DESIGN, SYNTHESIS, AND EVALUATION OF NEW DERIVATIVE OF 1,2,4-TRIAZOLE FOR ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY

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Original Article

ABSTRACT

Objective: The object of the study is to design, synthesize and biological evaluation of isoniazid derived 1,2,4-triazoles compounds.

Methods: Isoniazid based 1,2,4-triazoles derivatives have been synthesized by reaction of Isoniazid with carbon disulfide in basic medium (KOH) to form Potassium dithiocarbamate as salt and reaction with hydrazine hydrate converted into 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol. These compounds were reacted with seven different benzaldehyde to form 4-[(substituted phenyl)-methylene]-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol. The final compounds were synthesized by reaction with four different acetanilide to form 4-[(substituted phenyl)-methylene]-amino-3-(N-substituted-carboxamidomethylthio)-5-(pyridin-4-yl)-4H-1,2,4-triazole derivatives. All these compounds were characterized by IR, 1H-NMR, 13C-NMR and elemental analysis. The antimicrobial activity was determined by using carrageenan-induced rat paw edema model. Acute anti-inflammatory activity determined by using carrageenan-induced rat paw edema model.

Results: Series PJ-A4, PJ-A7 and PJ-A13 showed more than 90% of the zone of inhibition against both Gram positive and Gram negative organisms. The antifungal study suggested that among synthesized compounds series PJ-A4, A7, A9, A11 and A13 showed more than 90% of zone of inhibition, A2, A10 and A12 showed more than 90% of the zone of inhibition. Anti-inflammatory study data indicate that compounds PJ-A4, PJ-A8, PJ-A9 and PJ-A13 produced 70 to 76% of paw edema inhibition compared to standard drug Ibuprofen which showed 83.3% after 5 h.

Conclusion: Results suggested that the isoniazid based 1,2,4-triazole derivatives have significant antibacterial, antifungal and anti-inflammatory activity.

Keywords: 1,2,4-triazole, Zone of inhibition, Anti-inflammatory activity, Antimicrobial activity

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), primarily important for the treatment of inflammation and pain in arthritis. NSAIDs act by inhibiting PGs synthesis by blockage of enzyme cyclooxygenase-1. Two isomers recognized as cyclooxygenase COX-1 and COX-2. COX-1, is an isomer found in kidneys, GI tract, and platelets and used for hemostatic maintenance of the kidney and GI tract; but COX-2 is produced during injury. The drawback of 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 (NSAID) [Mishra et al. 2008]. 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 carboxyl acid group and inhibition of prostaglandin biosynthesis in the GIT.

Isoniazid is used as first-line treatment of tuberculosis and shown to be more effective as 1,2,4-triazoles derivatives to encounter inflammation, antibacterial and antimicrobial agents. This hurdle is to rectify by synthesis of the 1,2,4-triazoles derivatives that have more stable in structure. 1,2,4-triazoles has shown anti-tubercular, antimicrobial hypoglycemic, anti-inflammatory, antibacterial, antiallergic, antitumor, analgesic and antidepressant activities. A considerable amount of research activities are directed towards potent, more specific and less toxic anti-inflammatory agents and offers a challenging task in the development of novel synthetic strategies.

In the 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, thiadiazole and triazine derivatives of Indometacin have been synthesized and tested for anti-inflammatory activity. 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. Prompted by these findings, it seemed of interest to synthesize new derivatives of 1,2,4-triazole and investigate their anti-inflammatory activity.

The object of the current research is to synthesize new 1,2,4-triazoles derivatives of isoniazid as potent antimicrobial and anti-inflammatory agents. In continuation with the above researches we proposed to synthesized some triazole derivatives to design and synthesize new 1,2,4-triazole derivatives 4-[substituted phenyl]-methylene]-amino-3-[N-substituted-carboxamidomethylthio]-5-(pyridin-4-yl)-4H-1,2,4-triazoles which were expected to show 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. In the present design, we synthesized newer di-substituted 1,2,4-triazoles derived from isonicotinic acid hydrazides (isoniazid) by replacing a 4-carboxyhydrizide group of isoniazid by substituting 1,2,4-triazole in hope of getting a synergistic response of pyridine nucleus (A) and 1,2,4-triazole nucleus (B) towards antibacterial, antifungal 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 complementary to the two nitrogen atoms present at the 1 and 2 position of the triazole nucleus.

MATERIALS 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-toluic acid, p-nitrobenzaldehyde, Cinnamaldehyde) were...
purchased from the CDH, New Delhi, India. and Alpha-chloroacetanilides compounds (2-chloro-2,6-dimethylanilines, (4-chloroacetyl)-morpholine, 4-bromo-2,2'-dichloroacetanilides, 2-chloro-N,N-dimethylacetanilides) were 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 [jeolSX-102 (FAB) by ESI.

![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](image)

**Synthesis of potassium dithiocarbazinate salt**

Isocitonic acid hydrazide (0.10 mol) [1] was reacted with an ethanolic solution of potassium hydroxide (0.15 mol) along with carbon disulfide (0.15 mol) 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**

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 then lead acetate soaked filter paper convert white to black. The compound 3 exist in thione-thiol tautomeric forms, but our investigation showed that in this case, the thiol structure obtained was used in the next step without further purification.

**Synthesis of 4-[(substituted phenyl)-methylene]-amino-5-(pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (4a-4g)**

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-carboxamidimethyl thio)-5-(pyridine-4-yl)-4H-1,2,4-triazoles**

Compound 1,2,4-triazole-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 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]. In the case of aliphatic N-substituted-α-chloroacetanilides, the amide was added at room temperature.

**Pj-A1: 2,4-(benzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)(N-(2,6-dimethyl phenyl)acetamide**

Molecular formula: C24H22N6 OS; Molecular weight: 442.54; TLC (Rf value): 0.62; IR (cm-1, KBr): 3231(NH str), 3144(C-H str), 3028(C-H str), 1642.2(CONH str), 1396(SHC2 str), 1607.7(C=C str), 1439(C-H def), 1450(C-C str), 1650(C=C str), 1317(C-N str), 1530(C=N str), 688(C-S str), 442(SHC2), 1329.2-2.44 ([(CH3)2], 154.3(N=CH), 19.2 [-C(NH)2], 131.7 (C1 of 2,6-dimethylphenyl ring), 130.7 (C2 and C6 of 2,6-dimethylphenyl ring), 127.7 (C3 and C5 of 2,6-dimethylphenyl ring), 121.3 (C2 of pyridine), 149.8 (C3 and C5 of pyridine ring), 134.0 (C6 of pyridine ring), 150 (C of 1,2,4-triazole ring), 135.3 (C1 of benzene ring), 129.2 (C2 and C6 of benzene ring), 128.8 (C3 and C5 of benzene ring), 131.0 (C4 of benzene ring), 38.5 (SC2H) mass (m/z): 442.158

**Pj-A2: 2,4-(2-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)(N-(2,6-dimethyl phenyl)acetamide**

Molecular formula: C24H22N6 OS; Molecular weight: 476.94; TLC (Rf value): 0.65; IR (cm-1, KBr): 3231(NH str), 3109(C-H str), 3035(C-H str), 1646(CONH str), 1396(SHC2 str), 1609(C=C str), 1430(C-H def), 1450(C-C str), 1650(C=C str), 1317(C-N str), 1540(C-N str), 688(C-S str), 754.2(C-C str), 1HM (DMSO-d6, 6 ppm): 9.85 (NH); 9.0 (NH); 4.38 (SCH2); 2.20-2.24(C3,NH); 5.42 (C=O of NH2); 131.7 (C1 of 2,6-dimethylphenyl ring); 131 (C2 and C6 of 2,6-dimethylphenyl ring); 125 (C of pyridine ring); 121.3 (C2 of pyridine ring); 150 (C of 1,2,4-triazole ring); 135.3 (C1 of benzene ring); 129.2 (C2 and C6 of benzene ring); 128.8 (C3 and C5 of benzene ring); 131.0 (C4 of benzene ring); 38.5 (SC2H) mass (m/z): 442.158

**Pj-A3: 2,4-(3-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)(N-(2,6-dimethyl phenyl)acetamide**

Molecular formula: C24H22N6 OS; Molecular weight: 476.94; TLC (Rf value): 0.68; Elemental Analysis: Nitrogen % (Found/Calc): 17.62
PJ-A4: 2,4-(4-chlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2,6-dimethylphenyl)acetamide

Molecular formula: C24H12ClN6OS; Molecular weight: 476.17; TLC (Rf value): 0.65; IR (cm−1, KBr): 3213 (N-H str.); 3109 (C-H str.); 3033 (C-H str.); 1646 (C=N str.); 1396 (SCH2 str.); 1203 (C=Br str.); 1H NMR (DMSO-d6, δ ppm): 9.82 (N=CH); 9.04 (NH); 7.63-8.89 aromatic protons; 4.40 SCH2; 3.24 OCH3; 2.2-2.3 (CH3)2. 13C NMR (DMSO-d6, δ ppm): 154.3 (N=CH); 170.2 (C=O of amide); 153.1 (C1 of 36-dimethylphenyl ring); 130.7 (C2 and C6 of 2,6-dimethylphenyl ring); 127.5 (C3 and C5 of 2,6-dimethylphenyl ring); 134.1 (C1 of pyridine ring); 124.9 (C3 and C5 of pyridine ring); 150.9 (C=Br of pyridine ring); 139.8 (C2 and C6 of benzene ring); 131.8 (C3 and C5 of benzene ring); 140.4 (C4 of benzene ring); 40.5 (SCH2); 17.9 (CH3 of 2,6-dimethyl phenyl). Mass (m/z): 476.

PJ-A5: 2,4-(2-bromobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2,6-dimethylphenyl)acetamide

Molecular formula: C24H21BrN6OS; Molecular weight: 520.06; TLC (Rf value): 0.74; IR (cm−1, KBr): 3210 (N-H str.); 3035 (C-H str.); 1680 (C=N str.); 1503 (C=O of amide); 1458 (C-C str.); 1353 (C-N str.); 1538 (C=O of amide); 87.04 cm−1 C=O str.; 1H NMR (DMSO-d6, δ ppm): 10.22 (N=CH); 8.09 (NH); 7.3-8.2 aromatic protons; 4.40 SCH2; 2.0-2.12 (CH3)2. 13C NMR (DMSO-d6, δ ppm): 150.3 (N=CH); 172.2 (C=O of amide); 130.1 (C1 of 2,6-dimethylphenyl ring); 128.7 (C2 and C6 of 2,6-dimethylphenyl ring); 125.2 (C3 and C5 of 2,6-dimethylphenyl ring); 130.1 (C1 of pyridine ring); 120.3 (C2 of pyridine ring pyridine); 148.3 (C3 and C5 of pyridine ring); 151.2 (C2 of 1,2,4-triazole ring); 159.5 (C=Br of pyridine ring); 131.4 (C1 of benzene ring); 124.6 (C2 and C6 of benzene ring); 130.2 (C3 and C5 of benzene ring); 140.9 (C4 of benzene ring); 41.5 SCH2; 17.9 CH3 of 2,6-dimethyl phenyl. Mass (m/z): 472.56.

PJ-A9: 2,4-(2,4-dichlorobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2,6-dimethylphenyl)acetamide

Molecular formula: C24H20Cl2N6OS; Molecular weight: 511.10; TLC (Rf value): 0.49; IR (cm−1, KBr): 3210.15-H str.; 3037-H str.; 1680 C=O str.; 1503 C-N str.); 1538 (C=O of amide); 868 (C-S str.); 1H NMR (DMSO-d6, δ ppm): 10.22 (N=CH); 8.19 (NH); 7.3-8.2 aromatic protons; 4.40 SCH2; 2.0-2.12 (CH3)2. 13C NMR (DMSO-d6, δ ppm): 150.3 (N=CH); 172.2 (C=O of amide); 130.1 (C1 of 2,6-dimethylphenyl ring); 128.7 (C2 and C6 of 2,6-dimethylphenyl ring); 125.2 (C3 and C5 of 2,6-dimethylphenyl ring); 130.1 (C1 of pyridine ring); 120.3 (C2 of pyridine ring pyridine); 140.9 (C3 and C5 of pyridine ring); 147.2 (C2 of 1,2,4-triazole ring); 155 (C5 of 1,2,4-triazole ring); 125.4 (C1 of benzene ring); 126.6 (C2 and C6 of benzene ring); 122.2 (C3 and C5 of benzene ring); 138.4 (C4 of benzene ring); 35.5 SCH2; 20.3 CH3 of 2,6-dimethyl phenyl. Mass (m/z): 511.06.

PJ-A10: 2,4-(2,6-dimethylaminobenzylideneamino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2,6-dimethylphenyl)acetamide

Molecular formula: C24H21ClN6OS; Molecular weight: 511.10; TLC (Rf value): 0.48; IR (cm−1, KBr): 3218-N-H str.; 3038 C-H str.; 3020 C=H str.; 1682 CONH str.; 1589 C=O str.; 1622 C-N str.; 3037 C=O str.; 1458 C=O str.; 1353 C=O str.; 1538 C-N str.; 868 C-S str.; 787 C=O str.; 1H NMR (DMSO-d6, δ ppm): 10.22 (N=CH); 8.19 (NH); 7.3-8.2 aromatic protons; 4.42 SCH2; 2.2-2.3 (CH3)2. 13C NMR (DMSO-d6, δ ppm): 150.3 (N=CH); 172.2 (C=O of amide); 130.1 (C1 of 2,6-dimethylphenyl ring); 128.7 (C2 and C6 of 2,6-dimethylphenyl ring); 125.2 (C3 and C5 of 2,6-dimethylphenyl ring); 130.1 (C1 of pyridine ring); 121.3 (C2 of pyridine ring pyridine); 140.9 (C3 and C5 of pyridine ring); 147.2 (C2 of 1,2,4-triazole ring); 155 (C5 of 1,2,4-triazole ring); 125 (C1 of benzene ring); 126.2 (C2 and C6 of benzene ring); 122.2 (C3 and C5 of benzene ring); 138.4 (C4 of benzene ring); 35.5 SCH2; 20.3 CH3 of 2,6-dimethyl phenyl. Mass (m/z): 511.06.

PJ-A11: 2,4-(4-N dimethylamino-benzylidene) amino)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2,6-dimethyl phenyl)acetamide

Molecular formula: C26H21N7O7S; Molecular weight: 486; TLC (Rf value): 0.72; IR (cm−1, KBr): 3350-N-H str.; 3108 C-H str.; 3068 C-H str.; 1672 CONH str.; 1490 SCH2 str.; 1612 C-N str.; 1439 C=H def.; 1478 C=C str.; 1630 C=O str.; 1340 C-N str.; 1588 C-N str.; 695 C-S str.; 1H NMR (DMSO-d6, δ ppm): 10.3-N=CH; 8.19 (NH); 7.3-8.6 aromatic protons; 4.42 SCH2; 3.22 (CH3)2. 13C NMR (DMSO-d6, δ ppm): 158.3 (N=CH); 172.2 (C=O of amide); 138.1 (C1 of 2,6-dimethylphenyl ring); 128.7 (C2 and C6 of 2,6-dimethylphenyl ring); 132.2 (C3 and C5 of 2,6-dimethylphenyl ring); 135 (C1 of pyridine ring); 120.3 (C2 of pyridine ring); 150.9 (C3 and C5 of benzene ring).
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 benzaldehydes to form 4-[substituted phenyl]-methylene]-amino]-5-(pyridine-4-yl)-4H-1,2,4-triazole-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]-5-[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. Elemental analysis data of 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 and acetone.

1H NMR, 13C NMR, LC-MS (FAB) and elemental analysis. All compounds were shown the solubility in DMSO, ethanol and acetonitrile and least in methanol and acetone.

![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 ºC](image-url)

**J-A12**: 2,4-(3-Hydroxybenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thio)-N-(2,6-dimethyl phenyl)acetamide

Molecular formula: C24H23N6OS; Molecular weight: 459; TLC (Rf value): 0.69; IR (cm−1, KBr): 3420 O-H, Str.; 3350-N-H Str.; 3008 C-H str.; 2948 C-H str.; 1720 C=O of amide; 1703 C=O of amide; 1592 C=N str.; 1520 C=O of amide; 1381 C1 of 2,6-dimethylphenyl ring; 1257 C2 of benzene ring; 25.6 CH3 of 2,6 dimethyl phenyl; Mass (m/z): 459.02.

**PJ-A13**: 2,4-(3-Hydroxybenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thio)-N-(2,6-dimethyl phenyl)acetamide

Molecular formula: C24H22 FN6OS; Molecular weight: 462; TLC (Rf value): 0.70; IR (cm−1, KBr): 3150-N-H Str.; 3028 C-H str.; 2948 C-H str.; 1720 C=O of amide; 1703 C=O of amide; 1592 C=N str.; 1520 C=O of amide; 1381 C1 of 2,6-dimethylphenyl ring; 1257 C2 of benzene ring; 25.6 CH3 of 2,6 dimethyl phenyl; Mass (m/z): 462.02.

**Anti-inflammatory activity**

All the synthesized compounds were screened for acute anti-inflammatory activity by using carrageenan-induced rat paw edema model (Winter et al., 1962). Male albino rats of either sex weighing (170-220) g of either sex used. The animals were divided into four groups of six each. They were starved overnight with water ad libitum prior to the day of the experiment. Control groups received 1 ml of 0.5% sodium carboxymethylcellulose (sodium CMC), the standard group received 20 mg/kg i.p. 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:

\[ \% \text{edema inhibition} = \frac{1-V_t/V_c}{100} \]

Where: Vt and Ve were edema 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 benzaldehydes to form 4-[substituted phenyl]-methylene]-amino]-5-(pyridine-4-yl)-4H-1,2,4-triazole-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]-5-[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. Elemental analysis data of 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 and acetone.
Among all compounds, compound no. PJ-A4, PJ-A7 and PJ-A13 shows more than 50% and less than 70% of zone of excellent MIC against both Gram positive and Gram negative organisms compare to standard Norflaxacin. Data of antibacterial activity is shown in table 2.

**Antifungal activity**

Among all compounds, compound no. PJ-A4, A7, A9, A11 and A13 shows more than 90% of zone of inhibition against all fungi. A2, A10 and A12 shows more than 80% of zone of inhibition and rest of compounds shows more than 50% and less than 70% of inhibition against all organisms. Among all these compounds only A13, shows excellent MIC against all fungal strains compared to standard norflaxacin. Data of antifungal activity is shown in table 3.

**Anti-inflammatory activity**

Among all the synthesized compounds, compound no. PJ-A4, PJ-A8, PJ-A9 and PJ-A13 showed significant anti-inflammatory activity 70 to 76% of paw edema inhibition compared to standard ibuprofen which shows 83.3% after 5 h. Remaining compounds showed moderate to weak anti-inflammatory activity. The anti-inflammatory activity of these compounds attributed to the inhibition of cyclooxygenase enzyme which plays vital role in the inflammation process.
Table 4: Antifungal activity of the synthesized compounds at 100 µg/ml

| S. No. | Code No. | Zone of inhibition at concentration (20 µg/ml) | Aspergillus niger MTCC-1344 | Candida Albicans MTCC-227 | Fusarium oxysporum MTCC-129 |
|--------|----------|---------------------------------------------|-----------------------------|---------------------------|--------------------------------|
|        |          | In mm mean | % of Inhibition | In mm mean | % of Inhibition | In mm mean | % of Inhibition |
| 1      | PJ-A₁    | 12.00±1.0 | 54.45          | 13.33±3.0 | 56.52          | 11.00±3.0 | 59.20          |
| 2      | PJ-A₂    | 18.66±1.5 | 84.48          | 20.66±3.7 | 88.66          | 18.66±1.1 | 85.71          |
| 3      | PJ-A₃    | 17.33±2.0 | 78.77          | 18.33±0.5 | 78.26          | 16.33±1.5 | 75.46          |
| 4      | PJ-A₄    | 20.66±1.5 | 93.6            | 22.33±4.5 | 96.65          | 21.00±2.0 | 96.95          |
| 5      | PJ-A₅    | 15.33±0.5 | 69.6            | 16.66±2.5 | 71.50          | 15.00±2.6 | 69.4           |
| 6      | PJ-A₆    | 14.00±1.5 | 63.3            | 15.33±2.8 | 65.21          | 14.66±3.0 | 66.66          |
| 7      | PJ-A₇    | 20.66±1.0 | 93.8            | 22.00±1.0 | 95.56          | 20.33±1.0 | 93.98          |
| 8      | PJ-A₈    | 12.00±3.0 | 54.0            | 12.00±5.0 | 51.0           | 11.66±1.5 | 53.70          |
| 9      | PJ-A₉    | 21.00±2.0 | 95.48           | 22.33±1.5 | 95.65          | 20.66±1.1 | 95.23          |
| 10     | PJ-A₁₀   | 18.33±3.0 | 83.8            | 20.66±1.6 | 88.66          | 17.33±1.5 | 80.23          |
| 11     | PJ-A₁₁   | 20.33±2.5 | 92.40           | 21.66±2.0 | 92.70          | 20.00±2.6 | 92.59          |
| 12     | PJ-A₁₂   | 19.00±3.0 | 86.30           | 20.33±1.1 | 86.95          | 18.66±2.5 | 85.71          |
| 13     | PJ-A₁₃   | 20.66±2.3 | 93.63           | 21.66±0.5 | 92.96          | 20.66±2.5 | 95.3           |
|        | 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 5: Minimum inhibitory concentration of some selected compounds (antifungal activity)

| S. No. | Code No. | MIC in µg/ml | A. niger's (MTCC-1344) | C. albican (MTCC-227) | F. oxysporum (MTCC-129) |
|--------|----------|--------------|------------------------|-----------------------|------------------------|
| 1      | PJ-A₁    | 13           | 7                      | 11                    |                        |
| 2      | PJ-A₇    | 13           | 7.5                    | 12                    |                        |
| 3      | PJ-A₉    | 13           | 8                      | 12                    |                        |
| 4      | PJ-A₁₁   | 14           | 8                      | 12                    |                        |
| 5      | PJ-A₁₂   | 6            | 7                      | 12                    |                        |
| 6      | PJ-A₁₃   | 12           | 6                      | 10                    |                        |
|        | Clotrimazole | 12          | 6                      | 10                    |                        |

Table 6: Anti-inflammatory activity of synthesized final compounds

| Compound code | Change in paw edema volume after treatment in ml (± SEM) | Percentage inhibition of edema after treatment |
|---------------|--------------------------------------------------------|-----------------------------------------------|
|               | 3h                                                      | 5h                                            |
| Solvent control (0.5% CMC) [1 ml/kg] | 0.60±0.03 | 0.62±0.03 | - | - |
| Ibuprofen (20 mg/kg) b. o | 0.14±0.03 | 0.10±0.03 | 76.6 | 83.3 |
| PJ-A₁ (100 mg/kg) (200 mg/kg) | 0.42±0.02 | 0.40±0.03 | 30.0 | 33.33 |
| PJ-A₁ (100 mg/kg) p.o | 0.26±0.04 | 0.24±0.03 | 56.66 | 60.0 |
| 200 mg/kg p.o | 0.21±0.03 | 0.18±0.04 | 65.0 | 70.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.29±0.02 | 0.28±0.04 | 0.29±0.04 | 0.28±0.02 |
| 200 mg/kg p.o | 0.20±0.02 | 0.23±0.02 | 51.66 | 53.3 |
| PJ-A₁ (100 mg/kg) p.o | 0.20±0.02 | 0.17±0.03 | 66.66 | 71.6 |
| 200 mg/kg p.o | 0.18±0.02 | 0.15±0.02 | 70.0 | 75.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.32±0.05 | 0.30±0.03 | 46.6 | 50.0 |
| 200 mg/kg p.o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| PJ-A₁ (100 mg/kg) p.o | 0.36±0.02 | 0.30±0.03 | 40.0 | 45.0 |
| 200 mg/kg p.o | 0.32±0.05 | 0.30±0.03 | 46.6 | 50.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.24±0.04 | 0.21±0.05 | 60.0 | 65.0 |
| 200 mg/kg p.o | 0.21±0.03 | 0.18±0.04 | 65.0 | 70.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.24±0.04 | 0.21±0.05 | 60.0 | 65.0 |
| 200 mg/kg p.o | 0.21±0.03 | 0.18±0.04 | 65.0 | 70.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.18±0.02 | 0.15±0.02 | 70.0 | 75.0 |
| 200 mg/kg p.o | 0.32±0.05 | 0.30±0.03 | 46.6 | 50.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.26±0.02 | 0.23±0.02 | 56.6 | 61.6 |
| 200 mg/kg p.o | 0.35±0.02 | 0.33±0.03 | 41.6 | 45.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.32±0.05 | 0.30±0.03 | 46.6 | 50.0 |
| 200 mg/kg p.o | 0.29±0.05 | 0.26±0.04 | 51.6 | 56.6 |
| PJ-A₁ (100 mg/kg) p.o | 0.26±0.02 | 0.23±0.02 | 56.6 | 61.6 |
| 200 mg/kg p.o | 0.21±0.03 | 0.18±0.04 | 65.0 | 70.0 |
| PJ-A₁ (100 mg/kg) p.o | 0.18±0.04 | 0.14±0.23 | 70.0 | 76.6 |

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

Antibacterial activity

The data revealed that p-chloro and m-hydroxy substituted compounds shows excellent activity against all tested organisms. The presence of an electron withdrawing group such as chloro in para position enhances the liphophility of the molecule enabling it to penetrate the microbial cell more 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 the least activity.

Antifungal activity

The data reveals that p-chloro and o-hydroxy substituted compounds show excellent activity against all tested organisms. The presence of electron withdrawing group such as chloro in para position enhances 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 containing free SH group and therefore it shows good activity. Unsubstituted and methoxy substituted compounds shows the least activity. Electron withdrawing group in meta position shows less MIC compare to standard.

Anti-inflammatory activity

4-chloro, 2,4-dichloro, 2,6-dichloro and hydroxyl substituted on benzylidine ring shows high 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 benzylidine ring shows more activity compare to at ortho and meta position. Presence of two chlorine group at ortho and para position in 2,4-dichloro shows more activity. Unsubstituted benzylidine ring and presence of electron donating group on benzaldehyde ring of triazole moiety such as methoxy group as nitro in para position along with trifluoro methyl group on phenyl ring 3rd position of triazole moiety showed good activity. Compounds having high molecular weight showed more activity

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. All these synthesized compound evaluated for the antibacterial, antifungal and anti-inflammatory activity and shown potential candidate for treating diseases. Isoniazid based 1,2,4-triazole derivatives has shown good antibacterial, antifungal and anti-inflammatory activity.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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