Original Article

Synthesis And Docking Studies Of Innovative Pyrazole Hydrazides

S.Gunasekar*, M.Saamanthi, S.Aruna and R.Girija

Department of chemistry, Queen Mary's college. Chennai 6000004, Tamilnadu, India
Corresponding Authors : S. Gunasekar - sgsjain@gmail.com
S. Aruna- arunaschemqmc@gmail.com
DOI: 10.47750/pnr.2023.14.S02.22

Abstract

The heterocyclic chemistry is never ending and ever-growing subject. Pyrazole, a heterocyclic molecule, which leading the pharmaceutical business due to their immense therapeutic activities. Due to the potential biological activities of the pyrazole compounds, more research work is being conducted on these molecules and which further leads to the large investment on pyrazoles followed by invention of new potential drugs. An array of pyrazole benzamides (8a-8s) were prepared by reacting pyrazole hydrazides with different aldehydes and among the synthesized molecules 8g and 8i was the lead molecules. Structures of 8a-8s were confirmed by ^{1}H NMR, LC-MS and IR spectroscopy and its biological activities were studied by molecular docking study and study shows almost all molecules have the activity for rheumatoid arthritis disease.

INTRODUCTION

The heterocyclic chemistry is greatest prime concern and interesting part of the organic chemistry, this is due to its theoretical insinuations and its wide business applications. Heterocyclic molecules are inherently having some therapeutic activities, which further increasing while fusing with other ring systems which results in the enhancement of biological activity. Among the heterocyclic molecules, pyrazole moiety is special scaffold which demonstrates the diversity biological activities like anti-arthritic, anti-tuberculosis, anaesthetic, hypnotic, anti-viral, anti-inflammatory and much more. As the result, research scientists and research investors like NCE and NDD organizations (New chemical entities and new drug discovery respectively) are being showing very special interest towards the synthesis of heterocyclic molecules especially compounds containing pyrazole heterocycles. The pyrazole molecules are generally being prepared by the reaction of unsaturated aldehyde with hydrazine.

Organic molecules are massive significant in our daily life and it is being primarily used in pharmaceutical, agrochemicals and veterinary products etc. In the field of organic chemistry, Heterocyclic compounds are being account for the largest family of organic molecules. Pyrazoles of heterocyclic family dominantly lead the pharmaceutical industry, the drugs made up of pyrazole rings are used to treat different diseases and examples for the few are Celebrex (arthritis and acute pain), Crizotinib (non-small cell lung cancer), Difenamizole (Anti-inflammatory), Epirizole (Anti-inflammatory), Lonazolac (Rheumatic pains), Ruxolitinib (Myelotibrosis) and Tosasertib (Aurora kinase inhibitor). Before moving to clinical trials, it will be easier and time saving and money saving process would be "in silico" method.

To evade the drug development cost and to speed the early phase of drug discovery works, virtual screening is the best and cost-effective method. Structure based drug design method is the best among the exiting other kind of drug design methods due to its highest reliability. While discussing about the structure based drug design, the main and important tool to study the orientation of molecules is molecular docking, also it is largely being the more used method in the area of structure-based drug design. The relation of molecule and ligand protein is the bottle neck in the area of molecular docking. The data base for the molecular docking is Protein Data Bank (PDB), which clearly demonstrates the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. Based on the literature review, the protein data bank RCSB (PDB) chooses the 6NIX protein since the protein- ligand interaction of 6NIX is good for the synthesized molecules. 8g and 8i compounds were bound strongly with hydrogen bonds present in GLU-128, ILE-127 and VAL-159, LAL129, LEU-147 and THR-157 amino acid residues. The compound 8g and 8i docking with protein 6NIX having more docking score

such as -5.315 Kcal /mol and -4.561 Kcal /mol hence 8g and 8i are claimed more active compounds. In this paper we present, synthesis and docking studies of innovative pyrazole hydrazides and their biological activity study using molecular docking method.

EXPERIMENTAL

The prepared molecular were elucidated and confirmed by proton NMR , Infrared spectroscopy and by LC-MS and the analysis was done in private college. Melting points of synthesized molecules were measured in an uncorrected open capillary tubes and IUPAC nomenclature were followed for newly synthesized molecules. Molecular docking study for the synthesized molecules were screened using Schrodinger software at Queen Mary's college. As the preliminary assessment, progress of all below reactions were ascertained by TLC.

Synthesis of 1,1,3-tricyano-2-amino-1-propene (1). Dicyanomethane (50 g, 0.75 mmol) and ethyl alcohol (250 ml) was added to a clean RBF and it cooled to 5-10 ºC and portion wise KOH (14.025, 0.25 mmol) was added and heated to 50-55 ºC for 30 minutes, the mass was cooled to 25-30 ºC and solid was filtered. The solid was dissolved in water (200 mL), hydrochloric acid was added and pH adjusted to 2-3, the obtained solid was filtered to give compound **I.** Color of the solid; Off white and % of yield 45; ^{1}H NMR (400MHz DMSO-d6, δppm): 9.08 (s, 1H), 8.99 (s, 1H), (3.85, 2H) m/z: 131.0 [M]-

Synthesis of 5-Amino-3-(cyanomethyl)-1H-pyrazol-4-yl cyanide (2). To a stirred solution of compound I (45g, 0.34 mmol) in 450 ml of water, added hydrazine hydrate ~50% (25g, 0.39 mmol) slowly, the reaction mass temperature was raised to 90ºC and stirred for 3 h, after completion of the reaction, the solid was filtered at 25-30 ºC and washed with water to give 38g of Compound **2**; Color of the solid: Brown and % of yield 78. ^{1}H NMR (300MHz DMSO-d6, δppm): 12.00 (s, 1H) 6.5 (s, 2H) 3.94 (s, 2H) m/z: 149 [M] +

Synthesis of 3-amino-5-(carboxymethyl)-1H-pyrazole-4-carboxylic acid (3). Aq.Sodium hydroxide solution (12M, 60 mL) was added to Compound **2** (35 g, 0.24 mmol) at ambient temperature. The reaction was refluxed for overnight. The pH of the reaction was adjusted to ~2-3 further the obtained solid was filtered and filtrate was concentrated under vacuum to get 35g of compound **3** as grey solid with 66% yield.^{1}H NMR (300MHz DMSO-d6, δppm): 11.89 (m, 3H), 5.71 (m, 2H), 3.55 (m, 3H) m/z: 186 [M] +

Synthesis of 5-Amino-1H-pyrazol-3-ylacetic acid (4). Water (700 ml) was added to Compound **3** (28 g, 0.15 mmol) and the slurry was maintained under stirring condition at RT for 5 minutes, then reaction temperature raised to 60 ºC for 5h. The reaction was evaporated till complete concentration dryness to yield compound **4** and color of the solid is brown solid. ^{1}H NMR (300MHz DMSO-d6, δppm): 7.30 (m, 1H), 5.25 (s, 1H), 3.30 (s, 2H) mass: 142.3 [M] +

Synthesis the methyl ester of 5-Amino-1H-pyrazol-3-ylacetic acid hydrochloride (5). Methanol was charged to RBF having compound 4 (20g, 0.14 mmol) and to this stirred solution added thionyl chloride slowly (54.72 g, 0.45 mmol) at 0-5 ºC and stirred for 4 hours and filtered the material. ^{1}H NMR (300MHz DMSO-d6, δppm): 10.72 (m, 3H), 5.91 (s, 1H), 3.8 (s, 2H), 3.63 (s, 3H) m/z: 156.2 [M] +

Synthesis of methyl 2-(3-(4-chlorobenzamido)-1H-pyrazol-5-yl)acetate (6). DMF (110 mL), DIEA (22.04 g, 0.161 mmol) was stirred and to this clear solution added 2-fluoro-5-bromo benzoic acid (15.02g, 0.073 mmol) and the reaction mass was cooled to 0-5 ºC, in portion wise EDC.HCl (20.09g, 0.11 mmol) was added followed by HOBt (13.58g, 0.109 mmol). To this slurry, further compound 5 (amine) 13.781g, 0.073 mmol) was added and maintained for 30 minutes. The reaction was quenched with water and the product was extracted with ethyl acetate and it evaporated to dryness and further n-heptane was added and the solid was filtered to get Compound **6**, 11.01g; brown solid and % yield is 69, ^{1}H NMR (400MHz DMSO-d6, δppm): 12.62 (s, 1H), 10.87 (s, 1H), 8.02-7.93 (m, 2H), 7.58-7.55 (m, 2H), 6.50 (s, 1H), 3.74 (s,3H).

Synthesis of 4-chloro-N-(5-(2-hydrazinyl-2-oxoethyl)-1H-pyrazol-3-yl)benzamide (7). Hydrazine hydrate (20g, 0.62 mmol) was added Compound **6** (11g, 0.030 mmol) and the slurry was stirred for 30-45 minutes at 25±5 ºC, the solid was filtered to get compound **7** with 90% yield. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.27 (s, 1H) 10.82 (s, 1H), 9.22 (s, 1H), 8.01-7.99 (d, 2H), 7.57-7.55 (d, 2H), 6.50 (s, 1H),4.26(s,2H),3.40(s,2H);m/z:293.0[M] +

General Preparation technique for (8a- s): compound 7 (500mg, 1 eq) was added to ethanol followed by aromatic aldehydes (1.1 eq) were refluxed with small amount of CH3COOH and maintained for 3h at reflux. The precipitated solids were filtered.

^{1}H NMR and LC-MS of compound 8a-8s: (E)-3-chloro-N-(5-(2-(2-(2-hydroxybenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide) (8a). white solid. MP 166-173 ºC. Yield: 77 %. IR (KBr, cm^{-1}): 3305 (NH), 1672 (C=O); ^{1}H NMR (400MHz DMSO-d6, δppm): 12.41-12.34 (d, 1H), 11.88 (s, 1H) 11.46-11.06 (d, 1H), 10.93-10.89 (d, 1H), 10.07 (s, 1H) 8.43-8.32 (d, 1H), 7.96-7.94 (d, 1H), 7.74-7.72 (t, 1H), 7.55-7.50 (m, 3H), 7.31.7.22 (s, 1H) 6.92-6.85 (m. 1H) 6.60-6.54 (s, 1H), 4.02-3.64 (d, 2H), MS m/z: 474.10 [M] +

(E)-3-chloro-N-(5-(2-(2-(3-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (8b). Pale brown solid. MP 177-172 ºC. Yield: 77 %. IR (KBr, cm^{-1}): 3259 (NH), 1655(C=O); ^{1}H NMR (400MHz DMSO-d6, δppm): 12.39-12.35 (d, 1H), 11.60-11.47 (d, 1H), 10.93-10.90 (d, 1H), 9.63-9.62 (s, 1H) 8.12-7.93 (t, 3H), 7.64-7.62 (d, 2H), 7.26-7.22 (t, 2H), 7.17-7.07 (m, 2H), 6.83-6.81 (q, 1H) 6.59-6.55 (d, 1H), 4.03-3.61 (d, 2H); MS m/z: 398.4 [M] +

(E)-3-chloro-N-(5-(2-(2-(4-chlorobenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)benzamide (8c). White solid. MP 197-199 C. Yield: 75%. IR (KBr, cm^{-1}): 3310 (NH), 1676(C=O); ^{1}H NMR (400MHz DMSO-d6, δppm): 12.40-12.35 (d, 1H), 11.72-11.59 (d, 1H),

10.92-10.89 (d, 1H), 8.21 (s, 2H) 8.02 (s, 1H), 7.95-7.92 (t, 2H), 7.76-7.72 (t, 1H), 7.654-7.50 (m, 3H), 6.59-6.55 (d, 1H), 4.04-3.62 (d, 2H); MS m/z: 416.5 [M] +

(E)-3-chloro-N-(5-(2-(2-(4-methoxy-2,3-dimethylbenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (8d). Pale pink solid. MP 189-192 ºC. Yield: 55%.^{1}H NMR (400MHz DMSO-d6, δppm): 12.39-12.34 (d, 1H), 11.48-11.29 (d, 1H), 10.92-10.89 (d, 1H), 8.50 (s, 1H) 8.33 (s, 1H), 8.03 (s, 1H) 7.95-7.93 (d, 1H), 7.68-7.62 (m, 2H), 7.54-7.50 (m, 1H), 6.93-6.90 (m, 1H), 6.59-6.54 (s, 1H) 4.01-3.59 (d, 2H), 3.81 (s, 3H), 2.32-2.31 (d, 3H), 2.12 (s, 3H); m/z: 440.7 [M] +

(E)-N-(5-(2-(2-(4-bromo-3,5-dimethoxybenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)-3-chlorobenzamide (8e). Off white powder. MP 199-201 ºC. Yield: 63%.^{1}H NMR (400MHz DMSO-d6, δppm): 12.36 (s, 1H), 11.75-11.66 (d, 1H), 10.94-10.90 (d, 1H), 8.21-8.02 (m, 2H), 7.98-7.92 (m, 1H), 7.63-7.62 (d, 1H), 7.55-7.50 (m, 1H),7.28-7.27 (s, 1H), 7.05 (s, 2H) 6.55 (s, 1H) 4.05-3.63 (m, 8H); MS m/z: 520.8 [M] +

(E)-3-chloro-N-(5-(2-(2-(2,5-dichloro-4-methylbenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide 8(f). Gray solid. MP 200-203 ºC. percentage of Yield: 66%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.38-12.30 (d, 1H), 11.86-11.72 (d, 1H), 10.92-10.88 (d, 1H), 8.39-8.27 (d, 1H), 8.03-7.93 (t, 2H) 7.95-7.93 (d, 1H), 7.64-7.62 (d, 2H), 7.54-7.50 (m, 1H), 6.93-6.90 (m, 1H), 6.59-6.53 (s, 1H) 3.99-3.63 (d, 2H), 2.33 (s, 3H), MS m/z: 464.5 [M] +

(E)-3-chloro-N-(5-(2-(2-(2,3-dihydroxybenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide 8(g). Off white crystal. MP 211-213 ºC. Yield: 58%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.33 (s, 1H), 11.87-11.45 (d, 1H), 10.93-10.88 (t, 1H), 9.53-9.21 (t, 3H), 8.37-8.32 (s, 1H) 8.03-7.92 (m, 1H), 7.64-7.62 (d, 1H), 7.55-7.50 (m, 1H),7.17-7.15 (d, 1H) 6.97 (s, 1H), 6.97-6.95 (s, 1H), 6.85-6.80 (m, 1H), 6.68-6.66 (m, 1H), 6.56-6.50 (s, 1H) 3.99-3.63 (m, 8H), MS m/z: 414.7 [M] +

(E)-3-chloro-N-(5-(2-(2-(3,5-dichlorobenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)benzamide (8h) Brown solid. MP 203-204 ºC. Yield: 61%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.19 (s, 1H), 11.90-11.72 (d, 1H), 10.93-10.89(d, 1H), 8.18-8.03 (d, 2H), 7.98-7.93 (t, 1H), 7.78-7.75 (d, 2H) 7.67-7.62(d, 2H), 7.55-7.50 (m, 1H) 6.55-6.51 (d, 1H), 4.06-3.33 (d, 2H); MS m/z: 450.8 [M] +

(E)-3-chloro-N-(5-(2-(2-(4-hydroxy-3,5-dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (**8i**) Brown solid. MP 188-190 ºC. Yield: 71%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.17 (s, 1H), 11.49-11.39 (d, 1H), 10.92-10.88(d, 1H), 8.89-8.84 (d, 1H) 8.03-8.02 (d, 1H), 7.95-7.92 (t, 1H), 7.63-7.61 (m, 2H), 7.54-7.50 (m, 1H) 6.96 (s, 2H), 6.54 (s, 1H), 4.02-3.59 (d, 2H), 3.81-3.80 (d, 6H); MS m/z: 458.6 [M] +

(E)-3-chloro-N-(5-(2-(2-(2,3-dichlorobenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (**8j**) Brown solid. MP 200-201 ºC. Yield: 62%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.40-12.20 (d, 1H), 12.05-11.65 (d, 1H), 10.93-10.89 (d, 1H), 9.10-8.71(t, 1H) 8.58-8.53 (m, 1H), 8.34-8.26 (m, 1H), 8.18-8.14 (t, 1H), 8.04-8.03 (t, 1H), 7.96-7.93 (t, 1H), 7.55-7.50 (m, 2H), 7.18-7.08 (m, 1H) 6.5-6.54 (d, 1H), 4.07-3.65 (d, 2H); MS m/z: 450.8 [M] +

(E)-3-chloro-N-(5-(2-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (**8k**) off white solid. MP 187-190 ºC. Yield: 67%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.39-12.35 (d, 1H), 11.98-11.78 (d, 1H), 10.92-10.89 (d, 1H), 8.64-8.43 (d, 1H) 8.02-8.00 (d, 3H), 7.95-7.91 (m, 1H), 7.73-7.69 (m, 2H), 7.54-7.42 (m, 1H) 6.59-6.54 (d, 1H), 4.06-3.64 (d, 2H); MS m/z: 443.7 [M] +

(E)-3-chloro-N-(5-(2-(2-(2,4-dichlorobenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (**8l**) Brown solid. MP 198-199 ºC. Yield: 65%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.17 (s, 1H), 11.92-11.73 (d, 1H), 10.92-10.88 (d, 1H), 8.55-8.36 (d, 1H), 8.05-7.92 (m, 3H), 7.70-48 (m, 4H) 6.54-6.50 (d, 1H), 4.05-3.34 (d, 2H); MS m/z: 450.**7** [M] +

(E)-3-chloro-N-(5-(2-(2-(4-hydroxybenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide (8m). Pale brown solid. MP 180-182 ºC. Yield: 74 %. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.14 (s, 1H), 11.44-11.30 (d, 1H), 10.93-10.89 (d, 1H), 9.92-9.89 (s, 1H) 8.10-7.92 (q, 3H), 7.64-7.62 (d, 1H), 7.54-7.50 (m, 3H), 6.80-6.81 (d, 2H), 6.53-6.51 (d, 1H), 4.00-3.57 (d, 2H); MS m/z: 398.5 [M] +

(E)-N-(5-(2-(2-((2-bromopyridin-4-yl)methylene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)-3-chlorobenzamide (8n). Pale pink solid. MP 177-183 ºC. Yield: 66 %. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.20 (s, 1H), 12.05-11.90 (d, 1H), 10.94-10.91 (d, 1H), 8.44-8.43 (d, 1H) 8.18-8.03 (d, 1H), 7.97-7.72 (m, 3H), 7.64-7.62 (d, 1H), 7.55-7.50 (m, 2H), 6.54-6.52 (d, 1H), 4.08-3.66 (d, 2H); MS m/z: 462.1 [M] +

(E)-3-chloro-N-(5-(2-(2-(5-chloro-2-methoxybenzylidene) hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide 8(o). pink solid. MP 182-185 ºC. Yield: 57%. %. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.18 (s, 1H), 11.77-11.56 (d, 1H), 10.94-10.90 (d, 1H), 8.87-8.03 (t, 2H), 7.95-7.73 (m, 3H), 7.64-7.62 (d, 1H), 7.56-7.50 (m, 2H), 6.54-6.51 (d, 1H), 4.05-3.59 (d, 2H), 3.90-3.86 (t, 3H); MS m/z: 446.5 [M] +

(E)-N-(5-(2-(2-(4-(benzyloxy)benzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)-3-chlorobenzamide 8(p). Off white solid. MP 169-173 ºC. Yield: 68%. ^{1}H NMR (400MHz DMSO-d6, δppm): 12.34 (s, 1H), 11.52-11.39 (d, 1H), 10.92-10.89 (d, 1H), 8.16-8.03 (d, 1H), 7.97-7.93 (t, 2H), 7.67-7.62 (t, 3H), 7.54-7.32 (m, 6H), 7.15-7.07 (m,2H) 6.54 (s, 1H), 5.19-5.16 (d, 2H) 4.02-3.59 (d, 2H); MS m/z: 488.5 [M+] +

(E)-3-chloro-N-(5-(2-(2-(3,4-dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl) benzamide 8(q). Grey solid. MP 188-192 ºC. Yield: 67%.^{1}H NMR (400MHz DMSO-d6, δppm): 12.40 (s, 1H), 11.52-11.40 (d, 1H), 10.89 (s, 1H), 8.64-8.14 (m, 2H), 7.94-

7.92 (d, 1H), 7.63-7.62 (d, 1H), 7.55-7.49 (m, 1H),7.39-7.30 (s, 1H), 7.17-6.99 (m, 2H), 6.5 (s, 1H), 4.05-3.59 (m, 8H); MS m/z: 442.5 [M] +

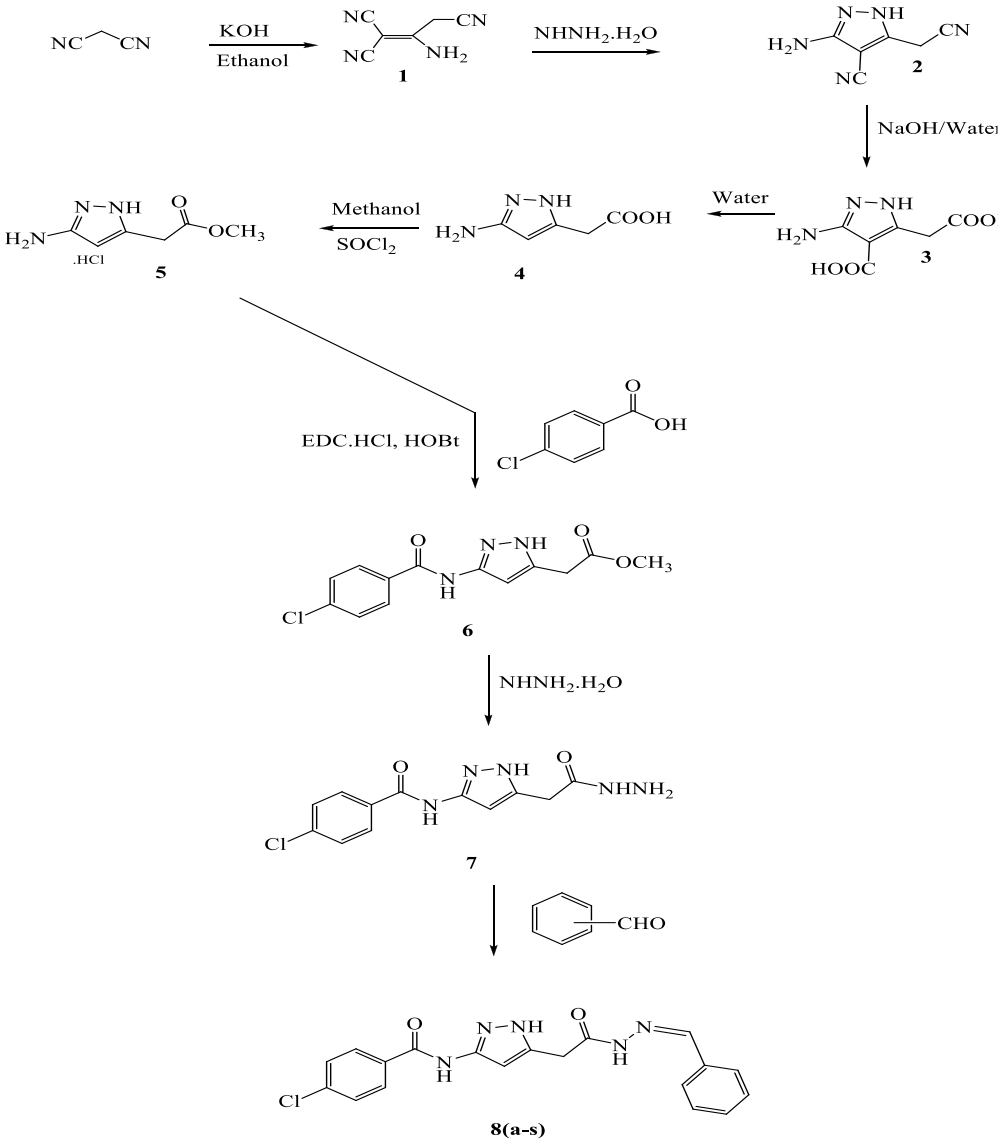
(E)-3-chloro-N-(5-(2-(2-(3-nitrobenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)benzamide (8r). Off white solid. MP 193-195 ºC. Yield: 75%.^{1}H NMR (400MHz DMSO-d6, δppm): 12.39-12.22 (d, 1H), 11.90-11.75 (d, 1H), 10.93-10.89 (d, 1H), 8.94-8.73 (m,2H) 8.57-8.51 (t, 1H), 8.40-8.30 (m, 2H), 8.26-8.14 (m, 2H), 8.03-7.50 (m, 3H), 6.54 (s,1H), 4.08-3.65 (d, 2H); MS m/z: 427.5 [M] +

(E)-N-(5-(2-(2-(5-bromo-2-nitrobenzylidene)hydrazinyl)-2-oxoethyl)-1H-pyrazol-3-yl)-3-chlorobenzamide (8s). White solid. MP 166-170 ºC. Yield: 69%.^{1}H NMR (400MHz DMSO-d6, δppm): 12.37 (s, 1H), 12.05-11.85 (d, 1H), 10.91(s, 1H), 8.90-8.29 (m, 2H), 8.08-7.93 (m, 4H), 7.64-7.62 (d,2H), 7.54-7.53 (m,1H), 6.53 (s,1H), 4.04-3.65 (d, 2H); MS m/z: 506.0 [M] +

RESULTS AND DISCUSSION

3.1 Chemistry: Dicyanomethane was dissolved with ethanol and to it added potassium hydroxide at ambient temperature to give Compound **1**, it was reacted with 80% solution of hydrazine hydrate at 25± 5º C for 60-120 minutes to get compound **2** and it reacted with aq.NaOH at 100º C for overnight to have compound **3** and to it charged H_2O and maintained for 4-6 hrs. at 50-55º C and it was evaporated to have compound **4.** To a slurry of Compound **4** and methyl alcohol, added $SOCl_2$ slowly to have **6**. To a DMF solvent, added Carboxylic acid, DIEA, N-(3-Dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride & Hydroxy benzotriazole and compound **5** and stirred for 4h at ambient temperature, to yield Compound **6**. Compound **6** was reacted with hydrazine hydrate in the ethanol solvent, post addition of hydrazine hydrate, The reaction mixture was maintained for 0.5h, to have Compound 7. Different aldehydes were refluxed with compound 7 in ethanol solvent media to have title molecules (pyrazole hydrazides). Synthetic scheme of 8 (a-s) is demonstrated below in **Synthetic scheme.**

Synthetic scheme



DOCKING SCREENING

Preparation of Ligands: Structures of ligands sketched and saved in SDF format were imported via selecting files. The imported ligands 8 a-s was set to minimize under force field OPLS3e. Minimization calculations can be performed on all structures of pyrazole derivatives.

Preparation of Protein: X-ray crystalline Structure of protein 6NIX was imported from Protein Data Bank (PDB) to workspace, which further set to preprocess followed by review and modification to remove unwanted chains and residues, further refined under force field of OPLS3e. The results were monitored in the job monitor.

Molecular Docking: The pyrazole compounds are selected for molecular docking. All the lead compounds showed good binding energy and exhibited interactions and better lower free energy values, indicating more thermodynamically favored interaction. As for Glide docking, crystal structures of 6NIX have been prepared by the protein preparation wizard in Schrodinger suite. Afterwards, the required receptor grids were made just before docking with the active site determined by the position of co crystal ligand. Crystal structures of 6NIX were imported into Glide, defined as the receptor structure and the location of active sites with a box. The PSLS3e force field was used for grid generation. The standard precision (SP) and the extra precision (XP) protocols were set for docking studies with crucial residues, in constrained binding to get accurate results. Binding affinity was retrieved running Prime MM-GBSA. All other parameters were maintained as default

The research aims to find more prospective lead compounds with a drug discovery system, in which

molecular docking studies achieve the logical drug design. Molecular docking is great tool and much being used technique in structure drug design due to its ability to predict the binding conformation of

small mole molecule ligands to the appropriate target binding site. Docking scores, glide scores and glide energy of synthesized pyrazole cules are shown in the below **table 1** and 2D and 3D interactions of

synthesized compounds are shown in **fig 1-18.**

Table 1: Glide Docking and binding energy scores

Title	Docking Score	Glide g score	Glide energy	Glide model
6NIX	- 5.505	-5.505	-48.561	-58.988
8g	- 5.315	-5.315	-44.561	-57.788
8i	- 4.561	-4.561	-41.442	-56.298
8k	-3.987	-3.987	-40.481	-54.867
8r	-3.923	-3.923	-41.344	-47.589
8b	-3.866	-3.866	-41.196	-54.896
8m	-3.571	-3.571	-36.51	-47.547
8a	-3.327	-3.327	-42.277	-57.014
8f	-3.151	-3.151	-43.084	-51.698
8e	-3.083	-3.083	-44.72	-51.411
8h	-3.024	-3.024	-42.615	-56.787
8c	-2.985	-2.985	-38.789	-48.78
8o	-2.915	-2.915	-42.48	-51.5
8l	-2.774	-2.774	-38.234	-55.803
8s	-2.655	-2.655	-41.704	-56.654
8j	-2.651	-2.651	-41.978	-55.02
8q	-2.633	-2.633	-35.397	-46.445

8n	-2.543	-2.543	-41.92	-59.364
8d	-2.414	-2.414	-37.257	-44.56
8p	-1.389	-1.389	-39.641	-56.392

Note: In the following figures 1 to 18, A and B with 2D and 3D interactions between ligands and receptor (hydrogen bonds are illustr arrows; C atoms are colored gray, N blue, and O red) and compound binding mode at the 6NIX active site. **Note:** In the following figures 1 A and B with 2D and 3D interactions between ligands and receptor (hydrogen bonds are illustrated as arrows; C atoms are colored gray, I and O red) and compound binding mode at the 6NIX active site.

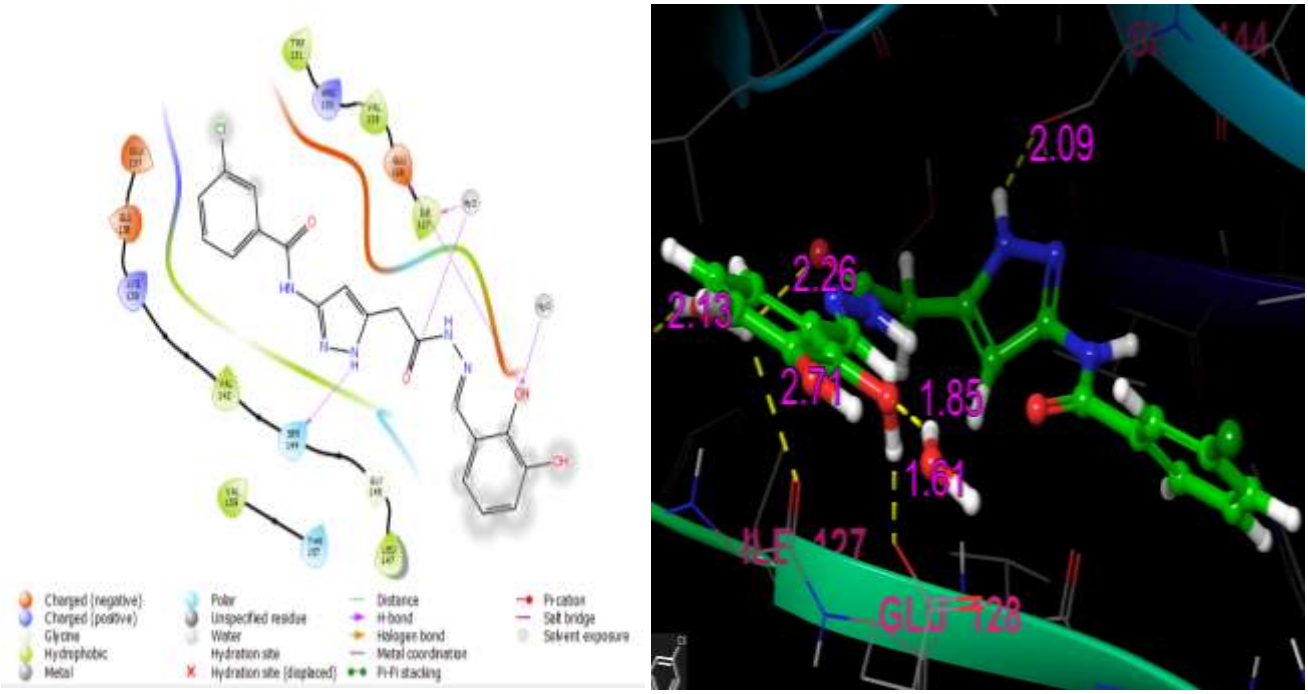


Figure 1: Compound 8g

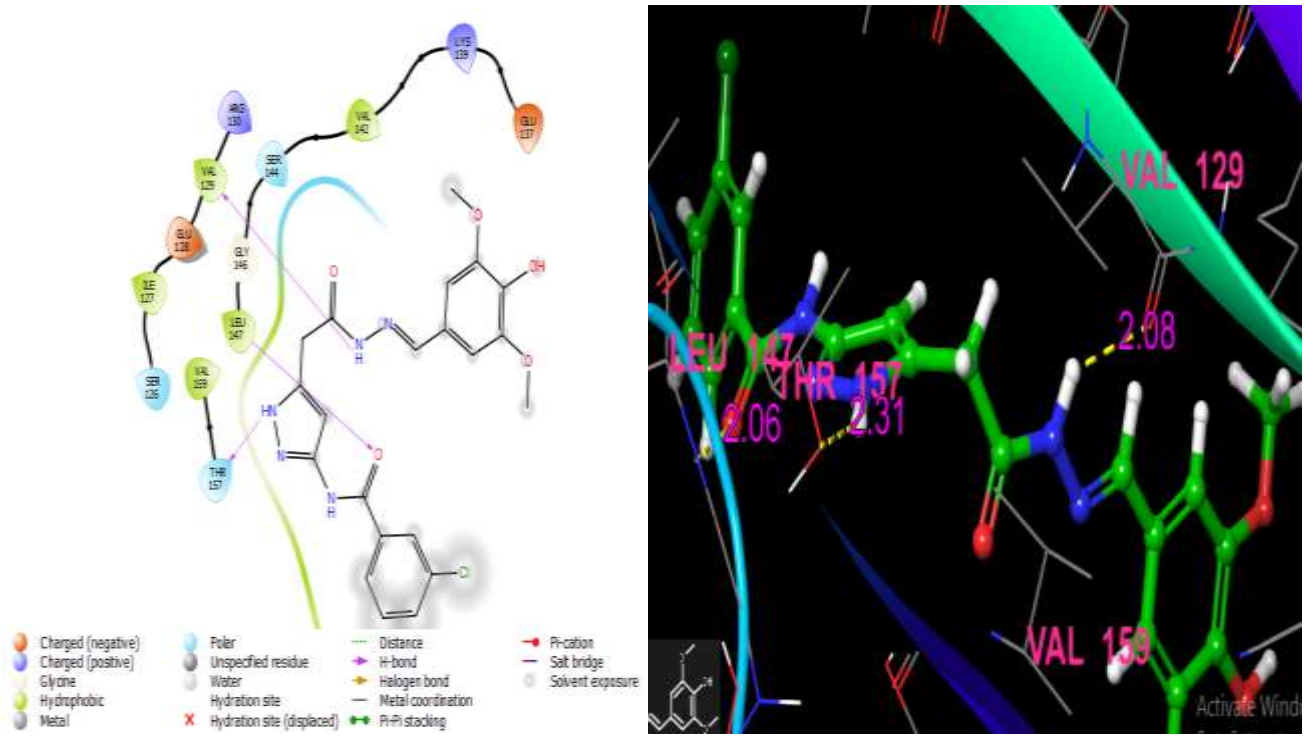


Figure 2: Compound 8i

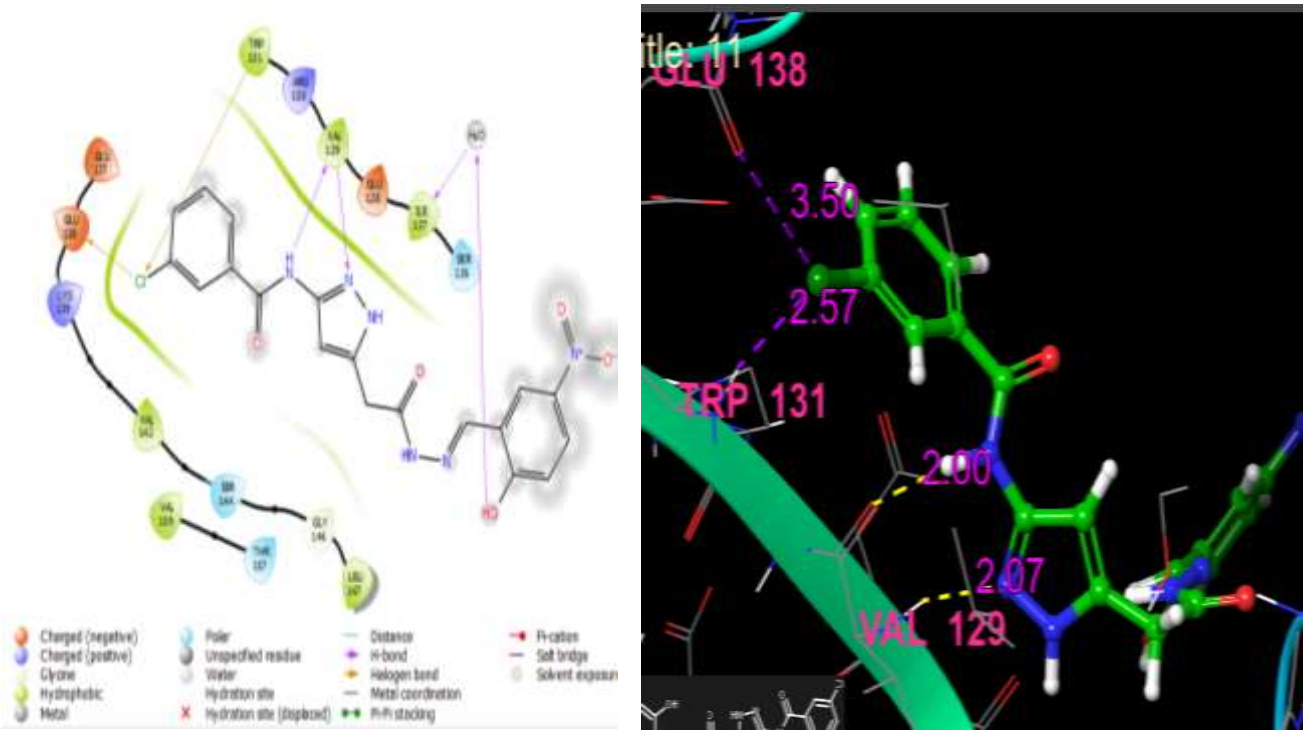


Figure 3: Compound 8k

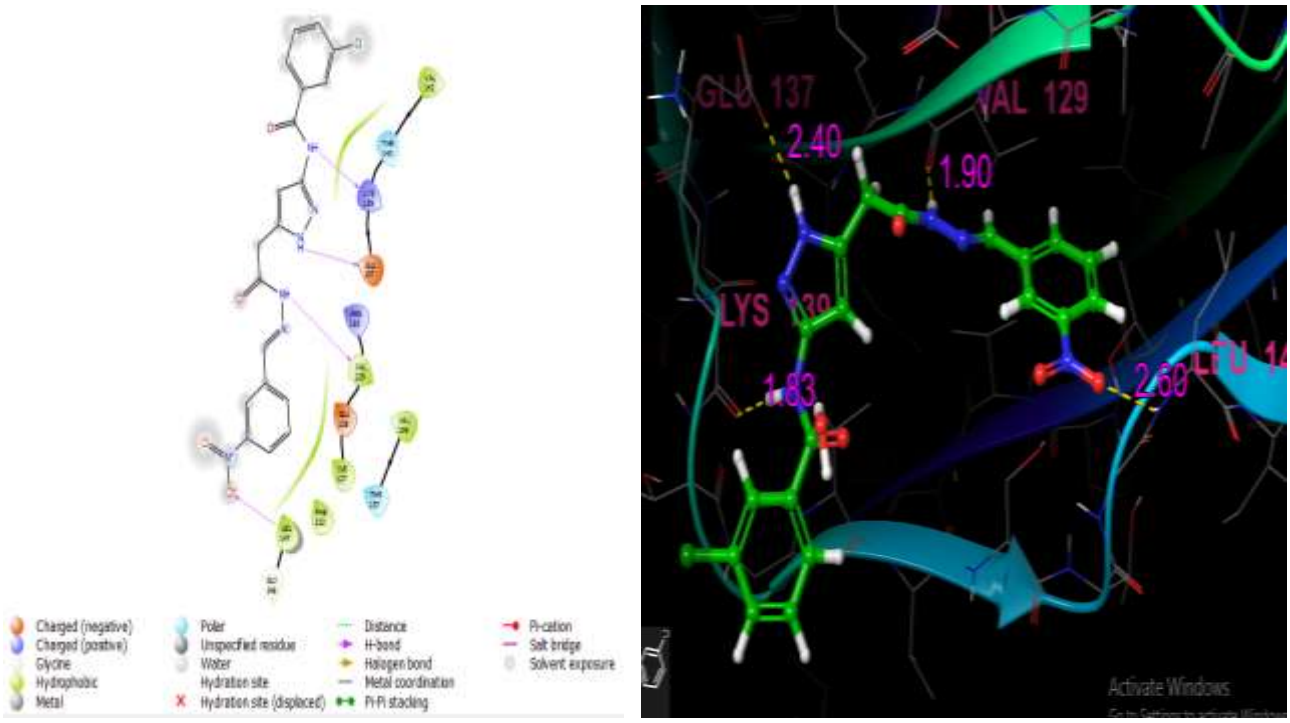


Figure 4: Compound 8r

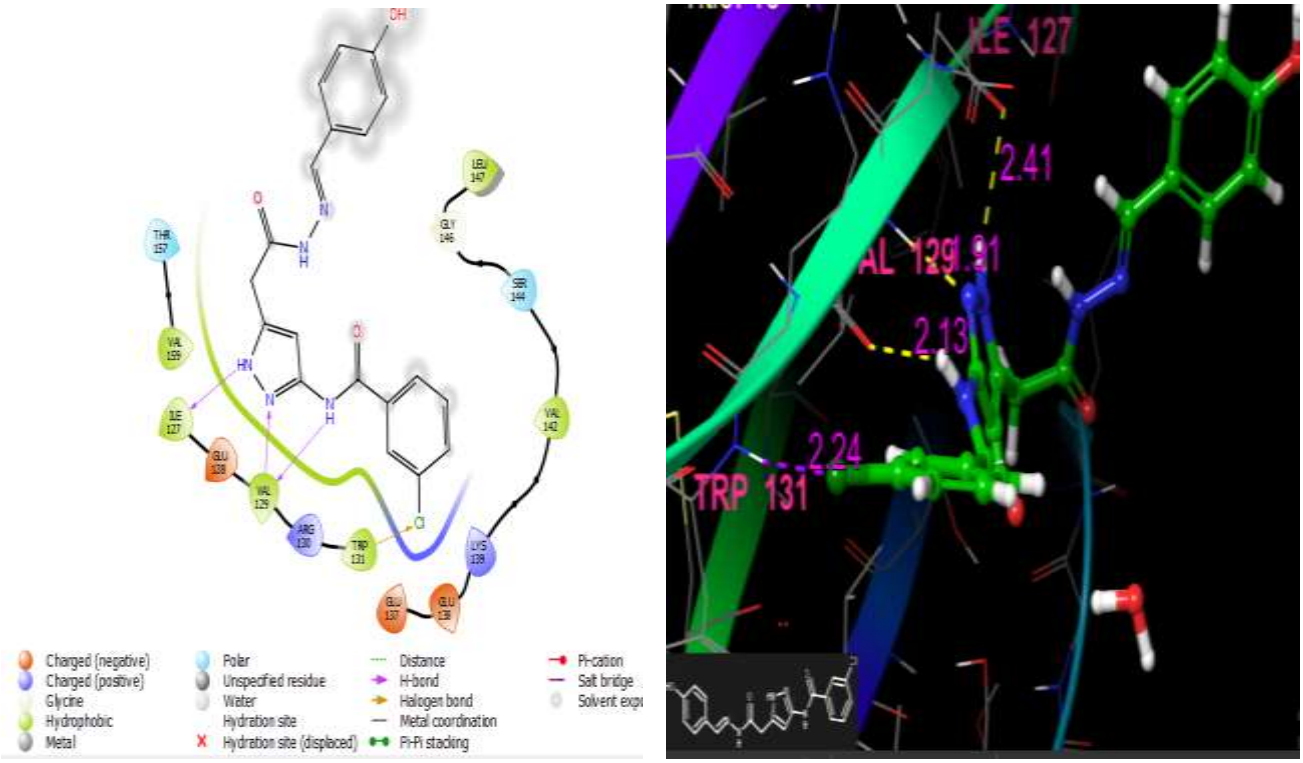


Figure 5: Compound 8m

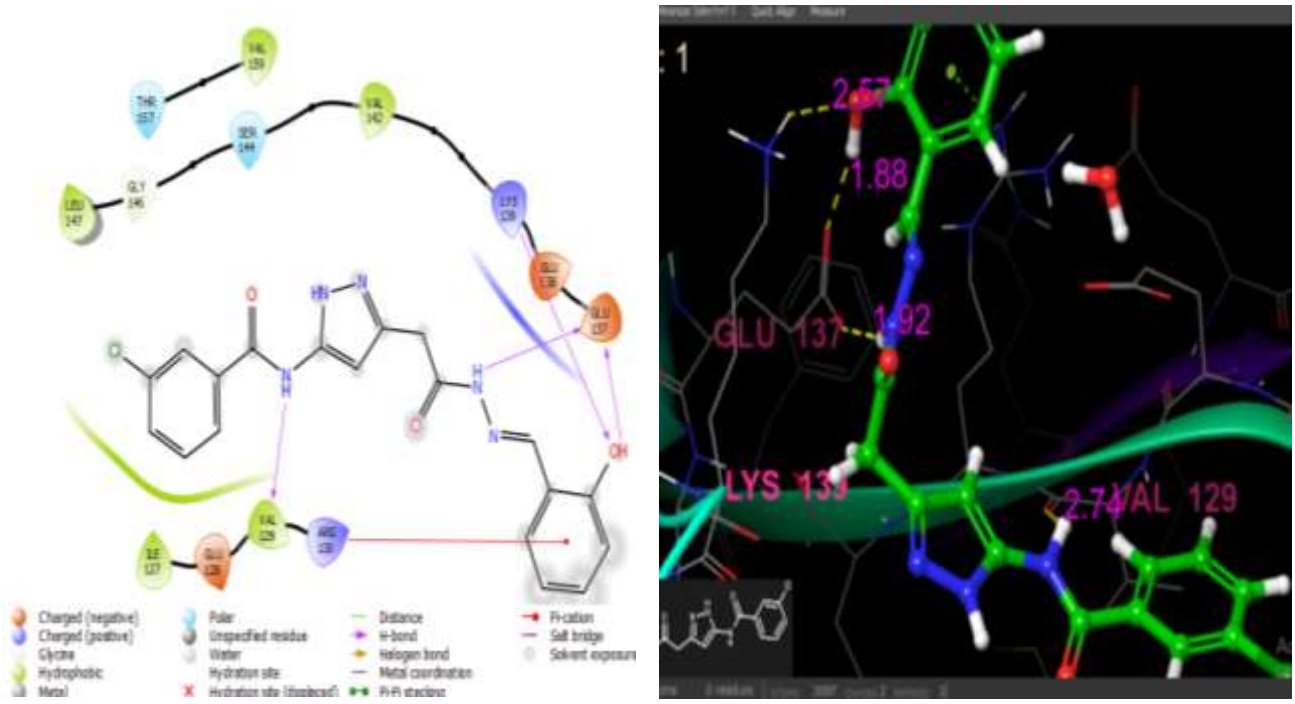


Figure 6: Compound 8a

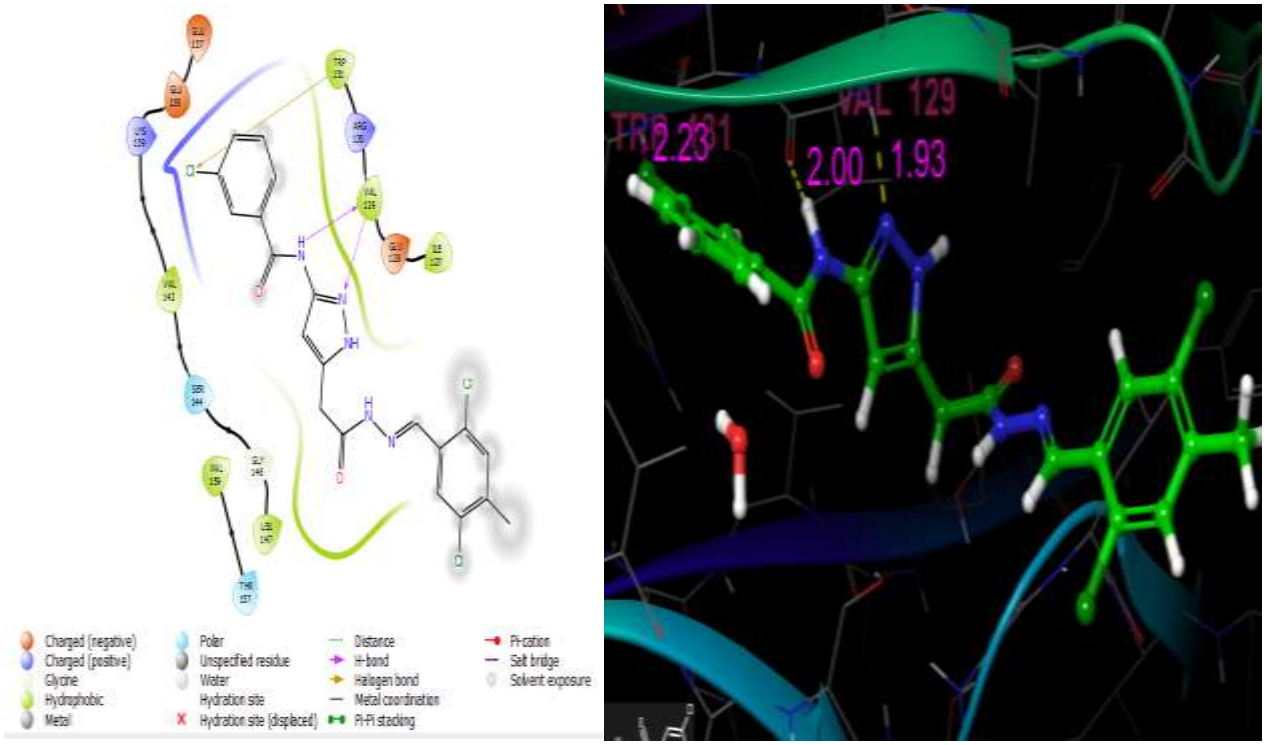


Figure 7: Compound 8f

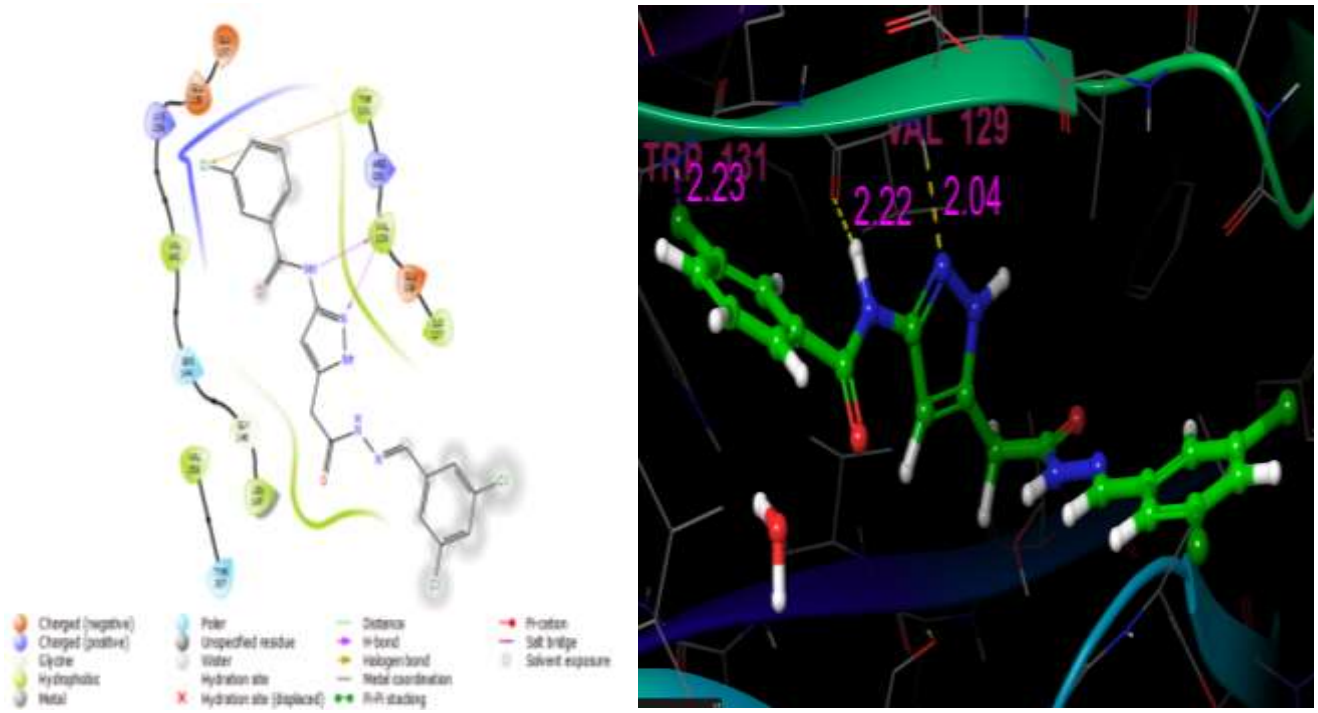


Figure 8: Compound 8h

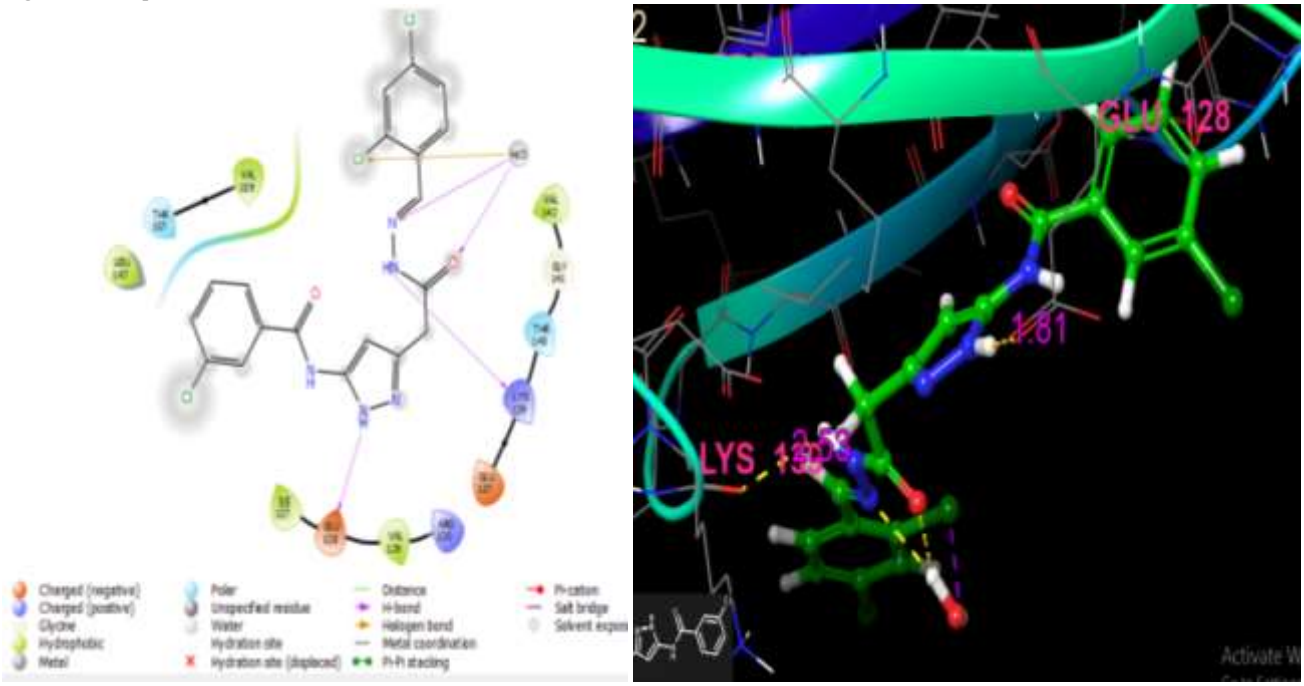


Figure 9: Compound 8l.

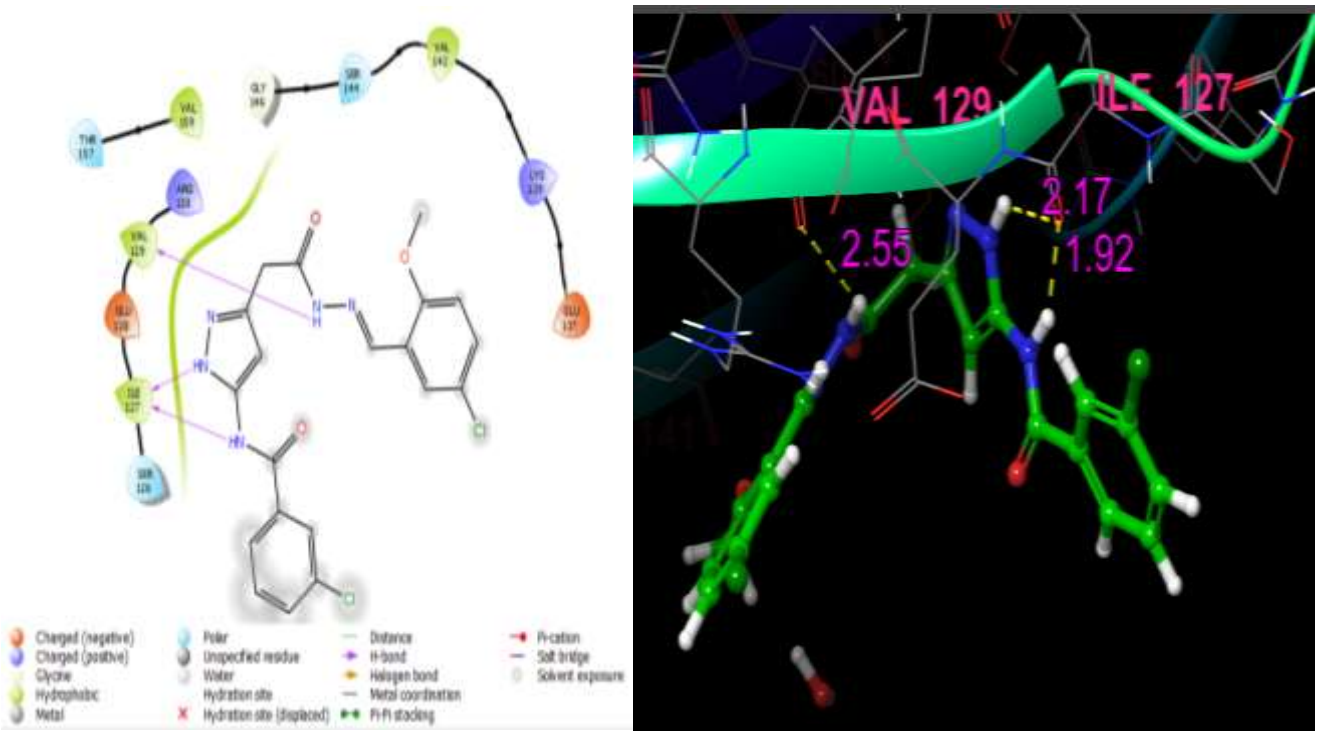


Figure 10: Compound 8o.

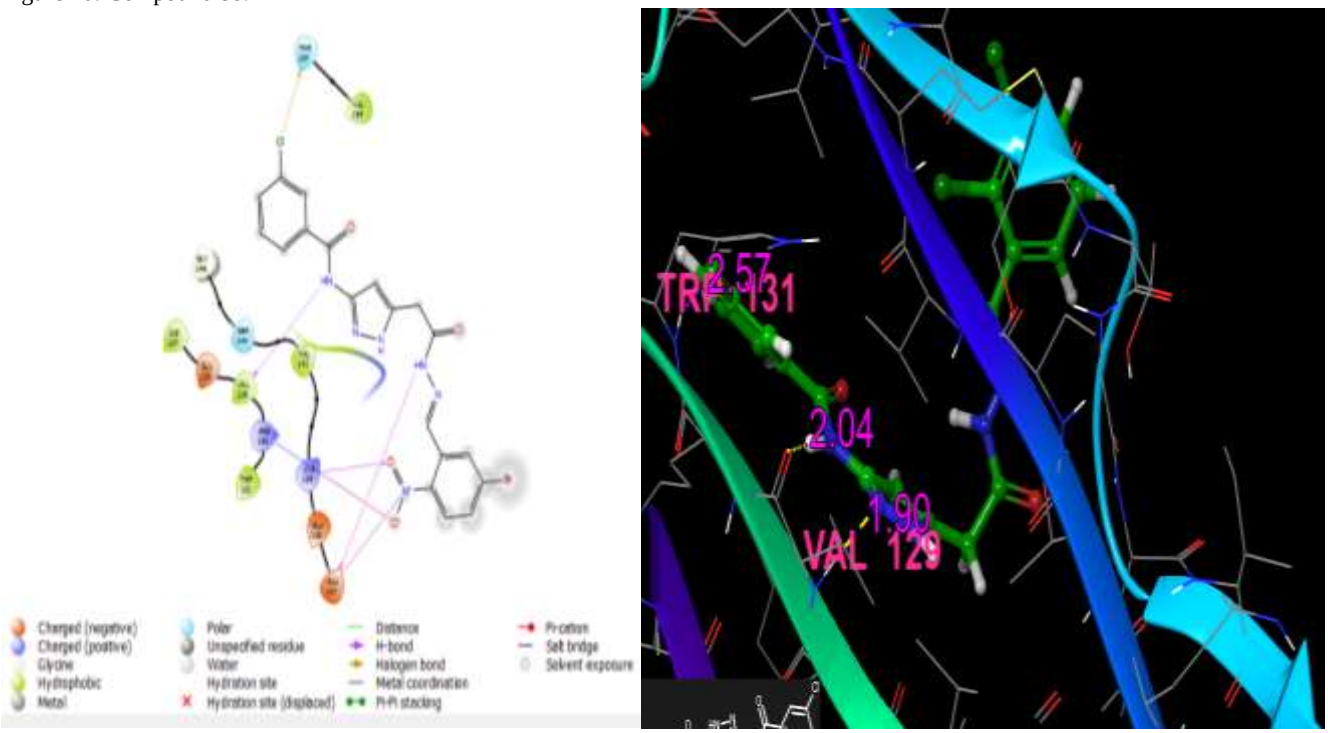


Figure 11: Compound 8s

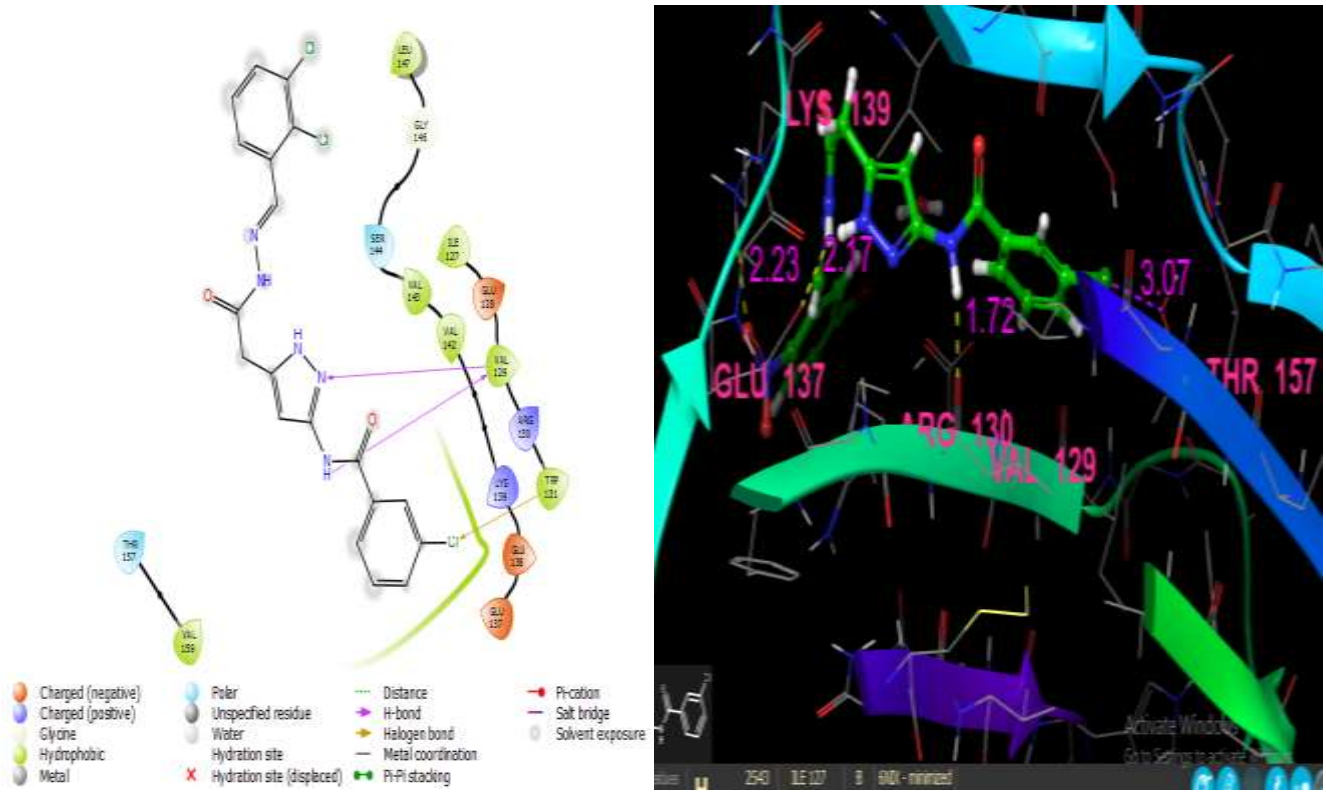


Figure12: Compound 8j

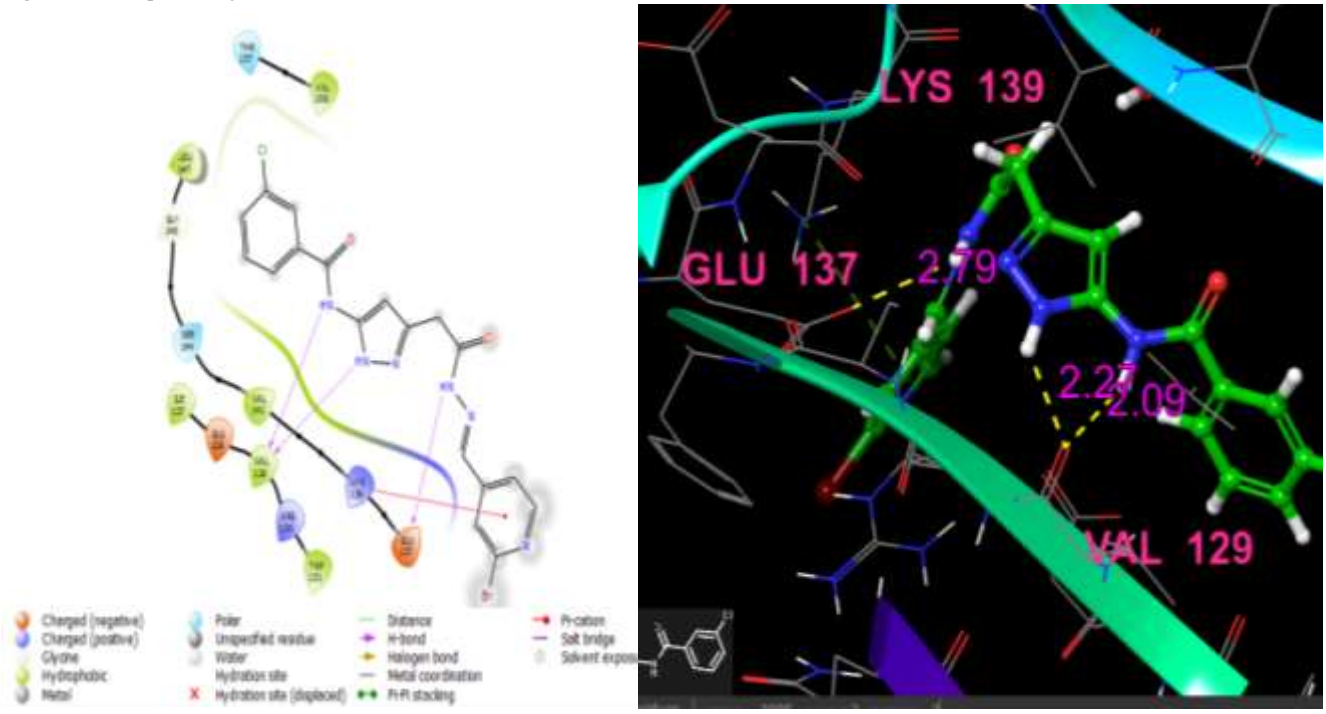


Figure 13: Compound 8n

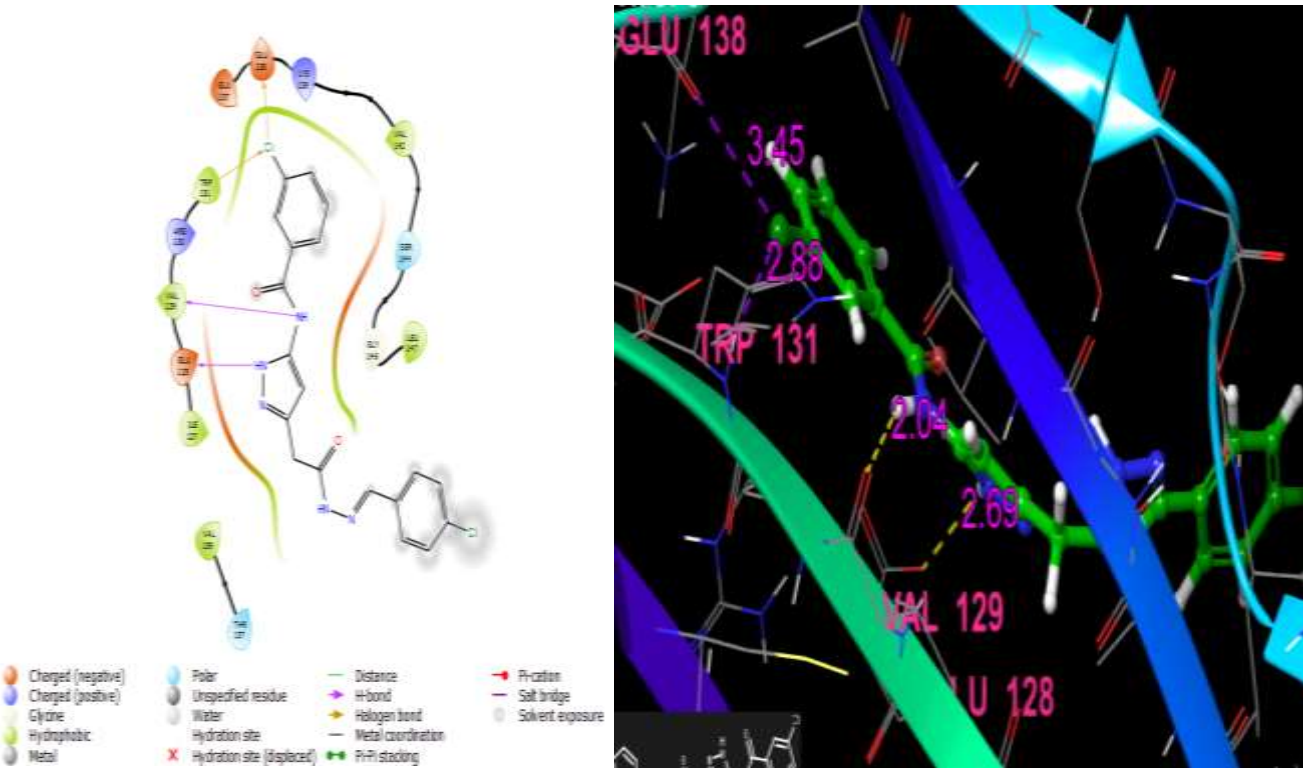


Figure 14: Compound 8c.

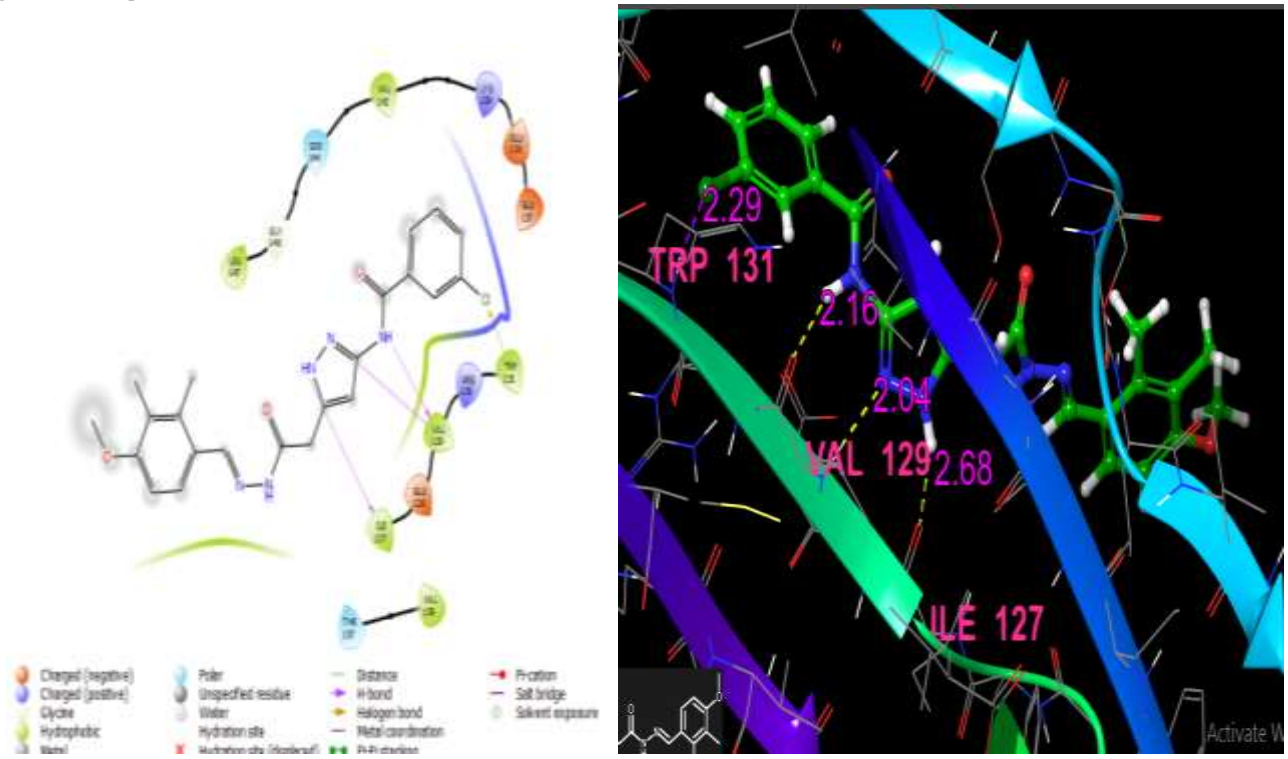


Figure 15: Compound 8d

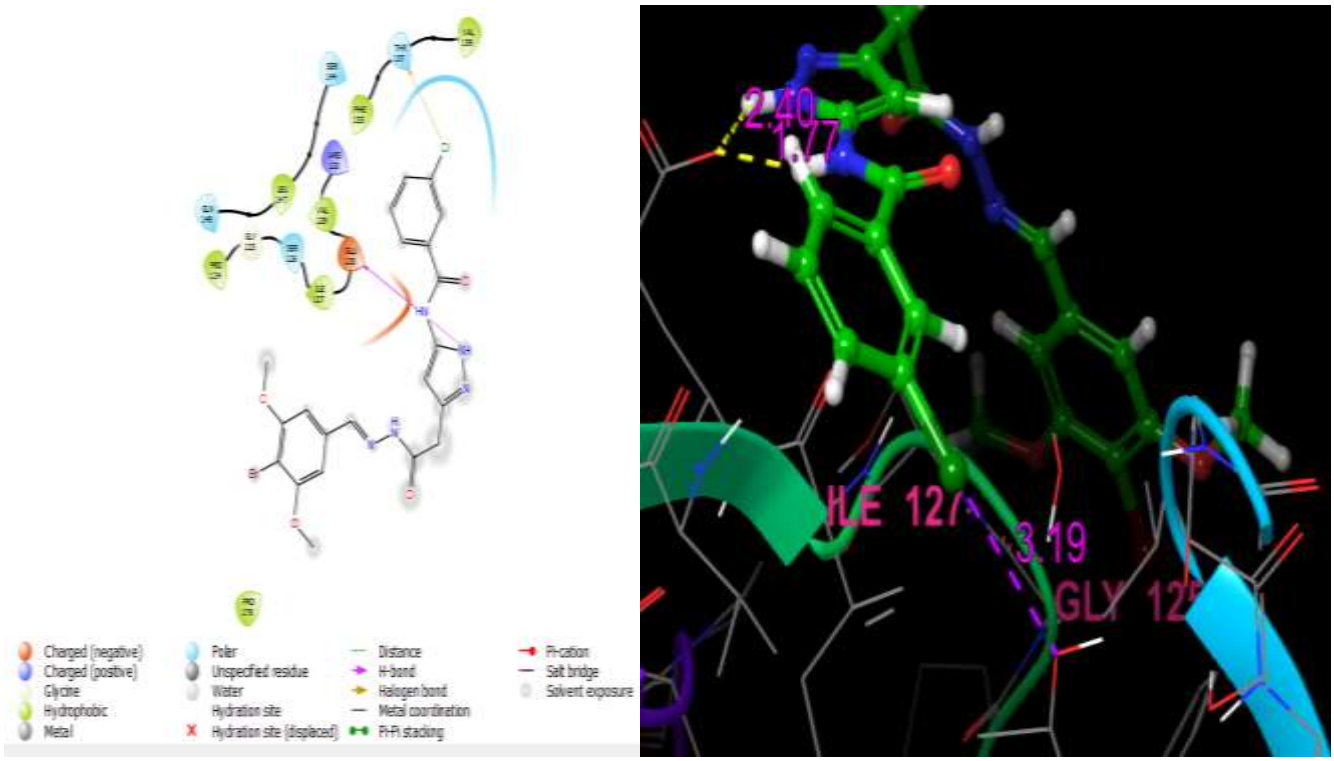


Figure 16: Compound 8e

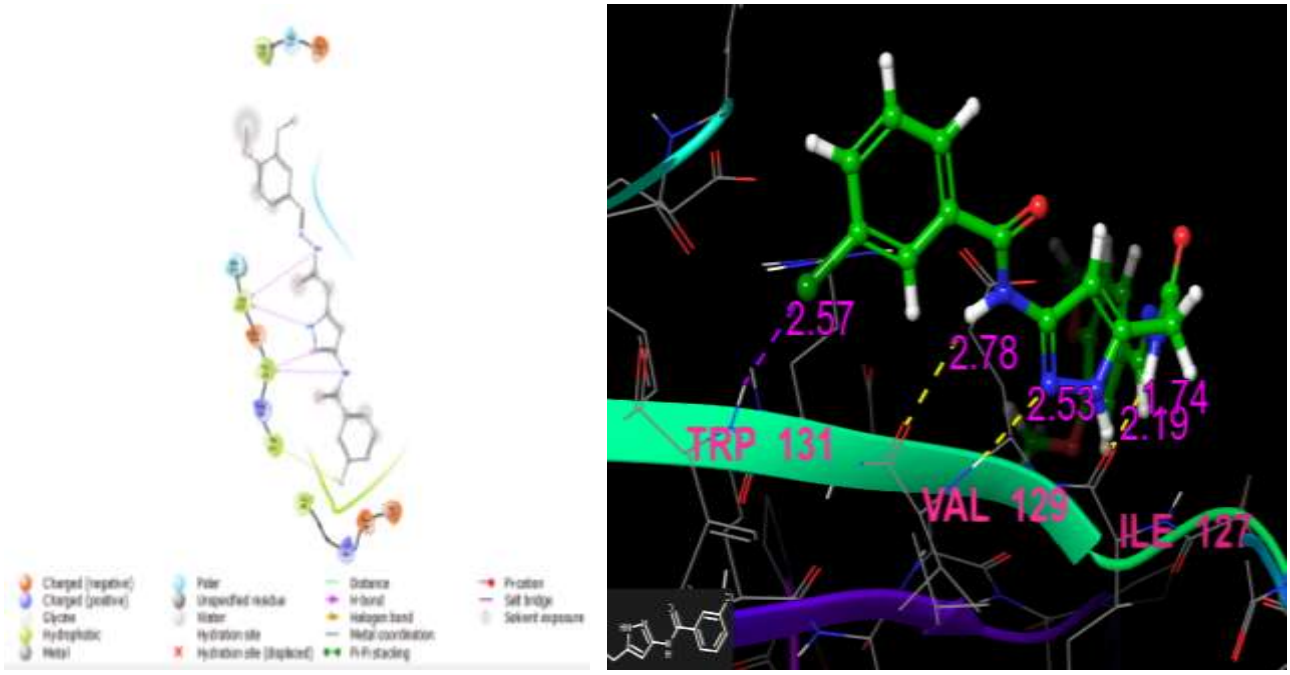


Figure 17: Compound 8q

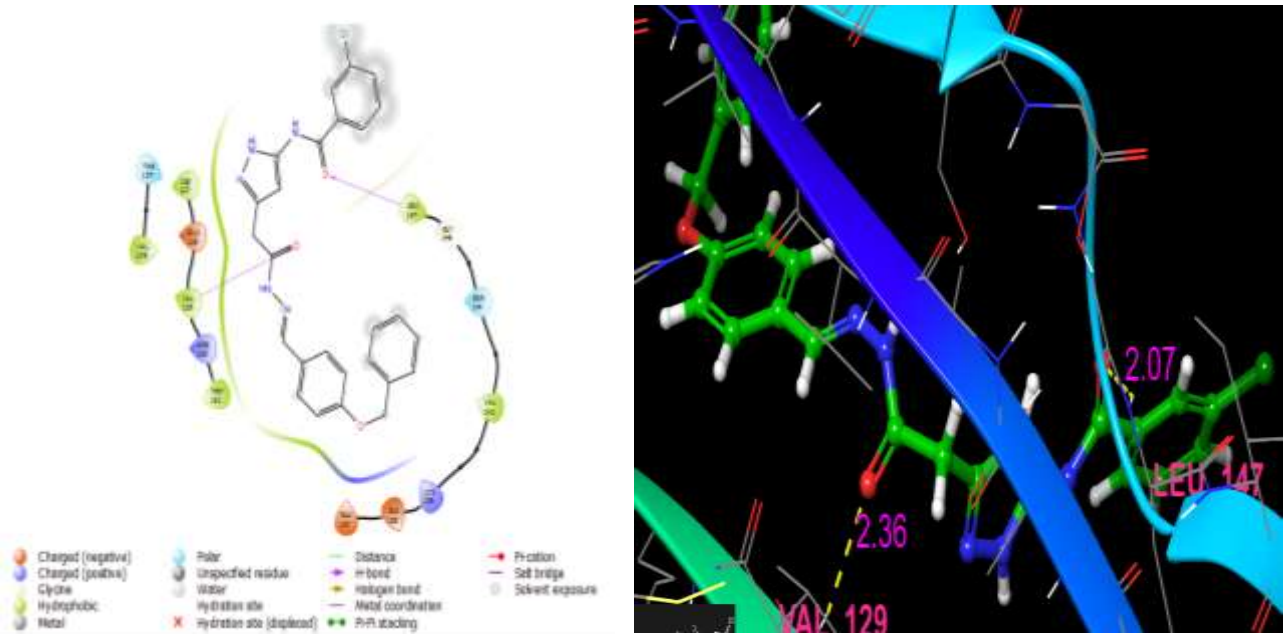


Figure 18: Compound 8p

CONCLUSION

A sequence of novel pyrazole compound was prepared by a general synthetic method and molecular docking for the synthesized molecules was performed and found that 8**g** and **8i** compounds are binds strongly with hydrogen bonds present in **GLU-128**, **ILE-127** and **VAL-129**, **THR-157, LEU-147** amino acid residues **(Figure 1-18).** The in silico molecular docking studies of synthesized pyrazole hydrazides shows that, almost all the synthesized compounds having therapeutic activities and among which, **8g** and **8i** having more ligand interactions when comparing with standard protein **8NIX.** With the help of Schrodinger software, an in vitro study of their **HLA class-II** inhibition for rheumatoid arthritis activity was performed and the research aim is to find more prospective lead compounds with a drug discovery system, in which molecular docking studies achieved the logical drug design.

ACKNOWLEDGMENTS

Authors are thankful to Bioinformatics Infrastructure Facility Centre (BIFC), Queen Mary's College, Chennai. I am also thankful to Dr.R.Girija Co-Ordinator BIFC for their guidance and support. As a main author, it is my pleasure and covet to thank Dr.S.Aruna (My guide) for given me the opportunity to do Ph.D. under her supervising and further her help till completion of my Ph.D. Work.

REFERENCES

[1] Devendra Kumar, Vikramjeet judge, Rakesh Narang, Sonia Sangwan, Erik De clercq, Jan Balzarini, Balasubramanian Narasimhan, European journal of medicinal chemistry 2010, 45,2806-2816

[2] Vikas Kumar, Ashok Kumar, Shalabh Sharma & Netra pal Singh, Indian journal of chemistry, 2011, 50B, 1496-1503

[3] Kiran Bajaj, V K Srivastava and Ashok Kumar l, Indian journal of chemistry, 2003, 42, 1149-1155

[4] Mittel Bach, Martin; Monat Shefte fur chemie, 1985, 116, 689-692

[5] Brain E.Love and Jianhua Ren, Organic preparations and procedures international, 1999, 31, 399-405

[6] V.Koteswara Rao, S.Subbha Reddy, B.Sathesh Krishna, Green chemistry letters and reviews, 2010, 3, 217-223

[7] Swayansiddha Tripathy, Viswajanani j Sattigeri, S.K.Sahu, International journal of chemical and pharmaceutical review and research, 2015,1,6-9

[8] Manisha R.Bhosle, Dayanand Kawale, D.Khillare, Amarsinh R.Deshmukh, Chemistry & Biology interface, 2017,7, 245-254

[9] JC Sheehan, PA Cruickshank, GL Boshart, J.Org.Chem. 1961, 26, 2525

[10] Shiori Tet.Lett, 1996, 37, 2261

[11] C.A.G.N.Montalbetti and V.Falque, Tetrahedron, 2005, 61, 10827-10852

[12] Paul J.Erdman, Jimmy L.Gosse, Jamey A.Jacobson & David E.Lewis, synthetic communications, 2004, 34, 1163-1171

[13] S.Tumkevicius, V.Yakubkene, P.Vainilavicius, Chemistry of heterocyclic compounds, 1999, 35, 1334-1336

[14] Ahmad Shaabani, Sayyed Emad Hoosahmand Molecular diversity, 2018, 22, 207-224

[15] R.A. Carboni, D.D.Coffman and E.G. Howard, J.Am.Chem.Soc, 1958, 80, 2838-2840

[16] M.Green and D.M.Throp, J.chem.Soc.B.Phys.Org., 1967, 1067

[17] Maria E.Due-Hansen, Sunil K.Pandey, Elisabeth Christiansen, Rikke Andersen, Steffen V.F.Hansen, Trond Ulvan, Org.Biomol.Chem, 2016, 14, 430-433

[18] Vimal Bhat, S.D.Samant, Suhas Pednekar, Letters in organic chemistry, 2017, 14, 764-768

[19] Eric Valeur, Mark Bradle, chemical society reviews, 2009, 38, 606-631

[20] Moustafa A.Gouda, Moged A.Berghot, Ghada E.Abd El-Ghani, and Abd El-Galil M.khalil, Journal of heterocyclic chemistry, 2018, 55,1935-1941

[21] Sabah Perveen, Hotam Singh Chaudhary, Pharmacogn Mag, 2015, 11 (Suppl 4): S550-S555

[22] S Ekins, J Mestres and B Testa, British journal of pharmacology, 2007, 152 (1), 9-20

[23] Bashir Lawal, Yen-Lin Liu, Ntlotlang Mokgautsi, Harshita Khedkar, Maryam Rachmawati Sumitra, Alexander T. H. Wu, Hsu-Shan Huang Biomedicines. 2021 Jan; 9(1): 92

[24] Ah-Young Kim, Yi Na Yoon, Jiyeon Leem, Jee-Young Lee, Kwan-Young Jung, Minsung Kang, Jiyeon Ahn, Sang-Gu Hwang, Jeong Su Oh, Jae-Sung Kim

Front Oncol. 2020; 10: 571601.

[25]Anael Viana Pinto Alberto, Natiele Carla da Silva Ferreira, Rafael Ferreira Soares, Luiz Anastacio Alves, Front Pharmacol. 2020; 11: 01221.

[26] Md. Sorwer Alam Parvez, Md. Adnan Karim, Mahmudul Hasan, Jomana Jaman, Ziaul Karim, Tohura Tahsin, Md. Nazmul Hasan, Mohammad Jakir Hosen, Int J Biol Macromol. 2020 Nov 15; 163: 1787–1797.

[27] Zainab Ayaz, Bibi Zainab, Sajid Khan, Arshad Mehmood Abbasi, Mohamed S. Elshikh, Anum Munir, Abdullah Ahmed Al-Ghamdi, Amal H. Alajmi, Qasi D. Alsubaie, Abd El-Zaher M.A. Mustafa

Saudi J Biol Sci. 2020 Sep; 27(9): 2444–2451

[28] Kelton L. B. dos Santos, Jorddy N. Cruz, Luciane B. Silva, Ryan S. Ramos, Moysés F. A. Neto, Cleison C. Lobato, Sirlene S. B. Ota, Franco H. A. Leite, Rosivaldo S. Borges, Carlos H. T. P. da Silva, Joaquín M. Campos, Cleydson B. R. Santos, Molecules. 2020 Mar; 25(5): 1245

[29] Mohd Javed Naim, Ozair Alam, Farah Nawaz, Md. Jahangir Alam, and Perwaiz Alam[1]J Pharm Bioallied Sci. 2016 Jan-Mar; 8(1): 2–17.

[30] Khalid Karrouchi,1,2,3 Smaail Radi,2,* Youssef Ramli,1 Jamal Taoufik,1 Yahia N. Mabkhot,4,* Faiz A. Al-aizari,4 and Mohammed Ansar1Molecules. 2018 Jan; 23(1): 134.

[31] "Front Matter". Nomenclature of Organic Chemistry : IUPAC Recommendations and Preferred Names 2013 (Blue Book). Cambridge: The Royal Society of Chemistry. 2014. p. 141.

[32] Schmidt, Andreas; Dreger, Andrij (2011). "Recent Advances in the Chemistry of Pyrazoles. Properties, Biological Activities, and Syntheses". Curr. Org. Chem. 15 (9): 1423–1463

[33] A.M.VijeshaArun M.IsloorbSandeepTelkarcT., ArulmolidHoong-KunFune, Arabian Journal of ChemistryVolume 6, Issue 2, April 2013, Pages 197-204

[34] Arthington-Skaggs et al., 2000B.A. Arthington-Skaggs, M. Motley, D.W. Warnock, C.J. Morrison, Comparative evaluation of PASCO and National Committee for Clinical Laboratory Standards M27-A Broth Microdilution Method for Antifungal Drug Susceptibility Testing of Yeasts

J. Clin. Microbial., 38 (2000), pp. 2254-2260

[35] Bekhita and Abdel-Aziem, 2004, A.A. Bekhita, T. Abdel-Aziem, Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents, Bio-org. Med. Chem., 12 (2004), pp. 1935-1945

[36] Moristal.,1998G.M. Moris, D.S. Goosell, R.S. Hallday, R. Huey, W.E. Hart, R.K. Blew, A.J. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem., 19 (1998), pp. 1639-1662
[37] Sharma et al., 2010N.K. Sharma, K.K. Jha, Priyanka Molecular docking: an overview. Adv. Sci. Res., 1 (2010), pp. 67-72
[38] Yadav S, Pandey SK, Singh VK, Goel Y, Kumar A, Singh SM (2017) Molecular docking studies of 3-bromopyruvate and its derivatives to metabolic regulatory enzymes: Implication in designing of novel anticancer therapeutic strategies. PLOS ONE 12(5)
[39] H. R. Umesh., K. V. Ramesh & K. S. Devaraju Beni-Suef University Journal of Basic and Applied Sciences volume 9, Article number: 5 (2020)
[40] Gunasekar S., Saamanthi M., Aruna S. M; Synthesis and biological evaluations of new pyrazole hydrazides as potent anti-microbial agent; Material today: Proceedings. 45 (2021) 7132–7137