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Synthesis, characterization, molecular docking and evaluation of antibacterial, antiproliferative, and anti-inflammatory properties of new pyridinyl substituted triazole derivatives

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ABSTRACT

Ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives were synthesized by reacting 3-aminopyridines with ethyl-2-chloroacetoacetate followed by amination and reaction with different aldehydes under microwave conditions. The novel compounds were evaluated of their in vitro antibacterial, antiproliferative and anti-inflammatory activities against pathogens of medical importance. The newly synthesized compounds were subjected to molecular docking studies for the inhibition of the enzyme L-glutamine: D-fructose-6-phosphate amidotransferase [GlcN-6-P] (EC 2.6.1.16). The newly synthesized compounds were characterized by analytical, IR, ^{1}H NMR, ^{13}C NMR and LCMS. Among the screened compounds, Ethyl1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,5-difluorophenyl)-1H-1,2,4-triazole-3-carboxylate, Ethyl1-(2-bromo-5-fluoropyridin-3-yl)-5-(6-bromo pyridine-2-yl)-1H-1,2,4-triazole-3-carboxylate and Ethyl1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxylate, emerged as most active compounds against all the tests.

Keywords Ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate, 1,2,4-triazoles, antibacterial, antiproliferative, anti-inflammatory, molecular docking.

INTRODUCTION

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. 1,2,4-triazole moiety has been incorporated into a wide variety of therapeutically interesting drug candidates including antifungal, antibacterial, analgesics and anti-inflammatory, antineoplastic, antiviral, sedatives, anxiolytics, anticonvulsants, antimigraine, antihistaminics, CNS stimulants and other activities [1-14]. Triazole units are known in fields, such as medicinal and agrochemical research as well as in the material sciences due to their unique structure and properties [15]. Keeping in view of growing interest in the triazole chemistry, new derivatives of 1, 2, 4- triazole were synthesised and their characterization and properties were investigated.

A new series of compounds containing 1,3,5 substituted 1,2,4-Triazoles were synthesized and subjected the newly synthesized compounds to *in vitro*, *in vivo* and *in silico* biological activity screening. Results of their *in vitro* antibacterial, antiproliferative and anti-inflammatory activities against pathogens of medical importance were studied. The newly synthesized compounds were subjected to molecular docking studies for the inhibition of the enzyme L-glutamine: D-fructose-6-phosphate amidotransferase[GlcN-6-P] (EC 2.6.1.16). 3-amino pyridines were made to react with ethyl-2-chloroacetoacetate and the resulting chloro compound were reacted with ammonia. The obtained amino compound were reacted with different aldehydes under microwave conditions to get ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives.

MATERIALS AND METHODS

1. Experimental section

All chemicals were purchased from Sigma-Aldrich Co., and all solvents for column chromatography were of reagent grade, and were purchased from commercial sources. TLC experiments were performed on alumina-blocked silica gel 40 F254 plates. Melting points were recorded on Electro thermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Jeol 400/100 MHz Perkin Elmer spectrometer at IISc, Bangalore, Karnataka, India and Bruker NMR 400/100MHz. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. ^{13}C (100 MHz) NMR spectra were recorded for approximately 0.03 M solutions in DMSO-d_6 at 100 MHz with TMS as internal standard. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer at IISc, Bangalore, Karnataka, India. Column chromatography was performed using a silica gel (230-400 mesh). Elemental analysis was carried out using VARIO EL-III (Elementar Analysensysteme GmBH) at Department of Chemistry, Mangalore University, Mangalore, Karnataka, India. FTIR spectra were recorded on Perkin Elmer IR spectrophotometer in KBr phase.

1.1: General procedure for Ethyl 2-chloro[2-(pyridin-3-yl) hydrazinylidene] ethanoate **(Scheme 1.1)**
Substituted 3-amino pyridine (0.1mol) was dissolved in 6 volumes of 6N HCl solution and cooled to 0^0C. Sodium nitrite (0.1mol) dissolved in 3 volumes of DM water was added drop wise to the reaction mass and stirred for 30 minutes at the same temperature. Later, ethyl-2-chloro actetoacetate (0.1mol) in 2 volume ethanol was added drop wise for one hour at O^0C. After 30 minutes, sodium acetate (0.3mol) in 3 volumes of water was added drop wise to the reaction mixture and stirred for 12 hrs. Product was extracted using 3 volumes of DCM, organic layer dried over anhydrous sodium sulphate, concentrated under vacuum to afford Ethyl 2-chloro[2-(pyridin-3-yl)hydrazinylidene]ethanoate **(2).**

1.2: General procedure for Ethyl 2-amino[2-(pyridin-3-yl) hydrazinylidene] ethanoate **(Scheme 1.1)**
Ammonia gas was bubbled through the solution of Ethyl 2-chloro[2-(pyridin-3-yl)hydrazinylidene]ethanoate **(2)** in THF at -30^0C for 30 minutes and stirred at RT for 2hrs. TLC indicated all starting material had been converted to amine . The solvent and excess of ammonia were removed under vacuum; the residue was dissolved in dry chloroform and filtered to remove NH_4Cl. The filtrate evaporated and triturated with diethyl ether to afford ethyl 2-amino[2-(pyridin-3-yl)hydrazinylidene]ethanoate (**3).**

1.3: General procedure for the synthesis of ethyl 1-(pyridin-3-yl)-1,2,4-triazole-3-carboxylates **(4a-l :Scheme 1.1)**
To a solution of ethyl 2-amino[2-(pyridin-3-yl)hydrazinylidene]ethanoate **3,** in dry acetic acid corresponding aldehyde (1.1 equiv.) was added under N_2 atmosphere. The reaction mixture was stirred at RT for 1h, and then heated to 120°C under microwave for 30 minutes. The reaction mixture cooled to RT and evaporated to dryness. The mass dissolved in DCM washed with 10% $NaHCO_3$ (10ml), H_2O (10ml), brine (10ml), dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using silica gel (230-400 mesh) with pet ether/ethyl acetate to grt pure ethyl 1-(pyridin-3-yl)-1,2,4-triazole-3-carboxylate.

1.3.1: *Ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,5-difluorophenyl)-1H-1,2,4-triazole-3-carboxylate* ***(4a).***
Elem. Analysis for $C_{16}H_{10}BrF_3N_4O_2$; cacld: C 44.94, H 2.34 , N 13.10; found: C 44.72 , H 2.37 , N 13.11. **IR (KBr, ν cm^{-1})**: 3437, 3076, 3023, 1731, 1594, 1573, 1503, 1432, 1232, 1216, 1155, 1023, 876, 610. **^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm:** 1.322-1.358 (t, 3H) and 4.389-4.443 (q, 2H) for ethoxy protons, 7.385-7.441 (m, 1H) and 7.472-7.542(m, 2H) for phenyl ring protons containing two fluorine atoms, 8.752-8.759 (d, 1H, J=2.8) and 8.500-8.527 (q, 1H, J=2.8) are due to protons in the pyridine ring containing fluorine atom. **^{13}C NMR (DMSO-d_6, 100 MHz) : δ ppm:** 13.967, 61.785, 115.096-115.356, 117.959, 118.222-118.599, 120.656-120.983, 126.450, 126.675, 133.593-133.922, 140.910-141.157, 151.512, 153.823, 154.907, 156.278-156.635, 158.663-158.848, 159.214 (16 C atoms).**LCMS :** m/z 427.7[M], 428.6[M+1].

1.3.2: *Ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(6-bromopyridin-2-yl)-1H-1,2,4-triazole-3-carboxylate****(4b).***
Elem. Analysis for $C_{15}H_{10}Br_2FN_5O_2$; cacld: C 38.20, H 2.12 , N 14.85; found: C 38.32 , H 2.17 , N 14.81.**IR (KBr, ν cm^{-1}) :** 3433, 3314, 2982, 1466, 1411, 694, 1716, 1583, 1560, 1297, 1327, 1017, 879, 1174. **^{1}H NMR (DMSO-d_6, 400 MHz) :δ ppm** : 8.796-8.803(1H, pyridine ring with fluorine), 8.433-8.440(1H, pyridine ring with fluorine), 8.281-8.302 (d, J=8.4Hz, 1H, pyridine), 7.956-7.995(t, 1H, pyridine),7.758-7.778 (d, J=8Hz,1H, pyridine), 1.348-1.366(t, 3H) and 4.332-4.412(q, 2H) for ethoxy protons. **^{13}C NMR (DMSO-d_6, 100 MHz) : δ ppm:** 13.984, 61.777, 122.405, 127.413, 130.118, 132.407-132.660, 140.033-140.928, 141.291, 145.101, 152.544, 154.285, 156.999-157.783, 158.715, 159.559, 159.888 (15 C atoms). **LCMS :** m/z 471.6[M+], 473.6[M+2], 474.6[M+3].

1.3.3: *Ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(pyridin-2-yl)-1H-1,2,4-triazole-3-carboxylate* ***(4e).***
Elem. Analysis for $C_{15}H_{11}BrFN_5O_2$; cacld: C 45.89, H 2.80, N 17.84; found: C 45.82, H 2.76 , N 17.81. **^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm :** 8.818-8.826 (1H, J=3.2, pyridine with fluorine), 8.599-8.626(1H, J=2.8, pyridine with fluorine), 8.737-8.742 (s, 1H, pyridine), 8.702-8.718(d, J=6.4Hz, pyridine), 7.496-7.518(t, 1H, pyridine), 7.861-7.891(d, J=12Hz, 1H, pyridine), 4.390-4.443(q, 2H) and 1.325-1.361(t, 3H) for ethoxy group. **^{13}C NMR (DMSO-d_6, 100 MHz) : δ ppm:** 13.989, 61.736, 122.307, 123.961-124.033, 127.068-127.92, 133.951-134.250, 135.670-135.741, 141.323-141.573, 148.385-148.485, 151.871, 154.352, 154.714 156.947, 158.814, 159.532(15 C atoms). **LCMS :** m/z 393.7[M+1], 394.7[M+2].

1.3.4: *Ethyl 5-(3-bromo-5-tert-butyl-2-hydroxyphenyl)-1-(2-bromo-5-fluoropyridin-3-yl)-1H-1,2,4-triazole-3-carboxylate* ***(4f).***
Elem. Analysis for $C20H_{19}Br_2FN_4O3$; cacld: C 44.26, H 3.50, N 10.32; found: C 44.12 , H 3.66 , N 10.12. **IR (KBr, ν cm^{-1})** :3454, 3059, 2958, 1737, 1600, 1517, 1500, 1481, 1258, 1227, 1155, 1077, 1033, 846, 663.**^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm :** 8.536-8.543(1H, J=2.8, Pyridine with fluorine), 8.284-8.311(1H, J=2.8, Pyridine with fluorine), 7.869(s, 1H, phenyl),8.00(s, 1H, phenyl),4.490-4.464(q,2H) and 1.345-1.380(t, 3H) for ethoxy protons,1.312(s, 9H, t-butyl),10.330(s, 1H, Phenolic proton). **^{13}C NMR (DMSO-d_6, 100 MHz) : δ ppm:** 13.994, 30.672, 34.611, 61.809, 115.470, 119.866, 120.833, 121.083, 125.521, 125.614, 134.535, 135.938-136.201, 149.507-149.800, 150.938, 152.489, 155.049, 156.183, 158.716(20 C atoms). **LCMS :** m/z 541.7[M+], 542.6[M+2], 544.6[M+3].

1.3.5: *Ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxylate* ***(4g).***
Elem. Analysis for $C_{16}H_{10}BrCl_2FN_4O_2$; cacld: C 41.73, H 2.17, N 12.17; found: C 41.62 , H 2.16 , N 12.18. **^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm :** 8.737(d, 1H, pyridine ring with Fluorine),8.521-8.541(dd, J=3.2Hz, 1H, pyridine ring with Fluorine),7.85(s, 1H, Phenyl),7.522-7.543(d, J=8.4Hz, 1 H, phenyl),7.656-7.678(d, J=8.8, 1H, phenyl),4.450-4.485(q, 2H, -OCH_2) and 1.320-1.368(t, 3H, -CH_3) for ethoxy group. **^{13}C NMR (DMSO-d_6, 100 MHz) : δ ppm:** 13.971, 61.752, 124.044, 126.763, 126.988, 127.686-127.878, 129.775-129.872, 133.246-134.012, 137.038, 140.940-141.187, 152.563, 153.621, 154.604, 156.511, 158.735, 159.091 (16 C atoms). **LCMS:** m/z 460.6[M+], 464.6[M+].

1.3.6: *Ethyl 1-(6-chloropyridin-3-yl)-5-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxylate* ***(4h).***
Elem. Analysis for $C_{16},H_{11},Cl_3,N_4O_2$, cacld: C 48.28, H 2.76 , N 14.08; found: C 48.32 , H 2.67 , N 14.11. **IR (KBr, ν cm^{-1}) :**1289, 1243, 1747, 1597, 1574, 804, 3433, 3086, 2959, 1554, 1470, 841, 1197. **^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm:** 1.31-1.35(t, J=16Hz, 3H) and 4.38-4.43(q, J=20Hz, 2H) for ethoxy protons, 8.470-8.477(1H, pyridine), 7.908-7.936 (2H, pyridine), 7.645 (s, 1H, phenyl), 7.672-7.694(d, J=8.4Hz, phenyl), 7.791-7.812(d, J=8.4Hz, phenyl). **^{13}C NMR (DMSO-d_6, 400 MHz) : δ ppm:** 13.996, 61.616, 124.872, 125.144, 128.189, 129.568, 132.770, 133.283, 133.984, 135.587, 137.076, 145.223, 150.670,152.612, 154.424, 158.864 (16 C atoms). **LCMS :** m/z 398.7[M+1] 400.7[M+3] 401.7[M+4].

1.3.7:Ethyl5-(3-bromo-4-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1H-1,2,4-triazole-3-carboxylate* ***(4i).
Elem. Analysis for $C_{16}H_{12}BrClN_4O_3$, cacld: C 45.32, H 2.83, N 13.21; found: C 45.11, H 2.87, N 13.19. **IR (KBr, ν cm^{-1}) :** 3444, 3057, 2963, 1735, 1621, 1585, 1568, 1508, 1263, 1218, 1111, 847,756. **^{1}H NMR (DMSO-d_6, 400 MHz) : δ ppm:** 10.996(s, 1H, -OH phenyl), 8.586(s, 1H, pyridine), 8.027-8.048(d, J=8.4Hz, 1H, pyridine), 7.734-7.755(d, J=8.4Hz, 1H, pyridine), 7.711(s, 1H, phenyl), 7.226-7.247(d, J=8.4Hz, 1H, phenyl), 6.973-6.996(d, J=9.2Hz, 1H, phenyl). **^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm:** 14.565, 61.923, 109.933, 116.842, 118.717, 125.573, 130.139, 134.164, 137.649, 147.285, 151.201, 154.473, 155.274, 156.855, 159.733(16 C atoms). **LCMS:** m/z 423.65[M+] 424.65[M+1] 427.65[M+4].

1.3.8: *Ethyl 1-(6-chloropyridin-3-yl)-5-(2,5-difluorophenyl)-1H-1,2,4-triazole-3-carboxylate****(4j).***
Elem. Analysis for $C_{16}H_{11}ClF_2N_4O$, cacld: C 52.63, H 3.01, N 15.35; found: C 52.61, H 3.97, N 15.29. **^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm:** 8.557-8.563(s, 1H, pyridine), 8.016-8.045(d, J=11.6Hz, 1H, pyridine), 7.701-7.723(d, J=8.8 Hz, 1H, pyridine), 7.546-7.599(m, 1H, phenyl containing fluorine), 7.495-7.536,(m, 1H, phenyl containing fluorine), 7.268-7.427(m, 1H, phenyl containing fluorine). **^{13}C NMR (DMSO-d_6, 100 MHz): δ ppm:** 159.018, 158.839, 156.607, 154.684, 153.673, 150.312, 138.364, 133.584, 124.188, 123.337, 120.437-120.764, 118.113-118.589, 117.865, 115.604-115.866, 61.620, 13.996 (16 C atoms).

1.3.9: *Ethyl 5-(3-bromo-5-tert-butyl-2-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1H-1,2,4-triazole-3-carboxylate* ***(4k).***
Elem. Analysis for $C_{20}H_{20}BrClN_4O_3$, cacld: C 50.02, H 4.16, N 11.67; found: C 49.98, H 4.13, N 11.59. **IR (KBr, ν cm^{-1}) :** 1213, 1172, 1607, 1574, 1751, 1113, 740, 843, 3430, 3100, 2979, 1460, 1430, 695. **^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm:** 9.915(s, 1H,-OH phenyl), 8.449-8.455 (s, 1H, pyridine), 7.908-7.937 (d, J=11.6Hz, 1H,

pyridine), 7.696-7.717 (d, J=8.4 Hz, 1H, pyridine), 7.656 (s, 1H, phenyl), 7.334(s, 1H, phenyl), 4.384-4.437 & 1.321-1.356 (q & t, 5H , ethoxy), 1.176(s, 9H, t-butyl). **^{13}C NMR (DMSO-d_6, 100 MHz) ppm: δ** 159.113, 158.716, 156.183, 155.049, 153.827, 150.355, 149.557, 145.354, 143.740, 135.736, 133.603, 132.609, 127.372, 124.956, 115.220, 111.592, 61.486, 33.905, 30.775, 14.043(20 C atoms). **LCMS:** m/z 479.7[M+], 480.7[M+1].

1.3.10: *Ethyl 5-(3-chloro-4-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1H-1,2,4-triazole-3-carboxylate* ***(4l).***
Elem. Analysis for $C_{16}H_{12}Cl_2N_4O_3$, cacld: C 50.63, H 3.16, N 14.76; found: C 50.78, H 3.13, N 14.9. **LCMS**: m/z 379.2[M+], 380.2[M+1].

1.4: Antibacterial activity
The antibacterial activity of synthesized compounds was carried out using agar well diffusion method. The *in vitro* antibacterial activity was carried out against 24 h culture of four bacterial strains *Staphylococcus aureus, Escherichia coli, pseudomonas aeroginosa* and *klebsiella pneumoniqe.* The stock solution of compounds was made at 50 µg/100 µl and desired concentrations were prepared by using stock solution. DMSO was used as a solvent to dissolve compounds. Nitrofurazone (50 µg in100 µl) was used as standard drug for antibacterial activities. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 ^{o}C for antibacterial activity . The zone of inhibition was recorded in mm.

1.5: Antiproliferative studies against Dalton's Ascites Lymphoma (DAL) and HeLa-ccl-13 cell lines
Cell culture and in vitro cytotoxicity assay (MTT assay). Cells were treated with compounds at a concentration range of 10µg/ ml and 20µg/ ml added to 96-well plates in antibiotic-free RPMI medium containing 10 % fetal calf serum. Compound treatment lasted for 48 h in 5 % CO_2 atmosphere at 37˚C with high humidity. After 48 h, 50µl of 1 mg/ml solution of MTT in RPMI-1640 medium was added to each well. The culture plates were gently shaken and incubated for four more hours. MTT was removed carefully and DMSO (100 µl) was added and shaken well. The absorbance was measured at 570 nm in an automated plate reader and the percentage of cell growth inhibition was calculated by means of the following formula:

Inhibitory rate (%) = Absorption control - Absorption test/Absorption test X 100.

1.6: Assessment of cell viability (LC50)
Cell viability was checked before and after treatment with compounds using the trypan blue exclusion method. Cells (5 9 104cells/well) were seeded in six-well plates prior to the addition of compounds. The cells were incubated with different doses (25, 50, 100 200, 30000 µg/mL) of compounds along with 1 % DMSO as the solvent control. Cultures were harvested and monitored for cell number by counting cell suspensions using a hemocytometer.

1.7: Anti-inflammatory activity
Anti-inflammatory activity of all synthesized compounds was determined by the carrageenean-induced rat paw oedema test as described by Winter *et al*. [16]. Albino rats of Wistar strain (150–200 g) of both sexes were divided into different groups (control, test and standard) containing six animals each. The animals were housed in standard polypropylene cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 2 °C). They had free access to standard commercial diet and water. Ethical guidelines for the investigations of animals used in experiments were followed in all tests and the institutional Ethical Committee Approval was provided. The test and standard compounds were suspended in 1% carboxymethyl cellulose and administered orally to each animal using a gastric gavage needle. The control group animals, however, received the same volume of vehicle (1% carboxymethyl cellulose). One hour after the compounds were administered, carrageenean was injected into the sub plantar surface of the right hind paw of animals. In this study, the animals were administered a 50 mg kg^{-1} (body mass) dose of the test drug and 10 mg kg^{-1} (body mass) dose of the standard drug indomethacin. The paw volume was measured immediately using a plethysmometer (initial paw volume) and thereafter the paw volume was measured 3 hours and 6 hours after the administration of carrageenean. Percent paw oedema inhibition is reported.

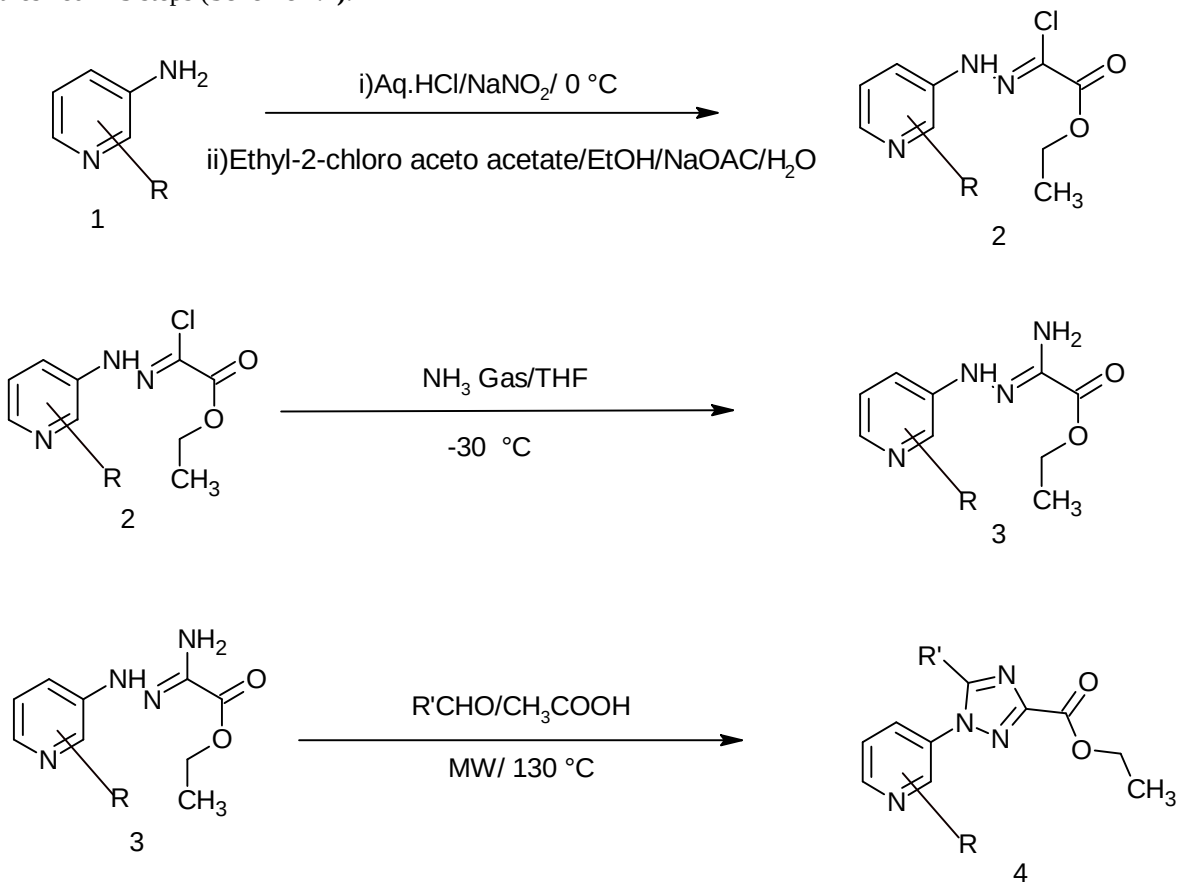
1.8: Docking studies
Docking studies were carried out with Hex (6.3 version) program. *Hex* is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. In *Hex*'s docking calculations, each molecule is modelled using 3D expansions of real orthogonal spherical polar basis functions to encode both surface shape and electrostatic charge and potential distributions. Essentially, this allows each property to be represented by a vector of coefficients (which are the components of the basis functions). *Hex* represents the surface shapes of proteins using a two-term surface skin plus Van der Waals steric density model. By writing expressions for the *overlap* of pairs of parametric functions, one can obtain an overall docking score as a function of the six degrees of freedom in a rigid body docking search. With suitable

scaling factors, this docking score can be interpreted as an interaction energy, which we seek to minimise. The more negative the e-value, the more efficient is the docking process.

RESULTS AND DISCUSSION

1. Chemistry

The new series of 5-substituted, ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives (**4)** were synthesized in 3 steps (**Scheme 1.1).**



Scheme 1.1
5-substituted, ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives (4)

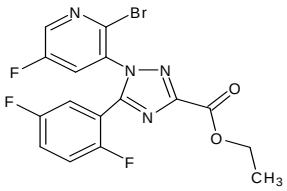
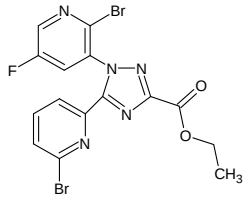
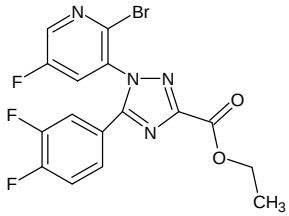
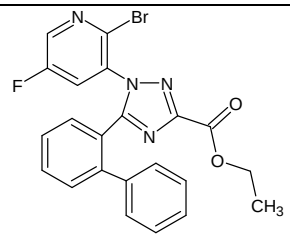
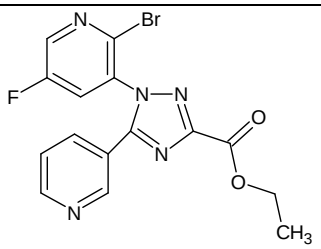
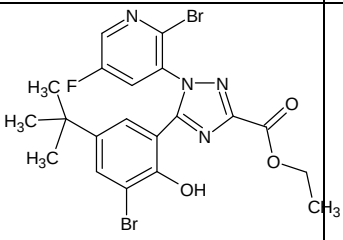
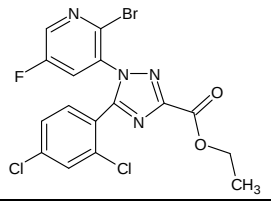
Substituted 3-aminopyridines (**1)** was reacted with ethyl-2-chloro acetoacetate and the obtained ethyl 2-chloro[2-(pyridin-3-yl)hydrazinylidene]ethanoate **(2)** is made to react with ammonia at low temperature to get ethyl 2-amino[2-(pyridin-3-yl)hydrazinylidene]ethanoate **(3)**. Different aldehydes were made to react with ethyl 2-amino[2-(pyridin-3-yl)hydrazinylidene]ethanoate under microwave for 30 minutes at 120ºC to get 5-substituted, ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives(**4**). The synthesized compounds are tabulated in **Table 1.1.**

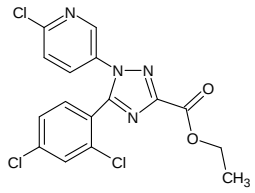
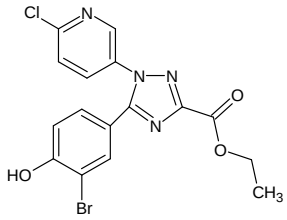
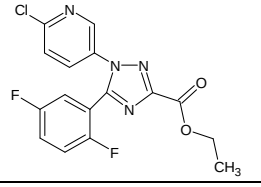
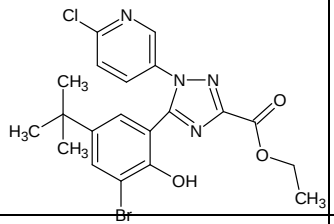
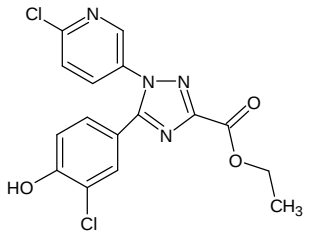
1.1: The formation of compounds **4a-l** was confirmed by recording Elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

1.1.1: Characterization data for ethyl 1-(2-bromo-5-fluoropyridin-3-yl) -5- (2, 5-difluorophenyl)-1*H*-1,2,4-triazole-3-carboxylate **(4a).**

In the IR (KBr, ν cm^{-1}) spectrum **,** absorption bands seen at 1232, 1216 represents C-N bond, strong absorption peak at 1731 was due to C=O, absorption peaks at 1594, 1573 were due to C=N stretch, absorption band seen at 610 was due to C-Br stretching, strong absorption at 1023 was the characteristic of C-F, bands at 3076, 3023, 1503, 1432, 876 are due to aromatic region and sharp absorption seen at1155 might be due to ethoxy group.

Table 1.1 5-substituted, ethyl 1-(pyridin-3-yl)-1 H-1,2,4-triazole-3-carboxylate derivatives (4a-l)

Compd.no	structure	M.Wt	Appearance	Yield	M.P Uncorrected
4a		427.18	White solid	75.8%	122.2°C
4b		471.08	Off white solid	65.3%	129.8° C
4c		427.18	Pale yellow solid	68%	136-137°C
4d		467.29	Light brown solid	70%	138-141°C
4e		392.18	Yellow solid	64.2%	121.1° C
4f		542.2	White solid	45%	132.1° C
4g		460.08	Yellow solid	59.7%	136.5° C

4h		397.64	Yellow solid	58.3%	133.2° C
4i		423.65	Brown liquid	68%	BP not determined
4j		364.73	Pale yellow solid	72%	138.1° C
4k		479.75	Off white solid	63.4%	138.2° C
4l		379.10	Off white solid	57.7%	139.6° C

The ^{1}H NMR of compound **4a,** showed signals at δ 1.322-1.358 ppm (t, J=14.4Hz, 3H) and δ 4.389-4.443 ppm (q, J=21.6Hz, 2H) a triplet and quartet for ethoxy protons. The splitting of signal for proton adjacent to nitrogen atom as a doublet in the region δ 8.752-8.759(J=2.8ppm) could be due to ortho coupling of fluorine present in the pyridine ring. Multiplet in the region δ 8.500-8.527(J=2.7ppm) was due to fluorine splitting of the remaining proton on the pyridine ring away from nitrogen atom. The multiple splitting observed in the region δ 7.385-7.441ppm (m, 1H) and 7.472-7.542(m, 2H) could be due to ortho-para coupling of the two fluorine atoms present in the phenyl ring. Overall, characteristic splitting due to ortho-para coupling of fluorine was observed and this supported the characterization.

It was further supported by recording **^{13}C** NMR spectrum and the signals appeared in the spectrum accounted for all the C-atoms present in the molecule **4a.** The signals seen at δ 13.967 & 61.785ppm were due to two carbons of ethoxy group. Two carbon atoms of the triazole ring resonated at δ 153.823 & 154.907ppm .Characteristic splitting of the signals were observed in the aromatic region due to fluorine coupling. The signal due to 4 carbons of the pyridine ring containing fluorine appeared as two doublets at δ 140.910 to 141.157(J=24.7Hz, 3rd carbon from nitrogen), 158.663-158.848(J=18.5Hz, adjacent carbon to nitrogen) and two triplets at 156.278-156.635(J=35.7Hz, bromo substituted carbon) & 133.593-133.922(J=32.9Hz, fluoro substituted epso carbon) and a singlet at δ151.512 was due to triazole substituted carbon . The signals of the phenyl ring carbon atoms appeared as multiplet in the aromatic region. This can be attributed to, one to four bond coupling of fluorines present in the ring.

LC mass spectrum of the compound **4a,** showed molecular ion peaks m/z 427.7[M], 428.6[M+1] and this confirmed the structure.

1.1.2: Characterization data for Ethyl 1-(6-chloropyridin-3-yl)-5-(2, 4-dichlorophenyl)-1*H*-1,2,4-triazole-3-carboxylate **(4h).**

In the IR (KBr, ν cm^{-1}) spectrum, absorption bands at 1289, 1243 represented C-N bond, strong absorption peak at 1747 was due to C=O, absorption peaks at 1597, 1574 were due to C=N, 804 was due to C-Cl, absorption bands appeared at 3433, 3086, 2959, 1554,1470, 841 were due to aromatic region, band at1197 might be due to ethoxy group.

The ^{1}H NMR of compound **4h,** showed signals at δ 1.31-1.35ppm (J=16Hz, 2H) a triplet and δ 4.38-4.43ppm (J=20Hz, 3H) a quartet for ethoxy protons, singlet at δ 8.47ppm for proton in the pyridine ring adjacent to nitrogen, two doublets at δ 7.90-7.91ppm (J=4Hz) and δ 7.93-7.94ppm (J=4Hz) might be due to remaining protons of the pyridine ring. A singlet resonated at δ 7.64ppm was due to proton on the phenyl ring between the two chlorine and two doublets at δ 7.67-7.69ppm (J=8Hz) and δ 7.79-7.81ppm (J=8Hz) may be attributed to the remaining protons on the phenyl group. These data confirmed the structure of the molecule.

It was further supported by recording **^{13}C** NMR spectrum and the signals appeared in the spectrum account for all the C-atoms present in the molecule **4h.** It is interesting to note that the signals in the aromatic region appeared as singlets unlike in the spectra of fluorine substituted compounds.

LC mass spectrum of the compound **4h,** showed molecular ion peaks m/z 398.7[M+1] 400.7[M+3] 401.7[M+4] and this is in agreement with its structure.

Similarly all the synthesized compounds were characterized by Elem.analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectra.

1.2: Biological evaluation

1.2.1: Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against bacterial strains by disc diffusion method. The zone of inhibition of tested compounds is given in **Table 1.2.** Among the tested compounds, ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,5-difluorophenyl)-1*H*-1,2,4-triazole-3-carboxylate **(4a),** ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(6-bromopyridin-2-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4b)** and ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(3,4-difluorophenyl)-1*H*-1,2,4-triazole-3-carboxylate (**4c)** emerged as the most active and ethyl 5-(biphenyl-2-yl)-1-(2-bromo-5-fluoropyridin-3-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4d),** ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(pyridin-2-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4e),** methyl5-(3-bromo-5-*tert*-butyl-2-hydroxyphenyl)-1-(2-bromo-5-fluoropyridin-3-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4f),** ethyl 1-(2-bromo-5-fluoropyridin-3-yl)-5-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-3-carboxylate **(4g)** and ethyl 5-(3-chloro-4-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1*H*-1,2,4-triazole-3-carboxylate (**4l)** showed moderate activity, whereas compounds Ethyl 1-(6-chloropyridin-3-yl)-5-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-3-carboxylate **(4h), E**thyl 5-(3-bromo-4-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4i)** and ethyl 5-(3-bromo-5-*tert*-butyl-2-hydroxyphenyl)-1-(6-chloropyridin-3-yl)-1*H*-1,2,4-triazole-3-carboxylate **(4k)** showed less activity.

Table 1.2 Antibacterial activity of 5-substituted, ethyl 1-(substitued-pyridin-3-yl)-1 *H*-1,2,4-triazole-3-carboxylate derivatives

Compound	*S.aureus (Recultured)*	*E.coli (Recultured)*	*P.aeroginosa (Recultured)*	*K.Pneumoniae (Recultured)*
4a	**34**	**39**	**27**	**29**
4b	**30**	**28**	**20**	**27**
4c	**37**	**32**	**32**	**20**
4d	**21**	**36**	**19**	**27**
4e	**19**	**29**	**23**	**23**
4f	**15**	**27**	**24**	**29**
4g	**25**	**30**	**18**	**22**
4h	**17**	**32**	**18**	**18**
4i	**21**	**21**	**16**	**17**
4j	**20**	**28**	**21**	**18**
4k	**18**	**20**	**20**	**18**
4l	**23**	**33**	**11**	**24**
DMSO	**NI**	**NI**	**NI**	**NI**
Nitrofurazone Standard Drug	**32**	**30**	**36**	**32**

Standard drug used: Nitrofurazone, (50μ g/mL). Compounds used (50 μg/mL). Control: DMSO (dimethyl sulfoxide 5%). Zone of inhibition in mm.

1.2.2: Antiproliferative studies

Antiproliferative studies were conducted against Dalton's Ascites Lymphoma (DAL) and HeLa-ccl-13cell lines. Cell lines were cultured and cytotoxicity was carried out. Cells were treated with compounds at a concentration range of 10 and 20µg/ml.

The percentage of cell growth inhibition was calculated by means of the following formula: Inhibitory rate (%) = Absorption control - Absorption test/Absorption test X 100 **(Table 1.3).**

Among the tested compounds**, 4a, 4c, 4h** and **4l**, showed very good inhibition of DAL cell lines and compounds, **4d, 4e** and **4g** showed moderate inhibition of DAL cell lines. Compounds **4a, 4b**, **4h** and **4l** showed very good inhibition of HeLa-ccl-13 cells and compounds **4c, 4d** and **4e** showed moderate inhibition of Hela-ccl-13 cells.

Table 1.3: Antiproliferative studies

Code	Dalton's Ascites Lymphoma (DAL)		HeLa-ccl-13	
	10µg/mL	**20 µg/mL**	**10 µg/mL**	**20 µg/mL**
4a	71.6261±6.851	82.37±5.37	68.5732±6. 21	82.55±3.45
4b	67. 735±5.297	71.41±4.67	75.45±6.2984	70.21±5.56
4c	76.522±4.812	79.18±5.31	64. 36±20.21	77.59±5.32
4d	68.45±5.60	74.39±4.59	62.53±2.2492	63.42±3.10
4e	74. 14±3. 21	72.63±6.26	69. 14±2. 478	60.52±5.25
4f	53.58±4. 57	60.22±4.67	57.35±5. 52	51.19±3.38
4g	71. 71±2.16	73.15±4.31	62. 24±4. 21	53.16±4.07
4h	75.13±4.22	81.43±5.17	79.21±2.55	81.17±3.56
4i	66.89±2.45	71.56±1.98	45.22±3.67	52.55±2.17
4j	52.32±5.23	60.12±2.46	53.28±2.78	54.22±3. 81
4k	50.18±4.45	72.34±7.78	60.67±6.98	45.78±1.79
4l	78.97±6.583	82.32±3.67	79.92±5.6732	67.92±6.18

1.2.3: Assessment of cell viability (LC50)

Cell viability was checked before and after treatment with compounds using the trypan blue exclusion method) (**Table 1.4)**. The cells were incubated with different doses (25, 50, 100 200, 30000 µg/mL) of compounds along with 1 % DMSO as the solvent control. Cultures were harvested and monitored for cell number by counting cell suspensions using a hemocytometer [17].

Compounds **4a, 4f, 4i, 4j** exhibited very effective LC50 values for 50% cell kill for DAL cell lines and compounds **4a, 4f** and **4k** exhibited very effective LC50 values for 50% kill for HeLa-ccl-13 cells.

Table 1.4: LC 50 values in µg/ml

Compounds	Dalton's Ascites Lymphoma (DAL)	HeLa-ccl-13
4a	23	59
4b	67	212
4c	142	134
4d	89	173
4e	67	78
4f	45	56
4g	103	72
4h	68	82
4i	41	78
4j	39	89
4k	107	33
4l	122	158

1.2.4: Anti-inflammatory activity

Anti-inflammatory activity of all synthesized compounds was determined by the carrageenean-induced rat paw oedema test. In this study, the animals were administered a 50 mg kg^{-1} (body mass) dose of the test drug and 10 mg kg^{-1} (body mass) dose of the standard drug indomethacin. The paw volume was measured immediately using a plethysmometer (initial paw volume) and thereafter the paw volume was measured 3 hours and 6 hours after the administration of carrageenean. Percent paw oedema inhibition is reported in **Table 1.5**. Indomethacin sodium a known anti-inflammatory drug is used as standard. Among the tested compounds, **4b** and **4g** showed good anti-inflammatory property, where as compounds, **4e, 4f, 4j** and **4k** showed moderate and compounds**, 4a** and **4h** showed less anti-inflammatory property. Compound **4i** showed no anti-inflammatory property.

Table 1.5: Anti-inflammatory studies

Compd. No.	Inhibition of paw oedema after 3 hours (%)	Inhibition of paw oedema after 6 hours (%)
4a	13.11 ± 0.02	11.41 ± 0.01
4b	40.51 ± 0.01	31.37 ± 0.01
4e	21.35 ± 0.01	15.60 ± 0.02
4f	23.24 ± 0.02	22.13 ± 0.02
4g	39.86 ± 0.01	36.37 ± 0.02
4h	17.60 ± 0.02	15.54 ± 0.02
4i	00.00	–4.16
4j	25.74 ± 0.02	21.83 ± 0.02
4k	17.60± 0.02	14.34 ± 0.02
Indomethacin	76.74 ± 0.01	58.33 ± 0.02
CONTROL	----------	--------

1.2.5: Molecular docking studies

The docking results for ligand molecules against glucosamine-6-phosphate synthase [PDB Id: 1jka], are documented in **Table 1.6.** Among the twelve molecules, **4g** and **4j** had shown very minimum binding and docking energy and can say as a good inhibitor of GlcN-6-P. Molecules, **4a, 4f** and **4k** also exhibited interesting E-total value.

Table 1.6: Results of Docking Studies

4a	-275.9
4b	-269.1
4c	-135.0
4d	-265.0
4e	-263.5
4f	-272.59
4g	-284.0
4h	-254.0
4i	-254.0
4j	-282.1
4k	-272.6
4l	-258.0

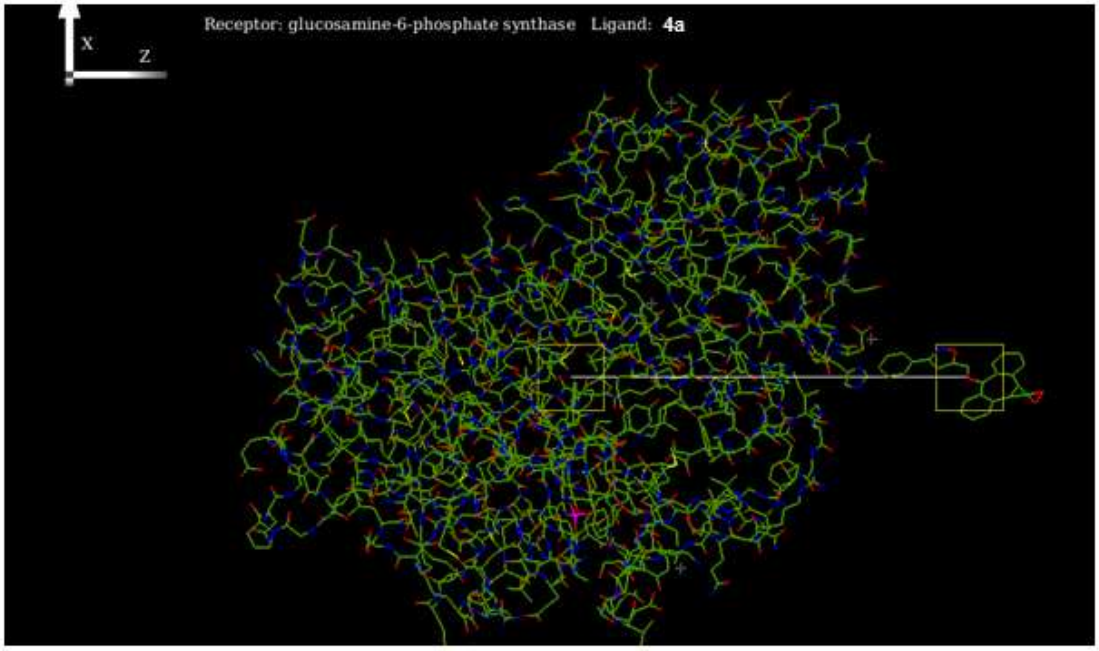


Fig 1: Interaction of compound 4a with glucosamine-6-phosphate synthase

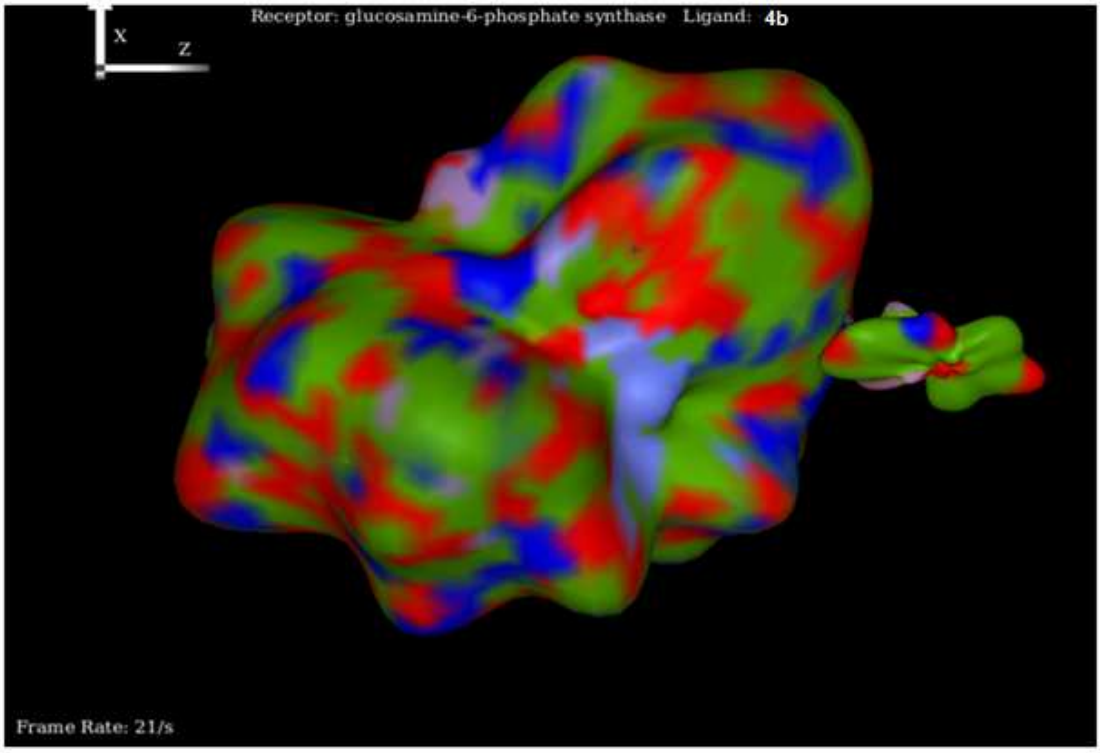


Fig 2: Interaction of compound 4b with glucosamine-6-phosphate synthase

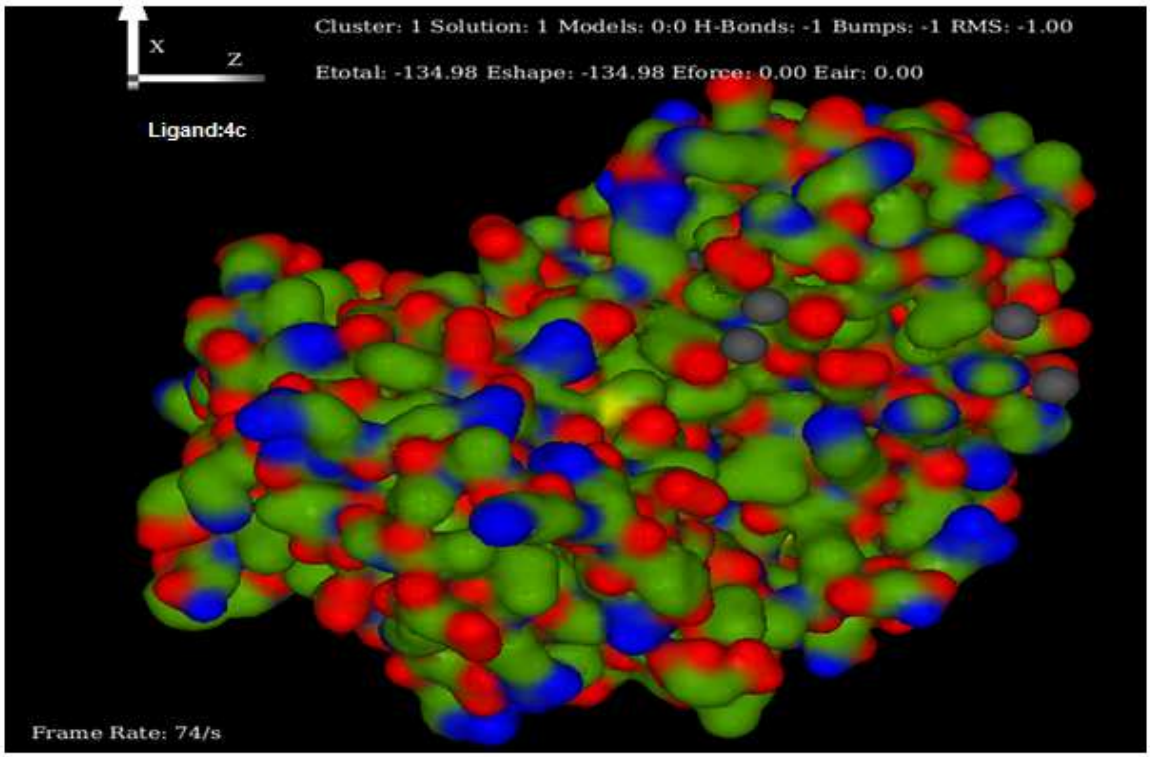


Fig 3: Interaction of compound 4c with glucosamine-6-phosphate synthase

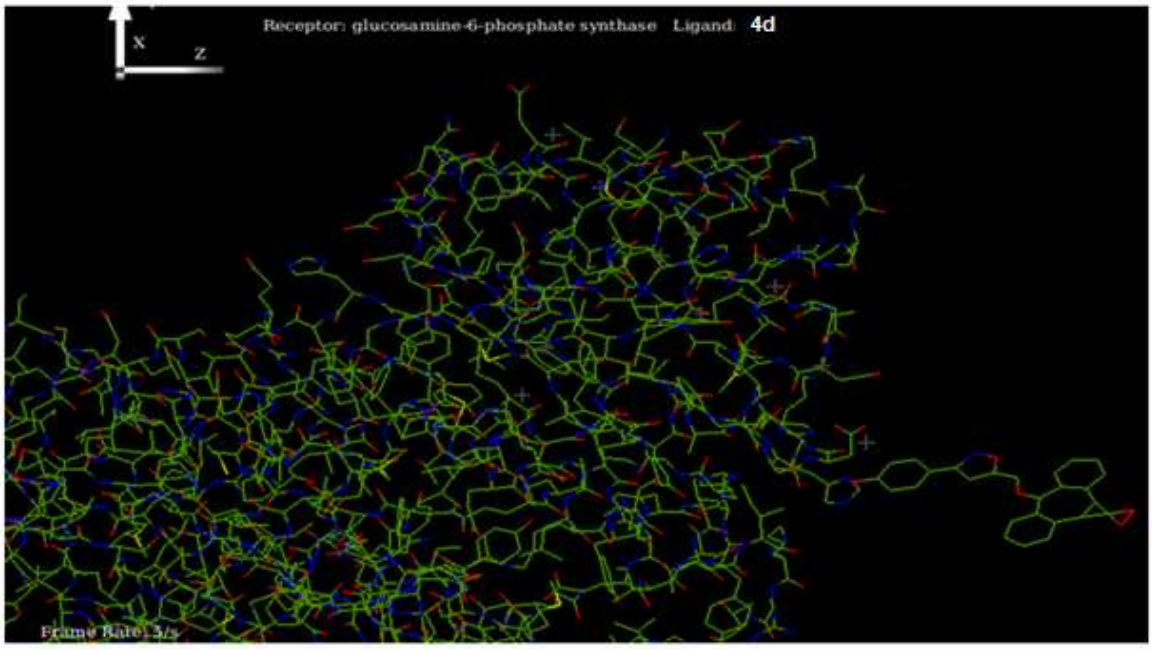


Fig 4: Interaction of compound 4d with glucosamine-6-phosphate synthase

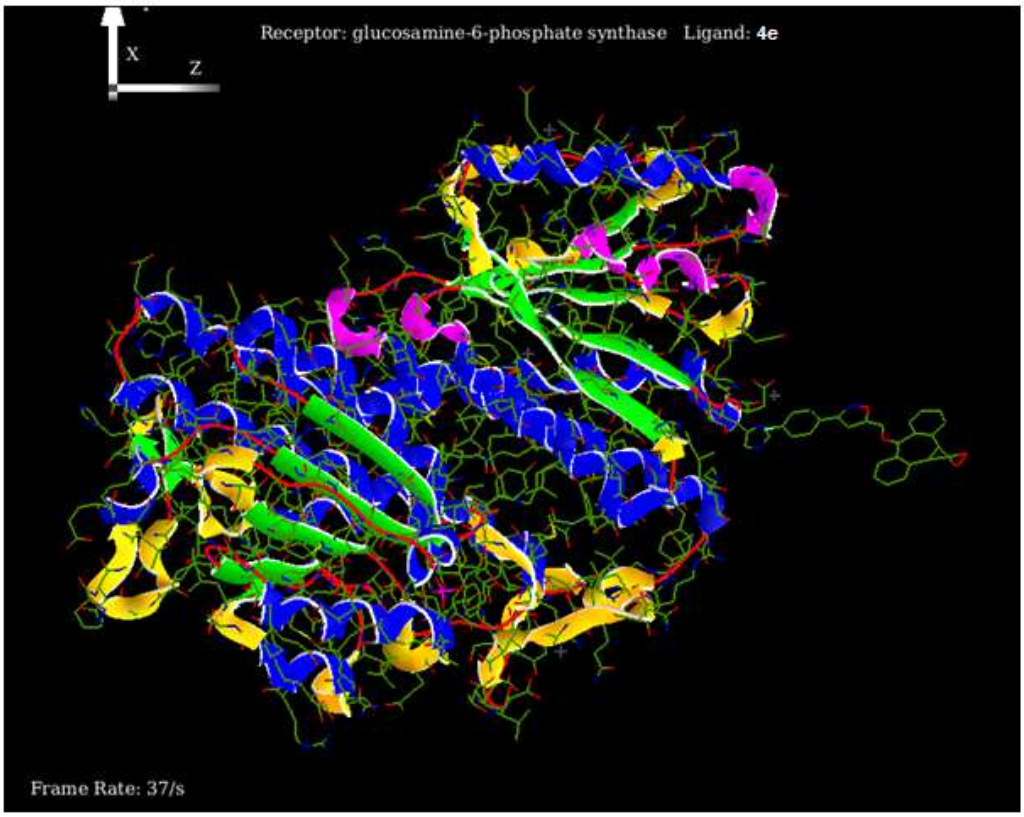


Fig 5: Interaction of compound 4e with glucosamine-6-phosphate synthase

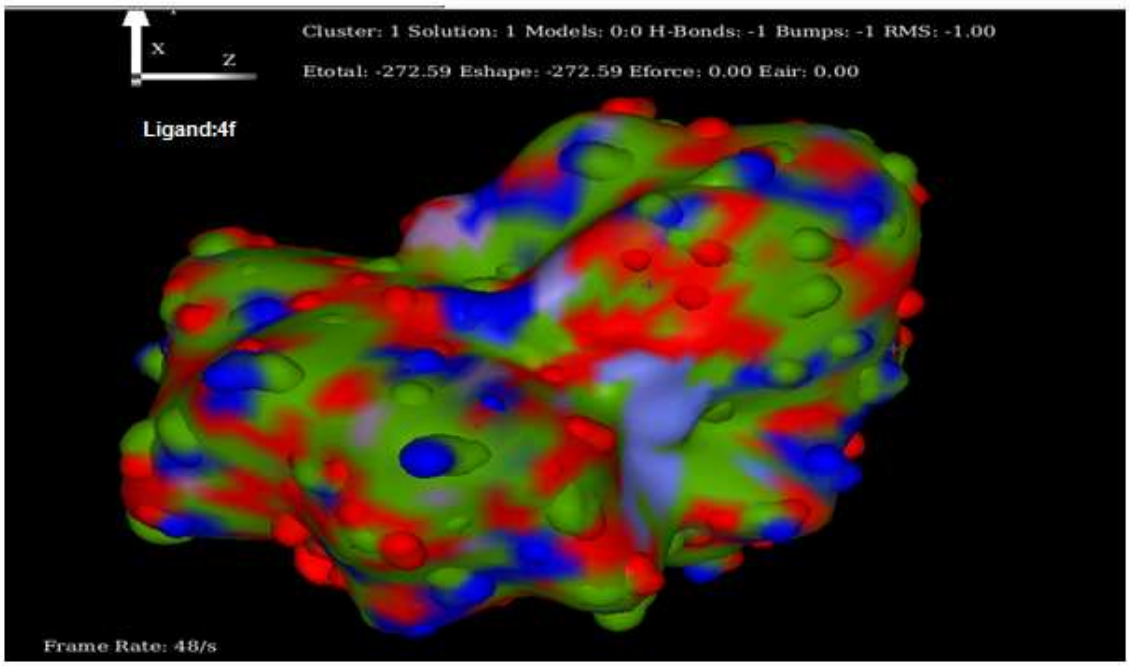


Fig 6: Interaction of compound 4f with glucosamine-6-phosphate synthase

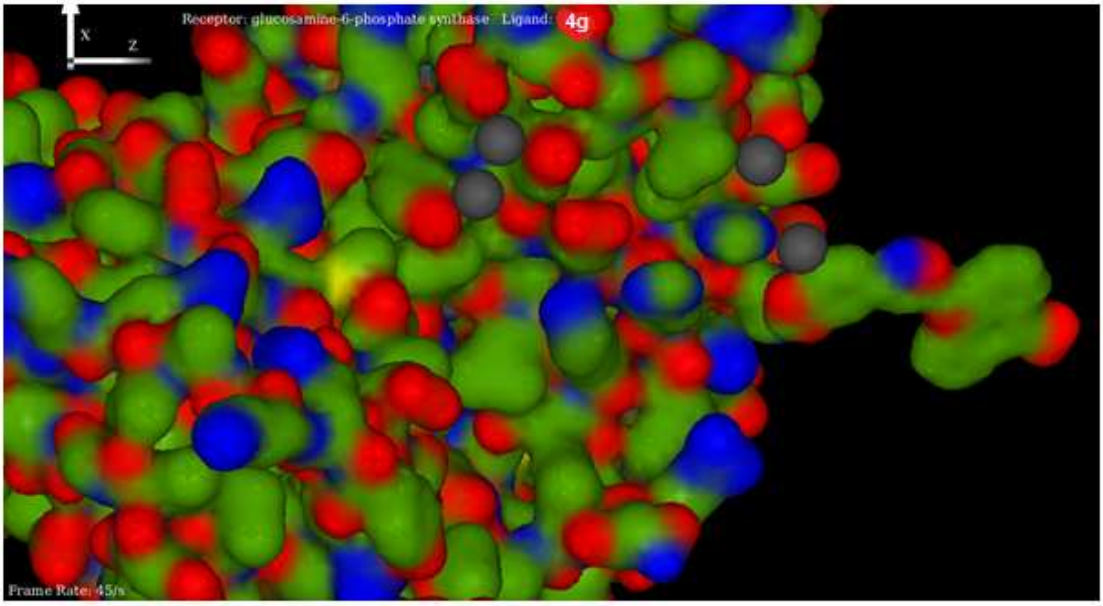


Fig 7: Interaction of compound 4g with glucosamine-6-phosphate synthase

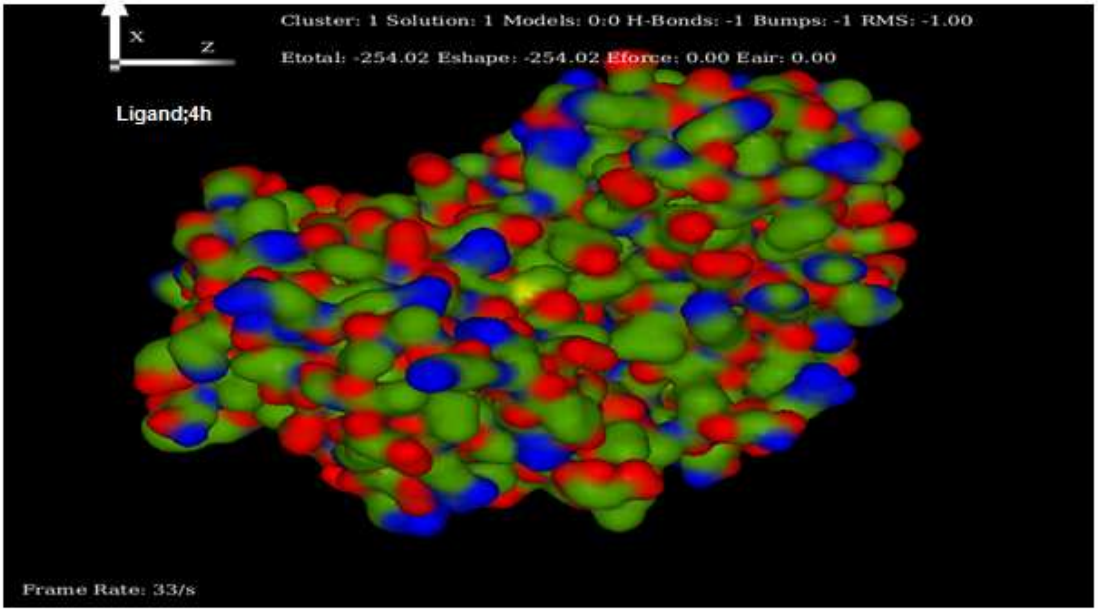


Fig 8: Interaction of compound 4h with glucosamine-6-phosphate synthase

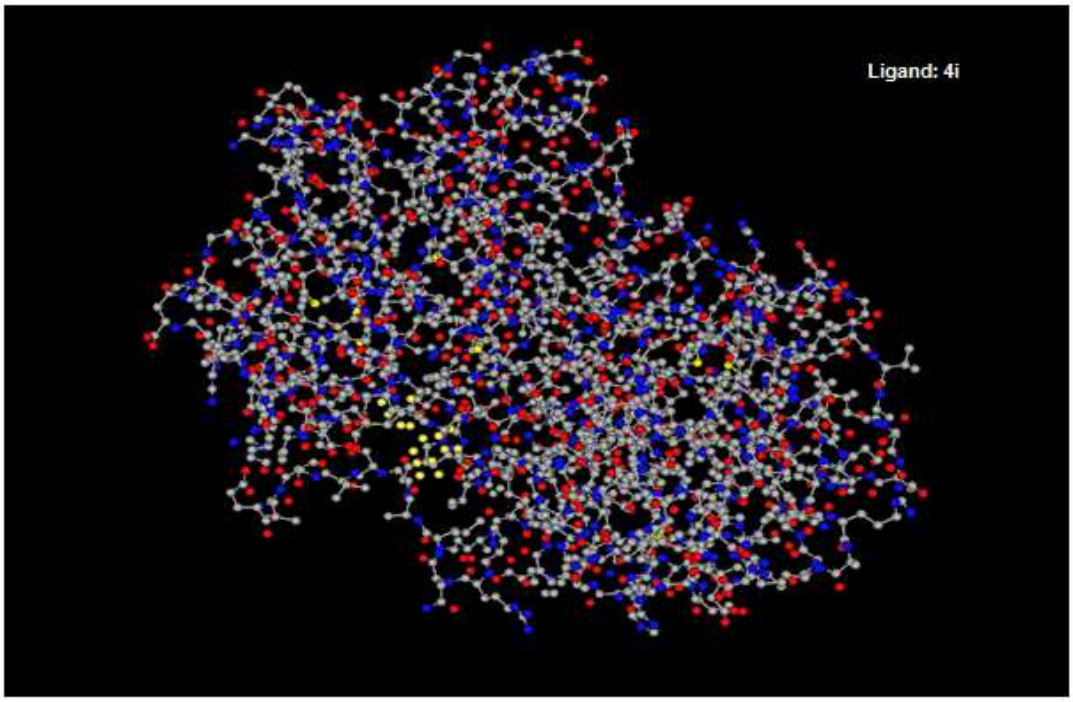


Fig 9: Interaction of compound 4i with glucosamine-6-phosphate synthase

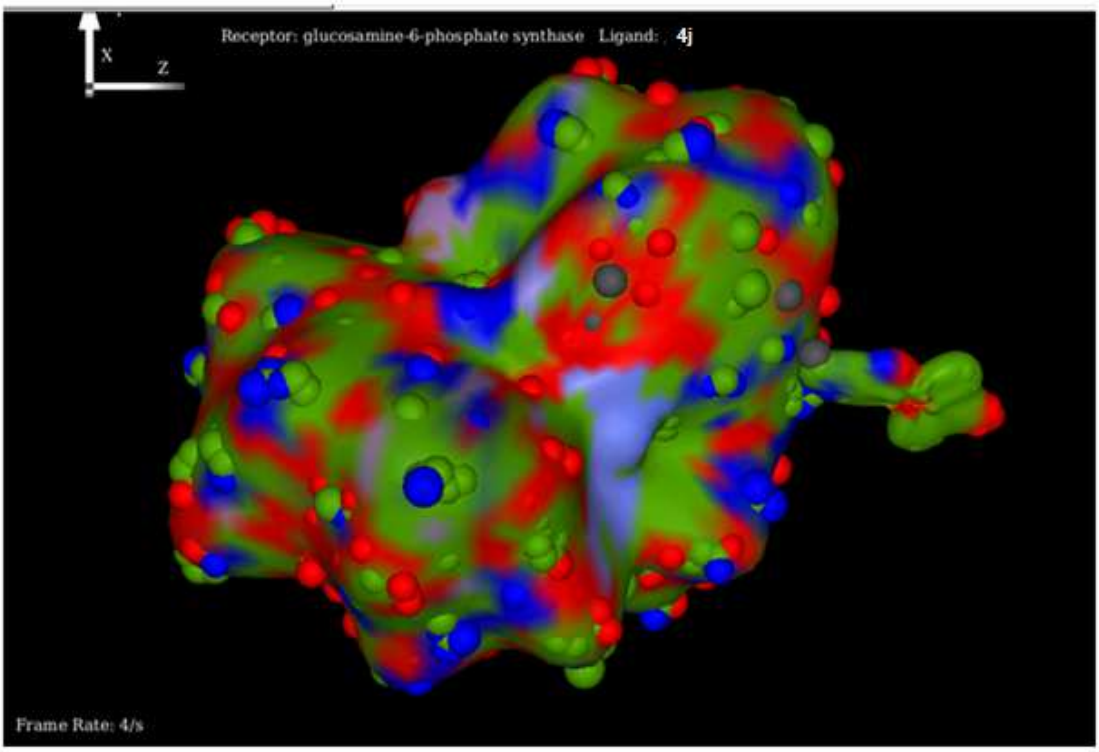


Fig 10: Interaction of compound 4j with glucosamine-6-phosphate synthase

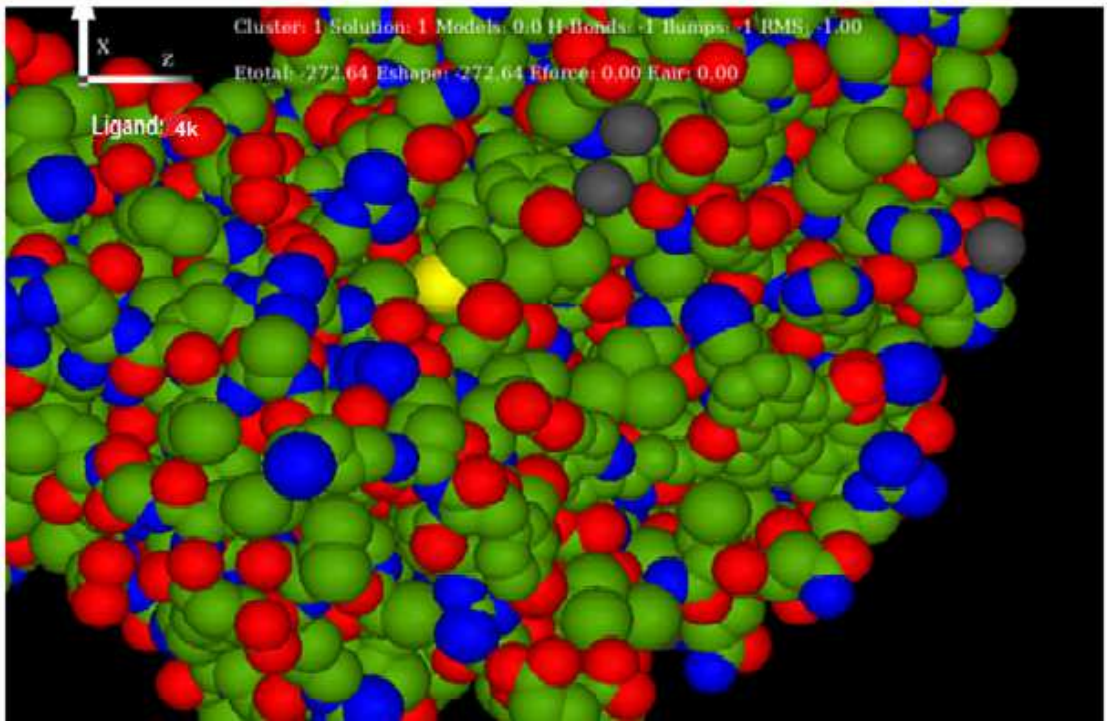


Fig 11: Interaction of compound 4k with glucosamine-6-phosphate synthase

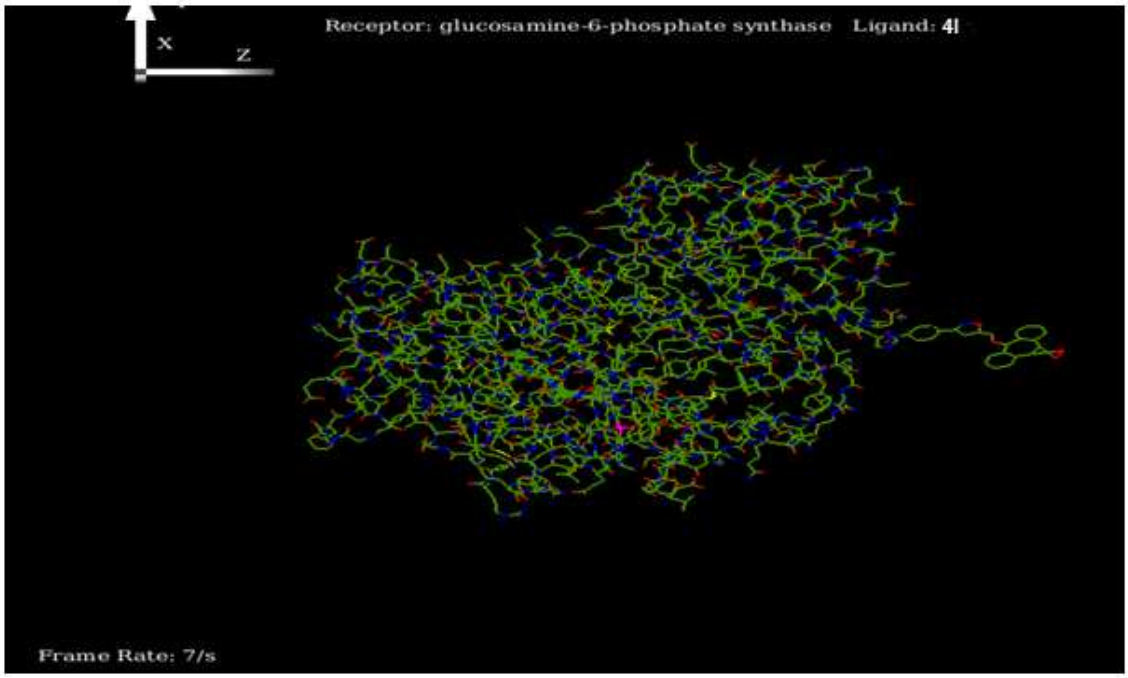


Fig 12: Interaction of compound 4l with glucosamine-6-phosphate synthase

CONCLUSION

- A new series of compounds containing 1,3,5 substituted 1,2,4-Triazoles were synthesized which are part of a wide variety of therapeutically interesting drug candidates.
- Among the tested compounds, **4a, 4b** and **4c** have emerged as most active against all tested bacterial strains**.**
- Molecular docking studies also revealed that compounds **4g** and **4j** has very minimum binding and docking energy and can say as a good inhibitor of GlcN-6-P. Hence this study has widened the scope of developing these triazole derivatives as promising antibacterial agents.
- Among the tested compounds, **4b** and **4g** showed good anti-inflammatory property, whereas compounds, **4e, 4f, 4j** and **4k** showed moderate anti-inflammatory property and compounds**, 4a** and **4h** showed less anti-inflammatory property. Compound **4i** showed no anti-inflammatory property.
- Among the tested compounds, **4a, 4c, 4h** and **4l,** showed good antiproliferative property, **4d, 4e** and **4g** showed moderate activity. The compounds, **4f, 4i, 4j** and **4k** showed less activity during antiproliferative activity studies.

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REFERENCES

[1] H. Miyauchi, K. Kozuki, T. Tanio, N. Ohashi. *Bioorg. Med. Chem. Lett.*, Vol 5(14), **1995**, pp 1479–1482.
[2] H. K. Nair, A. C. Peterson, P. T. Yazdi, R. Franzmair, *Bioorg. Med. Chem. Lett.*, Vol 7(18), **1997**, pp 2379–2382.
[3] P. M. Wood, L. W. L. Woo, J. Labrosse, M. N. Trusselle, S. Abbate, *J. Med. Chem*., Vol 51, **2008**, pp 4226–4238.
[4] A. T. Mavrova, D. Wesselinova, Y. A. Tsenov, D. Pavletta, *Eur. J. Med. Chem.*, Vol 44, **2009**, pp 63–69.
[5] V. Klimesova, L. Zahajska, K. Waisser, J. Kaustova, U. Mollmann, *Farmaco*, Vol 59, **2004**, pp 279-288.
[6] M. V. Pickering, J. T. Witkowski, R. K. Robins, *J. Med. Chem*., Vol 19(6), **1976**, pp 841-842.
[7] Y. Xia, Z. Fan, L. Peng, *Bioorg. Med. Chem.*, Vol 16, **2006**, pp 2693-2698.
[8] Zhang Y. Peng, I. W. Xin, S. M. Keenan, S. Arora, W. J. Welsh, *J. Med. Chem*., Vol 50, **2007**, pp 749-754.

[9] J. M. Kane, M. A. Staeger, C. R. Dalton, F. P. Miller, M. W. Dudley, P. A. Chmielewski, J. A. Miller, J. *Med. Chem*., Vol 37, **1994**, pp 125-132.
[10] F. Sternfeld, R. Baker, H .B. Broughton, A. R. Guiblin, R. A. Jelley, V. G. Matassa, A. J. Reeve, M. S. Beer, J. A. Stanton, R. J. Hargreaves, S. L. Shepheard, J. Longmore, Z. Razzaque, M. I. Graham, B. Sohal, L. J. Street, *Bioorg. Med. Chem. Lett*., Vol 6(15), **1996**, pp 1825–1830.
[11] C. A. Lipinski, *J. Med. Chem*., Vol 26(1), **1983**, pp 1–6.
[12] Y. Zhu, S. H. Olson, A. H. Vosatka, S. Wright, J. M. Balkovec, *Bioorg. Med. Chem. Lett*., Vol 18, **2008**, pp 3405-3411.
[13] I. Kucukguzel, S. Rollas, M. Kiraz, *Bioorg. Med. Chem. Lett.*, Vol 11,**2001**, pp 1703–1707.
[14] M. D. Rosa, H. W. Kim, W. Zhang, S. A. Lang, *Bioorg. Med. Chem. Lett*., Vol 16, **2006**, pp 4444-4449.
[15] U. Mothes-Wagner, H. K. Reitze, K. A. Seitz, *Exp. Appl. Acarolog*, Vol 8(2), **1990**, pp 27-40.
[16] C. A. Winter, E. A. Risely, G. N. Nuss, *Proc. Soc. Exp. Biol*., Vol 111, **1962**, pp 544–547.
[17] Y. Cetin, L. B. Bullerman, *J. Agric. Food. Chem*., Vol 5, **2005**, pp 6558–6563.