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Synthesis, characterization, molecular docking and evaluation of antimicrobial, antiproliferative, and anti-inflammatory properties of new 4-biphenyl substituted thiazolyl-pyridin-2-amine derivatives

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ABSTRACT

4-Biphenyl substituted thiazolyl-Pyridin-2-amine derivatives were prepared by Suzuki coupling reaction, between N-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine and substituted phenyl boronic acids. The new compounds were subjected to, antibacterial, antiproliferative, anti-inflammatory and molecular docking studies. Among the compounds tested, N-[4-(4'-fluorobiphenyl-4-yl)-1,3-thiazol-2-yl]pyridine-2-amine and N-{4-[4'-(trifluoromethyl) biphenyl-4-yl]-1,3-thiazol-2-yl}pyridine-2-amine showed promising properties. The newly synthesized compounds were characterized by analytical, IR, ^{1}H NMR, ^{13}C NMR and LCMS.

Keywords: Thiazolyl-pyridin-2-amine, thiazole, antibacterial, antiproliferative, anti-inflammatory, molecular docking.

INTRODUCTION

Pyridine and its derivatives are known to be pharmacophores which possess anticancer, analgesic, antimicrobial, and antidepressant activities [1-4].

It was reported that the series of N-(1,3-thiazol-2-yl)pyridin-2-amine KDR kinase inhibitors were developed that possess optimal properties. The compounds were reported to exhibit excellent *in vivo* potency. The amine substituted compounds found to have a favorable kinase selectivity profile that could be accentuated with appropriate substitution. Molecular modeling has postulated an interesting conformational preference and binding mode for these compounds in the active site of the enzyme [5-6].

The Kinase insert Domain containing Receptor (KDR), alternatively referred to as VEGFR-2, is a receptor for Vascular Endothelial Growth Factors (VEGFs) and functions as a key regulator of angiogenesis, the process by which new capillaries are created from pre-existing blood vessels. The induction of angiogenesis, or the "angiogenic switch," is a critical step in tumor progression, and inhibitors of KDR have been demonstrated both to induce tumor regression and reduce metastatic potential in preclinical models. In the last few years, medicinal chemists have expanded the kinase selectivity profile of known inhibitor classes to include KDR, and also identified novel classes of KDR inhibitors.

Gentles *et al* reported SAR study on a series of apamin-displacing 2-aminothiazole $K_{Ca}2$ channel blockers, Potent inhibitors such as *N*-(4-methylpyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine, and for select members of the series, the relationship between the observed activity in a thallium flux, a binding and a whole-cell electrophysiology assay was also presented [7].

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries [8]. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required [9]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease [10]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions [11]. However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes [12–14]. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates [15-16].

A new series of 4-biphenyl substituted thiazolyl-pyridin-2-amine derivatives were synthesisedand 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).4-Biphenyl substituted thiazolyl-pyridine-2-amine derivatives were prepared using Suzuki coupling reaction. Different aryl boronic acids were used to prepare the above said derivatives. 1,1′-Bis(diphenylphosphino) ferrocene]dichloropalladium(II) was used to catalyze the coupling between N-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine and substituted phenyl boronic acids.

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 MicromasszQ 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 (ElementarAnalysensystemeGmBH) at Department of Chemistry, Mangalore University, Mangalore, Karnataka, India. FTIR spectra were recorded on Perkin Elmer IR spectrophotometer in KBr phase.

1.1: **Synthesis of [4-(4-Bromo-phenyl)-thiazol-2-yl]-pyridin-2-yl-amineb[Scheme 1]**

A equimolar mixture of 2-Bromo-1-(4-bromo-phenyl)ethanone and Pyridin-2-yl-thiourea in absolute alcohol was refluxed for 16 h. The reaction mixture was evaporated under reduced pressure and the residue was suspended in 10% sodium bicarbonate solution. The solids thus precipitated was filtered, washed with water and dried under suction to afford the title compound as a white solid.

1.1.1: [4-(4-Bromo-phenyl)-thiazol-2-yl]-pyridin-2-yl-amine **(3)**

Elem. Anal. for $C_{14}H_{10}BrN_3S$, cacld; C 50.51 , H 3.0 , N 12.64; found; C 50.20 , H 2.95 , N 12.38. **IR (KBr, ν cm^{-1}):** 3393, 3064, 1649, 1616, 1587, 1265, 1159, 1198, 1071, 1002, 830, 767, 490. **^{1}H NMR (DMSO-d_6, 400 MHz) δppm:** 11.44(s, 1H, N-H), 7.50(s, 1H, thiazole proton), 8.31-8.32(d, J=5 Hz, Pyridine), 7.71-7.75 (t, J=16Hz, 1H, pyridine), 6.93-6.96 (t, J=16Hz, 1H, pyridine), 7.85-7.87(d, J=8.8 Hz 1H,pyridine), 7.10-7.12(d, J=8.4Hz, 2H, phenyl), 7.60-7.7.62(d, J=8.4Hz, 2H, phenyl). **^{13}C NMR (DMSO-d_6, 400 MHz) δppm:** 107.253, 111.691, 116.354, 120.658, 127.749, 131.516, 133.463, 139.485, 144.603, 147.603, 150.831, 159.845 (14C-atom).**LCMS:**molecular ion peak m/z 331.9[M+] 333.6[M+2], 335.9[M+4].

1.2: General procedure to synthesis (4-substituted biphenyl-4-yl-thiazol-2-yl)-pyridin-2-yl-amine. [Scheme 1]

To a solution of [4-(4-Bromo-phenyl)-thiazol-2-yl]-pyridin-2-yl-amine (0.2 g, 1eq) in 1,4-dioxane (10ml) /water (2ml), substituted phenyl boronic acid (1.2 eq) and potassium carbonate (3eq) were added. The mixture was degasified by purging nitrogen for 5 min and then added [1,1′-Bis (diphenylphosphino) ferrocene] dichloropalladium(II) (0.05 eq). The resulting mixture was heated at 100° C in sealed tube for 15h. The reaction mass was cooled to RT and solvent was evaporated under vaccum. The residue was extracted with ethyl acetate (2 x 50ml), organic layer was washed with water, brine, dried over sodium sulphate and evaporated under vaccum. The

crude material was purified by flash chromatography using silica gel (230-400) and pet ether /ethyl acetate as gradient elution to afford the title compound.

1.2.1: *N-[4-(3'-methoxybiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine* ***(5a)***
Elem. Anal.for $C_{21}H_{17}N_3OS$, cacld: C 70.1 , H 4.7 , N 11.7; found: C 69.7, H 4.5 , N 11.5.**IR (KBr, ν cm^{-1}):**3376, 3420, 3105, 1614, 1603, 1583, 1534, 1480, 1332, 1162, 1128, 1073, 1100, 824, 774, 588. **^{1}H NMR (DMSO-d_6, 400 MHz) δppm :** 11.44 (s, 1H, N-H proton), 7.46(s, 1H, thiazole proton), 3.82 (s, 3H, methoxygrp), 8.29-8.30(d, J=4.4Hz ,1H Pyridine),7.675-7.728(m, 3H, pyridine), 6.91-6.93(d, J=8 Hz, 2H, Phenyl), 7.96-7.98(d, J=8Hz, 2H, phenyl), 7.34-7.38(t, J=16Hz, 1H, Phenyl), 7.22(s, 1H, phenyl), 7.25-7.27(d, J=8Hz, 1H, phenyl), 7.08-7.10(d, J=8Hz, 1H, phenyl).**^{13}C NMR (DMSO-d_6, 400 MHz) δppm:** 55.609, 106.624, 111.294, 112.351, 113.603, 116.450, 119.272, 126.547, 127.424, 130.441, 134.518, 138.354, 139.335, 141.678, 146.928, 148.706, 152.333, 160.072, 160.274 (21 C-atom). **LCMS :** m/z 360.45[M+1] 361.95[M+2].

1.2.2: *N-[4-(4'-fluorobiphenyl-4-yl)-1,3-thiazol-2-yl]pyridin-2-amine* ***(5b)***
Elem. Anal. for $C_{20}H_{14}FN_3S$, cacld: C 69.08 , H 4.02 , N 12.08; found: C 70.1 , H 4.01 , N 12.11. **^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm :** 11.388 (s, 1H, N-H proton), 7.483(s, 1H, thiazole proton), 8.307-8.317(d, J=4.0Hz ,1H Pyridine), 7.274-7.318(m, 2H, pyridine), 6.931(1H, Pyridine), 7.984-8.005(d, J=8.4Hz, 2H, biphenyl), 7.702-7.777(dd, J=8Hz, 4H, biphenyl), 7.101-7.122 (d, J=8.4 Hz, 2H, biphenyl).**^{13}C NMR (DMSO-d_6,400 MHz)ppm:** 106.097, 110.747, 115.540, 115.752, 115.921, 126.084, 126.733, 128.319, 128.400, 131.464, 133.820, 136.125, 137.822, 137.864, 146.398, 148.103, 151.787, 159.527, 160.577, 163.007(20 C-atom). **LCMS**: m/z 348[M+].

1.2.3:*N-[4-(4'-chlorobiphenyl-4-yl)-1,3-thiazol-2-yl]pyridin-2-amine* ***(5c)***
Elem. Anal. for $C_{20}H_{14}ClN_3S$, cacld: C 65.95 , H 3.84 , N 11.54; found: C 66.01 , H 3.91 , N 11.07.**^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm:** 11.447 (s, 1H, N-H proton), 7.561(s, 1H, thiazole proton), 8.303-8.319 (d, J=6.4Hz, 1H Pyridine), 7.740-7.814 (t, J=29.6Hz, 1H, pyridine), 6.910-6.934(t, J=13.2Hz, 1H, Pyridine), 7.082-7.110(d, J=11.2Hz, 1H, pyridine), 8.027-8.055(dd, J=11.2Hz, 2H, biphenyl),7.842-7.931(dd , 4H, biphenyl), 7.689-7.715 (d, J=10.4, 2H, biphenyl).**^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm:** 106.288, 110.757, 115.937, 126.145, 126.752, 128.122, 128.821, 132.209, 134.184, 137.509, 137.830, 138.420, 146.398, 148.047, 151.782, 159.552 (20 C-atom). **LCMS:** m/z364[M],366[M+2],367[M+3].

1.2.4:*N-{4-[4'-(trifluoromethyl)biphenyl-4-yl]-1,3-thiazol-2-yl}pyridin-2-amine* ***(5d)***
Elem. Anal. for $C_{21}H_{14}F_3N_3S$, cacld: C 63.41 , H 3.52 , N 10.56; found: C 64.01 , H 3.61 , N 10.67.**^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm:** 11.446 (s, 1H, N-H proton), 7.369 (s, 1H, thiazole proton), 7.711-7.956(t, J=98Hz, 1H, pyridine), 8.314(1H, pyridine), 7.080-7.108(d, J=11.2Hz, 1H, Pyridine), 6.910-6.948(t, J=15.2Hz, 1H, pyridine), 7.990-8.017(d, J=10.8Hz, 2H, biphenyl) 7.646-7.674(d, J=11.2Hz, 2H, biphenyl). 7.489-7.531 (dd, 4H, biphenyl).**^{13}C NMR (DMSO-d_6,400 MHz) δ ppm:** 106.668, 110.768, 115.968, 122.978, 125.699, 126.209, 127.146, 127.219, 127.847, 134.806, 137.217, 137.861, 143.616, 146.408, 147.924, 151.771, 159.592 (21 C-atom). **LCMS**: m/z 398.2[M+1],399.2[M+2],400.2[M+3].

1.2.5: *N-[4-(3'-chlorobiphenyl-4-yl)-1,3-thiazol-2-yl]pyridin-2-amine* ***(5e)***
Elem. Anal. for $C_{20}H_{14}ClN_3S$, cacld: C 65.95 , H 3.84 , N 11.54; found: C 66.01 , H 3.61 , N11.67.**^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm:** 11.380 (s, 1H, N-H proton), 7.508 (s, 1H, thiazole proton), 7.685(1H, pyridine), 8.281(1H, pyridine), 7.082(1H, Pyridine), 6.904(1H, pyridine), 8.007(2H, biphenyl) 7.919(m, 2H, biphenyl). 7.788 (dd, 4H, biphenyl).

1.2.6: *N-{4'-[2-(pyridin-2-ylamino)-1,3-thiazol-4-yl]biphenyl-4-yl}acetamide* ***(5f)***
Elem. Anal.for $C_{22}H_{18}N_4OS$, cacld: C 68.31 , H 4.65 , N 14.49; found: C 68.01 , H 4.61 , N 14.67. **^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm:** 11.446 (s, 1H, N-H proton), 10.040(s, 1H, N-H Acetamido proton), 2.063(s, 1H, CH3CO), 7.369 (s, 1H, thiazole proton), 7.711-7.956(t, 1H, pyridine), 8.314(1H, pyridine), 7.080-7.108(d, J=11.2Hz, 1H, Pyridine), 6.910-6.948(t, J=15.2Hz, 1H, pyridine), 7.990-8.017(d, J=10.8Hz, 2H, biphenyl) 7.646-7.674(d, J=11.2Hz, 2H, biphenyl). 7.489-7.531 (dd, 4H, biphenyl).

1.3: Antibacterial activity
The newly synthesized thiazoles were screened for their antibacterial activity against bacterial strains by disc diffusion method. The discs measuring 6.25 mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140° C for an hour. The test compounds were prepared with different concentrations using dimethyl formamide. One millilitre containing 100 times the amount of chemical required in each disc was added to each bottle which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria

separately. The incubation was carried out at 37.8 C for 24 h. Nitrofurazone was used as a standard drug. Solvent and growth controls were kept. The zone of inhibition and minimum inhibitory concentrations [MIC] were noted.

1.4: Antiproliferative studies against HEPG2 and EAT cell lines

Cell culture and in vitro cytotoxicity assay (MTT assay). HEPG2 and EAT cell lineswere cultured and cytotoxicity was carried out [17-18]. Cells were treated with compounds at a concentration range of 10µg/ 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.

1.5: *In vivo* Anti-inflammatory activity assay

Anti-inflammatory screening was done using in vivo carrageenan induced rat paw edema model which is considered the most conventional one for acute inflammation [19]. Adult albino rats of both sexes weighing (120-200 gm) were randomly distributed in twelve groups of six animals each. The rats were kept fasted for 24 h prior to the experiment but allowed free access to water. The rats were injected intraperitoneally with 25µg/kg.bw doses of the tested compounds and reference drug (diclofenac sodium). One hour later, 0.05 ml of freshly prepared suspension of carrageenan (1% w/v in saline suspension) was injected subcutaneously into the subplantar region of the right hind paw. The left hind paw of each rat received a subplantar injection of equal volume of normal saline. Three hours after carrageenan injection, rats were sacrificed by cervical dislocation then the right and the left hind paws of each rat were cut at the tibiotarsic articulation and weighed. The difference in weight between right and left paws was recorded for each rat. The percentage increase in weight of the carrageenan-injected paw over the other paw was calculated and percentage reduction of edema was calculated.

1.6: Molecular 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

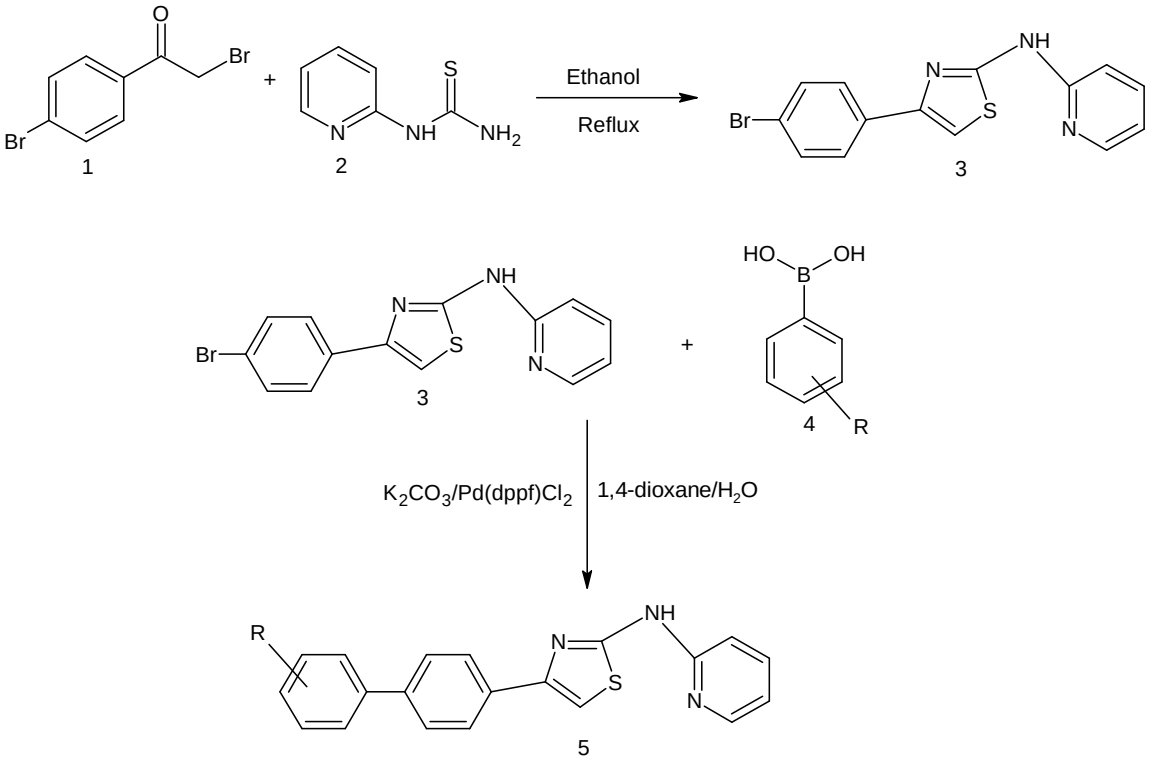
1. Chemistry

The new series of 4-biphenyl substituted thiazolyl-pyridin-2-amines **5a-g** were synthesised by the reaction of N-[4-(4-bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine with substituted phenyl boronic acid (**Scheme 1).**2-bromo-1-(4-bromophenyl) ethanone was reacted with pyridin-2-yl-thiourea to get N-[4-(4-bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine.The synthesized compounds are tabulated in **Table 1.**

The formation of new thiazolyl pyridine-2-amine derivatives was confirmed by analytical and recording IR, ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis of a few selected compounds.

The IR absorption spectrum of compound [4-(4-Bromo-phenyl)-thiazol-2-yl]-pyridin-2-yl-amine (**3)** showed strong absorption band in the region 3393cm^{-1} due to-NH group, the strong absorption band appeared at 1002 and 1071 cm^{-1} due to stretching of C-S bonds of the thiazole ring, absorption bands found in the region 1616 and 1587cm^{-1}were due to C=N of the thiazole ring, Bands observed in the region 1159 and 1198cm^{-1} were attributed to –N-C bonds of the thiazole ring, strong absortion peak at 490 cm^{-1} was due to C-Br bond of the phenyl ring.

Scheme 1: Synthesis of N-[4-(biphenyl substituted-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine(5)



R= 3-OCH_3; 4-F; 4-Cl ;4-CF_3; 3-Cl ; 4 - $NHCOCH_3$; 4-CN

The ^{1}H NMR spectrum of compound [4-(4-Bromo-phenyl)-thiazol-2-yl]-pyridin-2-yl-amine (**3)** showed signals at δ 11.44ppm , a singlet for N-H proton and another singlet at 7.50ppm for thiazole proton. A doublet at δ 8.31-8.32ppm (J=5Hz) could be attributed to proton next to the pyridine ring nitrogen. Triplets appeared at δ 7.71-7.75ppm (J=16.8Hz) and δ 6.93-6.96ppm (J=12.4Hz), doublet at δ 7.85-7.87ppm (J=8.8Hz) were assigned to the aromatic protons of pyridine ring. Doublets seen at δ 7.6-7.62ppm (J=8.4Hz) and δ 7.10-7.12ppm (J=8.4Hz) were assigned to phenyl ring containing bromine. The above data confirmed the structure of the compound **3**.
The ^{13} C NMR spectrum of compound **3** showed signals at δ 107.253, 111.691, 116.354, 120.658, 127.749, 131.516, 133.463, 139.485, 144.603, 147.603, 150.831, 159.845 which accounted for all the 14 carbon atoms.

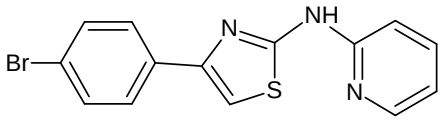
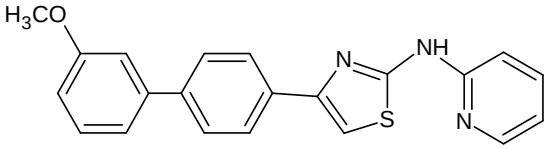
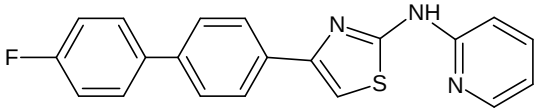
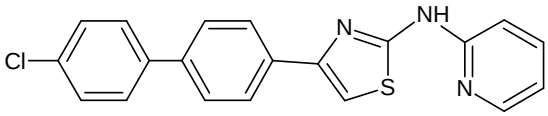
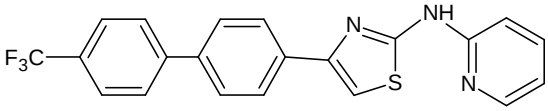
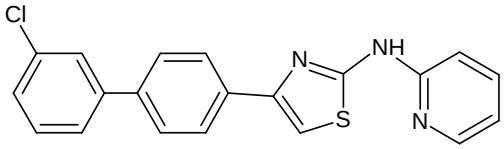
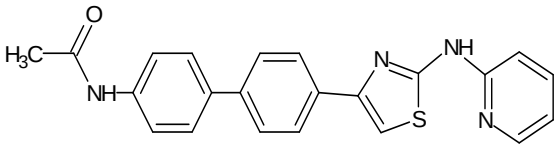
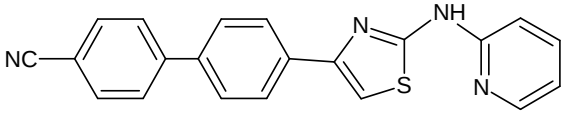
It was further supported by LC mass spectrum which showed molecular ion peak m/z 331.9[M+] 333.6[M+2], 335.9[M+4] which corresponded to the molecular formula $C_{14}H_{10}BrN_3S$.

The IR absorption spectrum of compound N-[4-(3'-methoxybiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine**(5a)** showed strong absorption band in the region 3376cm^{-1} due to -NH group, the strong absorption bands seen at 1073 and 1100 cm^{-1} were due to stretching of C-S bonds of the thiazole ring, absorption bands in the region 1603 and 1583cm^{-1} were due to C=N of the thiazole ring, Bands observed in the region 1162 and 1128cm^{-1} were due to –N-C bonds of the thiazole ring, strong absortion peak at 1332 cm^{-1} is due to –OCH_3 bond of the phenyl ring .

The ^{1}H NMR of compound **5a** showed signals at δ 11.44ppm a singlet for N-H proton and another singlet at 7.46ppm for thiazole proton. A singlet at δ 3.82ppm could be attributed to methoxy group. Doublet seen at δ 8.29-8.30ppm (J=4.4Hz) could be attributed to proton next to the pyridine ring nitrogen. Multiplet resonated at δ 7.675-7.728 ppm could be due to pyridine ring protons. Doublets at δ 6.91-6.93ppm (J=8Hz) and δ 7.96-7.98 ppm (J=8Hz) were due to phenyl ring. Triplet seen at δ 7.34-7.38ppm (J=16Hz), singlet at δ 7.22ppm, doublets at δ 7.25-7.27ppm (J=8Hz) and δ 7.08-7.10 ppm (J=8Hz) could be attributed to phenyl ring containing methoxy group. The above data confirmed the structure of compound **5a**.

The ^{13} C NMR spectrum of compound **5a** showed signals at δ 55.609 (methoxy group), 106.624, 111.294, 112.351, 113.603,116.450,119.272, 126.547, 127.424, 130.441, 134.518, 138.354, 139.335, 141.678, 146.928, 148.706, 152.333, 160.072, 160.274, which accounted for all the 21 carbon atoms.

Table 1: Characterization of N-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine (3), N-[4-(biphenyl substituted-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine (5a-g)

Compd No.	structure	M.Wt	Appearance	Yield	M.P Uncorrected
3		332.2	White solid	80%	121-124°C
5a		359.44	Off white solid	70%	131-134°C
5b		347.41	Off white solid	75%	161-164°C
5c		363.86	Tan solid	85%	136-138°C
5d		397.41	Light yellow solid	80%	127-128°C
5e		363.86	Pale yellow solid	78%	112-115°C
5f		386.46	White solid	68%	141-143°C
5g		354.42	Brown solid	58%	137-140°C

It was further supported by LC mass spectrum which showed molecular ion peak m/z 360.45[M+1] 361.95[M+2]. Similarly all the synthesized compounds were characterized by NMR and LC mass spectroscopy.

2. Biological evaluation

2.1: Antibacterial studies

The newly synthesized compounds were screened for their antibacterial activity against *E.coli*, *S.typhi*, *M. luteus*and *S. aureus* bacterial strains by disc diffusion method [20-21]. The MIC values of the tested compounds are given in **Table 2.** Among the tested compounds, the compound N-[4-(3'-methoxybiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine (**5a)** and **N**-[4-(4'-fluorobiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine **(5b)** showed excellent activity against all tested microorganisms, whereas N-[4-(4'-chlorobiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine (**5c),** N-[4-(3'-chlorobiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine **(5e)** and N-[4-(4'-acetamidobiphenyl-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine (**5f)** showed moderate activity, whereas N-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]-pyridin-2-amine **3** and N-{4-[4'-(trifluoromethyl) biphenyl-4-yl]-1,3-thiazol-2-yl}-pyridin-2-amine (**5d)** showed less activity.

Table 2:Minimum Inhibitory Concentrations (µg/ml) of compounds against tested Bacterial strains

Compounds	*E.coli*	*S.typhi*	*M.luteus*	*S.aureus*
3	125	500	250	62.5
5a	15.62	125	15.62	125
5b	31.25	125	15.62	125
5c	31.25	31.25	31.25	250
5d	62.5	125	62.5	15.62
5e	125	31.25	62.5	31.25
5f	31.25	125	31.25	31.25

2.2: Antiproliferative studies

MTT assay is a standard colorimetric assay can be used to determine the cytotoxicity of compounds [17-18].The newly synthesised compounds were subjected to antiproliferative studies against HEPG2 and EAT cell lines. Cells were treated with compounds at a concentration range of 10µg/ml and percentage cell growth inhibition was calculated. Concentration of all the compounds required for 50% inhibition was determined (**Table3).** All the newly synthesised compounds showed good antiproliferative property against HEPG2 and EAT cell lines. Among the tested compounds, bromo phenyl derivative (**3),** 3-methoxy biphenyl **(5b),** 4-fluoro biphenyl (**5c)** and 4-trifluoromethyl biphenyl (**5d)** showed very good inhibition of HEPG2 cells. Whereas compounds **(3), (5b)** and **(5f)** exhibited better activity for the inhibition of EAT cells. It is interesting to note that all the compounds exhibited very effective IC_{50} values. The chloro substituted derivative found to be effective at 11 µM and the acetamido substituted derivative was effective at 8 µM for 50% cell kill for HEPG2.The compound **5b** was found to be more potent against EAT cells with IC_{50} value 17µM and % inhibition 82.28±11.20.

Table 3: Antiproliferative studies against HEPG2 and EAT cell lines

Compounds	HEPG2 %INHIBITION	IC50µM	EAT %INHIBITION	IC50µM
3	87.42±8.15	25	92.33±6.77	87
5a	81.52±11.28	33	76. 74±7.91	39
5b	87.19±6.42	52	82.28±11.20	17
5c	86.33±8.39	11	63.21±6.72	21
5d	90.61±4.73	20	60.62±3.92	62
5e	71.82±8.42	42	72.53±8.8	110
5f	73.45±6.80	08	78.28±5.43	23

2.3: Anti-Inflammatory studies

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs alleviate pain by countering the cyclooxygenase (COX) enzyme. On its own, COX enzyme synthesizes prostaglandins, creating inflammation. In whole, anti-inflammatory drugs prevent the prostaglandins from ever being synthesized reducing or eliminating pain. Several medicinal chemistry and pharmacologic research revealed the biological potential of pyridine and thiazolecompounds. Hence it was decided to study anti-inflammatory property of the synthesised compounds.

Anti-inflammatory screening was done for the newly synthesised compounds using *in vivo* carrageenan induced rat paw edema model [21] which is considered the most conventional one for acute inflammation. The percentage increase in weight of the carrageenan-injected paw over the other paw was calculated and the percentage reduction of edema was calculated (**Table 4)**. Diclofenac sodium a well known anti-inflammatory drug was used as standard. Overall, one can say that all the newly tested compounds brought down the edema compared to control (31.65±1.72%). But compare to standard drug the % inhibition was moderate. N-[4-(4'-chlorobiphenyl-4-yl)-1,3-thiazol-2-yl]pyridin-2-amine **(5c)** emerged as potent one with (62.24±2.67) % inhibition. Compounds **3** and **5a**

showed good anti-inflammatory property, compound **5b** showed moderate and compounds **5d, 5e** and **5f** showed less anti-inflammatory property.

Table 4: Anti-inflammatory studies

Compound no.	Mean % increase in paw weight ± SE %	Inhibition of paw edema
Control	31.65±1.72	--------
Diclofenac	12.35±1.21	78.45±4.61
3	20.34±1.06	48.32±2.16
5a	18.17±0.84	53.34±1.45
5b	23.66±2.16	42.38±2.74
5c	14.14±2.21	62.24±2.67
5d	24.33±1.32	25.87±2.13
5e	25.26±2.34	26.49±1.28
5f	20.48±1.43	22.67±2.53

3. Molecular docking studies

The docking results for ligand molecules against glucosamine-6-phosphate synthase [PDB Id: 1jxa], are documented in **Table 5.** Among the seven molecules, **5b** and **5d,** showed minimum binding and docking energy among the docked compounds and can be concluded as good inhibitor of GlcN-6-P.The data represented as a total E-value rather than docking and binding energy. Theoretically all the seven molecules showed very good binding energy and docking energy.

Table 5: **Molecular docking results with glucosamine-6-phosphate synthase**

Compound	E-Total value
3	-244.9
5a	-251.6
5b	-259.5
5c	-243.7
5d	-262.1
5e	-248.4
5f	-250.7

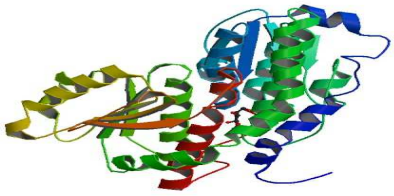


Fig 1:1JXa **glucosamine-6-phosphate synthase**

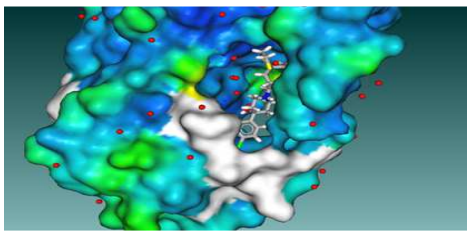


Fig 2: Binding of Drug with glucosamine-6-phosphate synthase

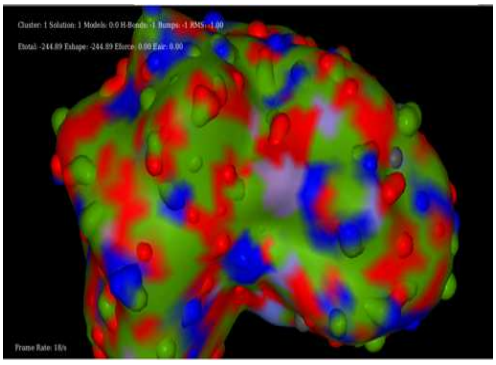


Fig 3: Interaction of compound 3 with GlcN-6-P

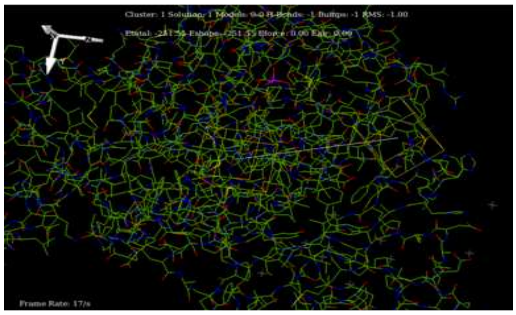


Fig.4: Interaction of 5a with GlcN-6-P

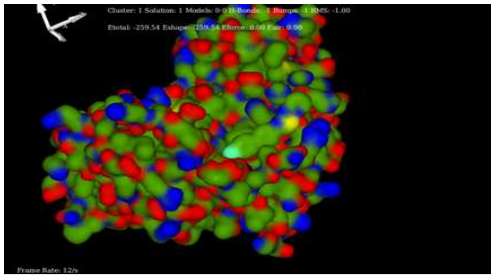


Fig 5: Interaction of 5b with GlcN-6-P

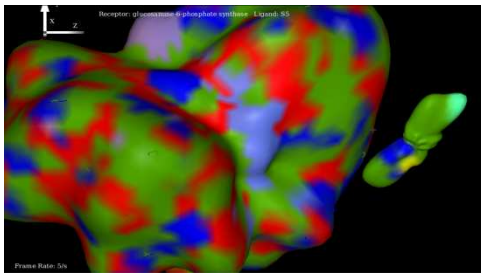


Fig 6: Interaction of 5c with GlcN-6-P

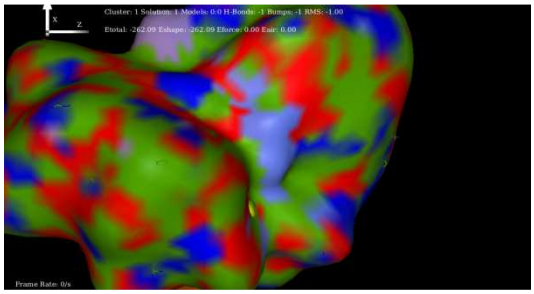


Fig 7: Interaction of 5d with GlcN-6-P

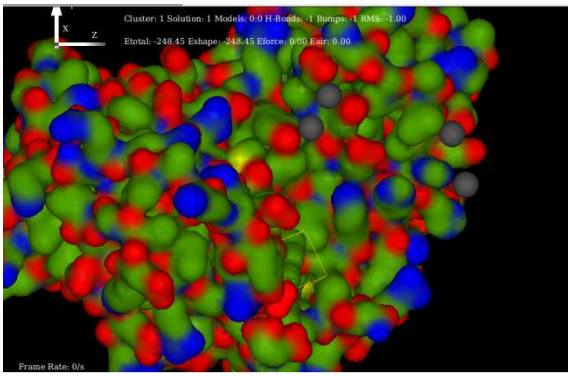


Fig 8: Interaction of 5e with GlcN-6-P

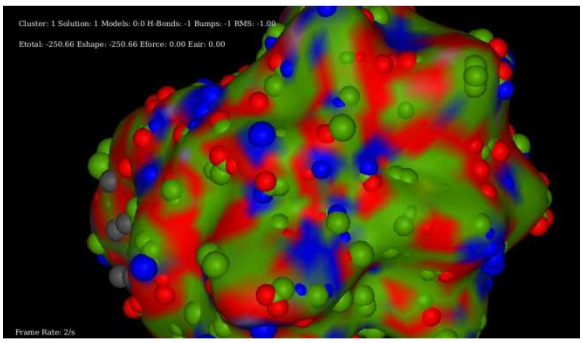


Fig 9: Interaction of 5f with GlcN-6-P

CONCLUSION

- New compounds N-[4-(biphenyl substituted-4-yl)-1,3-thiazol-2-yl]-pyridin-2-amine were synthesized by Suzuki coupling reaction in good yields .
- The newly synthesized compounds are characterized by analytical, IR, ^{1}H NMR, ^{13}C NMR and LCMS.
- The newly synthesized compounds were screened for antibacterial, antiproliferative and anti-inflammatory activities. Among the tested compounds, **3**, **5b, 5c** and 5**d** have emerged as most active compounds in all the tests.
- Molecular docking studies also revealed that compounds **5b** and **5d** showed lowest E-total value(minimum binding and docking energy) and among the docked compounds and can be concluded as good inhibitor of GlcN-6-P, antimicrobial target enzyme.

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