International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

Synthesis of novel 1, 2, 4 triazole derivatives as potent anticancer agent

[1]Nisha Anna Mathew, [2] Dr. Neha Kawathekar
Shri G.S Institute of Technology & Science, Indore M.P.
Shri G.S Institute of Technology & Science, Indore M.P.

Submitted: 01-01-2024 Accepted: 12-01-2024

ABSTRACT:2. novel series of 1,2,4 triazole derivatives were designed and synthesized as potential serine protease inhibitor.20 series of triazolidinedione derivative were designed , out of which 5 were synthesized as potential serine protease inhibitor 1GHZ receptor agonist . spectroscopic techniques like HNMR, IR and were performed to ascertain the structures of the synthesized compounds. On the human lung cell A549 the in vitro cytotoxicity tests were performed using the MTT assay. Further structure activity analysis was studied and pharmacological properties were studied. 2 of the designed compounds showed promising activity against the selected cancer cell line. Compound N2 showed promising activity with IC50 value less than the standard doxorubicin

KEYWORDS: Serine protease, triazolidinedione, MTT assay.

I. INTRODUCTION

Proteolysis is a crucial biological activity which is ascribed to a group of enzyme called proteases. The hydrolysis of the peptide bond by attacking the carbonyl group of peptide is attributed to the proteases which convert them into acyl enzyme intermediate. Owing to the key amino acid present at the active site of the protease and the method of cleavage of the peptide bond, proteases are classified into six divisions : Cysteine, serine, threonine, glutamic acid , aspartate and matrix metalloprotease. There are various cleaving mechanisms of the peptide bond. In serine proteases the key amino acid present at the active site is serine which executes it's proteolytic activity by the histidine residue activation that is generally present in the catalytic triad.

The involvement of proteases in Cancer growth

In normal cells proteases act as an agent performing indispensable biological activities like gene expression, differentiation and cell death. The advanced studies have revealed that the proteases explicit role in tumour growth and development and progression. It has been implicated that tumour cells propalgate the expression of proteases in the neighbouring non-neoplastic cells thus expanding the tumour. Usually the concentration of proteases is found to be high in neoplastic cells. Tumor expansion and metastasis is reliant on nutrient and oxygen supply which is supported by various proteases

The involvement of serine protease and it's regulation is critical for enduring the physiological activities of the cell. The dysregulation of serine protease may drive to pathological conditions like cancer.

[The serine protease usually contain serine residue at the active site. In fact there is a catalytic triad present at the active site which is of aspartate- histidine- serine. This catalytic triad is established in many enzymes- like subtilisin, chymotrypsin, carboxypeptidase and trypsin. The histidine acts as an activator of serine which cleaves the carbonyl group of the peptide bond. The aspartic acid stabilizes the 1GHZ protein is a a novel serine protease inhibitor motif in which binding is mediated by very short hydrogen bonds. There are many serine protease inhibitors like HAI-1, Serpins, cystatin etc which inhibits the serine protease. The 1GHZ paradigm is found in crystals like trypsin, thrombin , urokinase plasminogen activator that is being inhibited by the small molecules attached to it.

The triazolidinedione derivatives were docked against the serine protease inhibitor motif 1GHZ and the docking scores were calculated. 2 out of 5 synthesised compounds were subjected to in vitro MTT assay and their structural properties were studied for developing the novel drugs against the serine protease

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

Experimental

Molecular docking study using Schrodinger LLC

Structure based docking studies were performed utilising the poses estimated through docking using the glide module v5.6 program (Schrodinger LLC, New York, USA; http://www.schrodinger.com). All the compounds with the 1,2,4 triazole nucleus were docked against the protein (PDB Id:1GHZ a serine protease inhibitor). The molecular docking studies includes protein selection and preparation, receptor grid generation, ligand preparation, docking and further determination of docking studies

5.2 Ligand Preparation

All the compounds were build with ChemDraw ultra v8.0 (Cambridge Soft Corporation, Cambridge, MA ,USA; http://www.cambridgesoft.com) and their 3D structures were minimised with LigPrep v2.4 program (Schrodinger LLC, NewYork, USA; http://www.schrodinger.com), using the OPLS 2005 force field at 7.0 to generate single low energy 3D structure for each input structure and rest of the parameter values by default.

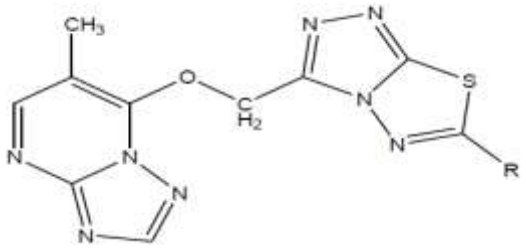
Protein selection & preparation

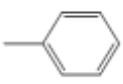
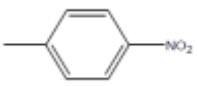
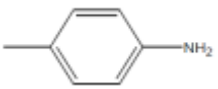
Various proteins were selected and from the protein data bank, they were downloaded out of which protein (PDB Id: 1GHZ a serine protease inhibitor) was selected. The drug is supposed to bind with this protein. The downloaded protein (PDB Id - 1GHZ a serine protease inhibitor) was imported, pre processed, optimised, it's extra water molecules were removed and it's het states were generated. This ultimately leads to the formation of a protein which is stable with low energy.

5.5 Docking Studies

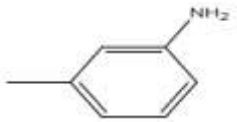
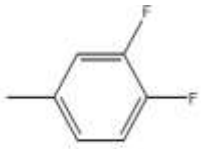
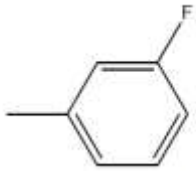
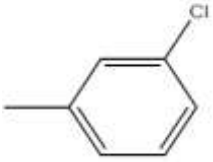
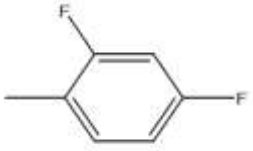
Standard precision docking was used for the examination of ligands. For the docking , flexible docking mode was chosen where the Glide program develops conformations internally. Extra precession docking was also performed. It leads to the formation of constructive ligand poses that are later inspected for the determination of the best ligand fit at the active site. The poses that succeed in passing the inspection are further sent for evaluation and minimisation of grid approximation. After this scoring was done on the poses with minimised energy for generating the Glide score

General structures and their substitutions

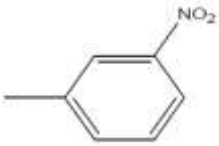
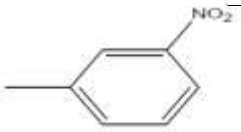
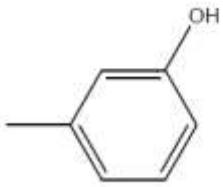
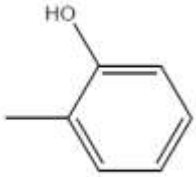
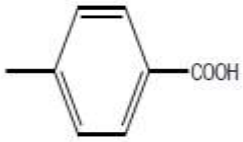
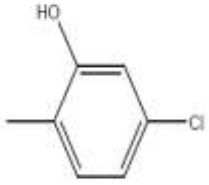


Code	R	IUPAC Name
N1		6-methyl-7-((6-phenyl [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)-[1,2,4]triazolo[1,5-a]pyrimidine
N2		6-methyl-7-((6-(4- nitrophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N3		6-methyl-4-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)benzenamine

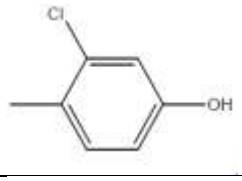
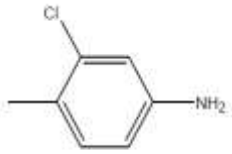
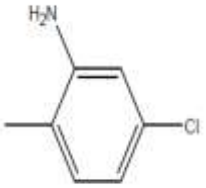
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

N4		6-methyl-3-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)benzenamine
N5		6-methyl-7-((6-(3,4- dichlorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N6		6-methyl-7-((6-(4- fluorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N7		6-methyl-7-((6-(3,4- difluorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N8		6-methyl-7-((6-(3- fluorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N9		6-methyl-7-((6-(3- chlorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N10		6-methyl-7-((6-(2,4- difluorophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

N11		6-methyl-7-((6-(4- methoxyphenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N12		6-methyl-7-((6-(3- nitrophenyl)- [1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3- yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine
N13		6-methyl-3-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)phenol.
N14		6-methyl-3-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)phenol
N15		6-methyl-2-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)phenol
N16		6-methyl-4-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)benzoic acid
N17		6-methyl-2-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)-5- chlorophenol

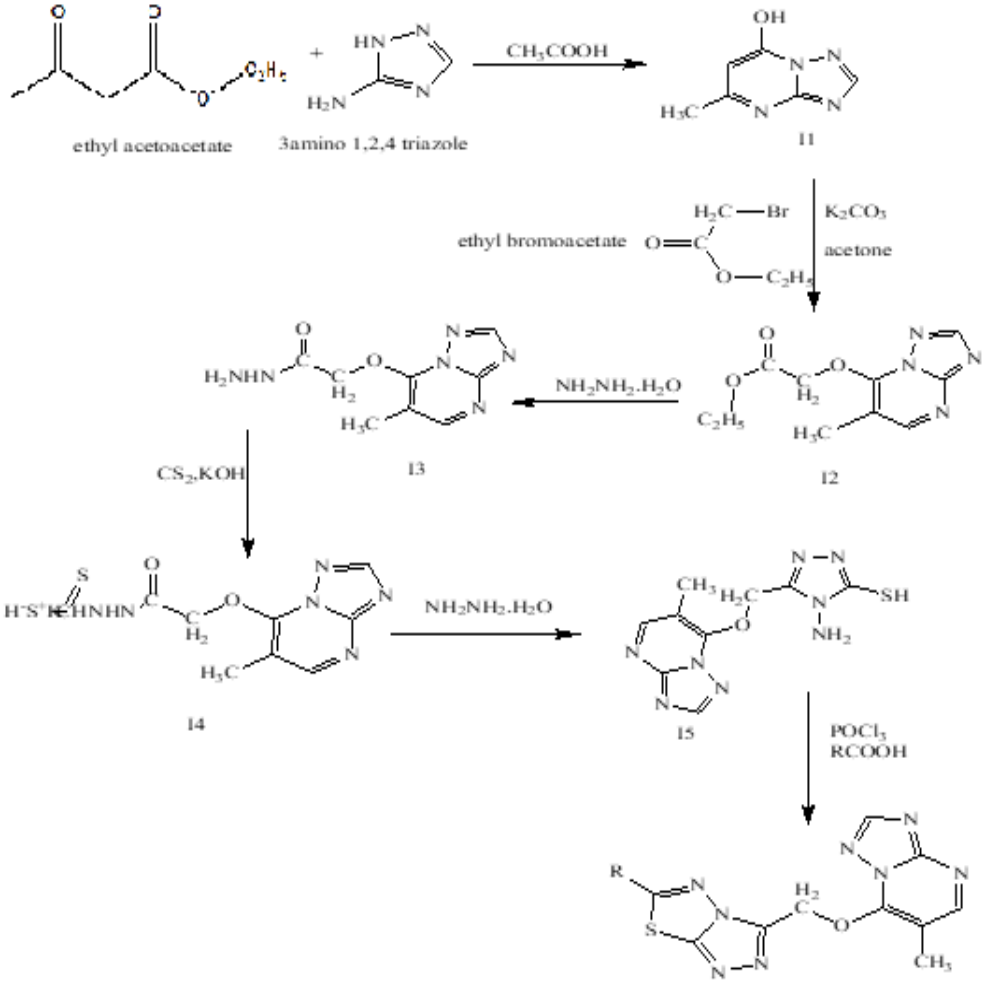
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

N18		6-methyl-4-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)-3- chlorophenol
N19		6-methyl-4-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)-3- chlorobenzenamine
N20		6-methyl-2-(3- (([1,2,4]triazolo[1,5- a]pyrimidin-7-yloxy)methyl)- [1,2,4]triazolo[3,4- b][1,3,4]thiadiazol-6-yl)-5- chlorobenzenamine

CHEMISTRY

1. 3 amino 1,2,4 triazole reacts with ethyl acetoacetate in mild acidic conditions at 120℃ to produce 5 methyl-[1,2,4] triazolo[1,5-a]pyrimidin-7-ol (I1). Here cyclisation of the ring takes place.
2. This intermediate reacts with ethyl bromoacetate in the presence of K2CO3 and acetone. The ether produced is refluxed with hydrazine hydrate in the presence of ethanol to form the hydrazide.
3. Potassium hydroxide in absolute ethanol containing hydrazide was cooled in ice bath and then CS2 was added with continuous stirring for 12 hours.
4. The precipitated potassium dithiocarbazinate was collected The potassium dithiocarbazinate derivative along with water, hydrazine hydrate was refluxed for 8 hours. This leads to the formation of a clear solution along with the evolution of H2S gas
5. The reaction mixture was diluted with cold water and acidified with HCl to give a white precipitate and was recrystallised by ethanol.
6. The precipitate was refluxed with aryl acid (RCOOH) and POCl3 for 10 hours. The reaction mixture was cooled to room temperature an then poured in crushed ice. The crude product obtained was filtered and neutralised with aqueous ammonia and recrystallised with ethanol.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781



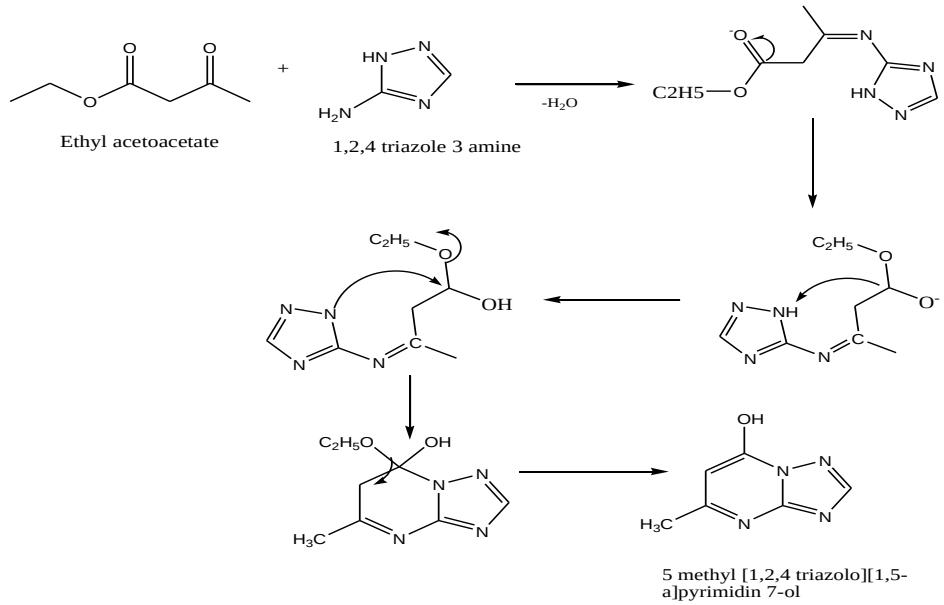
Reaction scheme of triazolothiadiazole derivatives

I1 = 5 methyl-[1,2,4] triazolo[1,5-a]pyrimidin-7-ol
I2 = ethyl 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetate
I3 = 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide
I4 = potassium disulphide salt of 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide
I5 = 4 amino- 5-((6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)methyl)-4H-1,2,4-triazole-3-thiol.

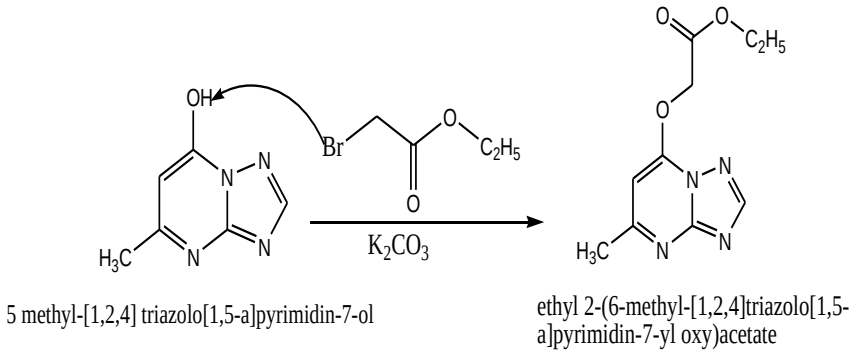
Mechanism of reaction

1) Proposed mechanism of synthesis of 5 methyl-[1,2,4] triazolo[1,5-a]pyrimidin-7-ol

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

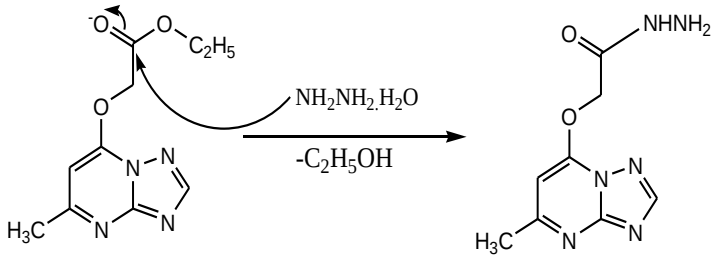


1) Proposed mechanism of synthesis of ethyl 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetate.



Proposed mechanism of synthesis of 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide

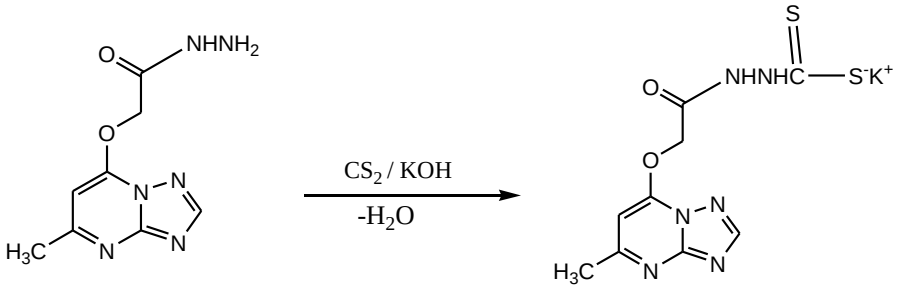
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781



ethyl 2-(6-methyl-[1,2,4]triazolo[1,5-a] pyrimidin-7-yl oxy)acetate

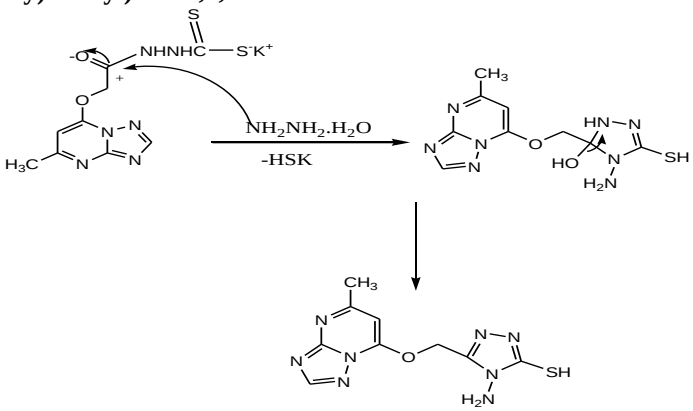
2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide

Proposed mechanism of synthesis ofpotassium disulphide salt of 2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide



2-(6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)acetohydrazide

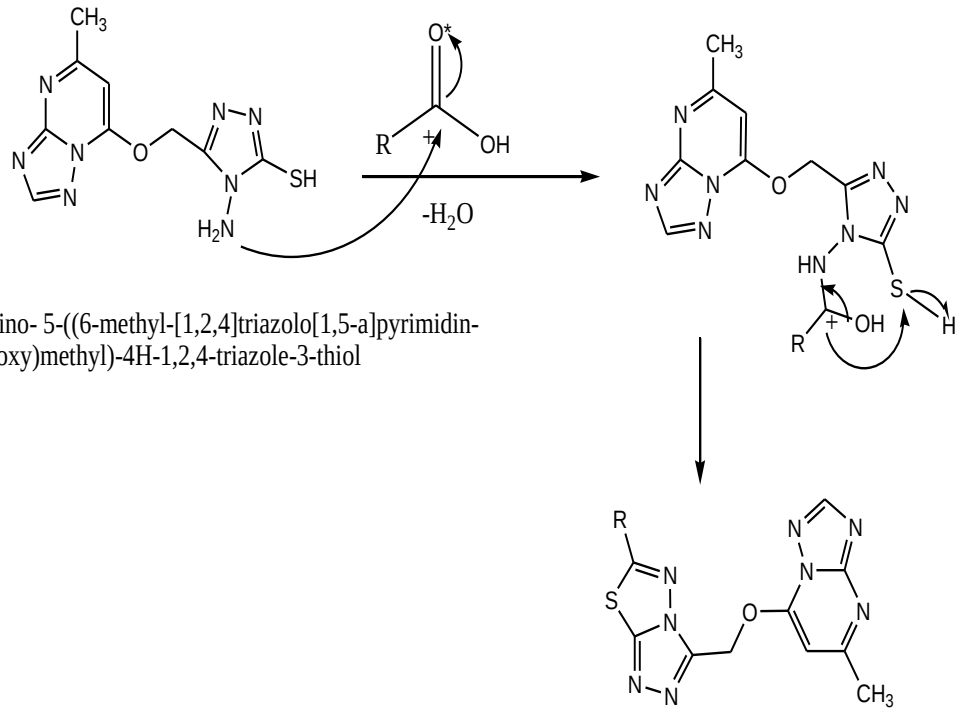
Proposed mechanism of synthesis of 4 amino- 5-((6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)methyl)-4H-1,2,4-triazole-3-thiol



4 amino- 5-((6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)methyl)-4H-1,2,4-triazole-3-thiol

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

Proposed mechanism of synthesis of 7-([1,2,4] triazolo[3,4-b][1,3,4] thiadiazol -3-yl methoxy)- [1,2,4] triazolo[1,5-a] pyrimidine derivative.



4 amino- 5-((6-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl oxy)methyl)-4H-1,2,4-triazole-3-thiol

7-([1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl-methoxy -[1,2,4]triazolo[1,5-a] pyrimidine derivative

Synthesis

Synthesis of 7-((6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy) -[1,2,4]triazolo[1,5-a]pyrimidine (N1)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was benzoic acid (0.0437 gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – pale yellow solid , MP = 240-250℃, % yield =30 %

Synthesis of 7-((6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy)- [1,2,4]triazolo[1,5- a]pyrimidine (N2)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was para nitro benzoic acid (0.0599 gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – light brown solid , MP = 260-270℃, % yield =45 %

Synthesis of 7-((6-(4-amino phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

yl)methoxy)- [1,2,4]triazolo[1,5- a]pyrimidine (N3)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was para amino benzoic acid (0.0491 gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – light brown solid , MP = 220-225℃, % yield =60 %

4 Synthesis of 7-((6-(3- amino phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy)- [1,2,4]triazolo[1,5- a]pyrimidine (N4)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was 3 amino benzoic acid (0.0490gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – light brown solid , MP = 220-225℃, % yield =40 %

.

Physical state – light brown solid , MP = 220-225℃, % yield =60 %

4 Synthesis of 7-((6-(3- amino phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy)- [1,2,4]triazolo[1,5- a]pyrimidine (N4)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was 3 amino benzoic acid (0.0490gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – light brown solid , MP = 220-225℃, % yield =40 %

Synthesis of 7-((6-(4-chloro phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy)- [1,2,4]triazolo[1,5- a]pyrimidine (N5)

In a 100 ml RBF a solution of intermediate 5 (100 mg, 0.000359 mole) was taken , added $POCl_3$ (0.35 ml. 0.0084 mole) dropwise. To it added was 4- chloro benzoic acid (0.0563 gm . 0.000359 mole) and refluxed for 10 hours at 60℃. It was cooled to room temperature and poured into crushed ice and adjusted pH to 8 with aqueous ammonia. The ppt was formed, filtered and recrystallised with ethanol.

Physical state – white solid , MP = 250℃, % yield =40 %

Characterisation of synthesised compounds

The characterisation of the synthesised compounds was performed to assure that the compounds were actually synthesised. The following are the characterisation methods that were carried out

- Thin layer chromatography
- Melting point determination
- IR Spectroscopy
- ^{1}HNMR
- **Thin Layer Chromatography**
- Thin layer chromatography is a technique in which a mixture is evaluated by separating different components in it. TLC is commonly used for identification of compounds, number of components and purity of compounds. TLC is also used for monitoring how far the reaction has proceeded by evaluating the appearance of product or disappearance of reactant. The silica gel plates were used as stationary phase and for mobile phase DCM: ethanol (9:1) was used in TLC. The R_f values of the components were determined. The variation of Rf value between the reactant and the product signifies the conversion of reactants into product and the purity of compounds. The R_f values of the synthesised compounds are depicted in table 4.

- **Melting point**

The melting point determination was carried out in an open capillary tube using liquid paraffin. The melting point of the synthesised compounds are depicted in table.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

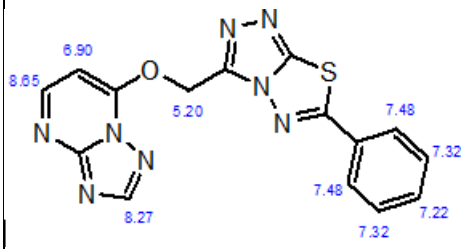
S.no	Compound code	Rf	Melting point
1.	I1	0.28	-
2.	I2	0.42	-
3.	I3	0.32	-
4.	I5	0.25	-
5.	N1	0.54	240-250°C
6.	N2	0.62	260-270°C
7.	N3	0.40	220-225°C
8.	N4	0.45	220-225°C
9.	N5	0.56	240-250°C

- **IR spectroscopy**

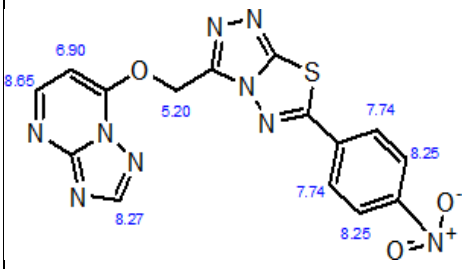
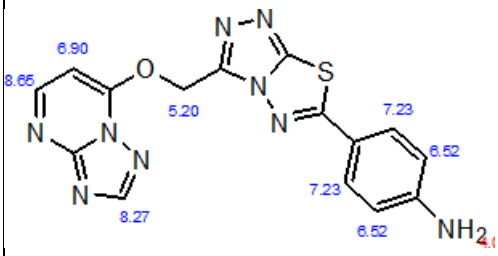
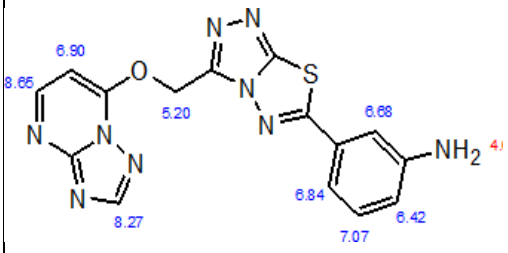
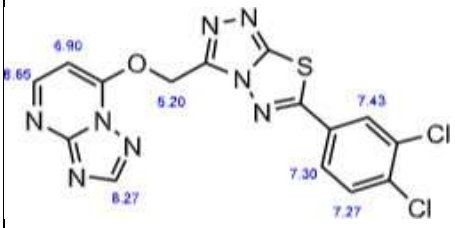
The infrared spectroscopy of all the synthesised compounds was recorded on IR AFFINITY-1 1400 using KBr pellet technique which are expressed in cm^{-1}. The IR values of the compounds are depicted in table 5.

- **^{1}HNMR Spectroscopy**

^{1}HNMR spectra was recorded using Bruker Advance II 300 MHz NMR spectrometers using Methanol -d6 solvent at Dr. Harisingh Gour University, Sagar. The NMR of the compounds are depicted in table.

Compoundcode	NMR Interpretation	IR values	Mol wt (gm)
N1		a sharp peak of ether at 1250 cm^{-1}, the aromatic double bonded carbon at 1500 cm^{-1}. an aromatic C-H streach at 3130 cm^{-1} and a nitrogen in plane ring bending vibration at 1280 cm^{-1}.	364.38

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

N2		a sharp peak of ether at 1250 cm^{-1}, the aromatic double bonded carbon at 1500 cm^{-1}. an aromatic C-H streach at 3130 cm^{-1} and a nitrogen in plane ring bending vibration at 1280 cm^{-1} and a strong nitro group vibration at 1440 cm^{-1}.	409.38
N3		a sharp peak of ether at 1250 cm^{-1}, the aromatic double bonded carbon at 1500 cm^{-1}. an aromatic C-H streach at 3130 cm^{-1} and a nitrogen in plane ring bending vibration at 1280 cm^{-1} and a medium amine group vibration at 1110 cm^{-1}.	379.4
N4		a sharp peak of ether at 1250 cm^{-1}, the aromatic double bonded carbon at 1500 cm^{-1}. an aromatic C-H streach at 3130 cm^{-1} and a nitrogen in plane ring bending vibration at 1280 cm^{-1} and medium amine group vibration at 1110 cm^{-1}.	379.4
N5		a sharp peak of ether at 1250 cm^{-1}, the aromatic double bonded carbon at 1500 cm^{-1}. an aromatic C-H streach at 3130 cm^{-1} and a nitrogen in plane ring bending vibration at 1280 cm^{-1} and a strong C-Cl peak at 620 cm^{-1}	433.27

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

The IR interpretation of N1 shows a sharp peak at 1250 cm $^{-1}$ which shows the presence of ether. A sharp peak at 1500 cm $^{-1}$ is of aromatic double bond carbon indicates the presence of a benzene ring. A streach at 3130 cm^{-1} indicates the presence of aromatic C-H bonds.

The IR interpretation of N5 shows a sharp peak at 1280 cm^{-1} the presence of ether bond. A peak at 620 cm^{-1} shows the presence of C-Cl bond. The objective of current work was to find 1,2,4 triazole derivatives acting as anticancer agents by inhibiting different enzymes. Twenty compounds were designed and synthesised. Glide score was obtained using GLIDE module (Grid- based Ligand Docking with Energetics, version Schrodinger 9.1) at CADD laboratory, Shri G.S. Institute of Technology and Science, Indore. Molecular Docking studies of the designed compounds were done to examine binding pattern of the structure with the protein. The docking studies were performed using protein PDB ID- 1GHZ

Docking results

The reasonable method of examining the binding conformation that is the correctness of a docking procedure is to determining how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode. Now the poses predicted by the Glide were used to study the structure based docking study. The ligand prepared were docked in the bindingsite of protein selected. Through the receptor-grid generation panel the binding site was demarcated. The glide program created conformations internally in the docking period as flexible docking mode was opted.

A maximum of 5 poses per ligand was formed during each docking. The glideX-score was used as a function of fitness and docking calculation were performed with XP mode . A perfect orientation for every low energy conformer was defined by the XP mode.

During docking the ligand's torsional degree are relaxed. In docking the conformation, orientation and position of the ligand was searched. The G- scorewhich is the scoring function was used to select the best conformation of ligandmolecule.

OPLS-2005 force field was used to evaluate the molecular simulation studies. The RMSD of the compounds were checked.

Docking score, Glide emodel energy and RMSD of (N1-N20) on a novel serineprotease inhibition motif involving a multicentered short hydrogen bonding network at the active site (PDB id- 1GHZ)

S.no	Compound Code	Docking Score	Glide emodel	RMSD
1.	N1	-6.097	-61.478	68.646
2.	N2	-6.838	-67.107	69.555
3.	N3	-6.757	-66.456	69.491
4.	N4	-7.163	-66.301	69.735
5.	N5	-6.985	-69.166	69.541
6.	N6	-6.101	-62.693	69.387
7.	N7	-6.667	-63.267	68.295
8.	N8	-6.549	-62.368	69.207
9.	N9	-6.177	-64.200	69.464
10.	N10	-6.281	-61.485	68.363
11.	N11	-6.394	-63.569	69.472
12.	N12	-6.022	-61.293	62.043
13.	N13	-7.094	-68.683	69.600

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

14.	N14	-7.706	-76.274	67.686
15.	N15	-7.679	-73.674	69.989
16.	N16	-6.329	-63.563	69.731
17.	N17	-7.018	-71.140	68.844
18.	N18	-7.211	-70.443	69.053
19.	N19	-6.867	-66.593	69.324
20.	N20	-7.521	-70.612	68.749

SAR Studies

1. The presence of electron withdrawing group activated the compound and enhanced it's anticancer activity.
2. The unsubstituted compound N1 showed a fair biological activity but the presence of NO2 group in N2 showed better anticancer activity than the standard doxorubicin used.
3. NO2 exhibited it's electron withdrawing effect by –M effect mainly.

Biological Activity

Determination of anticancer activity of the synthesised compounds

MTT assay

1. The MTT assay was performed at Deshpande Laboratories, Pvt.Ltd. on A549 lung cancer cells were taken and maintained under regulated conditions and were seeded in 96 well plates.
2. The compounds N1-(6-methyl-7-((6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3yl)methoxy)- [1,2,4]triazolo[1,5-a]pyrimidine and N2-6-methyl-7-((6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methoxy)-[1,2,4]triazolo[1,5-a]pyrimidine were selected and their docking scores were obtained sent for the MTT assay.
3. The synthesised compounds (N1 & N2) were dissolved in DMSO and were diluted to obtain the concentration of 10 ug/ml, 1ug/ml, 0.1 ug/ml, 0.01 ug/ml and 0.001 ug/ml.
4. The IC50 values were obtained for N1 and N2 taking doxorubicin as the standard drug

The IC50 value of N1 & N2

S.no	Concentration(µg/ml)	Percentage Inhibition(%)	
		N1	N2
1.	10	58.41	66.48
2.	1	32.75	53.28
3.	0.1	18.62	43.10
4.	0.01	12.48	7.85
5.	0.001	3.63	2.37
IC_{50} value (µM)		**5**	**0.20**
Doxorubicin		**0.22 µM**	

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 222-236 www.ijprajournal.com ISSN: 2249-7781

II. CONCLUSION-

This research work was oriented towards synthesis of triazolthiadiazolidine derivatives and their structure confirmation through IR, NMR and further performed the anticancer activity (through MTT assay).

Based on the docking study and the biological activity following conclusions were drawn:

- Presence of electron withdrawing group nitro (NO_2), Cyno (CN) attached at the para position of the phenyl ring enhances the anticancer activity.
- Triazoles act like strong anticancer agents when it possess electron-donating groups like 3,4,5-trimethoxy group in the phenyl ring at di-meta and para positions.
- Among the halogen withdrawing groups the substitution on phenyl ring at the para position by 4-chloro group exhibited better anticancer activity than the 4-fluro and 4-bromo derivatives.
- 1,2,4-triazole nucleus is stable to metabolism and behaves like a chief group by acting as at as hydrogen bond donor and as acceptor to the receptor's active site.

REFERENCES

[1]. Mahanti, S., Sunkara, S. and Bhavani, R., 2019. Synthesis, biological evaluation and computational studies of fused acridine containing 1, 2, 4-triazole derivatives as anticancer agents. Synthetic Communications, 49(13),pp.1729-1740.

[2]. Zhang, W., Zhangyuan, G., Wang, J., Jin, K., Liu, Y., Wang, F., Yu, W.,Zhang,H.,Li, G., Yu, D. and Chen, H., 2019. Effect of lymph nodes count in node- positive gastric cancer. Journal of Cancer, 10(23), pp.5646-5653

[3]. Hassan, A.Y., Sarg, M.T., Bayoumi, A.H. and El-Deeb, M.A., 2018. Synthesis and anticancer evaluation of some novel 5-amino [1, 2, 4] triazole derivatives. Journal of Heterocyclic Chemistry, 55(6), pp.1450-1478.

[4]. El-Sherief, H.A., Youssif, B.G., Bukhari, S.N.A., Abdelazeem, A.H., Abdel-Aziz, M. and Abdel-Rahman, H.M., 2018. Synthesis, anticancer activity and molecular modeling studies of 1, 2, 4-triazole derivatives as EGFR inhibitors. European journal of medicinal chemistry, 156, pp.774-789.

[5]. WANG, Y. and ZHOU, C., 2011. Recent advances in the researches of triazole compounds as medicinal drugs. Scientia Sinica Chimica, 41(9), pp.1429- 1456.

[6]. Saini, M.S. and Dwivedi, J., 2013. Synthesis and biological significances of 1, 2, 4-triazole and its derivatives: a review. International Journal of Pharmaceutical Sciences and Research, 4(8), p.2866.