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Structural Design, Synthesis and Molecular Docking Studies of 6-[5-(Substituted Phenyl)-4,5-Dihydro-1*H*-Pyrazol-3-Yl]Pyrrolo[2,1-F][1,2,4]Triazin-4-Amine Derivatives as Anti-Inflammatory Agents

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KEYWORDS
Anti-inflammatory activity, Molecular Docking, Binding affinity, Claisen Schmidt condensation, HRBC,

Pyrazole,

Pyrrolotriazine.

ABSTRACT:

Introduction: Fused heterocycles are very capable of designing new medications due to their propensity to provide selectivity.Pyrrolo[2,1-f] [1,2,4] triazine is a bicyclic heterocycle, containing N–N bond with bridgehead nitrogen possesses numerous activities against various therapeutic targets.Pyrazole and its derivatives are pharmacologically important active scaffold that possesses various pharmacological activities.

Objectives: To design, synthesize and evaluate anti-inflammatory activity of 6-[5-(Substituted phenyl)-4,5-dihydro-1*H*- pyrazol-3-yl]pyrrolo[2,1-f][1,2,4]triazin-4-amine and its derivatives.

Methods: Structural design of 6-[5-(Substituted phenyl)-4,5-dihydro-1*H*- pyrazol-3-yl]pyrrolo[2,1-f][1,2,4]triazin-4-amine derivatives were synthesized, and evaluated for their potential as anti-inflammatory agents. Pyrrolotriazine was first synthesized from pyrrole carbaldehyde and hydroxylamine via pyrrole-2-carbonitrile followed by acetylation with acetyl chloride to yield acetyl-pyrrolotriazine. Subsequent condensation with substituted benzaldehyde gives pyrrolotriazine-based chalcones, which were cyclized using hydrazine hydrate to obtain the targeted compounds.All the synthesized derivatives were characterized by using spectroscopic techniques and evaluated its *in-vitro* anti-inflammatory activity.

Results: The Swiss ADME results revealed that none of the designed compounds violated Lipinski's rule of five.All the designed compounds showed potential binding affinity with PDB ID 6JVH as anti-inflammatory activity. Designed compounds R13, R14 and R15 showed docked scores -10.3, -10.4, and -9.9 kcal/mol whereas the reference drug Ibuprofen has a binding affinity of -7.9 kcal/mol. *In-vitro* anti-inflammatory activity of synthesized compounds were evaluated using the HRBC (Human Red Blood Cell) membrane stabilization method against ibuprofen as the standard.The results revealed that several synthesized compounds, namely R1, R2, R13, R14 and R15 showed significant *in-vitro* anti-inflammatory activity.

Conclusions: Among all these derivatives R13 showed highest anti-inflammatory activity indicating as lead molecule for further development into effective therapeutic agent.

1. Introduction

Heterocyclic derivatives, which contain at least two distinct elements, are central to the development of pharmacologically active compounds.The ability of fused heterocycles to enhance selectivity makes them particularly effective in drug design. A fused heterocycle contains multiple heteroatoms located at specific sites that can form hydrogen bonds and interact with adjacent amino acid residues, unlike monocyclic heterocycles or those

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fused with phenyl rings[1]. Given their wide-ranging biological and pharmacological uses, heterocycles with elements such as sulfur, nitrogen, and/or oxygen including structures like thiophene, pyrazole, and imines – continue to captivate medicinal chemists and researchers alike.[2] Non-steroidal anti-infammatory drugs (NSAIDs) are one of the most common analgesics are considered one of the most appropriate families for treating various rheumatism and arthritis, as well as their use as analgesics. Moreover, aspirin (acetylsalicylic acid), which belongs to this family, has been used for more than 100 years [1,2]. Prostacyclins, prostaglandins, and thromboxanes that have essential roles in many important pathological and physiological reactions.COX-1 and COX-2 are the main isoforms of cyclooxygenase enzymes which are considered membrane-bounded enzymes.COX-1 enzyme is engaged in the production of many prostaglandins that are essential to preserve the functions of the gastrointestinal and cardiovascular systems, as well as the COX-2 enzyme is overexpressed in various pathophysiological conditions like inflammation. The long-term use of medications that mainly inhibit the COX-1 enzyme usually leads to GIT side efects such as ulcers, as well as may lead to kidney or liver damage, and because of that the researchers tried to develop selective NSAIDs such as valdecoxib, celecoxib, and rofecoxib to overcome the mentioned side efects. However, the long-term use of these agents leads to a decrease in the biosynthesis of prostaglandin which has developed cardiovascular side effects and because of that, there is a necessity for safer and more selective inhibitors. Studies showed that tricyclic derivatives had better COX-2/COX-1 ratios compared to conventional NSAIDs such as aspirin and ketoprofen . COX-2 enzyme is usually overexpressed in several sorts of human cancers, the biological studies consistently explained that COX-2 inhibitors compounds can inhibit the tumour progression and metastasis in several animal models of cancer. Several observations have also shown COX-2 inhibitors can act synergistically with currently used anticancer agents, moreover some studies have suggested that COX inhibitors, particularly COX-2 inhibitors, may have anti-tumor effects and could be used as a potential treatment for cancer . Rofecoxib and celecoxib are selective drugs that reached the market, and chemically they contain heterocyclic rings with COX inhibitory activity[3].Pyrrolo[2,1-f] [1,2,4] triazine ring is prominent structural motif found in natural and synthetic biologically active compounds.The potential of fused heterocycles to enable selectivity makes them highly desirable for medication creation. In contrast to monocyclic heterocycles or heterocycles fused with phenyl rings, fused heterocycles have several heteroatoms at precise places that allow for the formation of hydrogen bonds and other interactions with nearby amino acid residues used as anti-inflammatory agents [4].Pyrazole derivatives represent an important class of nitrogen-containing heterocycles that have gained substantial attention in medicinal chemistry due to their wide spectrum of pharmacological activities, particularly their anti-inflammatory potential. These compounds are characterized by a five-membered aromatic ring containing two adjacent nitrogen atoms,allowing them to interact effectively with various biological targets. Pyrazole derivatives used as anti-inflammatory agents, due to their effects primarily through the selective inhibition of cyclooxygenase-2 (COX-2), suppression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and down regulation of key signaling pathways including NF-κB [5,6].

2. Methods

In view of current scenario, 15 pyrrolotriazine containing pyrazole derivatives were designed, synthesized and evaluated them for anti-inflammatory activity as shown in Scheme I.

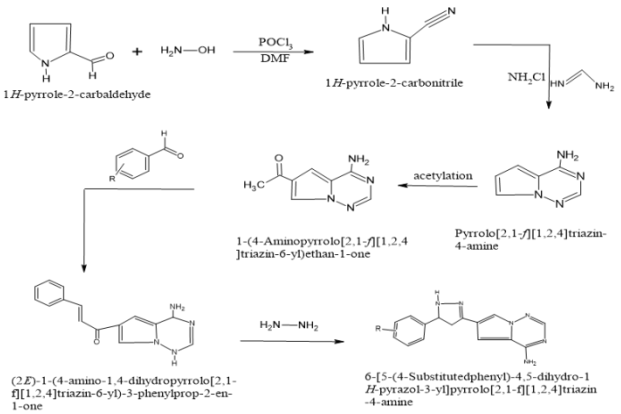

Fig.1: Scheme of synthesis of targeted compounds

Chemicals used are of laboratory or analytical-grade. Open capillary method was used to determine melting point. To monitor the progress of the reactions acetonitrile and benzene (3:1) were used as solvent systems. The developed TLC plates were visualized by exposing them to iodine

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vapors.The spectra were recorded using a Shimadzu UV-visible spectrophotometer model 1800. HPLC-grade ethanol was utilized as the solvent. The infrared (IR) spectra of the synthesized compounds were recorded using a Shimadzu IR Spirit L.UV and IR spectroscopic studies were carried out in Kamla Nehru College of Pharmacy, Butibori.The ^{1}H NMR and ^{13}C NMR spectra were recorded in methanol using NMR Bruker Advance NEO 500 MHz spectrometer from SAIF-PANJAB, Chandigarh chemical shifts in parts per million. HRMS and Mass spectra were recorded on an electron impact mass spectrometer at 70 eV ionizing beam and using a direct insertion probe from SAIF-PANJAB, Chandigarh.

2.1 Computational drug design

The synthesized compounds were investigated by computational drug design techniques for potential anti-inflammatory agent on its mechanism of action. The 2D structure of designed ligand were prepared by utilizing chemsketch software. Using PyRx and AutoDock Vina 4.2 software, target protein was docked with the designed ligands. The Pymol program was utilized to prepare the pdbqt file of the macromolecule and ligand interaction was exported to form PDB file. Utilizing the Discovery Studio Visualizer program,the docking outcomes of molecules were analyzed and visualized. Swiss ADME was employed to examine the physicochemical parameters of designed compounds.

2.1.1 Preparation of protein target and ligand

To expand anti-inflammatory activity of pyrrolotriazine containing pyrazole, docking analysis of target proteins was performed. The following proteins were utilized: 5X23, 6JVH and 6R6X based on the mechanism of action of anti-inflammatory agents. The protein's three-dimensional structure of proteins which was attained from the Protein Data Bank website (http://www.rcsb.org/pdb), was generated by eliminating heteroatoms and water molecules with the Discovery Studio Visualizer 2016 edition and then exported in the PDB file format to be utilized in molecular docking procedures. However, the drawing of geometrical 2D structure was performed using the ChemSketch program. The two-dimensional (2D) structures were transformed into 3D models using the Avogadro software and the ligand structures were saved in the PDB format [7].

2.1.2 Molecular docking and visualization

Molecular docking is used to evaluate interactions between macromolecules and ligands in order to explore the binding design as well as the functionality of the ligand. To generate the scoring functions (binding affinities) and observe the way the macromolecule interacts with the ligand, which consists of non-bonding polar and hydrophobic interactions, version 4.2 of AutoDock Vina with PyRx software and the 2020 edition of the software Discovery Studio Visualizer were employed [8].

2.1.3 ADMET Prediction

The designed derivatives and standard drug were further checked for drug-likeness properties according to Lipinski's rule. During drug development, it is necessary to predict the tolerability of selected derivative before being ingested by humans and animal models. The pharmacokinetic profile (ADME) and toxicity predictions of ligands were conducted using Swiss ADME predicting small-molecule pharmacokinetic properties using (http://biosig.unimelb.edu.au/pkcsm/prediction). [9].

2.3 Synthesis of Compounds (R1-R15)

2.3.1 Step 1: Synthesis of 1H-Pyrrole-2-Carbonitrile

A mixture containing 1 g of 1*H*-pyrrole-2-carbaldehyde, 2 g of hydroxylamine hydrochloride, were thoroughly mixed to form a uniform reaction mixture. Then the reaction mixture was subjected to microwave irradiation at a power of 300 W for 2 minutes using a laboratory microwave oven. Upon completion of the reaction, 2 ml dichloromethane was added to the round bottom flask and the mixture was stirred. It was filtered to remove any insoluble materials. The filtrate was collected and dried over anhydrous sodium sulphate. The solvent was evaporated to obtain the crude product. The crude product was recrystallized by methanol which was further purified by column chromatography [10-11].

2.3.2 Step 2: Synthesis of pyrrolo[2,1-f] [1,2,4] triazin-4-amine

In the round bottom flask, 1 g of pyrrole-2-carbonitrile and 0.85 g of chloroamine was added. The mixture was dissolved in 5 ml of methanol and subjected to microwave irradiation at 300 W for 2 minutes. To the cyclized reaction mixture, 0.72 g formamidine and 2 ml of methanol were

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added. The mixture was again irradiated under microwave conditions at 300 W for 2 minutes. Upon completion of the reaction, the crude product was collected by filtration and dried at room temperature. The solid was then purified by recrystallized by methanol to get the compound, pyrrolo[2,1-f][1,2,4]triazin-4-amine[12-13].

2.3.3 Step 3: Synthesis of 1-(4-aminopyrrolo[2,1-f] [1,2,4] triazin-6-yl) ethan-1-one In a round bottom flask, 0.245 g of pyrrolo[2,1-f][1,2,4]triazin-4-amine was added to 10 ml of dimethyl formamide (DMF). The mixture was stirred thoroughly until complete dissolution was achieved. Then 2ml acetyl chloride was added to the stirred solution. The flask was placed in a microwave oven and irradiated at 300 W for 3 minutes. The round bottom flask was removed from the microwave. The reaction mixture was cooled to room temperature. Then 2 ml of water was added to the reaction mixture and stirred to quench excess acetyl chloride. The mixture was transferred to a separatory funnel and extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate. The drying agent was removed by filtration which was further purified by column chromatography [14].

2.3.4Step 4: (2E)-1-(4-amino-1,4-dihydropyrrolo[2,1-f][1,2,4]triazin-6-yl)-3-phenylprop-2-en-1-one

To a round-bottom flask, 0.001 mol of the substituted aldehyde and 0.001 mol of 1-(4-aminopyrrolo[2,1-f][1,2,4]triazin-6-yl)ethanone were dissolved in 3 ml of ethanol. To this mixture, aqueous potassium hydroxide 0.003 mol was added slowly.The reaction mixture was then placed in a microwave oven set at 180 W and heated for 2–3 minutes under microwave irradiation. Upon completion of the reaction, the mixture was allowed to cool to room temperature. The resulting solid product was collected by filtration, washed with cold ethanol, and dried. The crude product was further purified by recrystallization by using methanol [15].

***2*.3.5 Step 5: Synthesis of 6-[5-(4-substitutedphenyl)-4,5-dihydro-1H-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine**

A quantity of 1 g of the previously synthesized (2E)-1-(4-amino-1,4-dihydropyrrolo[2,1-f][1,2,4]triazin-6-yl)-3-phenylprop-2-en-1-one was combined with 1 ml of acetic acid, followed by the careful addition of 3 ml of hydrazine hydrate. Subsequently, 3 ml of ethanol were added to the mixture. The complete solution was then subjected to microwave irradiation at a power of 300 W for a duration of 2 minutes. Upon completion of the heating process, the round bottom flask was removed from the microwave and allowed to cool to room temperature. The solid product that precipitated upon cooling was collected by filtration. The crude product was recrystallized by methanol and further purified by column chromatography [16].

2.4 Anti-inflammatory Screening

The compounds synthesized during the present work were subjected to *in-vitro* anti-inflammatory activity.

2.4.1 The Human Red Blood Cell (HRBC) Membrane Stabilization Method

The Human Red Blood Cell (HRBC) membrane stabilization method is used to assess compounds *in vitro* anti-inflammatory activity. The principle involves stabilizing the HRBC membrane, preventing its lysis under hypotonic conditions or heat stress. Reagents like phosphate buffer, hyposaline solution, and HRBC suspension are prepared, and the working procedure involves incubating samples with the test compound and controls, centrifuging, and measuring the haemoglobin content in the supernatant to determine the percentage of hemolysis.Blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks before the experiment. The collected blood was mixed with an equal volume of sterilized Alsever solution. The blood was centrifuged at 3000 rpm for 10 minutes, and the cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as a 10% v/v suspension with isosaline. The assay mixture contained 1 ml phosphate buffer (pH 7.4, 0.15 M), 2 ml hypo saline (0.36%),0.5 ml HRBC suspension (10% v/v), along with 0.5 ml of the sample and standard drug Ibuprofen at various concentrations (50, 100, 250, 500, 1000µg/ml). A control consisting of distilled water instead of hypo saline was used to produce 100% hemolysis. The mixtures were incubated at 37°C for 30 minutes and then centrifuged. The haemoglobin content in the suspension was estimated using a spectrophotometer at 560 nm [17,18].

The percentage of hemolysis of the HRBC membrane can be calculated as follows:

Journal of Chemical Health Risks

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JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

% Haemolysis= Absorbance of test sample/ Absorbance of control x 100

The percentage of HRBC membrane stabilization can be calculated as

% Protection = 1- Absorbance of test sample/ Absorbance of control x 100

3. Results

A series of 15 pyrazole derivatives (R1-R15) was synthesized through the [Scheme 1]. Characterization methods have indicated the synthesis of pyrazole derivatives (R1-R15). The synthesis began with 1*H*-pyrrole-2-carbaldehyde, which was reacted with hydroxylamine hydrochloride in the presence of phosphoryl chloride to obtain nitrile. This nitrile underwent cyclization to yield pyrrolo[2,1-f] [1,2,4] triazine-4-amine.The triazine-4-amine derivative was subjected to acetylation using acetyl chloride to form 1-(4-Aminopyrrolo[2,1-f] [1,2,4] triazine-6-yl) ethan-1-one which on reacted with various substituted aromatic aldehydes in the presence of base through an aldol condensation reaction, resulting in the formation of (E)-3-(Substituted phenyl)-1-(pyrrolo[2,1-f] [1,2,4] triazine-4-yl) prop-2-en-1-one derivatives. This undergoes cyclization using hydrazine hydrate in ethanol to afford 6-[5-(4-substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazine-4-amines.The targeted compounds rendered them promising candidates for anti-inflammatory activity as shown in Scheme 1 of Fig.1

3.1ADMET properties and Molecular Docking

The physicochemical properties are necessary for the design of new compounds that are intended to use as drugs. A drug-likeness profile can be evaluated via parameters of the molecule such as molecular weight (MW), Log p, hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), rotatable bonds, Topological polar surface areas (TPSA), Aqueous solubility and Human intestinal absorption[23]. These parameters were calculated for compounds R1-R15 and shown in Table 1.Lipinski's criteria (MW < 500; HBA ≤ 10 and HBD ≤ 5, rotatable bonds ≤ 10 and TPSA ≤ 140) were used to compute the drug-likeness profiles. It passes the Lipinski rule of five with an acceptable probability score of 85%. A score of 85% was obtained by all compounds R1–R15, indicating that they had good bioavailability and complied with all five requirements.

3.2 Molecular docking study

Discovery studio 2020 software program was utilized to evaluate the compounds (R1-R15) Docking score results were from (-7.7) kcal/mol to (-10.4) kcal/mol against (6JVH) PDBID. Ibuprofen docking result score was (-7.9 kcal/mol) as shown in Table 2

Table:1 *In silico* physicochemical parameters and Lipinski rule:

Sr No	MW≤500 (g/mol)	Log P ≤5	Rotatable Bond ≤7	HBA ≤10	HBD ≤5	BBB Absorption	GIT Absorption	Lipinski violation
1	264.28	2.09	3	3	1	YES	HIGH	0
2	298.73	2.40	3	3	1	YES	HIGH	0
3	280.28	1.76	3	4	2	NO	HIGH	0
4	310.31	2.32	4	5	2	NO	HIGH	0
5	336.39	3.14	7	4	1	NO	HIGH	0
6	294.31	2.44	4	4	1	NO	HIGH	0
7	309.28	1.81	4	5	1	NO	HIGH	0
8	292.34	2.55	3	3	1	YES	HIGH	0
9	343.18	2.42	3	3	1	YES	HIGH	0
10	293.32	2.18	4	3	2	NO	HIGH	0
11	309.28	1.78	4	5	1	NO	HIGH	0
12	309.28	1.80	4	5	1	NO	HIGH	0
13	296.15	2.42	3	3	1	YES	HIGH	0
14	343.18	2.51	3	3	1	YES	HIGH	0
15	298.73	2.40	3	3	1	YES	HIGH	0

Journal of Chemical Health Risks
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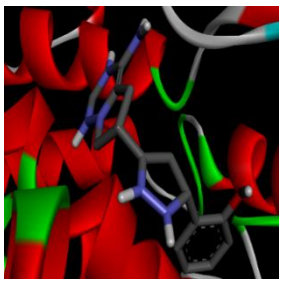
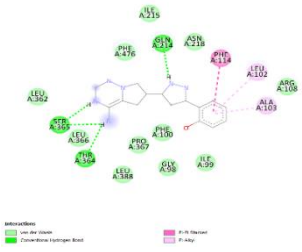
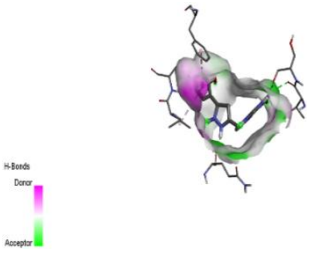
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

Reference drug								
15	298.73	1.98	4	2	4	YES	HIGH	0

Table 2: Binding affinity of designed compounds

Compound No	5X23(kcal/mol)	6JVH(kcal/mol)
1	**-9.5**	**-9.8**
2	**-9.6**	**-9.2**
3	**-9.6**	**-9.6**
4	**-8.3**	**-9.6**
5	**-9.7**	**-8.6**
6	**-9.5**	**-8.7**
7	**-9.5**	**-9.6**
8	**-9.3**	**-9.7**
9	**-9.5**	**-9.6**
10	**-9.5**	**-9.5**
11	**-9.7**	**-9.6**
12	**-8.9**	**-9.7**
13	**-9.7**	**-10.4**
14	**-9.7**	**-10.3**
15	**-9.3**	**-9.9**
Ibuprofen	**-8.1**	**-7.9**

Table 3: Ligand-receptor interaction along with the bond length and amino acid residues and hydrogen bond interaction against 5X23

Sr. No.	Ligand-Receptor Interaction	Amino Acid Residues	Hydrogen bond interaction
R1			

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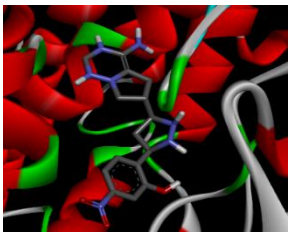
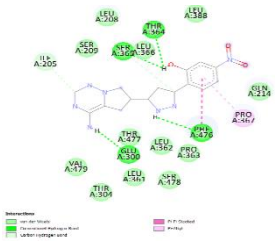
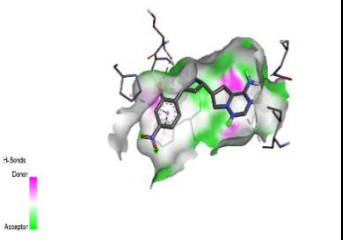
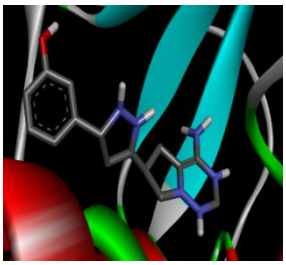
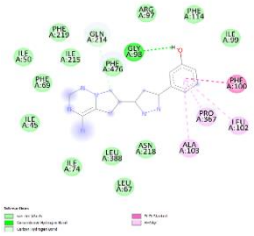
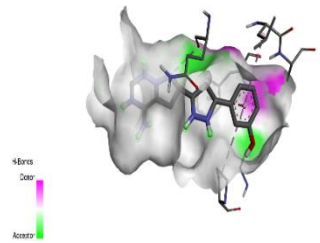
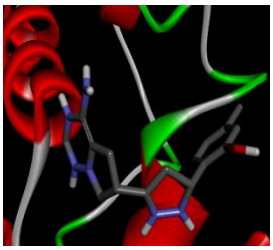
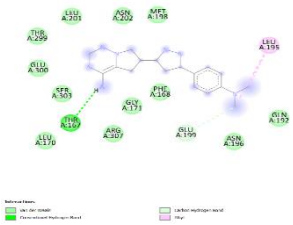
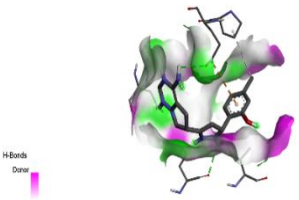
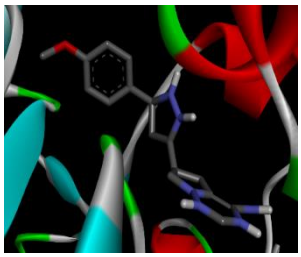
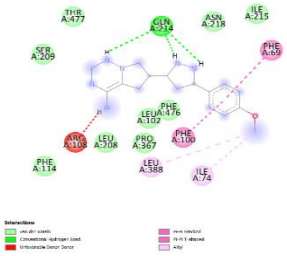
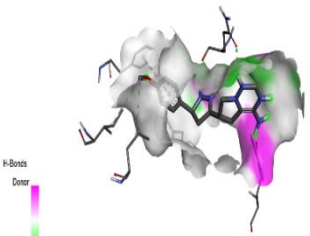
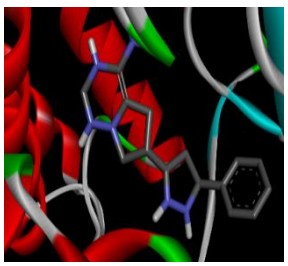
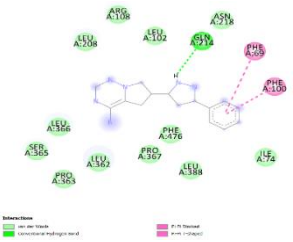
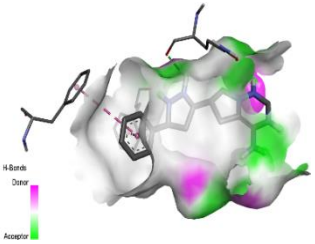
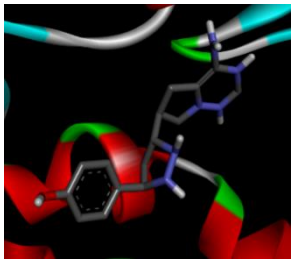
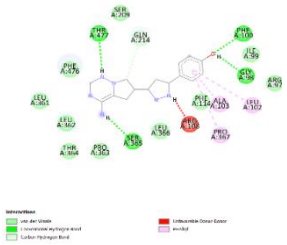
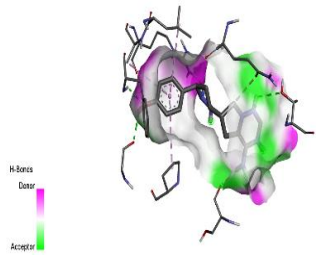
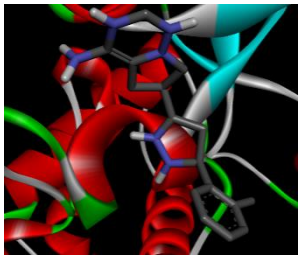
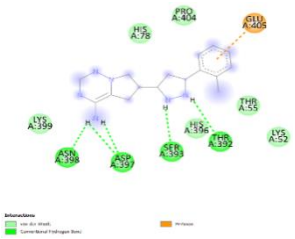
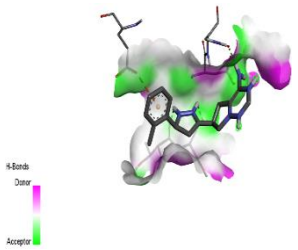
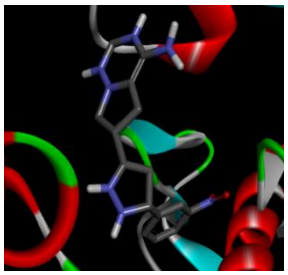
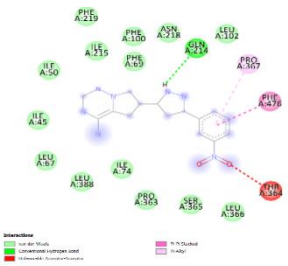
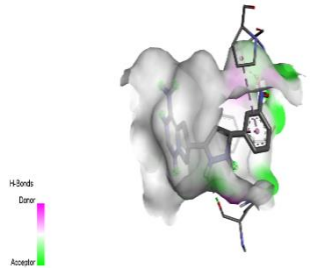
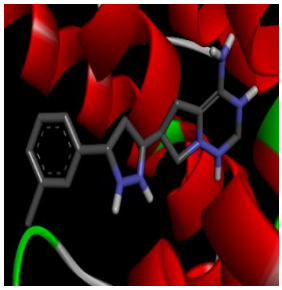
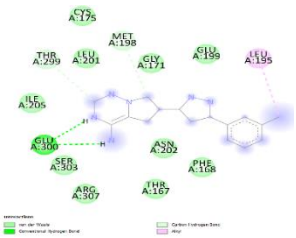
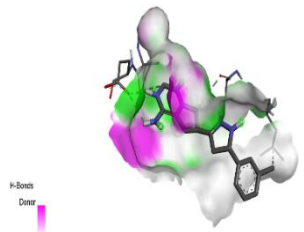
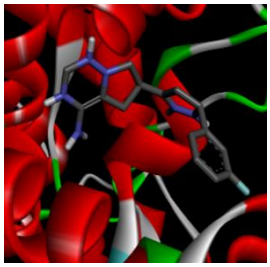
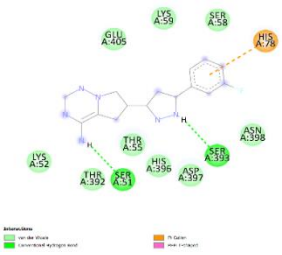
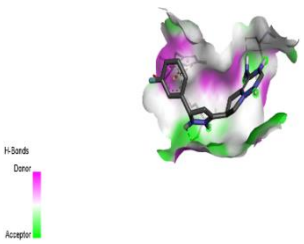
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R2			
R3			
R4			
R6			
R7			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R8			
R9			
R11			
R12			
R13			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

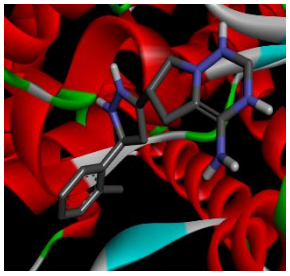
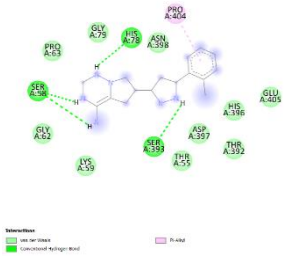
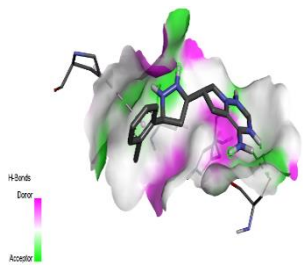
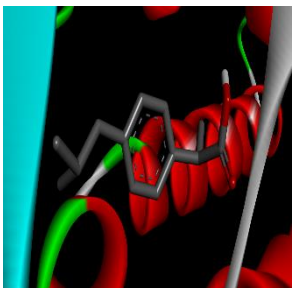
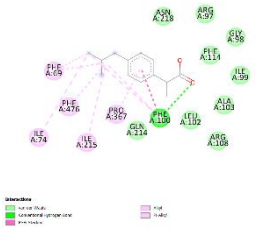
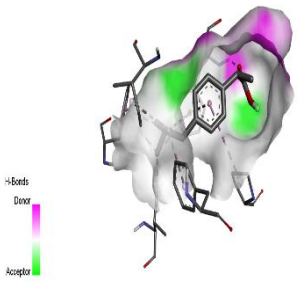
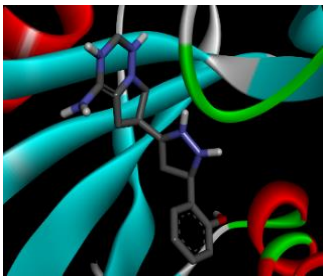
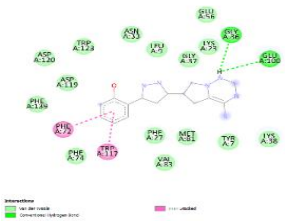
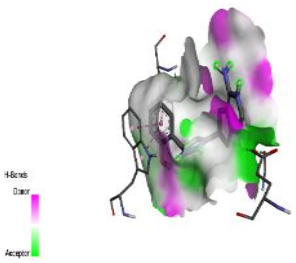
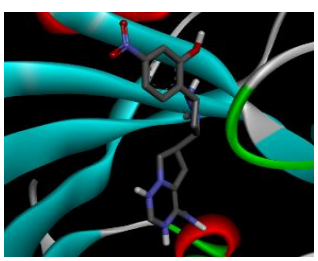
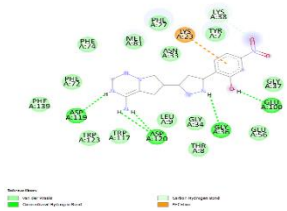
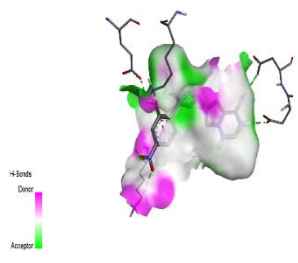
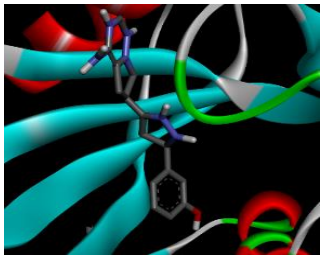
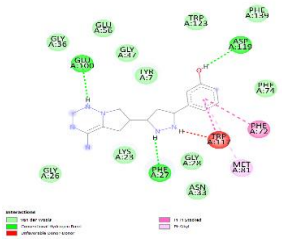
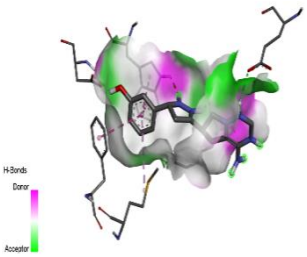
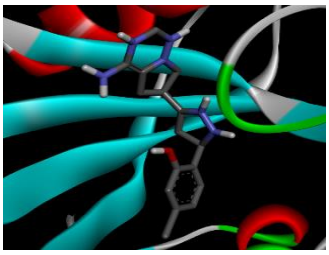
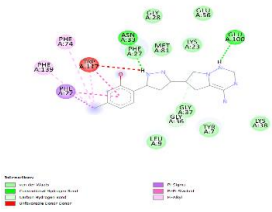
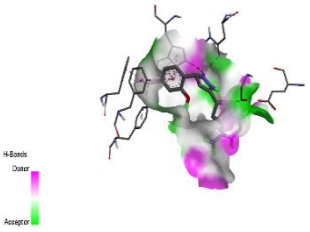
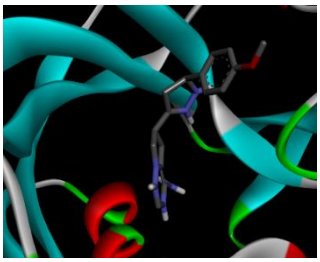
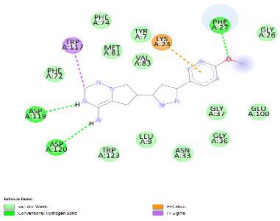
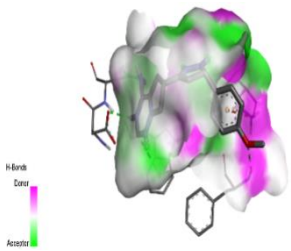
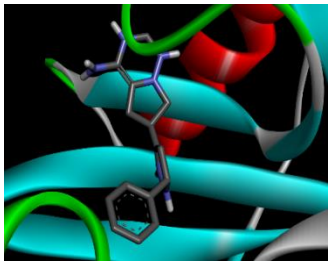
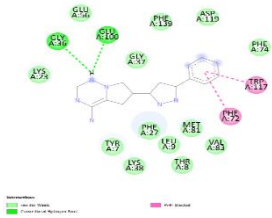
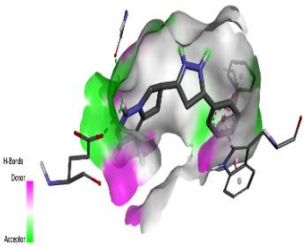
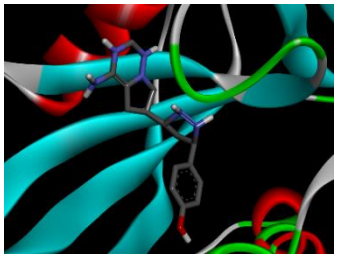
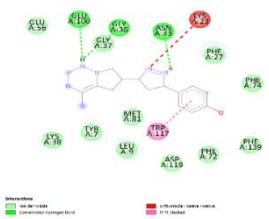
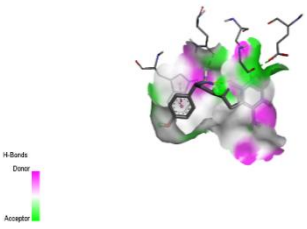
R14			
(Ibuprofen)			**Standard Drug**
1.			

Table 4: Ligand-receptor interaction along with the bond length and amino acid residues and hydrogen bond interaction against 6JVH

Sr. No.	Ligand-Receptor Interaction	Amino Acid Residues	Hydrogen bond interaction
R1			
R2			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R3			
R4			
R6			
R7			
R8			

Journal of Chemical Health Risks

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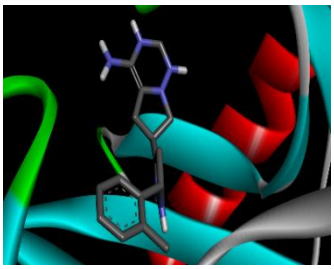
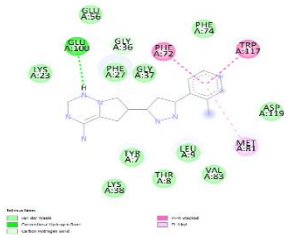
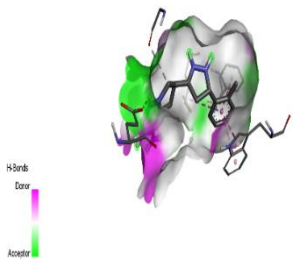
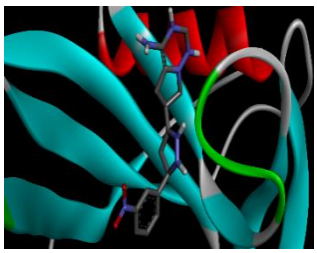
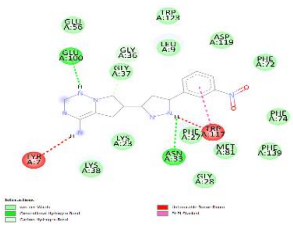
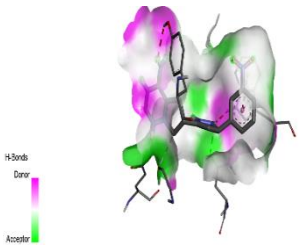
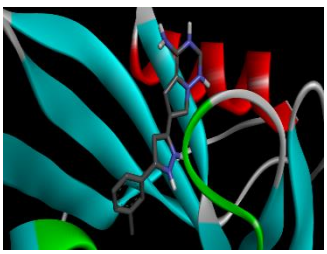
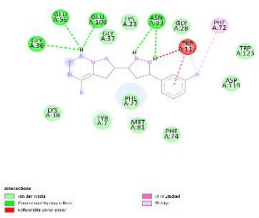
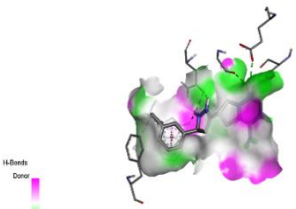
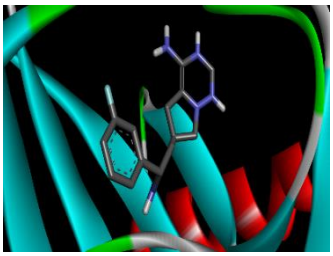
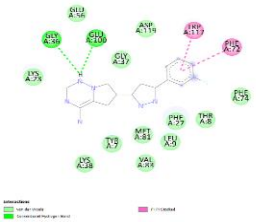
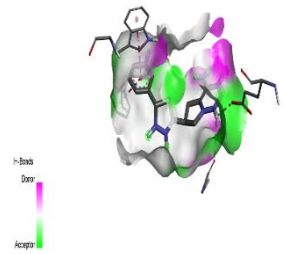
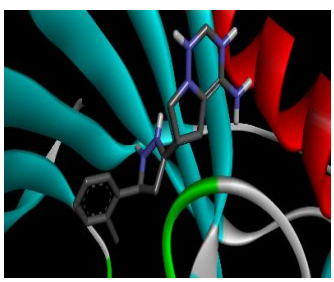
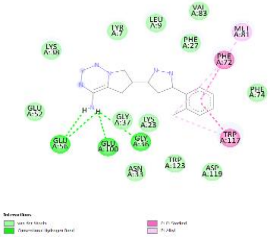
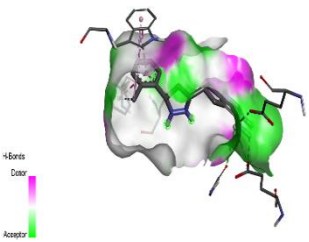
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R9			
R11			
R12			
R13			
R14			

Journal of Chemical Health Risks

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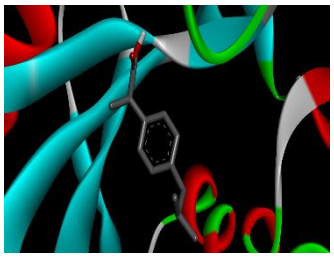
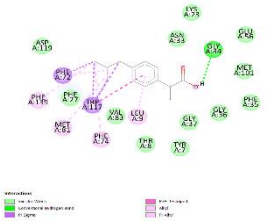
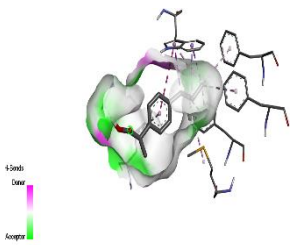
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

Standard Drug			
Ibuprofen			
1.			

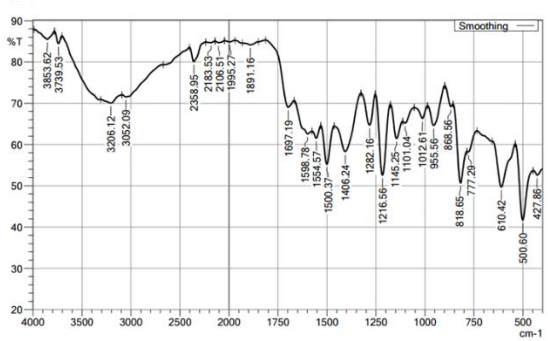


Fig.2:IR Spectrum of 6-[5-[4-(4-Fluorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine

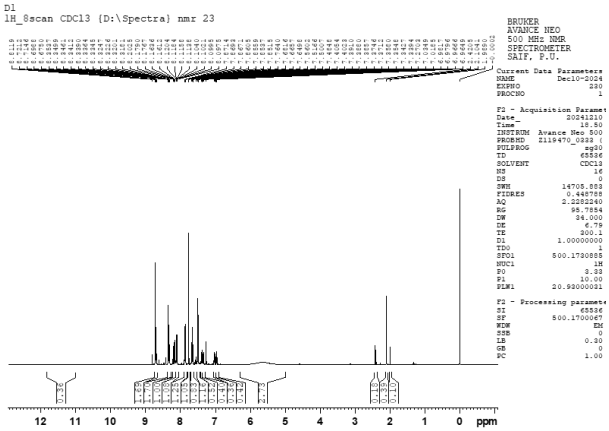


Fig.3:[1]HNMR Spectrum of 6-[5-[4-(4-Fluorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine

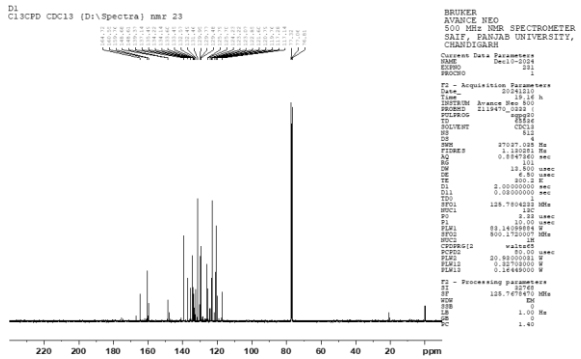


Fig.4: 13C NMR Spectrum of 6-[5-[4-(4-Fluorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine

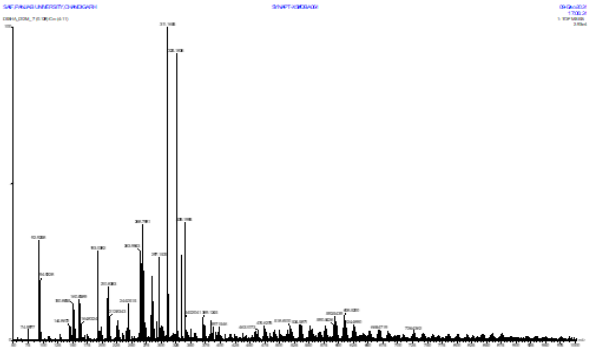


Fig.5: Mass Spectrum of 6-[5-[4-(4-Fluorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amin

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

3.3 Characterization of Synthesized Compounds

1. **6-[5-[(2-Hydroxyphenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R1)**

Yield:87%; colour: brown; molecular weight: 296.33; melting point: 202-204ºC; *Rf*: 0.75 (benzene and acetonitrile); λmax: 293.60; IR peak:(NH_2):3200.42,(-OH) 3686.76,(C=N) 1658.68,(C=C)1608.77 & 1486.11. ^{1}HNMR,(500MHz,$CDCl_3$):*d*-9.06(NH),8.59(OH),7.29-8.20(m,ArH),6.92(NH_2),3.92(CH_2).

^{13}CNMR(500MHz,$CDCl_3$):*d*76.17,77.91,77.23,117.71,132.57,133.49,147.71,153.31.MS:(m/z):Calcd for $C_{15}H_{16}N_6O$ (296.3312); Found :297.0501.

2. **6-[5-[(4-Nitrophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl]pyrrolo[2,1-f][1,2,4]triazin-4-amine (R2)**

Yield: 95%; colour: black; molecular weight: 323.40; melting point: 198-200ºC; *Rf*: 0.78 (benzene and acetonitrile); λmax: 265.60; IR peak: (-NH_2)3187.58,(-NO_2) 1558.85&1333.51,(C=N) 1658.68, (C=C)1608.77 & 1486.11, (C-H) 3089. ^{1}HNMR, (500MHz,$CDCl_3$):*d*-8.56(NH),7.23-7.85(m,Ar-H),6.02(NH_2),2.09(CH_2).

^{13}CNMR(500MHz,$CDCl_3$):*d*76.78,77.04,77.29,114.31,120.06,126.64,128.95,129.10,129.43,138.22,139.07,143.62,148.19,MS:(m/z):Calcd for $C_{15}H_{15}N_7O_2$ (323.3094); Found :324.9760

3. **6-[5-[4-(3-Hydroxyphenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R3)**

Yield: 82%; colour: yellow; molecular weight: 341.32; melting point: 210- 212ºC; *Rf*: 0.89 (benzene and acetonitrile); λmax: 204.80; IR peak: (-NH_2)3264.60, (-OH) 3616.87, (C=N) 1687.21,(C=C) 1597.36& 1459.01, (C-H) 3154.78.

4. **6-[5-[4-(4-Chlorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R4)**

Yield: 80.43%; colour: brown; molecular weight: 296.33; melting point:212- 214ºC; *Rf:* 0.75 (benzene and acetonitrile); λmax: 306.80; IR peak: (-NH_2)3264.60,(-Cl) 547.64, (C=N) 1627.31,(C=C) 1627.31 & 1383.43.

5. **6-[5-[4-(4-Bromophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R5)**

Yield: 76%; colour: brownish; molecular weight: 330.77; melting point: 204-206ºC; *Rf*: 0.76 (benzene and acetonitrile); λmax: 357.00; IR peak: (-NH_2):3338.76,(-Br) 557.65, (C=N) 1687.21, (C=C) 1620.18 & 1406.24, (C-H) 3171.89.

6. **6-[5-[(4-Methoxyphenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R6)**

Yield: 76%; colour: light brown; molecular weight: 323.40; melting point: 208-210ºC; *Rf*: 0.62 (benzene and acetonitrile); λmax: 306.20; IR peak: (-NH_2):3216.11, (-OH) 3216.11, (C=N) 1687.21, (C=C) 1621.66& 1463.29, (C-H) 3074.91, (C-O)1263.62

7. **6-[5-[Phenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R7)**

Yield: 75%; colour: yellow; molecular weight: 359.22; melting point: 184- 186ºC; *Rf*: 0.74 (benzene and acetonitrile); λmax: 300.20; IR peak: (-NH_2):3194.71,(C=N) 1682.93, (C=C) 1604.49& 1493.24, (C-H) 3023.57.

8. **6-[5-[(4-Hydroxyphenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R8)**

Yield: 72%; colour: yellow; molecular weight: 310.35; melting point: 210- 212ºC; *Rf*: 0.56(benzene and acetonitrile); λmax: 290.40; IR peak: (-NH_2)171.89, (-OH) 3618.30, (C=N) 1647.26, (C=C) 1647.26 & 1457.59, (C-H) 3049.24.

9. **6-[5-[(2-Methylphenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R9)**

Yield: 91%; colour: yellow; molecular weight: 280.33; melting point: 174- 176ºC; *Rf*: 0.56 (benzene and acetonitrile); λmax: 208.00; IR peak: (-NH_2)3304.53, (C=N) 1553.18, (C=C) 1553.18& 1400.54,(C-H) 3099.16, (-CH3)1400.54.

10. **6-[5-[(3-Bromophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R10)**

Yield: 77%; colour: grey; molecular weight: 296.33;

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

melting point: 186- 188ºC; *Rf*: 0.78(benzene and acetonitrile); λmax: 297.20; IR peak: (-NH_2)3206.12,(Br)496.32,(C=N) 1664.39, (C=C) 1608.77& 1407.64,(C-H) 3070.63, (-CH3)1400.54.

11. 6-[5-[(3-Nitrophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R11)

Yield: 89%; colour: black; molecular weight: 294.35; melting point: 210- 212ºC; *Rf*: 0.56 (benzene and acetonitrile); λmax: 214.20; IR peak: (-NH_2):3270.30, (-NO_2)1516.06 &1333.651, (C=N)1687.21, (C=C) 1687.21& 1410.52,(C-H) 3107.72.

12. 6-[5-[(3-Chlorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R12)

Yield: 32%; colour: brown; molecular weight: 294.35; melting point: 198- 200ºC; *Rf*: 0.75 (benzene and acetonitrile); λmax: 296.40; IR peak: (-NH_2)3201.85, (-Cl)544.81, (C=N) 1665.82, (C=C) 1610.19& 1481.83,(C-H)3013.59.

13. 6-[5-[(4-Fluorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R13)

Yield: 92%; colour: grey; molecular weight: 340.38; melting point: 192- 194ºC; *Rf*: 0.58 (benzene and acetonitrile);λmax: 299.40; IR peak: (-NH_2):3206.12,(-F)1216.56, (C=N) 1697.19, (C=C)1598.78& 1406.24,(C-H) 3052.09.^{1}HNMR,(500MHz,$CDCl_3$):*d*9.93(NH)7.207.33(m,ArH),3.34(NH_2),2.73(CH_2).^{13}CNMR(500MHz,$CDCl_3$): *d*76.01,77.06,77.29,117.40,117.28,119.76,120.79,125.70, 129.95,131.16,132.57,133.68,134.44,135.49,137.74,148.68,157.76,160.55,169.72.MS(m/z);Calcdfor$C_{15}H_{11}FN_6$(296.3023); Found :297.1531.

14. 6-[5-[(2-Chlorophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine (R14)

Yield: 89%; colour: brownish; molecular weight: 548.12; melting point: 204- 208ºC; *Rf*: 0.62 (benzene and acetonitrile); λmax: 306.60; IR peak:(-NH_2)3350.17,(-Cl)636.09, (C=N) 1657.26, (C=C) 1601.64 & 1430.49, (C-H) 3005.03. ^{1}HNMR,(500MHz, ($CDCl_3$)): *d* 8.09 (NH), 7.45-7.49 (m,Ar-H), 7.71(NH_2),3.12(CH_2).^{13}C NMR,(500MHz, ($CDCl_3$)): *d* 77.33, 126.02,MS-(m/z);Calcd for $C_{15}H_{15}ClN_6$(330.770); Found :332.1671

15. 6-[5-[(2-Bromophenyl)]-4,5-dihydro-1*H*-pyrazol-3-yl] pyrrolo[2,1-f] [1,2,4] triazin-4-amine(R15)

Yield: 87%; colour: yellow; molecular weight: 294.35; melting point: 172- 174ºC; *Rf*: 0.55 (benzene and acetonitrile); λmax: 307.20; IR peak:(-NH_2)3197.57, (-Br)513.44, (C=N) 1658.68, (C=C) 1603.06 & 1429.06, (C-H) 2999.32. ^{1}HNMR,(500MHz, ($CDCl_3$)): *d* 8.48 (NH), 7.03-7.33 (m,Ar-H), 6.94(NH_2),3.85(CH_2).^{13}C NMR-(500MHz, ($CDCl_3$)): *d* 45.76,76.,77.29,116.68,120.81,126.83,129.70,130.80,132.91,133.05,134.49,148.73,157.37,169.90.MS-(m/z) Calcd for $C_{15}H_{15}BrN_6$(357.2079); Found :359.1435

Table 5:*In-vitro* Antiinflammatory activity by HRBC Membrane Stabilization Method:

Sample No.		Concentration (µg/ml)				
		50	**100**	**250**	**500**	**1000**
R1	**Absorbance (560nm)**	0.897	0.823	0.741	0.702	0.438
	%hemolysis	90.88	83.38	75.05	71.12	44.37
	% inhibition	9.11	16.61	24.92	28.87	55.62

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R2	**Absorbance (560nm)**	0.902	0.85	0.731	0.622	0.562
	%hemolysis	91.38	86.11	74.06	63.01	56.94
	% inhibition	8.61	13.88	25.93	36.98	43.05
R3	**Absorbance (560nm)**	0.893	0.638	0.608	0.598	0.587
	%hemolysis	90.47	64.64	61.60	60.58	59.47
	% inhibition	9.52	35.35	38.39	39.41	40.52
R4	**Absorbance (560nm)**	0.912	0.893	0.806	0.789	0.638
	%hemolysis	92.40	90.47	81.66	79.93	64.64
	% inhibition	7.59	9.52	18.33	20.06	35.35
R5	**Absorbance (560nm)**	0.896	0.835	0.764	0.708	0.598
	%hemolysis	90.78	84.59	77.40	71.73	60.58
	% inhibition	9.21	15.40	22.59	28.26	39.41
R6	**Absorbance (560nm)**	0.859	0.806	0.786	0.736	0.698
	%hemolysis	87.03	81.66	79.63	74.56	70.71
	% inhibition	12.96	18.33	20.36	25.43	29.28
R7	**Absorbance (560nm)**	0.893	0.873	0.798	0.761	0.412
	%hemolysis	90.47	88.44	80.85	77.10	41.74
	% inhibition	9.52	11.55	19.14	22.89	58.25
	Absorbance (560nm)	0.874	0.806	0.741	0.598	0.497

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

R8	**%hemolysis**	88.55	81.66	75.07	60.58	50.35
	% inhibition	11.44	18.33	24.92	39.41	49.64
R9	**Absorbance (560nm)**	0.899	0.841	0.826	0.753	0.428
	%hemolysis	91.08	85.20	83.68	76.29	43.36
	% inhibition	8.91	14.79	16.31	23.70	56.63
R10	**Absorbance (560nm)**	0.901	0.893	0.841	0.741	0.498
	%hemolysis	91.28	90.47	85.20	75.07	50.45
	% inhibition	8.71	9.5	14.79	24.92	49.54
R11	**Absorbance (560nm)**	0.895	0.825	0.749	0.709	0.598
	%hemolysis	90.67	83.58	75.88	71.83	60.58
	% inhibition	9.32	16.41	24.11	28.16	39.41
R12	**Absorbance (560nm)**	0.941	0.892	0.821	0.562	0.537
	%hemolysis	95.33	90.37	83.18	57.54	54.40
	% inhibition	4.66	9.62	16.81	42.45	45.59
R13	**Absorbance (560nm)**	0.912	0.902	0.896	0.862	0.741
	%hemolysis	92.40	91.38	90.78	87.33	75.07
	% inhibition	7.59	8.61	9.21	12.66	24.92
R14	**Absorbance (560nm)**	0.743	0.756	0.569	0.429	0.419
	%hemolysis	75.27	71.52	70.71	50.86	50.45

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(6), 2675-2693 | ISSN:2251-6727

	% inhibition	24.72	28.47	29.28	49.13	49.54
R15	**Absorbance (560nm)**	0.796	0.756	0.569	0.429	0.419
	%hemolysis	80.64	76.59	57.64	43.46	43.45
	% inhibition	19.35	23.40	42.35	56.53	57.54
Standard						
Ibuprofen	**Absorbance (560nm)**	0.974	0.969	0.965	0.959	0.951
	%hemolysis	98.68	98.17	97.77	97.16	96.35
	% inhibition	1.31	1.82	2.22	2.83	3.64
Control Absorbance (560nm)						
1	0.987					

4. Discussion

Molecular docking study of pyrazole derivatives revealed that compounds interact with this enzyme in a sufficient way. This result was reinforced by the lower binding energy and strong binding length with the active sites of proteins through hydrogen bonding and hydrophobic interations. Targeted compounds and its derivatives (R1-R15) were synthesized. Mass spectroscopy, 1H-NMR, 13C-NMR, and FT-IR techniques were used to depicit the produced compounds. The characterization methods supported the proposed structures of all prepared compounds.Infra-red spectra of compounds which were prepared**(R1)** exhibited the presence of NH_2 :3200.42, -OH: 3686.76, C=N: 1658.68, C=C: 1608.77 & 1486.11, confirmed the presence of functional group in parent structure, **(R2)** whereas -NH_2 :3187.58, -NO_2: 1558.85&1333.51, C=N: 1658.68, C=C: 1608.77 & 1486.11, C-H: 3089.17. Similarly, **(R13)** showed peak at -NH_2 :3206.12, -F:1216.56, C=N: 1697.19, C=C: 1598.78& 1406.24, C-H: 3052.09. The ^{1}H NMR and ^{13}C NMR for compounds **(R1)** -(NH_2)-2.3799, (OH)-2.4721, (CH_2)-2.2759, ^{13}C NMR-(C-O)-77.03, (C=N)-164.72. **(R2)** ^{1}H NMR-(NH_2)-2.4166, (Ar-H)-7.2611-8.2387, (CH_2)-3.8310, ^{13}C NMR - (C-C)-77.04, (C=N)-148.46, (C-N)-21.51. **(R13)** -(NH_2)-2.4205, (CH_2)-1.9990, (Ar-H)-6.9649-8.3537,^{13}C NMR-(C-F)-134.22, (C-N)-129.95, (C=N)-159.76-164.72.NH. ^{1}H NMR spectra NH protons of ring was observed as singlet signals.Mass spectroscopy of the synthesized compounds **(R1)** showed actual observed molecular weight [M+1] at 297.0501, other fragments observed at: 208.0140, 166.0083, 134.8630, 122.0186, **(R2)** showed actual observed molecular weight [M+1]: 324.9760, other fragments observed at:208.0140, 166.0083, 134.8630, 122.0186. **(R13)** showed actual observed molecular weight [M+2]-297.1531, other fragments observed at: -74.9977, 94.9228, 164.8324. *In-vitro* Antiinflammatory activity of synthesized compounds were evaluated by HRBC Membrane stabilization method, compounds R1, R2, and R13 showed significant anti-inflammatory activity. Among this R13, 4-fluoro substituent possess highest activity due to lipohilicity, membrane permeability and balance between electronic and steric effect, hence suggested that the synthesized compounds would be useful as anti-inflammatory drugs.

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6.Declaration of Interest

There is not conflict of interest.

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8. Ethics Statements

The study doesn't need ethical approval.

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