar-C), 194.5 (C-C=O); Anal. calcd. (found) % for $C_{35}H_{33}N_5O_2S$ (587.7 g/mol): C, 71.52 (71.72); H, 5.66 (5.78); O, 5.44 (5.63); N, 11.92 (11.65); S, 5.46 (5.22); MS (*m/z*): 587.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10g): Yield 74%, IR (KBr, ν_{max}, cm^{-1}): 3483 and 3357 (asym. and sym. *str.* of -NH_2), 3012 (aromatic C-H *str.*), 2210 (-C≡N *str.*), 1675 (C=O *str.*), 1570 and 1460 (C=C *str.* of aromatic ring), 1198 (C-S-C *str.*), 718 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68-2.72 (m, 6H, 3×CH_2), 2.22 (s, 3H, CH_3), 4.41 (s, 1H, CH), 5.56 (s, 2H, NH_2), 6.90-7.68 (m, 14H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 18.4, 21.1, 36.7 (3×CH_2), 31.2 (C4), 117.8 (C-CN), 57.1, 111.3, 153.3, 167.6 (C=C), 119.5, 119.7, 122.7, 122.9, 125.3, 126.7, 129.2, 129.6, 129.9, 130.1, 131.8, 138.1, 141.7, 141.9, 147.5 (Ar-C), 198.2 (C-C=O); Anal. calcd. (found) % for $C_{32}H_{26}N_5OSCl$ (564.1 g/mol): C, 68.13 (68.22); H, 4.65 (4.51); O, 2.84 (2.98); N, 12.42 (12.55); S, 5.68 (5.40); Cl, 6.28 (6.34); MS (*m/z*): 563.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10h): Yield 79%, IR (KBr, ν_{max}, cm^{-1}): 3442 and 3376 (asym. and sym. *str.* of -NH_2), 3028 (aromatic C-H *str.*), 2220 (-C≡N *str.*), 1680 (C=O *str.*), 1554 and 1470 (C=C *str.* of aromatic ring), 1188 (C-S-C *str.*), 735 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.91 (s, 3H, CH_3), 1.72-2.62 (m, 6H, 3×CH_2), 2.16 (s, 3H, CH_3), 4.55 (s, 1H, CH), 5.66 (s, 2H, NH_2), 6.82-7.53 (m, 13H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 21.2 (CH_3), 19.4, 21.3, 36.5 (3×CH_2), 31.6 (C4), 117.5 (C-CN), 57.4, 111.3, 152.8, 167.4 (C=C), 119.0, 120.6, 123.2, 124.7, 125.4, 128.5, 129.0, 129.4, 129.7, 131.2, 131.3, 138.7, 138.9, 141.5, 147.3 (Ar-C), 198.4 (C-C=O); Anal. calcd. (found) % for $C_{33}H_{28}ClN_5OS$ (578.1 g/mol): C, 68.56 (68.50); H, 4.88 (5.04); O, 2.77 (2.98); N, 12.11 (12.32); S, 5.55 (5.31); Cl, 6.13 (5.85); MS (*m/z*): 577.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10i): Yield 71%, IR (KBr, ν_{max}, cm^{-1}): 3452 and 3375 (asym. and sym. *str.* of -NH_2), 3042 (aromatic C-H *str.*), 2202 (-C≡N *str.*), 1680 (C=O *str.*), 1534 and 1452 (C=C *str.* of aromatic ring), 1223 and 1045 (C-O-C asym & sym *str.* of -OCH_3), 1215 (C-S-C *str.*), 754 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68-2.52 (m, 6H, 3×CH_2), 2.22 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 4.67 (s, 1H, CH), 5.45 (s, 2H, NH_2), 6.82-7.58 (m, 13H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.9 (CH_3), 19.2, 21.7, 36.1 (3×CH_2), 31.7 (C4), 55.7 (OCH_3), 117.1 (C-CN), 57.5, 111.6, 153.5, 167.7 (C=C), 119.1, 119.7, 122.0, 123.5, 124.5, 125.3, 128.5, 128.9, 129.4, 130.4, 131.7, 132.4, 138.0, 141.5, 147.9 (Ar-C), 198.9 (C-C=O); Anal. calcd. (found) % for $C_{33}H_{28}N_5O_2SCl$ (594.1 g/mol): C, 66.71 (66.83); H, 4.75 (4.64); O, 5.39 (5.46); N, 11.79 (12.03); S, 5.40 (5.31); Cl, 5.97 (6.73); MS (*m/z*): 593.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10j): Yield 80%, IR (KBr, ν_{max}, cm^{-1}): 3450 and 3330 (asym. and sym. *str.* of -NH_2), 3062 (aromatic C-H *str.*), 2212 (-C≡N *str.*), 1675 (C=O *str.*), 1535 and 1480 (C=C *str.* of aromatic ring), 1215 (C-S-C *str.*), 1354 (*gem*-dimethyl *str.*), 775 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.76-2.62 (m, 4H, 2×CH_2), 2.13 (s, 3H, CH_3), 4.67 (s, 1H, CH), 5.68 (s, 2H, NH_2), 6.85-7.73 (m, 14H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.2 (CH_3), 27.3 (CH_3), 31.4, 33.8 (2×C4), 43.3, 51.5 (2×CH_2), 117.6 (C-CN), 57.3, 112.4, 153.6, 167.5 (C=C), 119.5, 119.9, 122.3, 123.0, 125.2, 126.8, 129.4, 129.7, 129.8, 130.0, 131.2, 138.8, 141.4, 141.7, 147.3 (Ar-C), 194.2 (C-C=O); Anal. calcd. (found) % for $C_{34}H_{30}N_5OSCl$ (592.2 g/mol): C, 68.96 (69.18); H, 5.11 (5.30); O, 2.70 (2.82); N, 11.83 (11.81); S, 5.41 (5.17); Cl, 5.99 (5.72); MS (*m/z*): 591.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10k): Yield 77%, IR (KBr, ν_{max}, cm^{-1}): 3444 and 3365 (asym. and sym. *str.* of -NH_2), 3070 (aromatic C-H *str.*), 2208 (-C≡N *str.*), 1688 (C=O *str.*), 1545 and 1425 (C=C *str.* of aromatic ring), 1196 (C-S-C *str.*), 1335 (*gem*-dimethyl *str.*), 740 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.76-2.57 (m, 4H, 2×CH_2), 1.90 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 4.72 (s, 1H, CH), 5.70 (s, 2H, NH_2), 6.82-7.60 (m, 13H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.2 (CH_3), 21.7 (CH_3), 27.2 (CH_3), 31.6, 33.3 (2×C4), 43.5, 51.2 (2×CH_2), 117.4 (C-CN), 57.2, 111.9, 153.2, 167.6 (C=C), 119.2, 119.7, 122.7, 124.2, 125.4, 126.5, 129.4, 129.5, 129.6, 129.9, 132.6, 138.4, 141.5, 142.2, 147.5 (Ar-C), 194.8 (C-C=O); Anal. calcd. (found) % for $C_{35}H_{32}N_5OSCl$ (606.2 g/mol): C, 69.35 (69.24); H, 5.32 (5.56); O, 2.64 (2.80); N, 11.55 (11.22); S, 5.29 (5.44); Cl, 5.85 (5.74); MS (*m/z*): 605.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10l): Yield 73%, IR (KBr, ν_{max}, cm^{-1}): 3445 and 3372 (asym. and sym. *str.* of -NH_2), 3058 (aromatic C-H *str.*), 2224 (-C≡N *str.*), 1690 (C=O *str.*), 1532 and 1446 (C=C *str.* of aromatic ring), 1215 and 1035 (C-O-C asym & sym *str.* of -OCH_3), 1212 (C-S-C *str.*), 1364 (*gem*-dimethyl *str.*), 756 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.78-2.64 (m, 4H, 2×CH_2), 2.21 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.68 (s, 1H, CH), 5.63 (s, 2H, NH_2), 6.82-7.56 (m, 13H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 27.6 (CH_3), 31.1, 33.7 (2×C4), 43.2, 51.4 (2×CH_2), 55.3 (OCH_3),117.3 (C-CN), 57.2, 111.4, 153.6, 167.9 (C=C), 118.8, 119.5, 122.6, 122.9, 125.6, 127.4, 129.1, 129.3, 129.4, 129.5, 132.3, 138.5, 141.4, 141.8, 148.4 (Ar-C), 194.1 (C-C=O); Anal. calcd. (found) % for $C_{35}H_{32}N_5O_2SCl$ (622.2 g/mol): C, 67.56 (67.42); H, 5.18 (5.28); O, 5.14 (5.21); N, 11.26 (11.42); S, 5.15 (5.13); Cl, 5.70 (5.54); MS (*m/z*): 621.2 (M^+).

2-Amino-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10m): Yield 71%, IR (KBr, ν_{max}, cm^{-1}): 3450 and 3345 (asym. and sym. *str.* of -NH_2), 3035 (aromatic C-H *str.*), 2211 (-C≡N *str.*), 1683 (C=O *str.*), 1574 and 1455

(C=C *str.* of aromatic ring), 1208 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.93 (s, 3H, C<u>H</u>$_3$), 1.80-2.50 (m, 6H, 3 × C<u>H</u>$_2$), 2.21 (s, 3H, C<u>H</u>$_3$), 4.57 (s, 1H, C<u>H</u>), 5.62 (s, 2H, N<u>H</u>$_2$), 6.90-7.68 (m, 14H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.2 (CH_3), 18.2, 21.5, 36.1 (3×CH_2), 21.3 (CH_3), 31.4 (C4), 117.7 (<u>C</u>-CN), 57.2, 111.3, 153.1, 167.7 (C=C), 119.0, 119.5, 122.7, 122.9, 125.3, 126.6, 129.1, 129.5, 129.6, 129.7, 131.4, 138.9, 141.6, 141.9, 147.7 (Ar-C), 198.2 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{33}H_{29}N_5OS$ (543.7 g/mol): C, 72.90 (73.15); H, 5.38 (5.23); O, 2.94 (2.75); N, 12.88 (12.77); S, 5.90 (6.10); MS (*m/z*): 543.2 (M^+).

2-Amino-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10n): Yield 80%, IR (KBr, ν_{max}, cm^{-1}): 3460 and 3375 (asym. and sym. *str.* of -NH_2), 3040 (aromatic C-H *str.*), 2214 (-C≡N *str.*), 1685 (C=O *str.*), 1535 and 1450 (C=C *str.* of aromatic ring), 1215 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.58-2.62 (m, 6H, 3×C<u>H</u>$_2$), 1.89 (s, 3H, C<u>H</u>$_3$), 1.95 (s, 3H, C<u>H</u>$_3$), 2.17 (s, 3H, C<u>H</u>$_3$), 4.80 (s, 1H, C<u>H</u>), 5.66 (s, 2H, N<u>H</u>$_2$), 6.80-7.65 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 18.4, 21.2, 38.3 (3×CH_2), 21.7 (CH_3), 21.1 (CH_3), 31.5 (C4), 119.3 (<u>C</u>-CN), 57.3, 111.4, 152.9, 167.4 (C=C), 119.6, 119.9, 123.2, 124.5, 125.6, 128.01, 129.5, 129.7, 130.4, 131.7, 131.9, 138.5, 138.7, 141.4, 147.3 (Ar-C), 198.7 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{34}H_{31}N_5OS$ (557.7 g/mol): C, 73.22 (73.43); H, 5.60 (5.52); O, 2.87 (3.06); N, 12.56 (12.34); S, 5.75 (5.65); MS (*m/z*): 557.2 (M^+).

2-Amino-1-(4-methoxyphenyl)-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10o): Yield 78%, IR (KBr, ν_{max}, cm^{-1}): 3463 and 3375 (asym. and sym. *str.* of -NH_2), 3048 (aromatic C-H *str.*), 2208 (-C≡N *str.*), 1688 (C=O *str.*), 1535 and 1455 (C=C *str.* of aromatic ring), 1230 and 1042 (C-O-C asym & sym *str.* of -OCH_3), 1210 (C-S-C *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.65-2.66 (m, 6H, 3×C<u>H</u>$_2$), 1.92 (s, 3H, C<u>H</u>$_3$), 2.18 (s, 3H, C<u>H</u>$_3$), 3.70 (s, 3H, OC<u>H</u>$_3$), 4.60 (s, 1H, C<u>H</u>), 5.58 (s, 2H, N<u>H</u>$_2$), 6.75-7.63 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4 (CH_3), 18.3, 21.5, 37.0 (3×CH_2), 21.3 (CH_3), 31.0 (C4), 56.6 (OCH_3), 117.2 (<u>C</u>-CN), 57.5, 111.7, 153.8, 167.0 (C=C), 119.9, 120.4, 122.6, 123.8, 124.4, 125.7, 128.5, 128.4, 129.6, 130.4, 131.8, 132.3, 138.0, 141.7, 147.9 (Ar-C), 197.1 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{34}H_{31}N_5O_2S$ (573.7 g/mol): C, 71.18 (71.03); H, 5.45 (5.49); O, 5.58 (5.76); N, 12.21 (12.40); S, 5.59 (5.32); MS (*m/z*): 573.2 (M^+)

2-Amino-7,7-dimethyl-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10p): Yield 75%, IR (KBr, ν_{max}, cm^{-1}): 3445 and 3367 (asym. and sym. *str.* of -NH_2), 3030 (aromatic C-H *str.*), 2215 (-C≡N *str.*), 1695 (C=O *str.*), 1557 and 1460 (C=C *str.* of aromatic ring), 1220 (C-S-C *str.*), 1366 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92 (s, 3H, C<u>H</u>$_3$), 0.97 (s, 3H, C<u>H</u>$_3$), 1.70-2.75 (m, 4H, 2×C<u>H</u>$_2$), 1.97 (s, 3H, C<u>H</u>$_3$), 2.11 (s, 3H, C<u>H</u>$_3$), 4.64 (s, 1H, C<u>H</u>), 5.60 (s, 2H, N<u>H</u>$_2$), 6.84-7.68 (m, 14H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 21.7 (CH_3), 27.1 (CH_3), 31.5, 33.1 (2×C4), 43.6, 51.8 (2×CH_2), 117.3 (<u>C</u>-CN), 57.5, 112.5, 153.7, 167.2 (C=C), 119.0, 119.6, 122.8, 123.2, 125.8, 126.4, 129.7, 129.8, 129.9, 130.7, 131.3, 138.5, 141.7, 141.9, 147.1 (Ar-C), 194.9 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{35}H_{33}N_5OS$ (571.7 g/mol): C, 73.53 (73.41); H, 5.82 (5.67); O, 2.80 (2.96); N, 12.25 (12.49); S, 5.61 (5.47); MS (*m/z*): 571.2 (M^+).

2-Amino-7,7-dimethyl-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10q): Yield 72%, IR (KBr, ν_{max}, cm^{-1}): 3460 and 3373 (asym. and sym. *str.* of -NH_2), 3045 (aromatic C-H *str.*), 2213 (-C≡N *str.*), 1690 (C=O *str.*), 1537 and 1445 (C=C *str.* of aromatic ring), 1202 (C-S-C *str.*), 1345 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, C<u>H</u>$_3$), 0.93 (s, 3H, C<u>H</u>$_3$), 1.57-2.70 (m, 4H, 2×C<u>H</u>$_2$), 1.92 (s, 3H, C<u>H</u>$_3$), 1.97 (s, 3H, C<u>H</u>$_3$), 2.14 (s, 3H, C<u>H</u>$_3$), 4.72 (s, 1H, C<u>H</u>), 5.67 (s, 2H, N<u>H</u>$_2$), 6.85-7.74 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.2 (CH_3), 21.1 (CH_3), 21.7 (CH_3), 27.5 (CH_3), 31.0, 33.5 (2×C4), 43.4, 51.8 (2×CH_2), 117.4 (<u>C</u>-CN), 57.2, 111.3, 153.7, 167.8 (C=C), 118.5, 119.6, 122.8, 123.4, 125.3, 126.8, 129.3, 129.5, 129.9, 130.3, 132.6, 138.2, 141.7, 142.2, 147.4 (Ar-C), 194.3 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{36}H_{35}N_5OS$ (585.8 g/mol): C, 73.82 (73.70); H, 6.02 (6.15); O, 2.73 (2.66); N, 11.96 (12.12); S, 5.47 (5.37); MS (*m/z*): 585.3 (M^+).

2-Amino-1-(4-methoxyphenyl)-7,7-dimethyl-4-(3-methyl-5-(phenylthio)-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10r): Yield 72%, IR (KBr, ν_{max}, cm^{-1}): 3446 and 3375 (asym. and sym. *str.* of -NH_2), 3053 (aromatic C-H *str.*), 2217 (-C≡N *str.*), 1681 (C=O *str.*), 1540 and 1465 (C=C *str.* of aromatic ring), 1230 and 1026 (C-O-C asym & sym *str.* of -OCH_3), 1198 (C-S-C *str.*), 1345 (*gem*-dimethyl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.94 (s, 3H, C<u>H</u>$_3$), 0.98 (s, 3H, C<u>H</u>$_3$), 1.74-2.58 (m, 4H, 2×C<u>H</u>$_2$), 1.95 (s, 3H, C<u>H</u>$_3$), 2.22 (s, 3H, C<u>H</u>$_3$), 3.65 (s, 3H, OC<u>H</u>$_3$), 4.72 (s, 1H, C<u>H</u>), 5.68 (s, 2H, N<u>H</u>$_2$), 6.86-7.72 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 21.4 (CH_3), 27.7 (CH_3), 31.4, 33.8 (2×C4), 43.4, 51.8 (2×CH_2), 55.7 (OCH_3), 117.4 (<u>C</u>-CN), 57.5, 111.6, 153.7, 167.3 (C=C), 118.5, 119.4, 122.2, 122.3, 125.8, 127.6, 129.9, 129.3, 129.6, 129.8, 132.2, 138.5, 141.0, 141.9, 148.3 (Ar-C), 194.6 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{36}H_{35}N_5O_2S$ (601.8 g/mol): C, 71.85 (71.68); H, 5.86 (5.93); O, 5.32 (5.43); N, 11.64 (11.56); S, 5.33 (5.40); MS (*m/z*): 601.3 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10s): Yield 81%, IR (KBr, ν_{max}, cm^{-1}): 3455 and 3347 (asym. and sym. *str.* of -NH_2), 3034 (aromatic C-H *str.*), 2205 (-C≡N *str.*), 1693 (C=O *str.*), 1567 and 1455 (C=C *str.* of aromatic ring), 1225 (C-S-C *str.*), 745 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.52-2.75 (m, 6H, 3×C<u>H</u>$_2$), 1.98 (s, 3H, C<u>H</u>$_3$), 2.19 (s, 3H, C<u>H</u>$_3$), 4.55 (s, 1H, C<u>H</u>), 5.68 (s, 2H, N<u>H</u>$_2$), 6.85-7.73 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.2 (CH_3), 18.1, 21.7, 36.3 (3×CH_2), 21.8 (CH_3), 31.4 (C4), 117.2 (<u>C</u>-CN), 57.4, 111.7, 153.1, 167.9 (C=C), 119.3, 119.7, 122.5, 122.8, 125.5, 126.4, 129.3, 129.7, 129.8, 129.9, 131.4, 138.5, 141.3, 141.6, 147.7 (Ar-C), 198.6 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{33}H_{28}N_5OSCl$ (578.1 g/mol): C, 68.56 (68.41); H, 4.88 (5.10); O, 2.77 (2.94); N, 12.11 (12.34); S, 5.55 (5.35); Cl, 6.13 (5.86); MS (*m/z*): 577.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10t): Yield 75%, IR (KBr, ν_{max}, cm^{-1}): 3455 and 3372 (asym. and sym. *str.* of -NH_2), 3045 (aromatic C-H *str.*), 2208 (-C≡N *str.*), 1673 (C=O *str.*), 1535 and 1467 (C=C *str.* of aromatic ring), 1197 (C-S-C *str.*), 730 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.67-2.71 (m, 6H, 3×C<u>H</u>$_2$), 1.90 (s, 3H, C<u>H</u>$_3$), 1.99 (s, 3H, C<u>H</u>$_3$), 2.24 (s, 3H, C<u>H</u>$_3$), 4.70 (s, 1H, C<u>H</u>), 5.57 (s, 2H, N<u>H</u>$_2$), 6.75-7.72 (m, 12H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 21.4 (CH_3), 21.9 (CH_3), 18.6, 21.6, 36.9 (3×CH_2), 31.3 (C4), 117.7 (<u>C</u>-CN), 57.6, 111.1, 153.0, 167.2 (C=C), 119.4, 120.7, 123.0, 124.4, 125.7, 128.2, 129.3, 129.6, 129.7, 131.5, 131.9, 138.5, 138.7, 141.2, 147.5 (Ar-C), 198.6 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{34}H_{30}N_5OSCl$ (592.2 g/mol): C, 68.96 (68.79); H, 5.11 (5.28); O, 2.70 (2.56); N, 11.83 (12.07); S, 5.41 (5.59); Cl, 5.99 (5.71); MS (*m/z*): 591.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-1-(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10u): Yield 72%, IR (KBr, ν_{max}, cm^{-1}): 3460 and 3376 (asym. and sym. *str.* of -NH_2), 3035 (aromatic C-H *str.*), 2214 (-C≡N *str.*), 1688 (C=O *str.*), 1525 and 1457 (C=C *str.* of aromatic ring), 1228 and 1055 (C-O-C asym & sym *str.* of -OCH_3), 1221 (C-S-C *str.*), 743 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62-2.71 (m, 6H, 3×C<u>H</u>$_2$), 1.93 (s, 3H, C<u>H</u>$_3$), 2.20 (s, 3H, C<u>H</u>$_3$), 3.66 (s, 3H, OC<u>H</u>$_3$), 4.72 (s, 1H, C<u>H</u>), 5.68 (s, 2H, N<u>H</u>$_2$), 6.77-7.63 (m, 12H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 19.5, 21.4, 36.5 (3×CH_2), 21.8 (CH_3), 31.3 (C4), 55.9 (OCH_3), 117.5 (<u>C</u>-CN), 57.1, 111.8, 153.1, 167.9 (C=C), 119.3, 119.8, 122.2, 123.6, 124.9, 125.0, 128.3, 128.7, 129.7, 130.9, 131.4, 132.7, 138.5, 141.3, 147.5 (Ar-C), 198.3 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{34}H_{30}N_5O_2SCl$ (608.2 g/mol): C, 67.15 (67.29); H, 4.97 (4.73); O, 5.26 (5.42); N, 11.52 (11.76); S, 5.27 (5.12); Cl, 5.83 (5.68); MS (*m/z*): 607.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10v): Yield 74%, IR (KBr, ν_{max}, cm^{-1}): 3442 and 3345 (asym. and sym. *str.* of -NH_2), 3055 (aromatic C-H *str.*), 2220 (-C≡N *str.*), 1691 (C=O *str.*), 1545 and 1486 (C=C *str.* of aromatic ring), 1203 (C-S-C *str.*), 1365 (*gem*-dimethyl *str.*), 748 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92 (s, 3H, C<u>H</u>$_3$), 0.97 (s, 3H, C<u>H</u>$_3$), 1.68-2.73 (m, 4H, 2×C<u>H</u>$_2$), 1.94 (s, 3H, C<u>H</u>$_3$), 2.23 (s, 3H, C<u>H</u>$_3$), 4.55 (s, 1H, C<u>H</u>), 5.82 (s, 2H, N<u>H</u>$_2$), 6.60-7.71 (m, 13H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 21.2 (CH_3), 27.8 (CH_3), 31.1, 33.6 (2×C4), 43.2, 51.1 (2×CH_2), 117.9 (<u>C</u>-CN), 57.2, 111.8, 153.4, 167.2 (C=C), 119.1, 119.7, 122.4, 123.6, 125.9, 126.5, 129.0, 129.3, 129.4, 129.8, 131.6, 138.9, 141.7, 141.9, 147.6 (Ar-C), 194.8 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{35}H_{32}N_5OSCl$ (606.2 g/mol): C, 69.35 (69.18); H, 5.32 (5.25); O, 2.64 (2.76); N, 11.55 (11.42); S, 5.29 (5.41); Cl, 5.85 (5.98); Found: C 69.18, H 5.25, O 2.76, N 11.42, S 5.41, Cl 5.98%; MS (*m/z*): 605.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-7,7-dimethyl-5-oxo-1-(*p*-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10w): Yield 77%, IR (KBr, ν_{max}, cm^{-1}): 3454 and 3369 (asym. and sym. *str.* of -NH_2), 3054 (aromatic C-H *str.*), 2212 (-C≡N *str.*), 1681 (C=O *str.*), 1535 and 1430 (C=C *str.* of aromatic ring), 1205 (C-S-C *str.*), 1347 (*gem*-dimethyl *str.*), 746 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, 3H, C<u>H</u>$_3$), 0.95 (s, 3H, C<u>H</u>$_3$), 1.64-2.68 (m, 4H, 2×C<u>H</u>$_2$), 1.92 (s, 3H, C<u>H</u>$_3$), 1.98 (s, 3H, C<u>H</u>$_3$), 2.15 (s, 3H, C<u>H</u>$_3$), 4.58 (s, 1H, C<u>H</u>), 5.74 (s, 2H, N<u>H</u>$_2$), 6.68-7.58 (m, 12H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 21.3 (CH_3), 21.9 (CH_3), 27.6 (CH_3), 31.2, 33.6 (2×C4), 43.6, 51.9 (2×CH_2), 117.8 (<u>C</u>-CN), 57.5, 111.2, 153.3, 167.7 (C=C), 119.5, 119.9, 122.2, 124.9, 125.8, 126.7, 129.2, 129.7, 129.8, 129.9, 132.9, 138.1, 141.4, 142.7, 147.1 (Ar-C), 194.2 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{36}H_{34}N_5OSCl$ (620.2 g/mol): C, 69.72 (69.56); H, 5.53 (5.72); O, 2.58 (2.65); N, 11.29 (11.40); S, 5.17 (5.10); Cl, 5.72 (5.57); MS (*m/z*): 619.2 (M^+).

2-Amino-4-(5-((4-chlorophenyl)thio)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazol-4-yl)-1-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (10x): Yield 82%, IR (KBr, ν_{max}, cm^{-1}): 3440 and 3363 (asym. and sym. *str.* of -NH_2), 3052 (aromatic C-H *str.*), 2210 (-C≡N *str.*), 1682 (C=O *str.*), 1537 and 1455 (C=C *str.* of aromatic ring), 1223 and 1026 (C-O-C asym & sym *str.* of -OCH_3), 1193 (C-S-C *str.*), 1351 (*gem*-dimethyl *str.*), 742 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, C<u>H</u>$_3$), 0.95 (s, 3H, C<u>H</u>$_3$), 1.65-2.54 (m, 4H, 2×C<u>H</u>$_2$), 1.97 (s, 3H, C<u>H</u>$_3$), 2.16 (s, 3H, C<u>H</u>$_3$), 3.58 (s, 3H, OC<u>H</u>$_3$), 4.76 (s, 1H, C<u>H</u>), 5.68 (s, 2H, N<u>H</u>$_2$), 6.78-7.74 (m, 12H, Ar-<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.4 (CH_3), 21.9 (CH_3), 27.2 (CH_3), 31.5, 33.2 (2×C4), 43.5, 51.7 (2×CH_2), 55.3 (OCH_3),117.7 (<u>C</u>-CN), 57.4, 111.8, 153.1, 167.3 (C=C), 118.2, 119.9, 122.1, 122.7, 125.3, 127.8, 129.3, 129.6, 129.7, 129.9, 132.9, 138.1, 141.3, 141.8, 148.7 (Ar-C), 194.4 (<u>C</u>-C=O); Anal. calcd. (found) % for $C_{36}H_{34}N_5O_2SCl$ (636.2 g/mol): C, 67.96 67.77(); H, 5.39 (5.47); O, 5.03 (5.19); N, 11.01 (11.05); S, 5.04 (5.16); Cl, 5.57 (5.36); MS (*m/z*): 635.2 (M^+).

RESULTS AND DISCUSSION

IR studies: The stretching or bending of the substituted and linkage functional groups is confirmed by the infrared spectra for all synthesized 24 compounds of pyrazole-quinoline hybrids. The medium intensity asymmetric and symmetric stretching bands of the primary amine (-NH_2) were observed in regions of 3485-3440 and 3380-3330 cm^{-1} for all compounds. Cyanide group (-C≡N) shows a weak stretching band in the region of 2224-2195 cm^{-1} in all compounds. The strong stretching between the carbon and oxygen of the carbonyl (-C=O-) group containing quinoline ring appeared at 1695-1673 cm^{-1} region. The stretching vibration of (-C=C-) aromatic ring also appeared at 1575-1532 and 1480-1425 cm^{-1} for all compounds. The strong carbon and sulphur stretching of the C-S containing thiophenoxy ring appeared at 1225-1188 cm^{-1}. The medium stretching of C-O-C appeared in –OCH_3 region of 1232-1215 cm^{-1} for compounds **10c**, **10f**, **10i**, **10l**, **10o**, **10r**, **10u** and **10x**. The medium stretching of carbon and chlorine (C-Cl) containing thiophenoxy ring appeared in the region of 775-718 cm^{-1} for compounds **10g-l** and **10s-x**.

^{1}H NMR studies: The structures of all 24 compounds were also confirmed by the proton magnetic resonance spectroscopy (^{1}H NMR). Two protons of the primary amine group appeared as a singlet in the region of δ 5.82-5.45 ppm; aromatic protons appeared as a multiplet in the region of δ 6.65-7.74 ppm, one aromatic chiral proton appeared as a singlet (s) in the region of δ 4.41-4.80 ppm for all compounds. R_1-substituted methyl protons attached to the phenoxy ring of pyrazole resonate in the region of δ 1.92-1.99 ppm as a singlet for compounds **10m-x**. And R_3-substituted methyl protons attached to the quinoline ring resonate in the region of δ 0.87-0.94 and 0.92-0.98 ppm as a singlet for compounds **10d-f**, **10j-l**, **10p-r** and **10v-x**. The R_4-substituted methyl protons attached to the phenoxy ring of quinoline resonate in the region of δ 1.86-1.93 ppm as singlet for compounds **10b**, **10e**, **10h**, **10k**, **10n**, **10q**, **10t** and **10w**. R_4-substituted methoxy protons attached to the phenoxy ring resonate at δ 3.65-3.82 ppm as singlet for compounds **10c**, **10f**, **10i**, **10l**, **10o**, **10r**, **10u** and **10x**. Methylene protons attached to the quinolone ring resonate at δ 1.52-2.75 ppm as a multiplet for all compounds.

^{13}C NMR studies: We also confirm the types of carbon in the present synthesized all the compounds, which are carried out by means of ^{13}C NMR. The methyl (-CH_3) carbon of R_1, R_3 and R_4-substitution showed a single line in the range of 12.1-27.9 ppm for the compounds **10d-f**, **10j-l** and **10m-x**. The cyclic methylene (-CH_2-) carbon of all synthesized compounds showed a single line at δ 18.0-51.9 ppm for the compounds **10a-x**. The methoxy carbon (-OCH_3) carbon of R_4-substitution showed a single line in the range of δ 55.0-56.9 ppm for compounds **10c**, **10f**, **10i**, **10l**, **10o**, **10r**, **10u** and **10x**. The range of δ 31.0-31.9 ppm was obtained as a single line for active methylene (C4) carbon. which was for all prepared compounds **10a-x**. The cynide (-CN) carbon of all synthesized compounds showed a single line at δ 117.0-118.9 ppm for compounds **10a-x**. The cyclic sp^2 (-C=C-) carbon of all synthesized compounds showed a single line in the range of δ 167.0-167.9 ppm for compounds **10a-x**. The cyclic keto (-C=O) carbon in all synthesized compounds showed a single line in the range of δ 194.3-198.9 ppm for compounds **10a-x**. The phenyl ring carbon showed fifteen lines in the range of δ 119.0-147.9 ppm for all twelve compounds **10a-x**.

Biological evaluation

Antiproliferation and EGFR inhibitory activity: All compounds were tested against EGFR kinase as well as against cancer cell A549 (adenocarcinomic human alveolar basal epithelial cell line) and Hep G2 (liver cancer cell line). Upon investigation of antiproliferative activity of compounds **10a-x**, it has been observed that compounds **10r** (IC_{50} = 1.41 ± 0.15 μM) and **10i** (IC_{50} = 1.20 ± 0.05 μM) against A549 as well as compounds **10i** (IC_{50} = 1.42 ± 0.14 μM) and **10x** (IC_{50} = 1.26 ± 0.15 μM) against Hep G2 showed most effective activity as compared to other compounds. Compounds **10r** (IC_{50} = 0.51 ± 0.05 μM) displayed the most potent inhibitory activity against EGFR as compared to other compounds and less comparable to the positive control erlotinib (IC_{50} = 0.032 ± 0.002 μM).

***E. coli* FabH inhibitory activity:** The *E. coli* FabH inhibitory potency of the synthesized **10a-x** derivatives was examined and the results are summarized in Table-1. Most of the tested compounds showed potent *E. coli* FabH inhibitory activity. Among them, compound **10i** showed the most potent inhibitory with IC_{50} of 3.1 μM.

Molecular docking study with EGFR: To gain better understanding on the potency of all compounds and guide further SAR studies, we proceeded to examine the interaction of those with EGFR (PDB code: 1M17) by molecular docking, which was performed by simulation of compounds into the ATP binding site in EGFR. The binding energy values of all the compounds is shown in Table-2. Of the compounds studied, compound **10r** was nicely bound into the active site of EGFR with minimum binding energy ΔG_b = -54.6913 kcal/mol. The binding model of compound **10r** and EGFR was depicted in Figs. 1a-b. The amino acid residues which had interaction with EGFR were labeled. In the binding mode, compound **10r** was nicely bound to the ATP binding site of EGFR through hydrophobic interaction and the binding was stabilized by three hydrogen bonds and one π-cation interaction. Among them one hydrogen bond forms between S atom of thiophenol group and ASP776 (distance: 3.26 Å), second one between O atom of methoxy group and LYS721 (distance: 3.16 Å) and third between N atom of pyrazole and CYS773 (distance: 2.98 Å), One π-cation bond forms between N atom of pyrazole ring and CYS773. From this binding model, it could be concluded that three hydrogen bonds and one π-cation interaction are responsible for the effective EGFR inhibitory of compound **10r**.

Molecular docking study with FabH: Similarly, to gain better understanding on the potency of all compounds and guide further SAR studies, molecular docking of compounds and *E. coli* FabH was performed on the binding model based on the *E. coli* FabH-CoA complex structure (PDB code: 1HNJ). The FabH active site generally contains a catalytic triad tunnel consisting of Cys-His-Asn, which is conserved in various bacteria. This catalytic triad plays an important role in the regulation of chain elongation and substrate binding. Since the alkyl chain of CoA is broken by Cys of the catalytic triad of FabH, interactions between Cys and substrate appear to play an important role in substrate binding. Of the compounds studied, compound **10i** was nicely bound to active site of the FabH with hydrogen bonds with minimum binding energy ΔG_b = -45.9125 kcal/mol. The binding energy of all the compounds is summarized in Table-2. The binding model of compound **10i** and FabH is depicted in Figs. 2a-b. Among them hydrogen bonds formed between nitrogen atom of cyanide and ASN230 (distance: 2.84 Å) and second hydrogen bond interaction is formed between nitrogen atom of amino group and MET207 (distance: 3.02 Å), One π-σ bond forms between phenyl ring and MET207 and second between phenyl ring and ARG36. From this binding model, it could be concluded that hydrogen bond interaction is responsible for the effective FabH inhibitory of compound **10i**.

Relationship of biological/molecular docking and DFT simulation

Frontier molecular orbitals (FMO) and biological activity: To understand the difference in the biological activity

TABLE-1
INHIBITION OF EGFR KINASE, ANTIPROLIFERATIVE AND *E. coli* FabH ACTIVITIES OF THE COMPOUNDS **10a-x**

Compd.	R_1	R_2	R_3	R_4	EGFR	A549	Hep G2	Lytic concentration 30%	
								E. coli FabH IC_{50} (μM)	Hemolysis LC_{30}[a] (mg/mL)
10a	H	H	H	H	12.05 ± 0.05	6.45 ± 0.05	9.21 ± 0.07	31.4	> 10
10b	H	H	H	CH_3	14.15 ± 0.14	16.04 ± 0.10	21.06 ± 0.15	5.6	> 10
10c	H	H	H	OCH_3	34.23 ± 0.12	12.21 ± 0.05	11.21 ± 0.10	4.8	> 10
10d	H	H	CH_3	H	21.17 ± 0.13	8.10 ± 0.14	11.60 ± 0.10	7.5	> 10
10e	H	H	CH_3	CH_3	8.23 ± 0.16	4.11 ± 0.11	5.03 ± 0.01	5.3	> 10
10f	H	H	CH_3	OCH_3	3.14 ± 0.11	8.24 ± 0.13	12.13 ± 0.08	13.2	> 10
10g	H	Cl	H	H	10.08 ± 0.04	7.21 ± 0.02	5.20 ± 0.06	6.4	> 10
10h	H	Cl	H	CH_3	11.14 ± 0.15	7.23 ± 0.24	9.23 ± 0.06	3.7	> 10
10i	H	Cl	H	OCH_3	0.91 ± 0.02	1.20 ± 0.05	1.42 ± 0.14	3.1	> 10
10j	H	Cl	CH_3	H	16.27 ± 0.13	4.15 ± 0.15	11.12 ± 0.15	5.8	> 10
10k	H	Cl	CH_3	CH_3	5.47 ± 0.12	5.08 ± 0.10	3.23 ± 0.17	7.1	> 10
10l	H	Cl	CH_3	OCH_3	11.10 ± 0.15	5.20 ± 0.32	9.65 ± 0.02	4.8	> 10
10m	CH_3	H	H	H	7.02 ± 0.15	10.02 ± 0.05	4.22 ± 0.05	5.9	> 10
10n	CH_3	H	H	CH_3	5.42 ± 0.10	9.28 ± 0.07	14.43 ± 0.05	6.5	> 10
10o	CH_3	H	H	OCH_3	13.11 ± 0.06	8.12 ± 0.16	11.52 ± 0.15	7.4	> 10
10p	CH_3	H	CH_3	H	2.03 ± 0.14	2.53 ± 0.10	3.12 ± 0.01	6.1	> 10
10q	CH_3	H	CH_3	CH_3	13.06 ± 0.12	11.10 ± 0.06	18.11 ± 0.10	8.7	> 10
10r	CH_3	H	CH_3	OCH_3	0.51 ± 0.05	1.41 ± 0.15	1.11 ± 0.05	3.9	> 10
10s	CH_3	Cl	H	H	7.68 ± 0.05	21.13 ± 0.12	8.08 ± 0.15	9.3	> 10
10t	CH_3	Cl	H	CH_3	5.12 ± 0.15	7.24 ± 0.09	20.10 ± 0.13	4.7	> 10
10u	CH_3	Cl	H	OCH_3	10.05 ± 0.12	11.07 ± 0.05	18.16 ± 0.11	4.9	> 10
10v	CH_3	Cl	CH_3	H	9.32 ± 0.10	4.11 ± 0.12	3.16 ± 0.15	6.3	> 10
10w	CH_3	Cl	CH_3	CH_3	8.45 ± 0.10	13.32 ± 0.05	6.11 ± 0.15	8.5	> 10
10x	CH_3	Cl	CH_3	OCH_3	1.08 ± 0.05	2.05 ± 0.04	1.26 ± 0.15	4.3	> 10
Erlotinib					0.032 ± 0.002	0.13 ± 0.01	0.12	–	–

TABLE-2
BINDING ENERGY OF COMPOUNDS **10a-x** WITH EGFR AND FabH

Compd.	Binding energy (Kcal/mol)	
	EGFR	FabH
10a	-44.4453	-38.4536
10b	-49.2342	-39.1327
10c	-42.5321	-37.6507
10d	-47.4522	-36.3422
10e	-46.3147	-37.0945
10f	-48.0234	-39.4563
10g	-48.2176	-41.5647
10h	-43.3553	-39.7645
10i	-51.1842	-45.9125
10j	-47.2673	-37.3045
10k	-49.9435	-38.4520
10l	-50.4321	-43.5638
10m	-48.8724	-40.4319
10n	-45.0170	-37.9563
10o	-52.5467	-41.5567
10p	-51.3749	-39.7640
10q	-47.4532	-37.8792
10r	-54.6913	-37.9023
10s	-46.7457	-39.5630
10t	-49.1846	-41.4562
10u	-47.8234	-40.5400
10v	-47.9845	-38.4562
10w	-45.2630	-42.2718
10x	-51.3491	-37.4129

of the prepared analogous compounds with respect to each other, the three-dimensional structure with their geometrical parameters and their energy in the isolated form were calculated using the quantum computational methods, where density functional theory (DFT) was employed with B3LYP level of the theory and def2-SVP as a basis set using ORCA [26]. The obtained optimized structures of the prepared analogues compounds were checked for their global minima, where absence of the imaginary frequency confirmed the stability of obtained geometry at obtained global minimum energy. To get the insight of the placement of the molecule in the pocket of the EGFR and FabH, the planes of the core constituents were observed and represented in Fig. 3 and their angle formation with respect to each other were calculated as twist angle. The twisting between the pyridine ring and pyrazole ring was noted as θ_1 while the twisting between pyrazole ring and phenyl ring attached to the pyridine nitrogen was noted as θ_2.

Geometry of the structure is a useful way of calculating the electronic property of the molecule which is dependent on the distribution of the charge on the individual atoms and also depends on the molecular orbital available to interact with the protein, these electronic properties of the compound is the base to the UV-vis reactivity or sensitivity of the molecule. It is well known that in general, the smaller energy gap value ΔE of the HOMO-LUMO is associated with the greater reactivity and stability of the molecule and *vice-versa* [27]. The energy gap calculated for the most active molecules and inactive molecules values, which varied between 5.885 eV to 5.536 eV. From

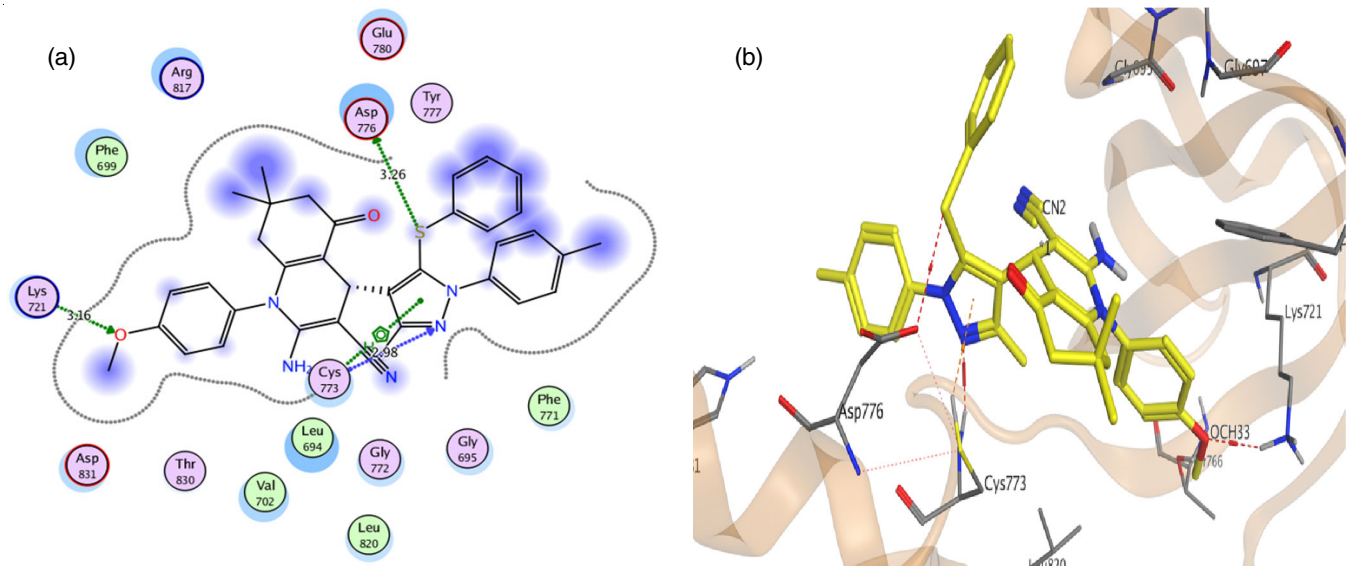


Fig. 1. 2D Binding model (a) and 3D binding model (b) of compound **10r** into the active pocket of EGFR

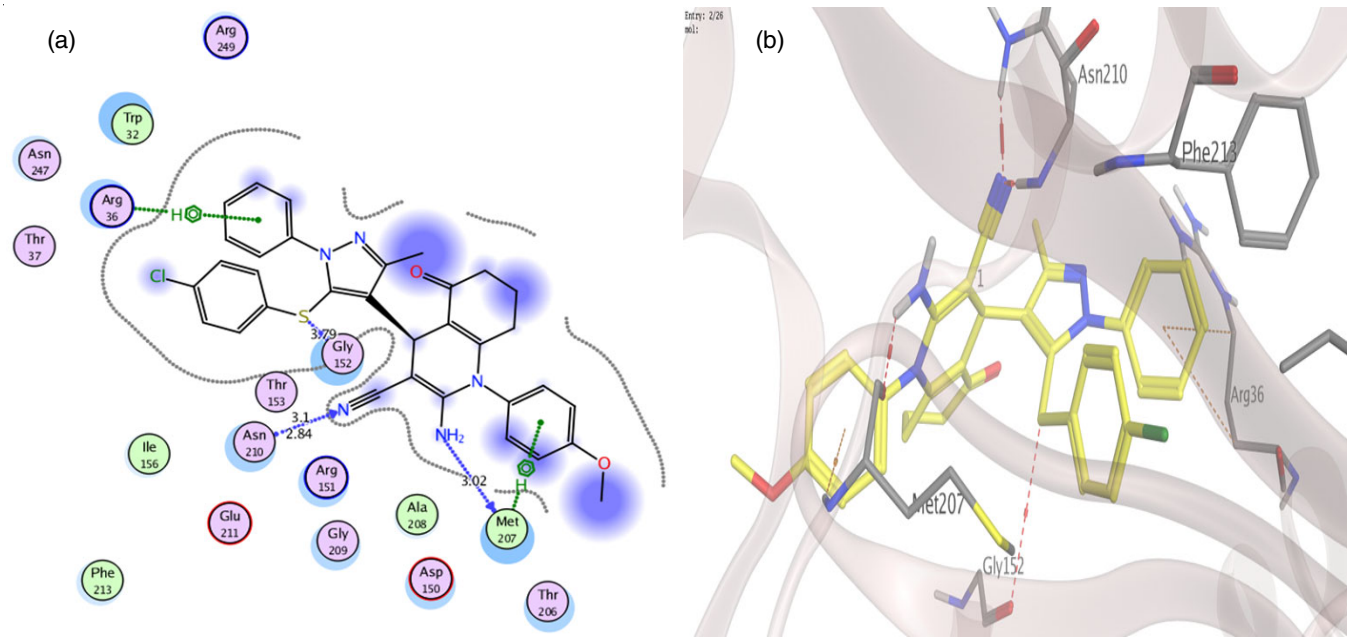


Fig. 2. 2D Binding model (a) and 3D binding model (b) of compound **10i** into the active site of FabH

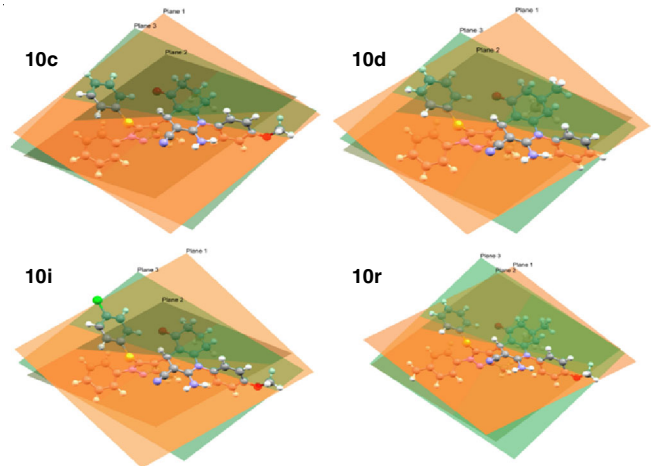


Fig. 3. Twist angel θ of compounds **10c**, **10d, 10i** and **10r**

the isodensity surface diagram of HOMO-LUMO of the molecules **10c**, **10d**, **10i** and **10r** (Fig. 4a-b), it is inferred that delocalization of the orbital is very broad, which can be seen by observing the number of nodal planes, indicating moderate overlap. The analogue molecules share the same nucleus but differ greatly in the four-substitution location, this change in the substitution and its effect on the electron density distribution can be easily observed from Fig. 4a-b of HOMO-LUMO, which in result affects energy gap value ΔE (Table-3). The energy gap values of compound **10c**, **10d**, **10i** and **10r** suggest that compound **10c** has the highest energy gap, while the compound **10i** has the lowest energy gap, which implies that compound **10c** should be most active and **10i** should be the least active among the synthesized compounds. Table-3 also represents such quantum chemical parameters which are

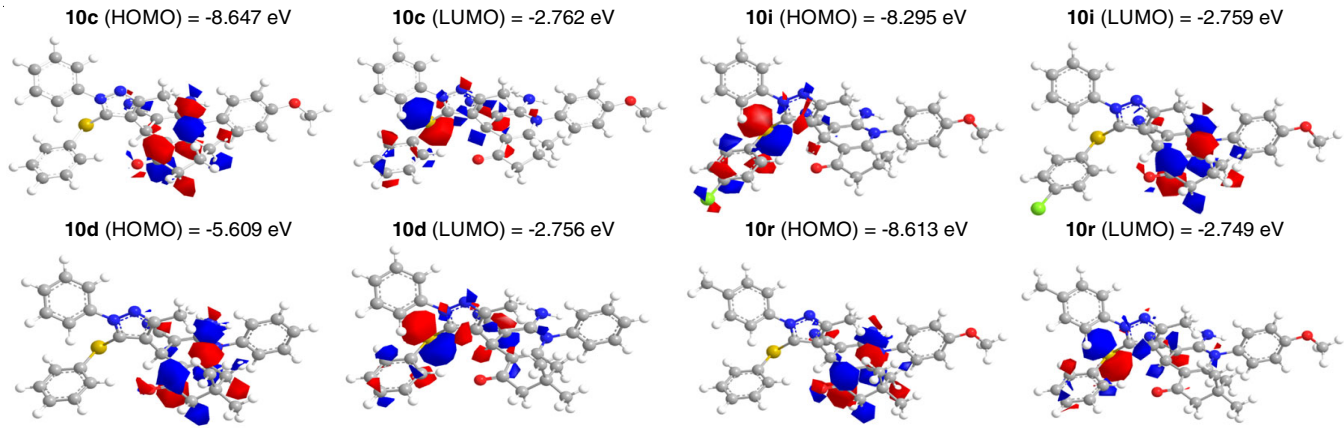


Fig. 4. HOMO-LUMO of compounds **10c**, **10d**, **10i** and **10r**

dependent on the energy gap values of HOMO and LUMO. Yet the EGFR kinase and antiproliferative activity and *E. coli* FabH activity showed that compound **10c** is the least effective and compound **10i** is the most effective. This contrary behaviour of the energy gap value and inhibition efficiency suggest that the evaluation of structural activity relationship of any molecule on the basis of its HOMO-LUMO energy gap is erroneous. Thus, its structural relationship activity must be related to the binding efficiency of the molecule in the pocket, which must be dependent on the geometry of the molecule in conjugation to the electron density at the terminals of the molecule.

Molecular geometry parameters and biological activity: EGFR kinase inhibition and the antiproliferative activity data obtained for all the prepared molecule compared to Erlotinib standard showed that the compounds **10r** and **10i** showed the highest activity against EGFR with 0.51 ± 0.05 at IC_{50} (μM) and 0.91 ± 0.02 at IC_{50} (μM) respectively, while the molecules **10c** and **10d** showed the lowest activity against EGFR with 34.23 ± 0.12 at IC_{50} (μM) and 21.17 ± 0.13 at IC_{50} (μM), respectively. If considering about compounds **10r** and **10c**, then these two differ to each other at only R_1 and R_3 substitution group and from the molecular docking data and optimized geometry it can be observed that when there is no substitution at R_1 and R_3 positions in molecule **10c**, the electron distribution does not allow the molecule to form any binding to the EGFR and hence it shows low score in docking simulation, which can be also justified from the twist angle (θ) where change in θ_1 between molecule **10c** and **10r** is 3.53 and θ_2 is 6.65 due to presence of electron donating group at R_1 and R_3, which allows the molecule **10r** to form hydrogen bond with LYS721 in plane 3, one arene proton interaction and hydrogen bond with CYS773 in plane 2, showing highest binding score.

Similarly, in case of compounds **10i** and **10d**, these two compounds differ to each other at R_2, R_3 and R_4 substitution group and from the molecular docking data and optimized geometry it can be observed that when there is no substitution at the R_2 and R_4 position in molecule **10d**, the electron distribution allows the molecule to form relatively low binding to the FabH and hence it shows low score in docking simulation, which can be also seen from the twist angle (θ) where change in θ_1

TABLE-3
CALCULATED CHARGES ON DONATING SITES AND ENERGY VALUES HOMO, LUMO, ENERGY GAP ΔE/eV, DIPOLE MOMENTS, ENERGIES, TWIST ANGLE (θ), HARDNESS (η), GLOBAL SOFTNESS (S), ELECTRO NEGATIVITY (χ), ABSOLUTE SOFTNESS (σ), CHEMICAL POTENTIAL (Pi), GLOBAL ELECTROPHILICITY (ω) AND ADDITIONAL ELECTRONIC CHARGE (ΔN_{max}) OF THE STUDIED COMPOUNDS **10c**, **10d**, **10i** AND **10r** BY USING DFT CALCULATIONS

Parameters		**10c**	**10d**	**10i**	**10r**
E_{HOMO} (eV)		-8.647	-8.609	-8.298	-8.613
E_{LUMO} (eV)		-2.762	-2.756	-2.759	-2.749
$I = -E_{HOMO}$		8.647	8.609	8.298	8.613
$A = -E_{LUMO}$		2.762	2.756	2.759	2.749
$\Delta E = I - A$ (eV)		5.885	5.853	5.536	5.864
Dipole moment (Debye)		11.41	9.82	12.48	10.76
Energy (a.u.)		-2094.91	-2059.04	-2554.31	-2212.68
Twist angle (θ)	θ_1	86.32	89.98	86.24	89.85
	θ_2	19.75	13.77	19.79	13.10
$\eta = (I - A)/2$		2.943	2.927	2.770	2.932
$\chi = (I + A)/2$		5.705	5.683	5.529	5.681
$\sigma = 1/\eta$		0.340	0.342	0.361	0.341
$S = 1/2\eta$		0.170	0.171	0.181	0.171
$Pi = -\chi$		-5.705	-5.683	-5.529	-5.681
$\omega = (Pi)^2/2\eta$		5.530	5.517	5.518	5.504
$\Delta N_{max} = \chi/\eta$		1.939	1.942	1.996	1.938

between molecule **10d** and **10i** is 3.74 and θ_2 is 6.02 due to presence of electron donating group at R_2 and R_4, as well as absence of such group at R_3 position, these allows the molecule **10i** to form hydrogen bond with MET769 in plane 1, one arene proton interaction with LYS721 and hydrogen bond with ASP831 about plane 2, showing highest binding score against *E. coli* FabH.

Conclusion

A new series of pyrazole-quinoline hybrids have been synthesized in one-pot multi-component reaction in the presence of base catalyzed using conventional method with slight modifications providing the good yield. The work provided two highly efficient bioactive nuclei in a single molecule showing relatively promising pharmacological activity compared to second generation therapeutic drug. Biological activity study of each component showed that the majority of the synthesized compounds showed significant antibacterial and anticancer activity. Among the synthesized 24 molecules, compounds **10r**, **10i**, **10x** and **10p** showed the most effective inhibitory activity against EGFR, while compounds **10i**, **10h**, **10r** and **10x** showed most effective against *E. coli* FabH, among all these compounds,**10i** and **10r** found to be effective against both EGFR and FabH. Docking studies also suggested that among all the synthesized compounds, **10r** showed lowest binding energy with EGFR and compound **10i** showed lowest binding energy with FabH. Hence, it is suitable to conclude that hybrid derivative with pyrazole and quinoline nucleus have become a dynamic spot of antibacterial and anticancer activity. Addition to the biological activity, the geometry of all the synthesized compounds were optimized using DFT method to evaluate the position of the terminal substitution and hybrid nucleus helping in understanding the docking simulation data.

ACKNOWLEDGEMENTS

The authors thank The Principal, Dr. (Prof.) Keyur Shah of Shri M. M. Patel Institute of Sciences and Researches, Department of Chemistry, Gandhinagar, India for providing the necessary research facilities.

CONFLICT OF INTEREST

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

REFERENCES

1. M.M. Heravi and V. Zadsirjan, *RSC Adv.*, **10**, 44247 (2020); https://doi.org/10.1039/D0RA09198G
2. N. Kerru, L. Gummidi, S. Maddila, K.K. Gangu and S.B. Jonnalagadda, *Molecules*, **25**, 1909 (2020); https://doi.org/10.3390/molecules25081909
3. K. Karrouchi, S. Radi, Y. Ramli, J. Taoufik, Y.N. Mabkhot, F.A. Al-aizari and M. Ansar, *Molecules*, **23**, 134 (2018); https://doi.org/10.3390/molecules23010134
4. S. Fustero, M. Sánchez-Roselló, P. Barrio and A. Simón-Fuentes, *Chem. Rev.*, **111**, 6984 (2011); https://doi.org/10.1021/cr2000459
5. A. Ansari, A. Ali, M. Asif and S. Shamsuzzaman, *New J. Chem.*, **41**, 16 (2017); https://doi.org/10.1039/C6NJ03181A
6. G. Steinbach, P.M. Lynch, R.K.S. Phillips, M.H. Wallace, E. Hawk, G.B. Gordon, N. Wakabayashi, B. Saunders, Y. Shen, T. Fujimura, L.-K. Su, B. Levin, L. Godio, S. Patterson, M.A. Rodriguez-Bigas, S.L. Jester, K.L. King, M. Schumacher, J. Abbruzzese, R.N. DuBois, W.N. Hittelman, S. Zimmerman, J.W. Sherman and G. Kelloff, *N. Engl. J. Med.*, **342**, 1946 (2000); https://doi.org/10.1056/NEJM200006293422603
7. J.M. Uslaner, S. Parmentier-Batteur, R.B. Flick, N.O. Surles, J.S.H. Lam, C.H. McNaughton, M.A. Jacobson and P.H. Hutson, *Neuropharmacology*, **57**, 531 (2009); https://doi.org/10.1016/j.neuropharm.2009.07.022
8. G. Friedrich, T. Rose and K. Rissler, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **766**, 295 (2002); https://doi.org/10.1016/S0378-4347(01)00514-X
9. C. Hampp, A.G. Hartzema and T.L. Kauf, *Value Health*, **11**, 389 (2008); https://doi.org/10.1111/j.1524-4733.2007.00281.x
10. I.M. Spitz, B.H. Novis, R. Ebert, S. Trestian, D. LeRoith and W. Creutzfeldt, *Metabolism*, **31**, 380 (1982); https://doi.org/10.1016/0026-0495(82)90114-7
11. D. Luttinger and D.J. Hlasta, *Annu. Rep. Med. Chem.*, **22**, 21 (1987); https://doi.org/10.1016/S0065-7743(08)61151-3
12. K. Tsutomu and N. Toshitaka, *Neuropharmacology*, **17**, 249 (1978); https://doi.org/10.1016/0028-3908(78)90108-9
13. J. García-Lozano, J. Server-Carrió, E. Escrivà, J.-V. Folgado, C. Molla and L. Lezama, *Polyhedron*, **16**, 939 (1997); https://doi.org/10.1016/S0277-5387(96)00346-4
14. J.Y. Lee, K.W. Jeong, S. Shin, J.U. Lee and Y. Kim, *Bioorg. Med. Chem.*, **17**, 5408 (2009); https://doi.org/10.1016/j.bmc.2009.06.059
15. E.J. Meuillet and E.G. Bremer, *Pediatr. Neurosurg.*, **29**, 1 (1998); https://doi.org/10.1159/000028677
16. F. Bernardi, I.G. Csizmadia and A. Mangini, Organic Sulfur Chemistry, Elsevier: Amsterdam, The Netherlands (1985).
17. E. Block, Block Copolymers – Overview and Critical Survey, In: Reactions of Organosulfur Compounds, Academic Press: New York (1978).
18. S. Patai and Z. Rappoport, The Chemistry of Organic Selenium and Tellurium Compounds, John Wiley & Sons: New York (1986).
19. M.D. McReynolds, J.M. Dougherty and P.R. Hanson, *Chem. Rev.*, **104**, 2239 (2004); https://doi.org/10.1021/cr020109k
20. D.J. Ager, *Chem. Soc. Rev.*, **11**, 493 (1982); https://doi.org/10.1039/cs9821100493
21. E.A. Ilardi, E. Vitaku and J.T. Njardarson, *J. Med. Chem.*, **57**, 2832 (2014); https://doi.org/10.1021/jm401375q
22. P. Sharma, R. Patel, R.R. Koshti, A. Vyas and C.B. Sangani, *Asian J. Chem.*, **34**, 3169 (2022); https://doi.org/10.14233/ajchem.2022.23981
23. L.F. Tietze and A. Steinmetz, *Synlett*, 667 (1996); https://doi.org/10.1055/s-1996-5548
24. C.J. Xu and Y.Q. Shi, *J. Chem. Crystallogr.*, **41**, 1816 (2011); https://doi.org/10.1007/s10870-011-0178-4
25. N.H. Sapariya, B.K. Vaghasiya, R.P. Thummar, R.D. Kamani, K.H. Patel, P. Thakor, S.S. Thakkar, A. Ray and D.K. Raval, *New J. Chem.*, **41**, 10686 (2017); https://doi.org/10.1039/C7NJ01962A
26. F. Neese, *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, **8**, e1327 (2017); https://doi.org/10.1002/wcms.1327
27. R. Kurtaran, S. Odabasioglu, A. Azizoglu, H. Kara and O. Atakol, *Polyhedron*, **26**, 5069 (2007); https://doi.org/10.1016/j.poly.2007.07.021