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# SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 4- [SUBSTITUTED PHENYL)-3-(N-SUBSTITUTED CARBOXAMIDOMETHYLTHIO)-5-(PYRIDINE-4-YL)-1,2,4-TRIAZOLES

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## ABSTRACT

***Objective:*** *The objective of the present work is to design some potential bioactive containing 1,2,4-triazole nucleus as well as pyrimidine nucleus and screening them for antimicrobial and anti-inflammatory activity.* ***Method:*** *Isonicotinic acid hydrazide based 1,2,4-triazoles derivatives has been synthesized by reaction of Isoniazid with carbon disulfide in basic medium (KOH) to form Potassium dithiocarbazinate salt and reaction with hydrazine hydrate converted into 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol. This compound was reacted with different benzaldehyde to form 4- [(substituted phenyl)-methylene]-amino-5-(pyridine-4-yl)-4H-1, 2, 4-triazol-3-thiol (4). The final compounds were synthesized by reaction with acetanilide to form 4- [substituted phenyl)-methylene]-amino-3-(N-substitutedcarboxamidomethylthio)-5-(pyridine- 4 – yl) -4H-1, 2, 4 - triazoles derivatives. Compounds were confirmed by IR, $^{1}H$ NMR, $^{13}C$ NMR and elemental analysis. Cup plate method were employed to find out the antimicrobial activity. Acute anti-inflammatory activity determined by using carrageenan induced rat paw edema model.* ***Result:*** *The antifungal activity of PJ-C2, PJ-C4, PJ-C7, PJ-C9 and PJ-C11 shows more than 90%, PJ-C3, PJ-C7 and PJ-C12 shows more than 80% and rest of compounds shows more than 50 and less than 70% of zone of inhibition. Among all these compounds PJ-C4 and PJ-C13 shows excellent MIC against all fungal strains compare to standard clotrimazole. The antibacterial activity of PJ-$C_4$ and PJ-$C_{13}$ shows more than 90%, PJ-$C_2$, PJ-$C_5$, PJ-$C_9$ and PJ-$C_{11}$ showed more than 80% and rest of compounds shows more than 50% and less than 70% of zone of inhibition against both Gram positive and Gram-negative organisms. Among all these compounds only PJ-$C_4$ and $C_{13}$ shows excellent MIC against both Gram positive and Gram-negative organisms compare to standard norfloxacin. Among all PJ-C4, PJ-C9 and PJ-C12 shows significant anti-inflammatory ranging from 70 to 80% edema inhibition compare to standard drug Ibuprofen among that PJ-C9 shows excellent activity that is 80% of edema inhibition.* ***Conclusion:*** *All these results suggested that the isoniazid based 1,2,4-triazole derivatives has shown good antibacterial, antifungal and anti-inflammatory activity.*

## KEY WORDS

*Potential bioactive nucleus, pyrimidine nucleus.*

## INTRODUCTION

Bacterial resistance, drug resistance and multiple drug resistance are the current issues of the scientific world dealing with the antimicrobial therapy. 1,2,4-triazoles has shown anti-tubercular[1,2] antimicrobial[3], anti-inflammatory[4], antibacterial[5-7], antioxidant[8], anticonvulsant[9], antifungal[10], anticancer[11], analgesic[12] etc**.** Non-steroidal anti-inflammatory drugs (NSAIDs) are primarily important for the treatment of inflammation and pain in arthritis. NSAIDs act by inhibiting PGs

synthesis by blockage of enzyme cyclooxygenase-1. In current scenario, microbial resistance is one of the hurdles and needs the development of newer agent to target the diseases. Literature survey indicates that triazole derivatives of isoniazid have been synthesized and tested for anti-inflammatory activity[13]. NSAIDs is on long exposure causes the gastric ulcer, bleeding and renal disorder. This is most likely due to the presence of free carboxyl group on Non-steroidal anti-inflammatory drugs (NSAIDs). The GIT mucosal injury problems produced by NSAIDs are commonly believed to be caused by two different mechanisms. One is local irritation produced by free carboxylic acid group and inhibition of prostaglandin biosynthesis in the GIT. The test compounds inhibited the induction of gastric mucosal lesions and their protective effects may be related to inhibition of lipid peroxidation in gastric mucosa.

1,2,4-triazoles simulate the three Nitrogen theory which make this nucleus basic and powerful antibacterial agent. Presence of one pyrimidine ring as present in the isoniazid, will further thought to enhance the antimicrobial potential (Fig. 1).

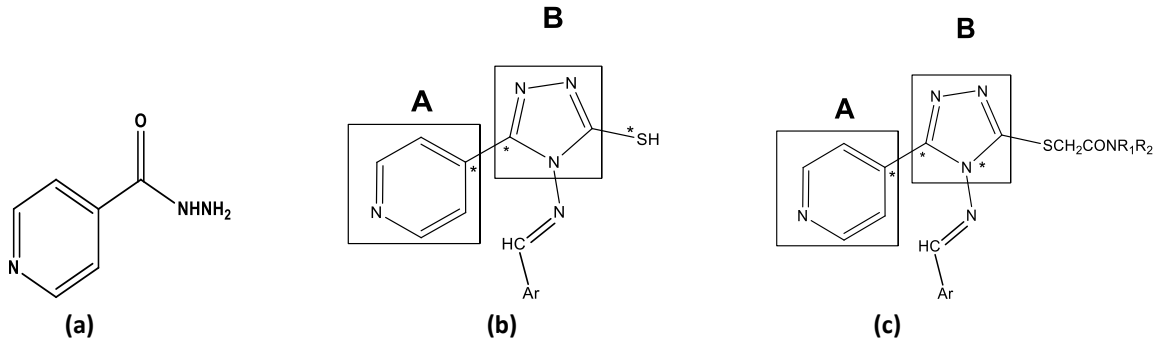


**Fig. 1: Proposed pictorial representation of the proposed hypothesis. a) Chemical structure of the Isoniazid b) and c) are proposed compounds scheme for the designing of the 1,2,4-triazole compounds.**

Mannich base derivatives and triazole fused with 6-membered rings were reported to possess significant antitubercular activity. The two nitrogen atoms of the hydrazide group of the isoniazid are complimentary to the two nitrogen atoms present at the 1 and 2 position of the triazole nucleus.

In continuation of our earlier research work we propose to develop some new bioactive 1,2,4-triazoles derivatives 4-[substitutedphenyl)-methylene]-amino-3-(N-substituted-carboxamidmethylthio)-5-(pyridine-4yl) - 4H-1, 2, 4-triazoles and analyze them for anti-microbial and anti-inflammatory properties.

## EXPERIMENTAL

### MATERIAL AND METHODS

Isoniazid (Isonicotinic acid hydrazides) was purchased from CDH (Chemical Drug House), India. Carbon-disulfide, potassium hydroxide, hydrazine hydrate, ethanol, methanol, glacial acetic acid, anhydrous ether, DMSO, aldehyde compounds (benzaldehyde, *p*-anisaldehyde, 4-bromobenzaldehyde, *p*-chlorobenzaldehyde, *p*-tolualdehyde, *p*-nitrobenzaldehyde, cinnamaldehyde) were purchased from the CDH, New Delhi, India. and Alpha-chloroacetanilides compounds 4-nitro-2-trifluoromethylacetanilide was purchased from the Sigma Aldrich, New Delhi, India. The chemical used for experimental work were synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on ALPHA (Bruker) FTIR Spectrometer. Elemental analysis was performed and found values were within 0.4% of theoretical values. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5 mm multi-nuclear inverse probe head, low and high-temperature facility and HRMAS accessory. Mass Spectra were recorded using Mass Spectrometers Jeol SX-102 (FAB) by ESI.

***Synthesis of potassium dithiocarbazinate salt[14]***

Isonicotinic acid hydrazide (0.10 mol) (1) was reacted with an ethanolic solution of potassium hydroxide (0.15 mol) along with carbon disulfide (0.15mol) was added slowly to it. The reaction mixture was diluted with absolute ethanol (50 ml) and stirred continuously for

16h at room temperature on a magnetic stirrer. The precipitated potassium dithiocarbazinate salt was collected by filtration, washed with anhydrous ether and dried. The potassium dithiocarbazinate salt (2) thus obtained was used in the next step without further purification.

***Synthesis of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol[15]***

Potassium dithiocarbazinate salt (2) (0.079 mol) reacted with aqueous hydrazine hydrate (12 mL, 0.24 mol) solution and refluxed for 4h, hydrogen sulfide ($H_2S$ gas) was evolved during the reaction was observed and indicated by the lead acetate solution (confirmatory test-turn lead acetate-soaked filter paper convert white to black). The reaction mixture was cooled to room temperature, diluted with ice-cold distilled water and subsequent acidification with dilute acetic acid. Obtained light yellow precipitate was filtered, washed with cold distilled water and dried. Recrystallization was done using absolute ethanol to get white crystals (3). The compound 3 exist in thione-thiol tautomeric forms, but our investigation showed that in this case, the thiol structure dominated in the solid state, as indicated by the IR and NMR data of the compound. Yield: 78%, m.p.: 214-216°C; IR (cm-1): 3160 (N-H), 3000 (C-H), 2582 (S-H), 1608 (C=N), 1571 (C=C), 709 (out of plane C-H bending), 689 (C-S). 1H NMR (ppm): 3.77 (s,1H, -NH2), 10.51 (Aromatic C-SH), 7.92 (d, 1H, Benzylidenimin), 8.59 (d, 1H, beta-pyridyl); 13C NMR (ppm): 151.1 (1,2,4-triazoles), 134.0 (C1-pyridine), 121.3 (C2 & C6-pyridine). 149.8 (C3 & C5-pyridine), LC-MS m/z: 193.23.

***Synthesis of 4-[(substituted phenyl)-methylene]-amino-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-thiol (4a-4g)[16]*** To a suspension of corresponding compound 1,2,4-triazole-3-thiol (3) (0.005 mol) in methanol (50 ml) and an equimolar quantity of aromatic aldehyde in methanol (20 ml) was mixed. This suspension was heated until a clear solution was obtained and refluxed for 3h in the presence of a few drops of concentrated hydrochloric acid in a water bath. The reaction solution was left undisturbed overnight. On the next day, the separated solid were filtered, washed with ethanol and recrystallized from ethanol to procure the product/compound (4).

***Synthesis of 4-[substituted phenyl)-methylene]-amino-3-(N-substituted-carboxamidmethyl thio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles[17]***

Compound 1,2,4-triazol-3-thiol (4) (0.01 mol) was dissolved in aqueous potassium hydroxide solution (0.61g in 100 ml distilled water) with stirring till a clear yellow color solution was obtained. It was filtered to remove any suspended impurities. Then various N-substituted-α-chloroacetanilides (0.01 mol) compound were dissolved in ethanol and added with shaking at 55°C stirrered for 4.5 h. Then the reaction mixture was left overnight and the next day, the separated solid was filtered and washed twice with cold distilled water to remove KCl, dried and recrystallized from dilute glacial acetic acid (5). In case of aliphatic N-substituted-α-chloroacetanilides, the amide was added at room temperature.

**Table 1: Elemental Analysis of synthesized final compounds**

| Compound code | Molecular formula | Molecular weight | Elemental analysis % found (calculated) | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| PJ-$C_1$ | $C_{24}H_{20}N_6O_2S$ | 456.52 | 63.14 (63.16) | 4.42 (4.41) | 18.41 (18.42) |
| PJ-$C_2$ | $C_{24}H_{19}ClN_6O_2S$ | 491.0 | 58.71 (58.74) | 4.42 (4.43) | 17.12 (17.15) |
| PJ-$C_3$ | $C_{24}H_{19}ClN_6O_2S$ | 491.0 | 58.71 (58.74) | 4.42 (4.43) | 17.12 (17.15) |
| PJ-$C_4$ | $C_{24}H_{19}ClN_6O_2S$ | 491.0 | 58.71 (58.74) | 4.42 (4.43) | 17.12 (17.15) |
| PJ-$C_5$ | $C_{24}H_{19}BrN_6O_2S$ | 535.0 | 53.84 (53.81) | 3.58 (3.56) | 15.70 (15.71) |
| PJ-$C_6$ | $C_{24}H_{19}BrN_6O_2S$ | 535.0 | 53.84 (53.81) | 3.58 (3.56) | 15.70 (15.71) |
| PJ-$C_7$ | $C_{25}H_{21}N_6O_2S$ | 471.0 | 63.81 (63.84) | 4.71 (4.69) | 17.86 (17.84) |
| PJ-$C_8$ | $C_{25}H_{21}N_6O_3S$ | 487.0 | 61.71 (61.73) | 4.56 (4.55) | 17.27 (17.28) |
| PJ-$C_9$ | $C_{24}H_{18}Cl_2N_6O_2S$ | 526.0 | 54.86 (54.83) | 3.45 (3.46) | 16.00 (16.02) |
| PJ-$C_{10}$ | $C_{24}H_{18}Cl_2N_6O_2S$ | 526.0 | 54.86 (54.83) | 3.45 (3.46) | 16.00 (16.02) |
| PJ-$C_{11}$ | $C_{26}H_{25}N_7O_2S$ | 500.0 | 62.51 (62.53) | 5.04 (5.03) | 19.63 (19.60) |
| PJ-$C_{12}$ | $C_{24}H_{20}FN_6O_2S$ | 474.5 | 60.75 (60.73) | 4.04 (4.02) | 17.71 (17.70) |
| PJ-$C_{13}$ | $C_{24}H_{20}N_6O_3S$ | 473.0 | 61.00 (61.02) | 4.27 (4.25) | 17.79 (17.78) |

***PJ-D1:2,4-(benzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoro methyl)-4-nitrophenyl)acetamide***

Molecular formula: C23H16F3N7O3S; Molecular weight: 527; TLC (Rf value): 0.66; IR (cm-1, KBr):3340 N-H str; 3150 C-H str; 2967 C-H str; 1720 C=O; 1420 SCH2 str; 1560 C=N str; 1400 C-H def; 1460 C-C str; 1589 C=C str; 1340 C-N str; 1580 C=N str; 1530 NO2 str; 685 C-S str; 1H NMR (DMSO-d6, δ ppm): 9.27 s,1H, -N=CH; 8.29s,1H,NH; 7.32-8.9 m, 12H, aromatic protons; 4.23 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 160.6 N=CH; 168.2

C=O of amide; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 134 C1 of benzene ring; 121 C2 & C6 of benzene ring; 142 C3 & C5 of benzene ring; 122 C4 of benzene ring; 40.3 SCH2; 116 Trifluro carbon; Mass (m/z): 527.

***PJ-D2: 2,4-(2-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoromethyl)4-nitrophenyl)acetamide***

Molecular formula: C23H15ClF3N7O3S; Molecular weight: 561; TLC (Rf value): 0.59; IR (cm-1, KBr): 3330 N-H str; 3150C-H str; 2960 C-H str; 1730C=O; 1420 SCH2 str; 1560 C=N str; 1300C-H def; 1420 C-C str; 1590 C=C str; 1310 C-N str; 1510 C=N str; 1550 NO2 str; 685 C-S str; 720 C-Cl str; 1H NMR (DMSO-d6, δ ppm): 9.07 s,1H, -N=CH; 8.69 s, 1H, NH; 7.82-8.8 m, 11H, aromatic protons; 4.3 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 165.6 N=CH; 172.2 C=O of amide; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 124 C1 of benzene ring; 121 C2 & C6 of benzene ring; 148 C3 & C5 of benzene ring; 122 C4 of benzene ring; 45.3 SCH2; 115 Trifluro carbon. Mass (m/z): 561.

***PJ-D3: 2,4-(3-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoro methyl)4-nitrophenyl) acetamide***

Molecular formula: C23H15ClF3N7O3S; Molecular weight: 561; TLC (Rf value): 0.60; IR (cm-1, KBr): 3330 N-H str; 3150 C-H str; 2960C-H str; 1730C=O; 1420 SCH2 str; 1560 C=N str; 1300 C-H def; 1420 C-C str; 1590 C=C str; 1310 C-N str; 1510 C=N str; 1330 NO2 str; 685 C-S str; 720 C-Cl str; 1H NMR (DMSO-d6, δ ppm): 9.07 s,1H, -N=CH; 8.69 s, 1H, NH; 7.82-8.8 m, 11H, aromatic protons; 4.3 s, 2H, SCH2. 13C NMR (DMSO-d6, δ ppm): 165.6 N=CH; 172.2 C=O of amide; 125. C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 124 C2 & C6 of benzene ring; 148 C3 & C5 of benzene ring; 122 C4 of benzene ring; 45.3 SCH2; 116 Trifluro carbon. Mass (m/z): 561.

***PJ-D4: 2,4-(4-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoro methyl)4-nitrophenyl)acetamide***

Molecular formula: C23H15ClF3N7O3S; Molecular weight: 561; TLC (Rf value): 0.60; IR (cm-1, KBr): 3350 N-H str;3150 C-H str; 2990 C-H str; 1730 C=O; 1420 SCH2 str; 1560 C=N str; 1300 C-H def; 1420 C-C str; 1590 C=C str; 1310 C-N str; 1510 C=N str; 1540NO2 str; 685 C-S str; 730 C-Cl str. 1H NMR (DMSO-d6, δ ppm): 9.06 s,1H, -N=CH; 8.60 s,1H,NH; 7.82-8.9 m, 11H, aromatic protons; 4.33 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 168.2 N=CH; 172.2 C=O of amide; 125.2 C1 of pyridine ring; 128.0 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 124 C2 & C6 of benzene ring; 148 C3 & C5 of benzene ring; 122 C4 of benzene ring; 45.3 SCH2; 118 Trifluro carbon; Mass (m/z): 561.

***PJ-D5: 2,4-(2-bromobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoro methyl)4-nitrophenyl)acetamide***

Molecular formula: C23H15BrF3N7O3S; Molecular weight: 606; TLC (Rf value):0.68; IR (cm-1, KBr): 3320 N-H str; 3080 C-H str; 2980 C-H str; 1710 C=O; 1410 SCH2 str; 1560 C=N str; 1420 C-C str; 1590 C=C str; 1310 C-N str; 1510 C=N str; 1330 NO2 str; 685 C-S str; 1230 C-Br str. 1H NMR (DMSO-d6, δ ppm): 9.08 s,1H, -N=CH; 8.70 s, 1H, NH; 7.62-8.9 m, 11H, aromatic protons; 4.33 s,2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 157.2 N=CH; 170.2 C=O of amide; 120.0

C1 of pyridine ring; 122.0 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring;152 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 134 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 144 C4 of benzene ring; 43.3 SCH2; 112 Trifluro carbon. Mass (m/z): 606.

***PJ-D6: 2,4-(3-bromobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoro methyl) 4-nitrophenyl) acetamide***

Molecular formula: C23H15BrF3N7O3S; Molecular weight: 606; TLC (Rf value) 0.68; IR (cm-1, KBr): 3320 N-H str; 3080 C-H str; 2980 C-H str; 1710 C=O; 1410 SCH2 str; 1560 C=N str; 1420 C-C str; 1590 C=C str; 1310 C-N

str; 1510 C=N str; 1330 NO2 str; 685 C-S str 1230 C-Br str; 1H NMR (DMSO-d6, δ ppm): 9.08 s,1H, -N=CH; 8.70 s, 1H, NH; 7.62-8.9 m, 11H, aromatic protons; 4.33 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 157.2 N=CH;170.2 C=O of amide; 120.0 C1 of pyridine ring; 122.0 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring 152 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 134 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 144 C4 of benzene ring; 43.3 SCH2; 112 Trifluro carbon. Mass (m/z): 606.

***PJ-D7: 2,4-(4-methylbenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoro methyl)4-nitrophenyl) acetamide***

Molecular formula: C23H15F3N7O3S; Molecular weight: 542; TLC (Rf value): 0.66; IR (cm-1, KBr): 3320 N-H str; 3090 C-H str; 2990 C-H str; 1720 C=O; 1420 SCH2 str; 1560 C=N str; 1420 C-C str; 1590 C=C str; 1310 C-N str; 1510 C=N str; 1530 NO2 str; 685 C-S str; 1H NMR (DMSO-d6, δ ppm): 10.0 s,1H, -N=CH; 8.90 s, 1H, NH; 7.62-9.2 m, 11H, aromatic protons; 4.30 s, 2H, SCH2; 3.15 s, 3H CH3; 13C NMR (DMSO-d6, δ ppm): 158.2 N=CH; 173.2 C=O of amide; 122.0 C1 of pyridine ring; 123.0 C2 of pyridine ring; 145 C3 & C5 of pyridine ring; 152 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 134 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 144 C4 of benzene ring; 43.3 SCH2; 116 Trifluro carbon. Mass (m/z): 542.

***PJ-D8: 2,4-(4-methoxybenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoro methyl)4-nitrophenyl) acetamide***

Molecular formula: C24H18F3 N7O4S; Molecular weight: 558; TLC (Rf value): 0.70; IR (cm-1, KBr): 3220 N-H str; 3190 C-H str; 3030 C-H str; 1682.0 C=O; 1420 SCH2 str; 1565 C=N str; 1420 C-C str; 1580 C=C str; 1312 C-N str; 1530 C=N str; 1330 NO2 str; 705 C-S str; 1H NMR (DMSO-d6, δ ppm): 8.98 s,1H, -N=CH; 8.60 s, 1H, NH; 6.82-8.69m, 11H, aromatic protons; 3.90 s, 2H, SCH2; 3.25 s, 3H OCH3; 13C NMR (DMSO-d6, δ ppm): 158.2 N=CH; 173.2 C=O of amide; 122.0 C1 of pyridine ring; 123.0 C2 of pyridine ring; 145 C3 & C5 of pyridine ring; 153 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 124 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 149 C4 of benzene ring; 43.3 SCH2; 116 Trifluro carbon. Mass (m/z): 558.

***PJ-D9: 2,4-(2,4-dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoro methyl)4-nitrophenyl)acetamide***

Molecular formula: C23H14Cl2F3N7O3S; Molecular weight: 597; TLC (Rf value): 0.64; IR (cm-1, KBr) 3210 N-H str; 3150 C-H str; 2930 C-H str; 1680.0 C=O; 1420 SCH2; 156.5 C=N str; 1430 C-C str; 1590 C=C str; 1312 C-N str; 1530 C=N str; 1330 NO2 str; 705C-S str; 751 C-Cl str; 1H NMR (DMSO-d6, δ ppm): 9.80 s,1H, -N=CH; 9.0 s, 1H, NH; 7.62-8.29 m, 10H, aromatic protons; 4.20 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 154.2 N=CH; 168.2 =O of amide; 132.0 C1 of pyridine ring; 133.0 C2 of pyridine ring; 145 C3 & C5 of pyridine ring; 153 C2 of 1,2,4-triazole ring; 150.2 C5 of 1,2,4-triazole ring; 120C1 of benzene ring; 124 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 145 C4 of benzene ring; 40.3 SCH2; 116 Trifluro carbon. Mass (m/z): 597.

***PJ-D10: 2, 4-(2,6-dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1, 2, 4-triazol-3-yl) thio)-N-(2-trifluoromethyl)4-nitrophenyl) acetamide***

Molecular formula: C23H14Cl2F3N7O3S; Molecular weight: 597; TLC (Rf value): 0.67; IR (cm-1, KBr); 3230 N-H str; 3160 C-H str; 2920 C-H str; 169.0 C=O; 1420SCH2 str; 1565 C=N str; 1430 C-C str; 1590 C=C str; 1310 C-N str; 1520 C=N str; 1330 NO2 str; 705 C-S str; 750C-Cl str; 1H NMR (DMSO-d6, δ ppm): 9.82 s,1H, -N=CH; 9.06 s, 1H, NH; 7.62-8.69 m, 10H, aromatic protons; 4.23 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 156.2 N=CH; 172.2C=O of amide; 132.0 C1 of pyridine ring; 133.0 C2 of pyridine ring; 148 C3 & C5 of pyridine ring; 151 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 122 C1 of benzene ring; 125 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 142 C4 of benzene ring; 40.9 SCH2; 118 Trifluro carbon. Mass (m/z): 597.

***PJ-D11: 2,4-(N, N-dimethylamino dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoromethyl)4-nitrophenyl) acetamide***

Molecular formula: C25H21F3 N8O3S; Molecular weight: 571; TLC (Rf value): 0.64; IR (cm-1, KBr): 3210 N-H str; 3160 C-H str; 2923 C-H str; 1710.0 C=O; 1420 SCH2 str; 1560 C=N str; 1430 C-C str; 1590 C=C str; 1320 C-N str; 1525 C=N str; 1335 NO2 str; 705 C-S str; 1H NMR (DMSO-d6, δ ppm): 8.09 s,1H,-N=CH; 9.1 s, 1H, NH; 7.2-8.9 m, 11H, aromatic protons; 4.23s, 2H, SCH2; 3.6S, 6H, N(CH3)2; 13C NMR (DMSO-d6, δ ppm): 152.2 N=CH; 170.2 C=O of amide; 130.0C1 of pyridine ring; 132.0 C2 of pyridine ring; 148 C3 & C5 of pyridine ring; 158 C2 of 1,2,4-triazole ring; 156 C5 of 1,2,4-triazole ring; 126 C1 of benzene ring; 128 C2 & C6 of benzene ring; 140 C3 &

C5 of benzene ring; 148 C4 of benzene ring; 38.9 SCH2; 116 Trifluro carbon; Mass (m/z): 571.

***PJ-D12: 2,4-(4-flurodichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(2-trifluoromethyl)4-nitrophenyl)acetamide***

Molecular formula; C23H15F4 N7O3S; Molecular weight: 546; TLC (Rf value): 0.71; IR (cm-1, KBr): 3320 N-H str; 3100 C-H str; 2990 C-H str; 1720.0 C=O; 1420 SCH2 str; 1560 C=N str; 1430 C-C str; 1490 C=C str; 1340 C-N str; 1525 C=N str; 1330 NO2 str; 700 C-S str; 1H NMR (DMSO-d6, δ ppm): 8.9 s,1H, -N=CH; 8.66s, 1H, NH; 7.02-8.90m, 11H, aromatic protons; 4.63 s, 2H, SCH2; 13C NMR (DMSO-d6, δ ppm): 157.2 N=CH; 160.2 C=O of amide; 132.0 C1 of pyridine ring; 130.0 C2 of pyridine ring; 148 C3 & C5 of pyridine ring; 153 C2 of 1,2,4-triazole ring; 150 C5 of 1,2,4-triazole ring; 127 C1 of benzene ring; 128 C2 & C6 of benzene ring; 140 C3 & C5 of benzene ring; 149 C4 of benzene ring; 40.9 SCH2; 118 Trifluro carbon. Mass (m/z): 546.

***PJ-D13: 2,4-(3-hydroxybenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl) thio)-N-(2-trifluoromethyl)4-nitrophenyl) acetamide***

Molecular formula: C23H15F4N7O3S; Molecular weight: 544; TLC (Rf value): 0.72; IR (cm-1, KBr): 3420 OH str; 3340 N-H str; 3200; C-H str; 2980; C-H str; 1710.0 C=O; 1420 SCH2 str; 1520 C=N str; 1430 C-C str; 1490 C=C str; 1340 C-N str; 1520 C=N str; 1330 NO2 str; 700 C-S str. 1H NMR (DMSO-d6, δ ppm): 8.98 s,1H, -N=CH; 8.72s, 1H, NH; 7.12-8.60 m, 11H, aromatic protons; 4.63 s, 2H, SCH2; 5.3 s, 1H, OH. 13C NMR (DMSO-d6, δ ppm): 158.2 N=CH; 162.2 C=O of amide; 128.0 C1 of pyridine ring; 130.0 C2 of pyridine ring; 146 C3 & C5 of pyridine ring; 152

C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 129 C1 of benzene ring; 132 C2 & C6 of benzene ring; 142 C3 & C5 of benzene ring; 138 C4 of benzene ring; 41.9 SCH2; 119 Trifluro carbon. Mass (m/z): 544.

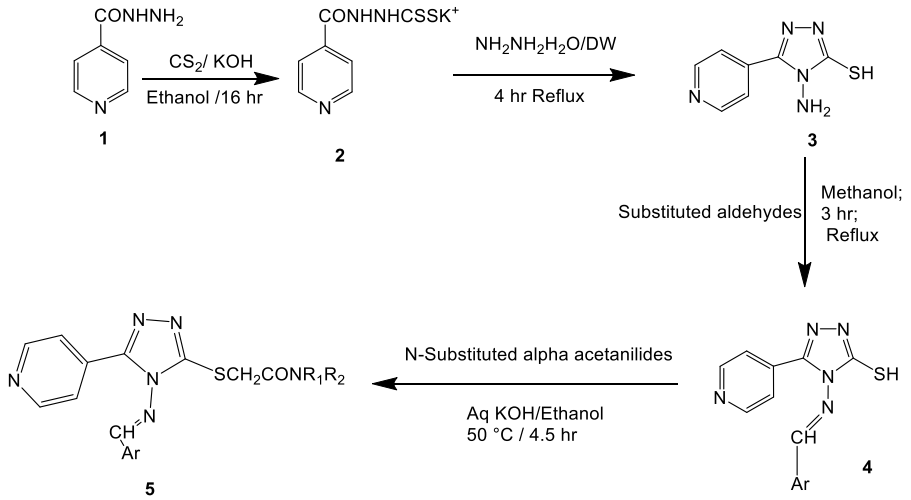


**Fig 2: Scheme for the synthesis; Reagent and reaction condition: I) $CS_2$, ethanolic KOH, reflux 16 h; II) $NH_2$-$NH_2.H_2O$, reflux, 4h; III) aromatic aldehyde, methanol, reflux, 3h; IV) acetanilide, Aq. KOH, ethanol, 55°C**

## BIOLOGICAL ACTIVITY

***Antibacterial and Antifungal activity[18]***

The antibacterial activity was determined by the cup plate method. Microbial strains (ATCC No): *Staphylococcus aureus* (12598); *Bacillus subtilis* (6051); *Pseudomonas aeruginosa* (25619); *Escherichia coli* (25922) is used for antibacterial activity. In this method sterilized molten nutrient agar media (20 ml) was poured aseptically and spread on the sterilized petri dishes (10 cm). The bacterial culture (CFU-$10^7$-$10^9$/ml; 0.1 ml) was added to it and mixed by swirl motion and kept aside. After setting of the culture media, a sterilized glass tube (5 mm diameter) was used aseptically to scoop out the media to make wells. Two drops (0.1 ml) of the sample solution were transferred to these wells aseptically. These were then incubated at 37±1°C for 24 hours. Control cups contained DMSO only. The experiments were carried out in triplicate. The result (mean value n=3) were recorded by measuring the zone of growth inhibition around the cups in mm at 100 µg/ml concentration and compared with standard drug norfloxacin. The statistical analysis was carried out using student t-test, and the % of growth inhibition was calculated by taking norfloxacin as positive control with 100% inhibitions for Gram +ve and Gram-ve bacteria. Some of the compounds showed very good activity as

compare to the standard drug were selected for determination of MIC. This same procedure adopted for antifungal activity. Fungal strain (ATCC No.) including *Aspergillus niger* (9029); *Candida albicans* (2091); *Aspergillus fumigates* (36607) has been utilized for antifungal activity. The zone of inhibition (% inhibition) and MIC for standard drug i.e. clotrimazole and for synthesized compounds were determined by using same procedure as described under antibacterial screening.

***Anti-inflammatory activity*[19]**

All the synthesized compounds were screened for acute anti-inflammatory activity by using carrageenan induced rat paw edema model[20]. Male albino rats of either sex weighing (170-220) g of either sex used. The animals were divided in to four groups of six each. They were starvated overnight with water ad libitium prior to the day of experiment. Control groups received 1ml of 0.5% sodium carboxymethyl cellulose (sodium CMC), standard group received 20 mg/kg ibuprofen and test groups were received 100, 200 mg/kg of synthesized compounds orally. One hour later; a sub planar injection of 0.05 ml of 1% solution of carrageenan in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 0, 1, 2, 3, 4, and 5 hr. after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as % edema inhibition = 1-Vt/Vc x 100

Where: Vt and Vc were oedema volume in the drug treated and the control groups respectively. The results were expressed as percentage inhibition of edema over the untreated control group.

## RESULTS

***Chemistry***

The Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in the scheme of synthesis (Fig. 2). Potassium dithiocarbazinate salt (2) was obtained from the reaction of isonicotinic acid hydrazides (1) with carbon disulfide in basic medium (KOH) and converted into 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (3) by the treatment with hydrazine hydrate. The synthesis of the other compounds was performed by the reaction of 2 with seven different benzaldehyde to form 4 [(substituted phenyl)-methylene]-amino-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-thiol (4). The final compounds were synthesized by the reaction of 3 with four different acetanilide resulting in the formation of 4-[substituted phenyl)-methylene]-amino-3-(N-substituted carboxamidomethylthio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles (5). The elemental analysis data of synthesized compounds are given in Table 1. Synthesized compounds were characterized by IR, $^{1}$HNMR, $^{13}$CNMR, LC-MS (FAB) and elemental analysis. All compounds were shown the solubility in DMSO, ethanol , and acetonitrile and least in methanol & acetone.

***Antibacterial activity***

*Among all compounds PJ-D4, PJ-D9, PJ-D11 and PJ-D13 shows more than 90% and remaining shows more than 50% and less than 70% of zone of inhibition against both Gram positive and Gram-negative organisms. Among all these compounds PJ-D1, PJ-D4, PJ-D7, PJ-D9, PJ-D11 and PJ-D13 shows excellent MIC against both Gram positive and Gram-negative organisms compare to standard Norflaxacin. All Data of Antibacterial activity of synthesized compounds was depicted in Table 2 & 3.*

**Table 2: Antibacterial Activity of Synthesized Compound at 100 µg/mL**

| S. No. | Code No. | *S. aureus* (ATCC-12598) | | *B. subtilis* (ATCC-6051) | | *P.aeruginosa* (ATCC-25619) | | *E. coli* (MTCC-25922) | |
|---|---|---|---|---|---|---|---|---|---|
| | | In mm mean | % of Inhibition | In mm mean | % of Inhibition | In mm mean | % of Inhibition | In mm mean | % of Inhibition |
| 1. | PJ-$C_1$ | 18.0±2.0 | 64.4 | 19.66±1.15 | 63.1 | 17.00±2.00 | 62.0 | 16.66±1.15 | 63.1 |
| 2. | PJ-$C_2$ | 24.3±0.5 | 85.3 | 25.66±0.57 | 86.48 | 23.33±1.15 | 84.57 | 24.66±1.52 | 93.8 |
| 3. | PJ-$C_3$ | 25.0±1.0 | 87.23 | 26.00±1.00 | 89.65 | 24.00±2.00 | 82.7 | 25.00±0.57 | 94.9 |
| 4. | PJ-$C_4$ | 26.6±1.5 | 94.7 | 22.33±1.15 | 74.2 | 21.00±1.00 | 72.6 | 19.66±2.08 | 74.5 |
| 5. | PJ-$C_5$ | 24.6±1.1 | 82.00 | 25.66±3.05 | 84.1 | 25.00±2.00 | 90.38 | 24.33±0.57 | 93.7 |
| 6. | PJ-$C_6$ | 20.0±2.6 | 67.1 | 21.66±1.15 | 72.1 | 20.00±1.73 | 74.1 | 18.66±2.08 | 70.0 |
| 7. | PJ- $C_7$ | 18.6±0.5 | 62.2 | 20.00±2.00 | 67.40 | 19.33±0.57 | 69.88 | 18.33±1.15 | 69.23 |
| 8. | PJ- $C_8$ | 19.0±1.0 | 67.5 | 21.66±2.08 | 65.5 | 18.66±0.58 | 66.6 | 17.66±1.15 | 65.0 |
| 9. | PJ- $C_9$ | 24.6±1.1 | 84.00 | 25.00±2.00 | 84.7 | 23.66±1.52 | 85.15 | 22.00±2.00 | 85.6 |
| 10. | PJ-$C_{10}$ | 21.3±0.5 | 75.08 | 24.00±1.00 | 82..5 | 22.66±2.64 | 81.4 | 23.33±2.30 | 88.41 |
| 11. | PJ-$C_{11}$ | 24.3±0.5 | 82.0 | 22.33±0.57 | 56.3 | 19.66±1.52 | 70.3 | 18.33±2.30 | 69.8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12. | PJ-$C_{12}$ | 21.3±2 | 75.08 | 21.33±0.57 | 74.6 | 18.33±1.53 | 66.26 | 19.00±1.00 | 72.6 |
| 13. | PJ-$C_{13}$ | 26.6±0.5 | 94.7 | 26.66±1.15 | 89.0 | 25.66±3.21 | 92.5 | 25.33±2.88 | 96.16 |
| Norfloxacin | | 28.6±1.1 | 100.0 | 29.67±1.15 | 100.0 | 27.66±1.15 | 100.0 | 26.33±0.57 | 100.0 |
| DMSO | | 8.33±1.15 | 10.3 | 8.33±0.57 | 14.1 | 7.33±1.00 | 10.3 | 8.00±1.00 | 14.1 |

**Table 3: Minimum Inhibitory Concentration of Some Selected Compounds (Antibacterial Activity)**

| S. No | Code No. | MIC in μg/ml | | | |
|---|---|---|---|---|---|
| | | ***S.aureus*** **(ATCC-12598)** | ***B.subtilis*** **(ATCC-6051)** | ***P.aeruginosa*** **(ATCC-25619)** | ***E.coli*** **(MTCC-25922)** |
| 1. | PJ-$C_4$ | 20 | 30 | 50 | 45 |
| 2. | PJ-$C_7$ | 35 | 45 | 65 | 80 |
| 3. | PJ-$C_9$ | 25 | 40 | 60 | 50 |
| 4. | PJ-$C_{11}$ | 35 | 40 | 65 | 55 |
| 5. | PJ-$C_{12}$ | 25 | 35 | 45 | 12 |
| 6. | PJ-$C_{13}$ | 4 | 6 | 55 | 60 |
| Norfloxacin | | 20 | 30 | 50 | 50 |

***Anti-inflammatory activity***

In the synthesized compounds, compound no. PJ-C4, PJ-C9 and PJ-C12 shows significant anti-inflammatory ranging from 70 to 80% edema inhibition compare to standard drug Ibuprofen among that PJ-C9 shows excellent activity that is 80% of edema inhibition. Remaining showed moderate to weak activity.

**Table 4: Anti-inflammatory activity of synthesized compounds**

| Compound code | | Change in paw odema volume after treatment in mL (± SEM) | | Percentage inhibition of odema after treatment | |
|---|---|---|---|---|---|
| | | 3h | 5h | 3h | 5h |
| Solvent control (0.5 % CMC) (1ml/kg) | | 0.60±0.03 | 0.62±0.03 | - | - |
| Ibuprofen (20mg/kg)b.o | | 0.14±0.03 | 0.10±0.03 | 76.6 | 83.3 |
| PJ-$C_1$ | 100mg/kg p,o | 0.40±0.02 | 0.37±0.03 | 33.3 | 38.3 |
| | 200 mg/kg p.o | 0.35±0.02 | 0.33±0.03 | 41.6 | 45.0 |
| PJ-$C_2$ | 100mg/kg p,o | 0.29±0.02 | 0.22±0.04 | 51.0 | 63.3 |
| | 200 mg/kg p,o | 0.23±0.03 | 0.20±0.03 | 61.6 | 66.6 |
| PJ-$C_3$ | 100mg/kg p,o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| | 200 mg/kg p,o | 0.26±0.02 | 0.23±0.03 | 56.6 | 61.6 |
| PJ-$C_4$ | 100mg/kg p,o | 0.21±0.03 | 0.18±0.04 | 65.0 | 70.0 |
| | 200 mg/kg p,o | 0.17±0.02 | 0.13±002 | 70.0 | 78.0 |
| PJ-$C_5$ | 100mg/kg p,o | 0.23±0.03 | 0.20±0.03 | 61.6 | 66.6 |
| | 200 mg/kg p,o | 0.21±0.02 | 0.18±0.03 | 65.0 | 70 |
| PJ-$C_6$ | 100mg/kg p,o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| | 200 mg/kg p,o | 0.26±0.02 | 0.23±0.03 | 56.6 | 61.6 |
| PJ-$C_7$ | 100mg/kg p,o | 0.26±0.02 | 0.23±0.03 | 56.6 | 61.6 |
| | 200 mg/kg p,o | 0.23±0.03 | 0.19±0.03 | 61.6 | 68.0 |
| PJ-$C_8$ | 100mg/kg p,o | 0.23±0.03 | 0.20±0.03 | 61.6 | 66.6 |
| | 200 mg/kg p,o | 0.19±0.03 | 0.17±0.02 | 68.0 | 70 |
| PJ-$C_9$ | 100mg/kg p,o | 0.18±0.04 | 0.14±2.3 | 70.0 | 76.6 |
| | 200 mg/kg p,o | 0.16±002 | 0.12±002 | 73.3 | 80.0 |
| PJ-$C_{10}$ | 100mg/kg p,o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| | 200 mg/kg p,o | 0.23±0.03 | 0.20±0.03 | 61.6 | 66.6 |
| PJ-$C_{11}$ | 100mg/kg p,o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| | 200 mg/kg p,o | 0.26±0.03 | 0.22±0.03 | 56.6 | 63 |
| PJ-$C_{12}$ | 100mg/kg p,o | 0.23±0.03 | 0.20±0.03 | 61.6 | 66.6 |
| | 200 mg/kg p,o | 0.19±0.03 | 0.17±0.02 | 68.0 | 70.0 |

| | | | | | |
|---|---|---|---|---|---|
| PJ-$C_{13}$ | 100mg/kg p,o | 0.21±0.02 | 0.18±0.03 | 65.0 | 70 |
| | 200 mg/kg p,o | 0.17±0.02 | 0.13±002 | 70.0 | 78.3 |

**Values are mean ± SEM, no of animals in each group are (n = 6); *P value <0.05**

***Anti-Fungal Activity***

The antifungal activity of PJ-C2, PJ-C4, PJ-C7, PJ-C9 and PJ-C11 shows more than 90%, PJ-C3, PJ-C7 and PJ-C12 shows more than 80% and rest of compounds shows more than 50 and less than 70% of zone of inhibition. Among all these compounds PJ-C4 and PJ-C13 shows excellent MIC against all fungal strains compare to standard clotrimazole. Antifungal activity of synthesized compounds was depicted in Table 5 & 6.

**Table 5: Antifungal Activity of the Synthesized Compounds at 100 μg/mL**

| S. No. | Code No. | Zone of Inhibition at concentration (100 μg/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***Aspergillus niger*** **MTCC-1344** | | ***Candida Albican*** **MTCC-227** | | ***Fusarium oxysporum*** **MTCC-129** | |
| | | **In mm mean** | **% of Inhibition** | **In mm mean** | **% of Inhibition** | **In mm mean** | **% of Inhibition** |
| 1. | PJ-$C_1$ | 15.0±1.0 | 68.0 | 13.33±3.0 | 56.52 | 13.00±3.0 | 61.90 |
| 2. | PJ-$C_2$ | 20.6±1.5 | 93.63 | 21.66±3.7 | 92.96 | 19.66±1.1 | 90.41 |
| 3. | PJ-$C_3$ | 18.33±2.0 | 83.3 | 19.33±0.5 | 82.96 | 17.33±1.5 | 80.23 |
| 4. | PJ-$C_4$ | 21.66±1.5 | 98.0 | 22.66±4.5 | 97.25 | 21.00±2.0 | 97.22 |
| 5. | PJ-$C_5$ | 15.33±0.5 | 69.98 | 16.66±2.5 | 74.70 | 15.00±2.6 | 71.4 |
| 6. | PJ-$C_6$ | 13.33±1.5 | 60.05 | 14.66±2.8 | 62.97 | 13.66±3.0 | 61.90 |
| 7. | PJ- $C_7$ | 19.0±1.0 | 86.0 | 20.66±1.0 | 88.66 | 18.66±1.0 | 85.71 |
| 8. | PJ- $C_8$ | 12.00±3.0 | 52.13 | 12.66±5.0 | 54.33 | 11.66±1.5 | 52.38 |
| 9. | PJ- $C_9$ | 21.00±2.0 | 95.0 | 22.33±1.5 | 95.83 | 20.66±1.1 | 96.71 |
| 10. | PJ-$C_{10}$ | 17.0±3.0 | 77.27 | 16.66±1.6 | 74.70 | 16.33±1.5 | 76.19 |
| 11. | PJ-$C_{11}$ | 20.3±2.5 | 92.0 | 21.66±2.0 | 92.96 | 20.00±2.6 | 92.59 |
| 12. | PJ-$C_{12}$ | 18.00±3.0 | 81.81 | 19.66±1.1 | 84.37 | 18.66±2.5 | 85.71 |
| 13. | PJ-$C_{13}$ | 21.66±2.3 | 98.45 | 22.66±0.5 | 97.25 | 20.66±2.5 | 95.23 |
| Clotrimazole | | 22.00±1.0 | 100.0 | 23.3±0.57 | 100.0 | 21.66±2.082 | 100.0 |
| DMSO | | 8.0±1.0 | 18.8 | 7.66±0.58 | 16.3 | 8.33±0.57 | 21.3 |

**Table 6: Minimum Inhibitory Concentration of Some Selected Compounds (Antifungal Activity)**

| S. No | Code No. | MIC in μg/ml | | |
|---|---|---|---|---|
| | | ***A. nigers*** **(MTCC-1344)** | ***C. albican*** **(MTCC-227)** | ***F. oxysporum*** **(MTCC-129)** |
| 1. | PJ-$C_4$ | 60 | 30 | 50 |
| 2. | PJ-$C_7$ | 35 | 40 | 60 |
| 3. | PJ-$C_9$ | 70 | 40 | 60 |
| 4. | PJ-$C_{11}$ | 70 | 45 | 60 |
| 5. | PJ-$C_{12}$ | 65 | 35 | 55 |
| 6. | PJ-$C_{13}$ | 60 | 30 | 50 |
| Clotrimazole | | 12 | 6 | 10 |

## DISCUSSION

***Antibacterial activity***

The data revealed that *p*-chloro and *m*-hydroxy substituted compounds shows excellent activity against all tested organisms. The presence of electron withdrawing group such as chloro in para position enhance the liphophilicity of the molecule enabling it to penetrate the microbial cell ore easily and shows the good activity. The presence of OH group on aromatic ring increases the hydrogen bonding of the compound with bacterial cell wall proteins containing free SH group and therefore it shows good activity. Un-substituted

and methoxy substituted compounds shows least activity.

***Anti-inflammatory activity***

4-chloro, 2,4-dichloro, 2,6-dichloro and hydroxyl substituted on benzyledene moiety shows weak degree of anti-inflammatory activity. It may be because of the presence of trifluoromethyl along with nitro group in para-position of benzene ring which is present on 3rd position of triazole moiety. Presence of electron withdrawing groups like chloro in para position in benzelidine ring shows more activity compare to at ortho and meta position. presence of two chlorine group at ortho and para position ie 2,4-dichloro shows more activity. Unsusbstituted benzelidine ring and presence of electron donating group on benzelidine ring of triazole moiety such as methoxy group shows decreases in activity. Compounds with bulkier substitution such as nitro in para position along with trifluoro methyl group on phenyl ring 3rd position of triazole moiety showed moderate to weak activity.

***Anti-Fungal Activity***

The data revealed that *p*-chloro and *o*-hydroxy substituted compounds shows excellent activity against all tested organisms. the presence of electron withdrawing group such as Chloro in para position enhance the liphophilicity of the molecule enabling it to penetrate the microbial cell more easily and shows the minimum inhibitory concentration compare to standard clotrimazole and almost equal to standard. The presence of OH group on aromatic ring increases the hydrogen bonding of the compound with fungal cell wall proteins containg free SH group and therefore it shows good activity. Un-substituted and methoxy substituted compounds shows least activity. Electron withdrawing group in meta position shows less MIC compare to standard.

## CONCLUSION:

The isoniazid based 1,2,4-triazoles derivatives has been synthesized and spectral analysis data denoted that the compound is synthesized as they design. These entire synthesized compounds evaluated for the antibacterial and anti-inflammatory activity. Isoniazid based 1,2,4-triazole derivatives has shown good antibacterial and but weak anti-inflammatory activity.

## REFERENCES:

1. Ahmadi F, Ghayahbashi MR, Sharifzadeh M, Alipoiur E, Ostad SN, Vosooghi M, khademi HR, Amini M. Synthesis and evaluation of anti-inflammatory and analgesic activities of new 1,2,4-triazole derivatives. Med Chem. 2014;11(1):69-76.
2. Aktay G, Tozkoparan B, Ertan M, Investigation of antioxidant properties of some 6-(α-aminobenzyl)-thiazolo[3,2-b]-1,2,4-triazole-5-ol compounds. J Enzyme Inhib Med Chem, 2009;24(3):898-902.
3. Bayrak H, Demirbas A, Demirbas N, Karaoglu SA. Synthesis of some new 1,2,4-triazoles starting from isonicotinic acid hydrazide and evaluation of their antimicrobial activities. *Eur J Med Chem,* 2009;44:4362–4366.
4. El-Sherief HAM, Youssif BGM, Bukhari SNA, Abdel-Aziz M, Abdel-Rahman HM. Novel 1,2,4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. Bioorg Chem. 2018; 76:314-325.
5. Fan Z, Yang Z, Zhang H, Mi N, Wang H, Cai F, Zuo X, Zheng Q, Song H. Synthesis, Crystal Structure, and Biological Activity of 4-Methyl-1,2,3-thiadiazole-Containing 1,2,4-Triazolo[3,4-b][1,3,4] thiadiazoles. J Agric Food Chem, 2010,58(5):2630-2636.
6. Gajanan S, Sandyavalli MS., Manjunath E, Kavitha NV. Synthesis and Anti-inflammatory Activity of 3,5-Diphenyl 4-amino 1,2,4 -Triazole Derivatives. World Journal of Pharmaceutical Research, 2017;6(8):1744-1754.
7. Hany MD, Khaled E, Ali ZS, Ahmmed AS. Synthesis and biological activities of some new spiro 1,2,4-triazole derivatives having sulfonamide moiety. Organic communications, 2017; 10(4):280-287.
8. Hasan MJ, Adnan IM, Abass SJ. Antibacterial Activity Importance of 1,2,3-triazole and 1,2,4-triazole by Click Chemistry. Journal of Chemistry and Chemical Sciences, 2015;5(6):317-324.
9. Joshi SD, Vagdevi HM, Vaidya VP, Gadaginamath GS. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular agents. Eur J Med Chem, 2008;43:1989-1996.
10. Kumar A, Kashaw V, Amit V., Kashaw SK, Sahu P, Mishra V. Synthesis, characterization and anti-mycobaterial activity of some newer 1,2,4-triazole derivatives. International Journal of Pharmacy and Biological Research, 2015; 5(2):379-392.
11. Kumar A, Mishra V, Kashaw V. Synthesis and antimicrobial activity some new 1,2,4-triazoles. Asian Journal of Chemistry, 2017:1317-1322.
12. Ledeti I, Bercean V, Alexa A, Foica C, Futa LM, Dehelean C, Trandafirescu C, Muntean D, Licker M, Fulia A.

Preparation and Antibacterial Properties of Substituted 1,2,4-Triazoles. Journal of Chemistry, 2015;5:879-886.

13. Li-Jung Min, Yan-Xia Shi, Hong-Ke Wu Sun, Xing-Hai Liu, Yong-Gang Zhang. Microwave assisted synthesis and antifungal activity of some Novel thioethers containg 1,2,4-triazole Moiety. Applied Sciences, 2015;5:1211-1220.
14. Mehdi K, Mahboobeh S, Iraj N, Seyedeh RB. Synthesis, Characterization and Antibacterial Activity of some Novel Thiosemicarbazides, 1,2,4-Triazol-3-thiols and their *S*-substituted Derivatives. Iranian Journal of Pharmaceutical Research, 2015;14 (1):67-75.
15. Paprocka R, Wiese M, Eljaszewicz A, Helmin-Basa A, Gzella A, Modzelewska-Banachiewicz B, Michalkiewicz J. Synthesis and anti-inflammatory activity of new 1,2,4-triazole derivatives. Bioorg Med Chem Lett, 2015;25(13):2664-7.
16. Rajoriya V, Kashaw V, Kashsaw SK. Design, Synthesis, Characterization and Antitubercular Screening of some New 1,2,4-Triazoles Derived from Isonicotinic Acid Hydrazides. Letters in Drug Design & Discovery, 2018;15(5):451-462.
17. Silvia S, Olga B, Angelo R, Francesco B and Walter F. Synthesis and anti-inflammatory activity of esters derived from 5-aryl-1,2-dihydro-2-(2-hydroxyethyl)-3H-1,2,4-triazole-3-thiones. Il Farmaco 53, 1998, 590-593.
18. Singh S, Kashaw SK, Mishra VK, Mishra M, Rajoriya V, Kashaw V. Design and Synthesis of New Bioactive 1,2,4-Triazoles, Potential Antitubercular and Antimicrobial Agents. Indian J Pharm Sci, 2018;80(1):36-45.
19. Somani RR, Toraskar MP, Mali KK, Naik PP, Shirodkar PY. Synthesis of some Antifungal and Anti-tubercular 1, 2, 4-Triazole Analogues. Int J ChemTech Res, 2009; 1(2):168-173.
20. Yasmin K, Mohammad S, Vijender S, Mohammad S. Synthesis, Characterization and Anticonvulsant Activity of Some Novel 4,5-Disubstituted-1,2,4-Triazole Derivatives. Journal of Applied Pharmaceutical Science, 2017;7(7):158-167.

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