



SYNTHESIS, ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF SOME BIOACTIVE 1, 2, 4-TRIAZOLES ANALOGUES

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ABSTRACT

Objective: The objective is to screen antimicrobial and anti-inflammatory activity of some novel 1, 2, 4-triazole based isonicotinic acid hydrazide derivatives. **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 compounds 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 4-nitro-2-trifluoro methyl acetanilide to form 4-[substituted phenyl]-methylene]-amino-3-(N-substitutedcarboxamidomethylthio)-5-(pyridine-4-yl)-4H-1, 2, 4-triazoles derivatives. All these compounds characterized by IR, ¹H NMR, ¹³C NMR and elemental analysis. The antibacterial and antifungal activity was determined by the cup plate method. Acute anti-inflammatory activity determined by using carrageenan induced rat paw edema model. **Result:** Antibacterial activity of 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 compare to standard Norfloxacin. In Antifungal activity compound no. PJ-D4, PJ-D9, PJ-D11 and PJ-D13 showed more than 80% and rest of compounds shows more than 50 and less than 70% of zone of inhibition against clotrimazole. Among the compounds PJ-D2, PJ-D4, PJ-D5, PJ-D7, PJ-D8, PJ-D9, PJ-D10, PJ-D11 and PJ-D13 shows excellent anti-inflammatory activity up to 70 to 78% of paw edema inhibition compare to standard drug Ibuprofen which shows 83.3% after 5 hr. Among all these compounds only PJ-D13 shows excellent MIC against all fungal strains compare to standard drug. **Conclusion:** All these results suggested that the isoniazid based 1,2,4-triazole derivatives has shown good antibacterial and anti-inflammatory activity.

KEY WORDS

1,2,4-triazoles, Norfloxacin, antifungal, anti-inflammatory, elemental analysis, Zone of inhibition.

INTRODUCTION

Bacterial resistance to antimicrobial is recognized by the WHO as major health threat of the 21st century. Microbial infections are one of the leading cause of death worldwide. Drug used to treat T.B., malaria, leprosy etc. are becoming less effective with time. Bacterial resistance is driven by the continued use of antimicrobial and it is unlikely that the treat of resistance can be managed if we do not discover new compounds. In this work we synthesized some triazole

derivatives 1, 2, 4-triazoles derivatives 4- [substitutedphenyl)-methylene]-amino-3-(N-substituted-carboxamid methylthio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles in hope to get anti-microbial and anti-inflammatory properties. This paper discusses the most common and useful procedure for synthesizing 4-amino-3-mercapto-1,2,4-triazoles.

Literature survey indicates that triazole derivatives of isoniazid have been synthesized and tested for anti-inflammatory activity¹. The test compounds inhibited the induction of gastric mucosal lesions and their

protective effects may be related to inhibition of lipid peroxidation in gastric mucosa². Prompted by these findings, it seemed of interest to synthesize new derivatives of 1,2,4-triazole and investigate their anti-inflammatory activity.

We synthesized di-substituted 1,2,4-triazoles derive from isonicotinic acid hydrazides were used as starting material by replacing 4-carbohydrazide group of isoniazid by substituting 1,2,4-triazole. This was done to

get combination response of pyridine nucleus (A) and 1,2,4-triazole nucleus (B) towards antibacterial³ and anti-inflammatory activity (Fig. 1). 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 positions of the triazole nucleus.

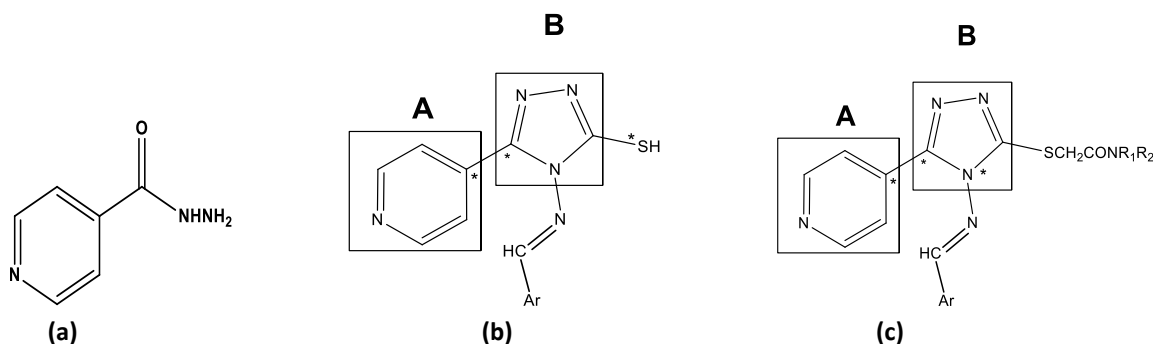


Fig. 1: Proposed pictorial representation of the proposed hypothesis. a) Chemical structure of the Isoniazid b) and c) are proposed compounds scheme for the designing of the 1,2,4-triazole compounds.

EXPERIMENTAL

MATERIAL AND METHODS

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 4-acetamido acetophenone 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. ¹H NMR and ¹³C 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 salt⁴

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-thiol⁵

Potassium dithiocarbazinate salt (2) (0.079 mol) reacted with aqueous hydrazine hydrate (12 mL, 0.24 mol) solution and refluxed for 4h, hydrogen sulfide (H₂S 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)

⁶ 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-triazoles⁷

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 4-acetamido acetophenone (0.01 mol) compound were dissolved in ethanol and added with shaking at 55°C stirred 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).

Table 1: Elemental Analysis of synthesized compounds

Compound code	Molecular formula	Molecular weight	Elemental analysis % found (calculated)		
			C	H	N
PJ-D ₁	C ₂₃ H ₁₆ F ₃ N ₇ O ₃ S	527.5	52.37 (52.36)	3.06 (3.05)	18.59 (18.60)
PJ-D ₂	C ₂₃ H ₁₅ ClF ₃ N ₇ O ₃ S	543.0	49.16 (49.19)	2.69 (2.66)	17.45 (17.43)
PJ-D ₃	C ₂₃ H ₁₅ ClF ₃ N ₇ O ₃ S	543.0	49.16 (49.19)	2.69 (2.66)	17.45 (17.43)
PJ-D ₄	C ₂₃ H ₁₅ ClF ₃ N ₇ O ₃ S	543.0	49.16 (49.19)	2.69 (2.66)	17.45 (17.43)
PJ-D ₅	C ₂₃ H ₁₅ BrF ₃ N ₇ O ₃ S	606.37	45.56 (45.55)	2.49 (2.48)	16.17 (16.18)
PJ-D ₆	C ₂₃ H ₁₅ BrF ₃ N ₇ O ₃ S	606.37	45.56 (45.55)	2.49 (2.48)	16.17 (16.18)
PJ-D ₇	C ₂₄ H ₁₈ F ₃ N ₇ O ₃ S	541.51	53.23 (53.20)	3.35 (3.34)	18.11 (18.10)
PJ-D ₈	C ₂₄ H ₁₈ F ₃ N ₇ O ₄ S	557.5	51.70 (51.68)	3.25 (3.23)	17.59 (17.56)
PJ-D ₉	C ₂₃ H ₁₄ Cl ₂ F ₃ N ₇ O ₃ S	596.37	46.32 (46.30)	2.37 (2.36)	16.44 (16.42)
PJ-D ₁₀	C ₂₃ H ₁₄ Cl ₂ F ₃ N ₇ O ₃ S	596.37	46.32 (46.30)	2.37 (2.36)	16.44 (16.42)
PJ-D ₁₁	C ₂₅ H ₂₁ F ₃ N ₈ O ₃ S	570.56	52.63 (52.60)	3.71 (3.69)	19.64 (19.63)
PJ-D ₁₂	C ₂₃ H ₁₅ F ₄ N ₇ O ₃ S	545.47	50.64 (50.61)	2.77 (2.75)	17.97 (17.96)
PJ-D ₁₃	C ₂₃ H ₁₆ F ₃ N ₇ O ₃ S	543.48	50.83 (50.84)	2.97 (2.98)	18.04 (18.02)

PJ-D1:2,4-(benzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2-trifluoromethyl)-4-nitrophenyl)acetamide

PJ-C1:2,4-(benzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetyl phenyl) acetamide

Molecular formula: C₂₄H₂₀N₆O₂S; Molecular weight: 456; TLC (R_f value): 0.66; IR (cm⁻¹, KBr): 3250 N-H str; 3050 C-H str; 2990 C-H str; 1720 C=O; 1660 C=O str; 1420 SCH₂ str; 1586 C=N str; 1435 C-H def; 1480 C-C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 680 C-S str; ¹H NMR (DMSO-d₆, δ ppm): 7.78 s, 1H, -N=CH; 6.9 s, 1H, NH; 7.25-8.17 m, 13H, aromatic protons; 4.20 s, 2H, SCH₂; 3.0 s, 3H CH₃; ¹³C NMR (DMSO-d₆, δ ppm): 152.2

N=CH; 162.2 C=O of amide; 182.2 C=O of methyl; 136 C1 of pyridine ring; 122 C2 of pyridine ring; 152 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 135 C1 of benzene ring; 138 C2 & C6 of benzene ring; 129 C3 & C5 of benzene ring; 128 C4 of benzene ring; 42.5 SCH₂; 28.2 CH₃; Mass (m/z): 456

PJ-C2: 2,4-(2-chlorobenzylideneamino)-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₁₉Cl N₆O₂S; Molecular weight: 490; TLC (R_f value) 0.66; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2980 C-H str; 1700 C=O; 1660 C=O str; 1410 SCH₂ str; 1586 C=N str; 1430 C-H def; 1480 C-

C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 680 C-S str; 750 C-Cl str; 1H NMR (DMSO-d₆, δ ppm): 6.78 s, 1H, -N=CH; 6.5 s, 1H, NH; 7.25-8.97 m, 12H, aromatic protons; 4.20 s, 2H, SCH₂; 3.5 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 157.2 N=CH; 166.2 C=O of amide; 184.2 C=O of methyl; 136.2 C1 of pyridine ring; 127.1 C2 of pyridine ring; 152.2 C3 & C5 of pyridine ring; 148 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 135.3 C1 of benzene ring; 113 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 128 C4 of benzene ring; 42.75 SCH₂; 28.2 CH₃; Mass (m/z): 490.

PJ-C3: 2,4-(3-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₁₉Cl N₆O₂S; Molecular weight: 490; TLC (R_f value): 0.66; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2980 C-H str; 1700 C=O str; 1660 C=O str; 1410 SCH₂ str; 1586 C=N str; 1430 C-H def; 1480 C-C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 680 C-S str; 750 C-Cl str; 1H NMR (DMSO-d₆, δ ppm): 6.78 s, 1H, -N=CH; 6.5 s, 1H, NH; 7.25-8.97 m, 12H, aromatic protons; 4.20s, 2H, SCH₂; 3.5 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 157.2 N=CH; 166.2 C=O of amide; 184.2 C=O str.; 136.2 C1 of pyridine ring; 127.1 C2 of pyridine ring; 152.2 C3 & C5 of pyridine ring; 148 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 135.3 C1 of benzene ring; 113 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 128 C4 of benzene ring; 42.75 SCH₂; 28.2 CH₃. Mass (m/z): 490.

PJ-C4: 2,4-(4-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₁₉Cl N₆O₂S; Molecular weight: 490; TLC (R_f value): 0.69; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2990 C-H str; 1710 C=O; 1670 C=O str; 1410 SCH₂ str; 1586 C=N str; 1430 C-H def; 1480 C-C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 680 C-S str; 750 C-Cl str; 1H NMR (DMSO-d₆, δ ppm): 6.9s, 1H, -N=CH; 7.25-8.90 m, 12H, aromatic protons; 4.25 s, 2H, SCH₂; 3.7 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 158.2 N=CH; 168.2 C=O of amide; 180.2 C=O of methyl; 135.2 C1 of pyridine ring; 128.1 C2 of pyridine ring pyridine; 152.2 C3 & C5 of pyridine ring; 150 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 135 C1 of benzene ring; 113 C2 & C6 of benzene ring; 138 C3 & C5 of benzene ring; 128 C4 of benzene ring; 42.7 SCH₂; 28.2 CH₃; Mass (m/z): 490

PJ-C5: 2,4-(2-bromobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl)acetamide

Molecular formula: C₂₄H₁₉BrN₆O₂S; Molecular weight: 534; TLC (R_f value): 0.65; IR (cm⁻¹, KBr): 3360 N-H str; 3150 C-H str; 2980 C-H str; 1710 C=O; 1678 C=O str; 1420 SCH₂ str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 690 C-S str; 650 C-Br str; 1H NMR (DMSO-d₆, δ ppm): 6.97 s, 1H, -N=CH; 7.19 s, 1H, NH; 7.20-8.19 m, 12H, aromatic protons; 4.3 s, 2H, SCH₂; 3.08 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 159.2 N=CH; 172.2 C=O of amide; 190.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring pyridine; 145.2 C3 & C5 of pyridine ring; 150.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 123 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122 C4 of benzene ring; 40.0 SCH₂; 28.0 CH₃; Mass (m/z): 534

PJ-C6: 2,4-(3-bromobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₁₉BrN₆O₂S; Molecular weight: 534; TLC (R_f value): 0.67; IR (cm⁻¹, KBr): 3360 N-H str; 3150 C-H str; 2980 C-H str; 1710 C=O; 1678 C=O str; 1420 SCH₂ str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1650 C=C str; 1390 C-N str; 1580 C=N str; 690 C-S str; 650 C-Br str; 1H NMR (DMSO-d₆, δ ppm): 6.98 s, 1H, -N=CH; 7.18 s, 1H, NH; 7.20-8.2 m, 12H, aromatic protons; 4.32 s, 2H, SCH₂; 3.08 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 159.2 N=CH; 172.2 C=O of amide; 190.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 123 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122 C4 of benzene ring; 40.0 SCH₂; 28.0 CH₃; Mass (m/z): 534.

PJ-C7: 2,4-(4-methylbenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₅H₂₂N₆O₂S; Molecular weight: 470; TLC (R_f value): 0.73; IR (cm⁻¹, KBr): 3060 N-H str; 3150 C-H str; 2980 C-H str; 1680 C=O; 1710 C=O str; 1420 SCH₂ str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1550 C=C str; 1340 C-N str; 1580 C=N str; 690 C-S str; 1H NMR (DMSO-d₆, δ ppm): 8.97 s, 1H, -N=CH; 7.89 s, 1H, NH; 7.02-8.6 m, 12H, aromatic protons; 3.82s, 2H, SCH₂; 2.88 s, 3H CH₃; 3.12s, 3H CH₃ of benzene; 13C NMR (DMSO-d₆, δ ppm): 158.6 N=CH; 170.2 C=O of amide;

192.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 123 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 40.75 SCH2; 28.0 CH3, Mass (m/z): 470.

PJ-C8: 2,4-(4-methoxybenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₅H₂₂N₆O₃S; Molecular weight: 487; TLC (R_f value): 0.65; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2990 C-H str; 168 C=O; 1720 C=O str; 1420 SCH2 str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1550 C=C str; 1340 C-N str; 1580 C=N str; 690 C-S str; 1H NMR (DMSO-d₆, δ ppm): 8.90 s, 1H, -N-CH; 8.6 s, 1H, NH; 7.02-8.6 m, 12H, aromatic protons; 3.82 s, 2H, SCH2; 2.88 s, 3H CH₃; 3.32 s, 3H of OCH₃; 13C NMR (DMSO-d₆, δ ppm): 160.6 N=CH; 170.2 C=O of amide; 192.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 145.2 C3 & C5 of pyridine ring; 150.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 130 C1 of benzene ring; 122 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 43.5 SCH2; 28.0 CH₃, Mass (m/z): 487.

PJ-C9: 2,4-(2,4-dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₅H₂₂Cl₂N₆O₂S; Molecular weight: 526; TLC (R_f value): 0.67; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2980 C-H str; 1660 C=O; 1700 C=O str; 1420 SCH2 str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1570 C=C str; 1340 C-N str; 1580 C=N str; 680 C-S str; 720 C-Cl; 1H NMR (DMSO-d₆, δ ppm): 9.70 s, 1H, -N=CH; 8.59s, 1H, NH; 7.62-8.19 m, 11H, aromatic protons; 4.62 s, 2H, SCH2; 3.28 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 157.6 N=CH; 168.2 C=O of amide; 196.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 155.2 C3 & C5 of pyridine ring; 152.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 130 C1 of benzene ring; 122 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 42.5 SCH2, 28.8 CH₃, Mass (m/z): 526

PJ-C10: 2,4-(2,6-dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₅H₂₂Cl₂N₆O₂S; Molecular weight: 526; TLC (R_f value): 0.68; IR (cm⁻¹, KBr): 3350 N-H str; 3100 C-H str; 2980 C-H str; 1660 C=O; 1700 C=O

str; 1420 SCH2 str; 1570 C=N str; 1430 C-H def; 1480 C-C str; 1570 C=C str; 1340 C-N str; 1580 C=N str; 680 C-S str; 720 C-Cl str; 1H NMR (DMSO-d₆, δ ppm): 9.72 s, 1H, -N=CH; 8.59 s, 1H, NH; 7.62-8.19 m, 11H, aromatic protons; 4.62 s, 2H, SCH2; 3.28 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 157.6 N=CH; 168.2 C=O of amide; 196.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 155.2 C3 & C5 of pyridine ring; 152.2 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 122 C2 & C6 of benzene ring; 158 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 42.5 SCH2; 28.8 CH₃, Mass (m/z): 526.

PJ-C11: 2,4-(N,N-dimethyl aminobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₆H₂₅N₇O₂S; Molecular weight: 500; TLC (R_f value): 0.71; IR (cm⁻¹, KBr) : 3350 N-H str; 3100 C-H str; 3068 C-H str; 1680 C=O; 1710 C=O str; 1480 SCH2 str; 1570 C=N str; 1450 C-C str; 1589 C=C str; 1340 C-N str; 1589 C=N str; 695 C-S str; 1H NMR (DMSO-d₆, δ ppm): 8.72 s, 1H, -N=CH; 8.09 s, 1H, NH; 7.62-8.69 m, 11H, aromatic protons; 4.2 s, 2H, SCH2; 3.28 s, 3H CH₃; 3.22 s, 6H N(CH₃)₂; 13C NMR (DMSO-d₆, δ ppm): 158.6 N=CH; 172.2 C=O of amide; 186.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 155.2 C3 & C5 of pyridine ring; 150.8 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 122 C2 & C6 of benzene ring; 152 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 42.5 SCH2; 28.6 CH₃; 26.3 N-CH₃; Mass (m/z): 500

PJ-C12: 2,4-(4-fluorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₁₉FN₆O₂S; Molecular weight: 474; TLC (R_f value): 0.76; IR (cm⁻¹, KBr): 3250 N-H str; 3150 C-H str; 3128 C-H str; 1680 C=O; 1700 C=O str; 1410 SCH2 str; 1570 C=N str; 1430 C-H def; 1440 C-C str; 1589 C=C str; 1320 C-N str; 1589 C=N str; 655 C-S str; 1200 C-F str; 1H NMR (DMSO-d₆, δ ppm): 9.72 s, 1H, -N=CH; 8.1 s, 1H, NH; 7.32-8.79 m, 11H, aromatic protons; 4.1 s, 2H, SCH2; 3.2 s, 3H CH₃; 13C NMR (DMSO-d₆, δ ppm): 157.6 N=CH; 168.2 C=O of amide; 186.2 C=O of methyl; 138.2 C1 of pyridine ring; 123.3 C2 of pyridine ring; 155.8 C3 & C5 of pyridine ring; 152.8 C2 of 1,2,4-triazole ring; 148 C5 of 1,2,4-triazole ring; 136 C1 of benzene ring; 123 C2 & C6 of benzene ring; 132 C3 & C5 of benzene ring; 122 C4 of benzene ring; 42.5 SCH2; 28.6 CH₃ methyl ketone; Mass (m/z): 474

PJ-C13: 2,4-(3-hydroxybenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(4-acetylphenyl) acetamide

Molecular formula: C₂₄H₂₀N₆O₃S; Molecular weight: 472; TLC (R_f value): 0.78; IR (cm⁻¹, KBr): 3420 O-H str; 3350 N-H str; 3100 C-H str; 2987 C-H str; 1660 C=O; 1710 C=O str; 1410 SCH₂ str; 1570 C=N str; 1400 C-H def; 1470 C-C str; 1589 C=C str; 1340 C-N str; 1580 C=N str; 680 C-S str; ¹H NMR (DMSO-d₆, δ ppm): 9.77 s, 1H, -N=CH; 8.59

s, 1H, NH 7.62-8.19 m, 11H, aromatic protons; 4.6 s, 2H, SCH₂; ¹³C NMR (DMSO-d₆, δ ppm): 155.6 N=CH; 162.2 C=O of amide; 180.2 C=O of methyl; 125.2 C1 of pyridine ring; 128.3 C2 of pyridine ring; 155.2 C3 & C5 of pyridine ring; 150.8 C2 of 1,2,4-triazole ring; 149 C5 of 1,2,4-triazole ring; 132 C1 of benzene ring; 122 C2 & C6 of benzene ring; 152 C3 & C5 of benzene ring; 122.7 C4 of benzene ring; 40.5 SCH₂; 28.6 CH₃; Mass (m/z): 472.

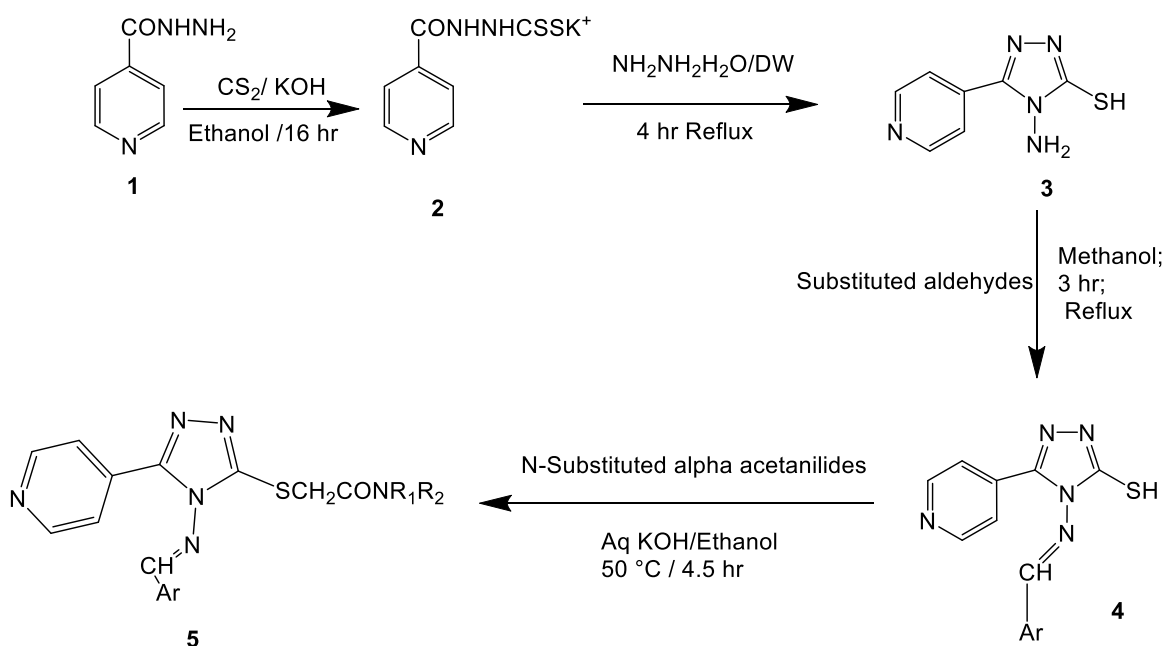


Fig 2: Scheme for the synthesis; Reagent and reaction condition: I) CS₂, ethanolic KOH, reflux 16 h; II) NH₂-NH₂·H₂O, reflux, 4h; III) aromatic aldehyde, methanol, reflux, 3h; IV) acetanilide, Aq. KOH, ethanol, 55°C

BIOLOGICAL ACTIVITY

Antibacterial and Antifungal activity⁸

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-10⁷-10⁹/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. This same procedure adopted for antifungal activity. Fungal strain (ATCC No.) *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 activity⁹

All the synthesized compounds were screened for acute anti-inflammatory activity by using carrageenan

induced rat paw edema model¹⁰. 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 libitum 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 = $1 - \frac{V_t}{V_c} \times 100$

Where: V_t and V_c 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 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 acetanilide resulting in the formation of 4-[substituted phenyl]-methylene]-amino-3-(N-substitutedcarboxamidomethylthio)-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, ¹HNMR, ¹³CNMR, 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-C4 and PJ-C13 shows more than 90%, PJ-C2, PJ-C5, PJ-C9 and PJ-C11 shows 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 PJ-C4 and PJ-C13 shows excellent MIC against both Gram positive and Gram-negative organisms compare to standard Norfloxacin. 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.	<i>S. aureus</i> (ATCC-12598)		<i>B. subtilis</i> (ATCC-6051)		<i>P. aeruginosa</i> (ATCC-25619)		<i>E. coli</i> (MTCC-25922)	
		In mm mean	% of Inhibition	In mm mean	% of Inhibition	In mm mean	% of Inhibition	In mm mean	% of Inhibition
1.	PJ-D ₁	16.3±1.5	57.30	15.33±0.57	51.66	18.66±1.52	66.6	17.33±0.57	65.9
2.	PJ-D ₂	21.3±2	75.08	25.66±1.15	86.3	24.33±1.15	87.96	23.66±1.52	88.5
3.	PJ-D ₃	20.3±2.5	67.91	22.66±1.15	75.80	21.00±2.00	75.9	22.66±2.08	84.7
4.	PJ-D ₄	25.0±1.5	87.22	22.33±1.15	75.26	23.00±1.00	83.10	24.66±2.08	93.5
5.	PJ-D ₅	20.3±1.1	67.91	25.66±3.05	86.20	24.00±2.00	86.8	24.33±0.57	92.7
6.	PJ-D ₆	20.0±2.6	67.03	21.66±1.15	72.4	18.00±1.73	65.07	20.66±2.08	83.0
7.	PJ-D ₇	17.6±0.5	59.31	18.00±2.00	62.0	16.33±0.57	59.3	18.33±1.15	69.23
8.	PJ-D ₈	18.0±1.0	62.80	20.66±2.08	67.2	17.66±0.58	62.9	18.66±1.15	71.0
9.	PJ-D ₉	23.6±1.	82.18	25.00±2.00	84.23	22.66±1.52	81.0	23.00±2.00	87.6
10.	PJ-D ₁₀	22.3±0.5	77.91	18.0±1.0	62.80	17.66±0.58	62.9	18.33±1.15	69.23
11.	PJ-D ₁₁	23.3±0.5	82.14	22.33±0.57	77.0	21.66±1.52	77.7	19.33±2.30	73.0
12.	PJ-D ₁₂	19.0±2.0	66.29	21.33±0.57	71.89	20.33±1.53	74.0	19.00±1.00	73.6
13.	PJ-D ₁₃	23.3±0.5	82.14	26.66±1.15	89.06	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±1.1	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			
		<i>S. aureus</i> (ATCC-12598)	<i>B. subtilis</i> (ATCC-6051)	<i>P. aeruginosa</i> (ATCC-25619)	<i>E. coli</i> (MTCC-25922)
1.	PJ-D ₁	50	60	75	65
2.	PJ-D ₄	45	55	80	70
3.	PJ-D ₇	40	50	70	60
4.	PJ-D ₉	45	55	80	70
5.	PJ-D ₁₁	30	40	60	50
6.	PJ-D ₁₃	30	40	65	60
	Norfloxacin	4	16	10	8

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 (\pm SEM)		Percentage inhibition of odema after treatment	
	3h	5h	3h	5h
Solvent control (0.5 % CMC) (1ml/kg)	0.60 \pm 0.03	0.62 \pm 0.03	-	-
Ibuprofen (20mg/kg)b.o	0.14 \pm 0.03	0.10 \pm 0.03	76.6	83.3
PJ-D ₁ 100mg/kg p,o	0.36 \pm 0.05	0.33 \pm 0.04	40.0	45.0
200 mg/kg p,o	0.30 \pm 0.06	0.28 \pm 0.03	50.0	53.0
PJ-D ₂ 100mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
200 mg/kg p,o	0.20 \pm 0.03	0.17 \pm 0.02	66.6	70.0
PJ-D ₃ 100mg/kg p,o	0.29 \pm 0.05	0.26 \pm 0.04	51.6	56.6
200 mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
PJ-D ₄ 100mg/kg p,o	0.20 \pm 0.03	0.17 \pm 0.02	66.6	70.0
200 mg/kg p,o	0.17 \pm 0.02	0.13 \pm 0.02	70.0	78.3
PJ-D ₅ 100mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
200 mg/kg p,o	0.20 \pm 0.03	0.16 \pm 0.02	66.6	73.3
PJ-D ₆ 100mg/kg p,o	0.29 \pm 0.05	0.26 \pm 0.04	51.6	56.6
200 mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
PJ-D ₇ 100mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
200 mg/kg p,o	0.19 \pm 0.03	0.17 \pm 0.02	68.0	70.0
PJ-D ₈ 100mg/kg p,o	0.20 \pm 0.03	0.17 \pm 0.02	66.6	70.0
200 mg/kg p,o	0.19 \pm 0.03	0.16 \pm 0.02	68.0	73.3
PJ-D ₉ 100mg/kg p,o	0.20 \pm 0.03	0.17 \pm 0.02	66.6	70.0
200 mg/kg p,o	0.16 \pm 0.02	0.13 \pm 0.02	73.3	78.3
PJ-D ₁₀ 100mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
200 mg/kg p,o	0.19 \pm 0.03	0.16 \pm 0.02	68.0	73.3
PJ-D ₁₁ 100mg/kg p,o	0.23 \pm 0.03	0.20 \pm 0.03	61.6	66.6
200 mg/kg p,o	0.20 \pm 0.03	0.16 \pm 0.02	66.6	73.3
PJ-D ₁₂ 100mg/kg p,o	0.26 \pm 0.03	0.22 \pm 0.03	56.6	63
200 mg/kg p,o	0.20 \pm 0.03	0.19 \pm 0.03	66.6	68.0
PJ-D ₁₃ 100mg/kg p,o	0.19 \pm 0.03	0.16 \pm 0.02	68.0	73.3
200 mg/kg p,o	0.16 \pm 0.02	0.13 \pm 0.02	73.3	78.3

Values are mean \pm 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)					
		<i>Aspergillus niger</i> MTCC-1344		<i>Candida Albican</i> MTCC-227		<i>Fusarium oxysporum</i> MTCC-129	
		In mm mean	% of Inhibition	In mm mean	% of Inhibition	In mm mean	% of Inhibition
1.	PJ-D ₁	10.66±2.0	48.45	11.33±1.1	47.8	10.66±1.5	47.61
2.	PJ-D ₂	16.00±2.0	72.27	16.33±2.5	69.56	15.00±1.0	69.44
3.	PJ- D ₃	14.00±0.0	63.63	15.66±2.0	67.21	13.66±1.5	61.0
4.	PJ-D₄	18.33±3.5	83.31	19.33±3.6	82.83	18.66±1.5	85.71
5.	PJ-D ₅	12.33±0.5	56.04	14.33±0.5	60.08	13.00±2.0	61.0
6.	PJ-D ₆	10.33±0.5	46.95	13.33±1.5	57.0	12.33±3.0	56.09
7.	PJ-D ₇	17.00±1.0	77.27	18.00±1.0	77.25	17.00±3.0	78.48
8.	PJ-D ₈	11.66±1.0	53.0	12.66±3.2	53.3	10.66±2.3	47.76
9.	PJ-D₉	19.33±1.5	87.86	20.66±3.2	88.66	18.66±3.2	85.71
10.	PJ-D ₁₀	17.00±1.0	77.27	17.33±3.7	74.37	16.66±1.5	76.1
11.	PJ-D₁₁	19.33±0.5	87.86	20.66±3.2	88.66	19.66±3.00	90.41
12.	PJ-D ₁₂	17.0±1.0	77.27	19.66±0.5	84.37	17.66±1.5	80.95
13.	PJ-D₁₃	19.0±1.0	86.36	20.66±1.5	88.66	18.66±1.2	85.71
	Clotrimazole	22.00±1.0	100.0	23.3±0.57	100.0	21.66±2.08	100.0
	DMSO	8.0±1.0	18.8	7.66±0.58	16.3	8.33±0.57	21.3

Table 5: Minimum Inhibitory Concentration of Some Selected Compounds (Antifungal Activity)

S. No	Code No.	MIC in µg/ml		
		<i>A. nigers</i> (MTCC-1344)	<i>C. albican</i> (MTCC-227)	<i>F. oxysporum</i> (MTCC-129)
1.	PJ-D ₁	80	50	60
2.	PJ-D ₄	60	45	65
3.	PJ-D ₇	70	45	65
4.	PJ-D ₉	70	45	65
5.	PJ-D ₁₁	80	50	65
6.	PJ-D ₁₃	60	30	11
	Clotrimazole	-	12	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 lipophilicity 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

The data revealed that 4-chloro, 2,4-dichloro, 2,6-dichloro and hydroxyl substituted on benzylidene

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 benzylidene ring shows more activity compare to at ortho and meta position. presence of two chlorine group at ortho and para position i.e. 2,4-dichloro shows more activity. Unsubstituted benzylidene ring and presence of electron donating group on benzylidene 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 lipophilicity 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 contain free SH group and therefore it shows good activity. Unsubstituted 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, antifungal and anti-inflammatory activity. Isoniazid based 1,2,4-triazole derivatives has shown good antibacterial and but weak anti-inflammatory activity.

REFERENCE:

1. 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.
2. Santhosh S, Sini TK, Anandan R, Mathew PT. Effect of chitosan supplementation on antitubercular drugs.induced hepatotoxicity in rats. *Toxicology*, 2006;219:53-59.
3. Noha RM, Sh.El-Etrwy A. Design, Synthesis and Biological evaluation of some 1,2,4-triazole derivatives based on Schiff's base bearing Benzimidazole moiety *Asian Journal of Pharmaceutical analysis and medicinal chemistry*, 2015;3(2):62-75.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. Toma A, Mogoşan C, Vlase L, Leonte D, Zaharia V, Heterocycles. Synthesis, characterization and evaluation of the anti-inflammatory activity of thiazolo[3,2-b][1,2,4]triazole derivatives bearing pyridin-3/4-yl moiety. *Med Chem Res.*, 2017;39: 1-12.
10. 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.

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