SYNTHESIS AND *IN-VITRO* ANTI-TUBERCULAR ACTIVITY SCREENING OF NOVEL 1,2,4-TRIAZOLE DERIVATIVES

Honnalli S.S, Godinho Sybil E.M., Ronad P.M., Pradeep Kumar M.R*., Prateek A Angadi

Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Vidyanagar,

Hubli-580031 (A Constituent unit of KAHER, Belagavi)

*Corresponding Author-

Dr. Pradeep Kumar M.R.

Assistant Professor

Department of Pharmaceutical Chemistry

,KLE College of Pharmacy, Hubli 580031 INDIA

(A Constituent unit of KLE academy of Higher Education and Research, Belagavi)

E-mail: pradeepmrpk@yahoo.co.in

Mob.No: +91 8050106921

Abstract: The objective of this research work was to create new 1,2,4 Triazole derivatives and assess their anti-tubercular properties. Using a suitable synthetic approach, aniline and benzaldehyde were reacted to produce Schiff base, which was refluxed with urea in the presence of an acid in order to produce a new series of 1,2,4-triazole derivatives. Melting point and TLC revealed that every synthesized chemical was pure. All of these compounds structures were verified using mass spectrum analysis, NMR, and FTIR. By comparing the synthesized compounds (II-IIg) to the standard medicine isoniazid, the MABA (Microplate Alamar Blue Assay) method was used to test all of the compounds for antitubercular activity against the Mycobacterium Tuberculosis H37Rv strain. When compared to the conventional medication, compound Ild and Ile showed extremely good anti-tubercular activity. Compounds IIa, IIg, and Ilf had moderate activity, in contrast. The current study offers information to medicinal chemists so they can begin their research on creating anti-tubercular medicines. The activity can be further enhanced by making slight modifications in the ring substituent.

Keywords: Schiff Base, 1,2,4 Triazole, MABA Method, Anti-Tubercular activity.

Introduction: Bacteria is the root cause of many infectious diseases and responsible for an increase in the mortality rate.Numerous varieties of bacteria exist, and they are very resistant to antibiotics. One of the microorganisms is tuberculosis, which primarily affects the lungs and is brought on by Mycobacterium tuberculae. When TB patients cough, sneeze, or spit, the disease is transferred through the air. About ten million people contract tuberculosis (TB) annually. TB is the leading infectious disease killer in the world, taking the lives of 1.5 million people year despite being a preventable and curable illness. Eight countries- Bangladesh, China, India, Indonesia, Nigeria, Pakistan, Philippines, and South Africa- are home to around half of all TB patients. An estimated of about 25% of people worldwide are thought to be TB-positive. [1]

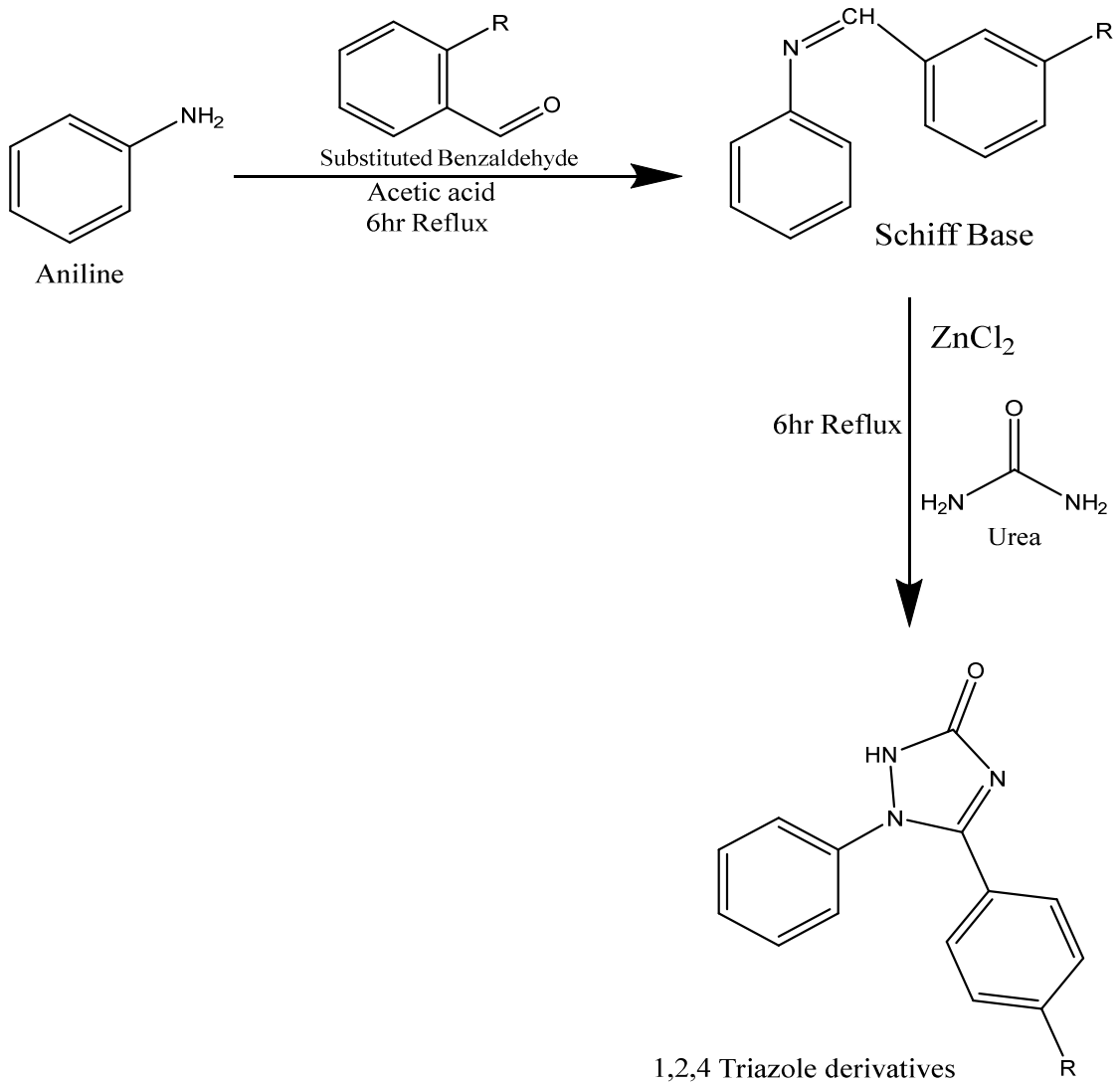
[2]Triazoles are a unique class of heterocyclic compounds that exhibit a wide range of biological activities, including insecticidal, antimycotic, anti-mycobacterial, anticancer, antiprotozoal, antimalarial, and anti-ulcer properties. They are also cytotoxic, antihistaminic, anticonvulsant, analgesic, and anti-inflammatory. Triazoles have a five-membered ring structure made up of two carbon and three nitrogen atoms. There are two isomeric chemical compounds that are known as triazoles: 1,2,3 and 1,2,4 triazole. Because of their possible anti-TB strength, triazole derivatives are thought to represent a new class of potent anti-TB medications. Therefore, compounds with a triazole moiety may have encouraging anti-TB properties both in vitro and in vivo.[3]

A Schiff base is a chemical having the general structure R2C=NR. Depending on their structure, Schiff bases are classified as secondary ketamines or secondary aldehydes within the imine class. The word is frequently used interchangeably with Azomethine condensation of an amino compound. Carbonyl compounds are utilised extensively in industry and have a variety of biological activities, such as antifungal, antibacterial, anti-malarial, anti-inflammatory, antiviral, and antipyretic qualities.This work represents an attempt to synthesise 1,2,4-triazoles and assess their anti-tubercular efficacy.[4]

In continuation to our research work on novel heterocyclic compounds with potent anti-tubercular activity [5-14], here we have synthesized novel 1,2,4 triazole derivatives to screen for their anti-tubercular activity

Methodology:

Scheme:



Chemistry:

Preparation of Schiff Base:

- Schiff base was synthesized by mixing 2 grams of benzaldehyde and 2 grams of aniline (0.018 and 0.021 respectively) in 25 ml of absolute alcohol with 0.5 ml of acetic anhydride (0.0048). The mixture was then refluxed for 6 hours in a water bath and allowed to cool. [5]

- The solvent was extracted under pressure. After obtaining a crude product, it was cleaned with cold water and re-crystallized in a 1:2 ratio with ethyl actetate and cyclohexane. TLC was used to compare the synthesised compound's purity. [15]

Preparation of 1,5-diphenyl-1*H*-1,2,4-triazole-3-(2H)-one:

- After refluxing for six hours, in presence of zinc chloride the schiff base (0.01mol) and urea (0.001mol) react in dioxin (25 ml).The reaction was cooled before being stirred and poured over crushed ice.

- The resultant solid, 1,5-diphenyl-1H-1,2,4-triazole-3-(2H)-one, was filtered and rinsed with sodium bicarbonate to eliminate any remaining unreacted substances. After being dried, the substance was crystallized again using ethanol. % yield 80, M.P. 76c, Rf value 0.67, ethyl acetate:cyclohexane (1:2) solvent system was utilized. [16]

Biological evaluation:

Anti-tubercular activity:

- The antitubercular activity of all the synthesized compounds was evaluated using mycobacterium tuberculosis H37RV Strain. The results of the antitubercular activity demonstrate that compound IId and IIe exhibited extremely good activity against standard drug compared to compounds II,IIa,IIb,IIc,IIf, and IIg. [17]

- MABA(Microplate Alamar Blue Assay) method was used to verify the anti-tubercular action. The standard medications that were compared included ethambutol, isoniazid, streptomycin, rifampicin, and pyrazinamide. The standard MIC was used to compare the activity of the synthesized triazoles. Isoniazid (1.6µg/ml), Ethambutol (1.6µg/ml), Pyrazinamide (3.125µg/ml), streptomycin, and rifampicin (0.8µg/ml) are the standards and their respective values. [18]

Results and Discussion:

Physicochemical data of 1,2,4 Triazole derivatives:

Sl No	Compound	R	% Yield	Melting Point (in ^oC)	Molecular Weight	Molecular Formula	Rf Value
1	II	-	84	149	251.29	$C_{14}H_{11}N_3O$	0.7
2	IIa	4-OH	73	160	253.26	$C_{14}H_{11}N_3O_2$	0.62
3	IIb	4-$N(CH_3)2$	68	187	278.31	$C_{16}H_{14}N_4O$	0.65
4	IIc	3,4,5-CH_3OH	65	196	234.24	$C_{14}H_8N_3O$	0.69
5	IId	4-Cl	75.3	167.5	271.7	$C_{14}H_{10}N_3ClO$	0.72
6	IIe	4-NO_2	77.6	176	282.26	$C_{14}H_{10}N_4O_3$	0.64
7	IIf	2-Cl	70	162	271.7	$C_{14}H_{10}N_3ClO$	0.75
8	IIg	2-NO_2	68.4	170	282.26	$C_{14}H_{10}N_4O_3$	0.73

MIC for anti-tubercular activity

Conc(µg/ml)	Activity of compounds												
	INH	EMB	PZA	RIF	STM	II	IIa	IIb	IIc	IId	IIe	IIf	IIg
100	S	S	S	S	S	S	S	S	S	S	S	S	S
50	S	S	S	S	S	S	S	S	S	S	S	S	S
25	S	S	S	S	S	S	S	S	S	S	S	S	S
12.5	S	S	S	S	S	S	S	S	S	S	S	S	S
6.25	S	S	S	S	S	S	R	S	S	S	S	S	S
3.2	S	S	S	S	S	S	R	R	R	S	S	S	S
1.6	S	S	R	S	S	S	R	R	R	S	S	S	S
0.8	R	R	R	S	S	R	R	R	R	S	S	S	S
0.4	R	R	R	R	R	R	R	R	R	R	R	R	R
0.2	R	R	R	R	R	R	R	R	R	R	R	R	R

INH-Isoniazid, PZA-Pyrazinamide, STM-Streptomycin, EMB-Ethambutol, RIF-Rifampicin.
R-Resistance (growth present), S-Sensitive (no growth).

Infrared Spectrum: The IR spectrum of the synthesized derivatives are as follows:

- 1,5-diphenyl-1h-1,2,4-triazole-3-(2H)-one.(II)
  IR (KBr) cm: 3413.88 (NH stretching), 1736.44 (C=O), 1626 (NH, scissoring). 1239.14 (C-O).

- 5-(4-hydroxypheny)-1-phenyl-1H-1,2,4-triazole-3-(2H)-one.(IIa)
  IR (KBr) cm: 3427.08 (NH stretching),2671.89 (OH),1576.89(C=0), 1283.80 * (C-O).

- 5-(4-dimethlyamino)phenyl)-1-phenyl-1H-1,2,4-triazole-3-(2H)-one.(IIb)
  IR (KBr) cm: 3419.92 (NH stretching), 2730.62 (CH3),1283.24 (C-O)

- 1-phenyl-5-(3,4,5-tri-methoxyphenyl)-1H-1,2,4-triazole-3-(2H)-one.(Ilc).
  IR (KBr) cm: 3337.29 (NH stretching), 2936.29(OH),1121.46 (C-O)

- 5-(4-chlorophenyl)-1-phenyl-1H-1,2,4-triazole-3-(2H)-one.(IId)
  IR (KBr) cm: 3416.22 (NH stretching), 1623.61 (C-O), 1395.51 (C-O),827.03 (CI).

- 1-(4-nitophenyl)-5-phenyl-1H-1,2,4-triazole-3-(2H)-one.(Ile)
  IR (KBr) cm: 3382.06 (NH stretching), 1344.20 (NO2), 1182.58 (C-O).

- 5-(2,6-dichlorophenyl)-1-phenyl-1H-1,2,4-triazole-3-(2H)-one.(IIf)
  IR (KBr) cm:3403.59 (NH stretching), 1635.54 (C-0),1194.73 (C-0),763.59 (CI)

- 5-(2-nitophenyl)-1-phenyl-1H-1,2,4-triazole-3-(2H)-one.(IIg)
  IR (KBr) cm:3398.96 (NH stretching), 1675.37(C=O), 1521.33 (NO2), 1186.42 (C-O)

Conclusion: The above 1,2,4 triazole derivatives were synthesized by using Schiff bases. Comparing these compounds to standard drugs, they demonstrated a notable anti-tubercular effect. Thus by considering these compounds as lead molecules further modification in the basic structure can yield better compounds

References:

(1) https://www.who.int/teams/global-tuberculosis-programme/data

(2) Tian, G.; Song, Q.; Liu, Z.; Guo, J.; Cao, S.; Long, S. Recent Advances in 1,2,3- and 1,2,4-Triazole Hybrids as Antimicrobials and Their SAR: A Critical Review. *Eur. J. Med. Chem.* **2023**, *259* (June), 115603. https://doi.org/10.1016/j.ejmech.2023.115603.

(3) Azim, T.; Wasim, M.; Akhtar, M. S.; Akram, I. An in Vivo Evaluation of Anti-Inflammatory, Analgesic and Anti-Pyretic Activities of Newly Synthesized 1, 2, 4 Triazole Derivatives. *BMC Complement. Med. Ther.* **2021**, *21* (1), 1–15. https://doi.org/10.1186/s12906-021-03485-x.

(4) Keri, R. S.; Patil, S. A.; Budagumpi, S.; Nagaraja, B. M. Triazole: A Promising Antitubercular Agent. *Chem. Biol. Drug Des.* **2015**, *86* (4), 410–423. https://doi.org/10.1111/cbdd.12527.

(5) M.R. Pradeep Kumar, S. D. Joshi, S. R. Dixit and V. H. Kulkarni. Synthesis, antibacterial and antitubercular activities of some novel quinoline derivatives. Indian Journal of Heterocyclic Chemistry, Apr-Jun, 2014, 23, 353-358.

(6) M.R. Pradeep Kumar, Shrinivas D. Joshi, S. R. Dixit and V. H. Kulkarni. Synthesis, antibacterial and antitubercular activities of some novel *N1*-[2-(substituted phenylamino)acetyl]-4-(1H-pyrrole-1-yl)-benzohydrazide derivatives. Indian Journal of Heterocyclic Chemistry. 2015, 25, 37-44.

(7) M.R. Pradeep Kumar, Shrinivas D. Joshi, S. R. Dixit and V. H. Kulkarni. Synthesis, antimicrobial, analgesic and anti-inflammatory activities of some novel pyrrole derivatives. Indian Journal of Heterocyclic Chemistry. 2015, 25, 75-82.

(8) Shrinivas D. Joshi, S. R. Dixit, M.R. Pradeep Kumar and V. H. Kulkarni. Molecular docking studies of pyrollyl benzohydrazide derivatives as enoyl ACP reductase inhibitors. Indo American Journal of Pharmaceutical Research. 2016, 6 (3). 4925-4933.

(9) Rajesh D. Hunashal and Pradeep Kumar M. R. Synthesis and screening of some novel *N*-(4-oxo-2-substituted phenyl- 1,3-thiazolidin-3-yl)-1,3-benzoxazole-5-carboxamide derivatives for antibacterial and antitubercular activities. Indian Journal of Heterocyclic Chemistry. 2016. 26, 1-6.

(10) Rajesh D. Hunashal and Pradeep Kumar M. R. Synthesis and antimicrobial activity screening of 2-[4-(substituted benzylidenamino)-5-(naphthalen-4-yloxy)methyl-*4H*-1,2,4-triazol-3-yl thio] acetic acid and 2-[4-(2-substituted benzylideneamino)-5-(naphthalen-4-yloxy)methyl-*4H*-1,2,4-triazol-3-yl] isonicotinyl hydrazide derivatives. Indian Journal of Heterocyclic Chemistry. 2017. 27, 165-172. 2456-4311

(11) Pradeep Kumar M.R and Rajesh D. Hunashal. Synthesis, spectral characterization and in-vitro screening of some novel tetrahydroquinoline derivatives for their antitubercular, antioxidant activities. Saudi Journal of Medical and Pharmaceutical Sciences. 2018. 4(1B), 151-155.

(12) Pradeep Kumar M.R and Rajesh D. Hunashal. Synthesis and in-vitro screening of some novel 6-substituted/ unsubstituted-2-phenyl-1,2,3,4-tetrahydro quinoline derivatives as potential antitubercular and antioxidant agents.. European Journal of Biomedical and Pharmaceutical Sciences 2018. 5(3), 240-244.

(13) Pradeep Kumar M.R and Rajesh D. Hunashal. Synthesis, spectral characterization and in-vitro screening of some novel tetrahydroquinoline derivatives for their antitubercular, antioxidant activities. Saudi Journal of Medical and Pharmaceutical Sciences. 2018. 4(9), 1014-1017.

(14) Manjoor Ahamad Syed, Pradeep Kumar M.R., Teshome Bekana, Firomsa Bekele, Manya B.S, Shivraj S.H. Synthesis, spectral characterization, *in-vitro* and *in-silico* anti-tubercular activity screening of novel series of imidazo [2,1-b] [1,3,4]-thiadiazole derivatives. European Chemical Bulletin, 2023, 12(4), 13982-13997.

(15) Ganesh Kumar, T. N. V.; Gautham Shenoy, G.; Kar, S. S.; Shenoy, V.; Bairy, I. Design, Synthesis and Evaluation of Antitubercular Activity of Novel 1,2,4-Triazoles Against MDR Strain of Mycobacterium Tuberculosis. *Pharm. Chem. J.* **2018**, *51* (10), 907–917. https://doi.org/10.1007/s11094-018-1714-8.

(16) Zhang, S.; Xu, Z.; Gao, C.; Ren, Q. C.; Chang, L.; Lv, Z. S.; Feng, L. S. Triazole Derivatives and Their Anti-Tubercular Activity. *Eur. J. Med. Chem.* **2017**, *138*, 501–513. https://doi.org/10.1016/j.ejmech.2017.06.051.

(17) Al-Masoudi, I. A.; Al-Soud, Y. A.; Al-Salihi, N. J.; Al-Masoudi, N. A. 1,2,4-Triazoles: Synthetic Approaches and Pharmacological Importance. (Review). *Chem. Heterocycl. Compd.* **2006**, *42* (11), 1377–1403. https://doi.org/10.1007/s10593-006-0255-3.

(18) Mali, R. K.; Somani, R. R.; Toraskar, M. P.; Mali, K. K.; Naik, P. P.; Shirodkar, P. Y. Synthesis of Some Antifungal and Anti-Tubercular 1, 2, 4-Triazole Analogues. *Int. J. ChemTech Res.* **2009**, *1* (2), 168–173.