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Research Article

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SUBSTITUTED (1*H*-TETRAZOL-1-YL) PYRIDINES

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ABSTRACT

Tetrazoles play a significant role in living organisms with a broad range of pharmacological activities. In the present study, a novel series of (1*H*-tetrazol-1-yl) pyridine derivatives were synthesized and characterized, evaluated their anti-inflammatory, analgesic, ulcerogenic potential, antioxidant, and antibacterial activities. Most of the compounds showed significant *in-vivo* anti-inflammatory and analgesic activities. Among the synthesized compounds, **5a** and **7a** were found to be the most potent, showed low ulcerogenic activity in rats and found reduced and equal activity against standard drugs diclofenac sodium and nimesulide. Compounds **5a, 5c** and **7b** exhibited good *in-vitro* antioxidant activity. The antibacterial screening was done by agar diffusion method against *Escherichia coli* and *Staphylococcus aureus.* Compounds **5a**, **7b** and **7c** were found to have good potential against *S. aureus*. Compound **5d** possessed good activity against *E. coli*.

Keywords: (1*H*-tetrazol-1-yl) pyridine derivatives, Anti-inflammatory, Analgesic, Ulcerogenic, antioxidant, Anti-bacterial potential, Structure Activity Relationship.

1. INTRODUCTION

Tetrazole nucleus has attracted the attention of many medicinal chemists due to its interesting [1] and a wide range of biological activities like anti-inflammatory [2], analgesic [3], antibacterial [4], antifungal [5], antiviral [5], anticholinergic [6], anti-asthmatic [7], anti-hypertensive [8], antiemetic activity [9], estrogen agonist and antagonist activities [10], and anticonvulsant activities [11]. In the last few decades, tetrazole moiety is the centre of research interest due to its stable molecular structure, multiple nitrogen atoms, and synthetic methods, chemical and physicochemical properties. Thus, tetrazole exhibit the extreme values of acidity, basicity, zwitter ions, complex formation and specific thermochemical properties [12]. On the other hand, pyridine is extensively studied due to their occurrence in living systems. Pyridine containing compounds have been reported as antibacterial, antifungal agents, herbicidal, bacteriostatic, antiviral, and antitumor [13], anti-human immunodeficiency virus (HIV) activity [14], anti-inflammatory activity [15] and analgesic [16], antiparkinsonian, anticonvulsant activity [17] and antihypertensive activity [18]. Tetrazole and pyridine ring alone having good and versatile biological activities already reported and fused heterocyclic ring also reported, but combined rings through carbon-carbon bond and small linkages not yet studied for anti-inflammatory, analgesic, antibacterial, antioxidant activities. Most of the anti-inflammatory agents Cyclo-oxygenase-I (COX-I) & Cyclooxygenase-II (COX-II) produces a severe side effect, for the gastrointestinal (GI) (*i.e.*, dyspepsia, ulcer, perforation, occlusion, and bleeding) and cardiovascular (CV) system (myocardial infarction, stroke, hypertension, sodium retention with edema and heart failure), which plausibly involve the inhibition of COX-1 and COX-2, respectively [19]. Novel pyridine containing tetrazole derivatives are based on the modification of the structures of the known potent non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) inhibitors. Tetrazole containing pyridine moiety possesses interesting biological activities because they form zwitter ions like phenomena. In addition, maybe toxicity is low as compared to other anti-inflammatory agents. The strategy is intended to obtain potent anti-inflammatory activity without ulcerogenic effects using traditional medicinal chemistry techniques motivated by the comparative pharmacophore modelling of COX-I & II. Therefore, we plan to design tetrazole containing pyridine moiety, which may solve the recent burden of

anti-inflammatory tragedy and fulfil the crises. We have synthesized (1*H*-tetrazol-1-yl) pyridine derivatives 2-(5-(2, 4 -disubstituted phenyl)-1*H*-tetrazol-1-yl) pyridines **5a-d** and 6-(5-(2, 4 - disubstituted phenyl)-1*H*-tetrazol-1-yl) pyridine-3-sulfonamide **7a-d** derivatives and evaluated for anti-inflammatory, analgesic, ulcerogenic, antioxidant, and antibacterial potential. The synthesized compounds **5a-d** and **7a-d** were characterized by IR, NMR and mass spectra.

2. MATERIAL AND METHODS

Melting points were uncorrected and measured on a Stuart™ melting point apparatus SMP3. The IR spectra were recorded on Bruker FT-IR spectrophotometer using KBr pellets. ^{1}H Nuclear magnetic resonance (NMR) spectra of the pure compounds in DMSO-d_6/$CDCl_3$ were recorded on Bruker 400 MHz NMR spectrophotometer. Mass spectral data of the prepared compounds were recorded on a Model: Waters ZQ-4000. The completion of the chemical reaction and the purity of the title compounds was determined by the help of thin layer chromatography (TLC) plates, pre-coated with silica gel G in solvent systems of toluene: ethyl acetate (6:4) and toluene: ethyl acetate (5:5) and chloroform: methanol (9.5:0.5). UV light was used to visualize the spots on TLC chromatograms.

2.1. Experimental

2.1.1. General preparation of 2, 4-disubstituted-N-(pyridine-2-yl) benzamide(**3**) *[20-23]*

2-aminopyridine **1** (5gm, 0.053 moles) was taken in 250 ml round bottom flask. To that 60ml of dichloromethane (DCM) was added and stirred for 15 minutes. Triethylamine (TEA) (11.03 ml, 0.079 moles) was added and stirred for 5 minutes. Substituted benzoylchloride **2** (5.74 ml, 0.053 moles) was added dropwise and the reaction mixture was stirred for overnight at room temperature. The reaction mixture was extracted with water and ethyl acetate by separating funnel. The organic layer was separated, dried over anhydrous magnesium sulphate, and evaporated under a rotary evaporator. Then DCM and ethyl acetate was evaporated using a vacuum rotary evaporator. The product was collected, recrystallized using methanol and dried using vacuum desiccators. Percentage yield (53.2%), Melting point (196-199°C) and R_f value (0.71) were found. The reaction was monitored using pre-coated TLC. (Mobile phase: Toluene: Ethyl acetate 6: 40).

2.1.2. Synthesis of 2,4-disubstituted-N-(pyridin-2-yl) benzimidoyl chloride (**4**) *[24-26]*

A solution of compound **3** (2 gm, 0.01 mole) was taken into a two necked 100 ml round bottom flask and PCl_5 (2.3 gm, 0.02 mole) was added and mixed with a glass rod. The reaction mixture was refluxed at 120°C on sand bath for 2 hours, attached with moisture trapped guard tube. Excess chlorine gas was removed under vacuum condition. Solid was washed with carbon tetrachloride to removed excess PCl_5. Percentage yield (46.3%), Melting point (60-63°C) and R_f value (0.67) were found. Reaction was monitored by TLC (Mobile phase: Toluene: Ethyl acetate 6: 40).

2.1.3. Synthesis of 2-(5-(2,4-disubstituted-phenyl)-1H-tetrazol-1-yl)pyridine (**5a-d**) **from** *2,4-disubstituted-N-(pyridin-2-yl)benzimidoyl chloride*(**4**) *[27-29]*

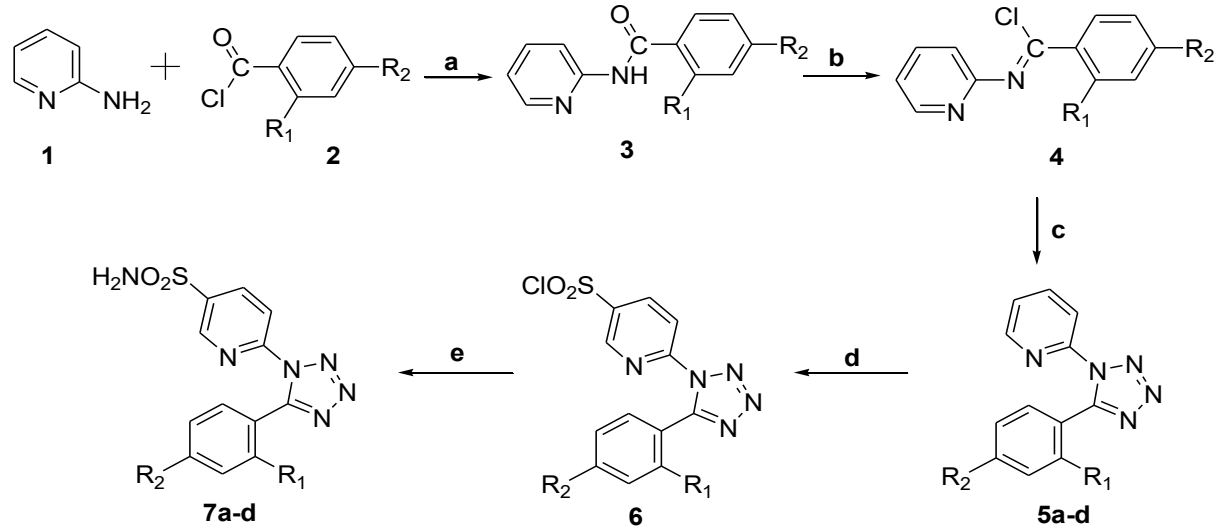
A mixture of compound **4** (3gm, 0.013 mole) was taken into a two necked 100ml round bottom flask and maintained the temperature at 0-5°C. Sodium azide and excess solution of sodium acetate was made in aqueous acetone at cold condition and stirred for overnight. The organic layer was separated using separating funnel and excess solvent was evaporated at room temperature (Scheme 1). The crude product was than washed with methanol and recrystallized with 95% ethanol to give **5a-d**. Reaction was monitored by TLC (Mobile phase: Toluene: Ethyl acetate 6: 40).

2.1.4. Synthesis of 6-(5-(2,4-disubstituted-phenyl) -1H-tetrazol-1-yl)pyridine-3-sulfonyl chloride (**6**) *from 2-(5-(2,4-disubstituted-phenyl)-1H-tetrazol-1-yl)pyridine* (**5**) *[30, 31]*

2-(5-(2, 4-disubstituted-phenyl)-*1H*-tetrazol-1-yl) pyridine **5**(400 mg, 0.074mole) was taken into 100 ml RBF and the temperature was maintained at below 15°C. Chlorosulphonic acid (1.4 ml, 0.002 mole) was added dropwise into the reaction mixture with stirring for 20 minutes. The resulting mixture was stirred and heated for 2 hours at 60°C. The reaction mixture was cooled and poured into the crushed ice and white product was obtained under vacuum, washed with cold water. Percentage yield (41%), Melting point (231°C) and R_f value (0.79) were recorded. The reaction was monitored by TLC (Mobile phase: toluene: ethyl acetate 5:5 and chloroform: methanol 9.5:0.5).

2.1.5. *Synthesis of 6-(5-(2,4-disubstitutedphenyl)-1H-tetrazol-1-yl)pyridine-3-sulfonamide* (7a-d) *from 6-(5-(2,4-disubstituted-phenyl)-1H-tetrazol-1-yl)pyridine-3-sulfonyl chloride* (6)

6-(5-(2,4-disubstituted-phenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonyl chloride **6** (20mg, 0.0005 mole) was taken into a 100 ml round bottom flask and excess amount of ammonia solution wasadded and refluxed for 2 hours. The reaction mixtures was cooled and acidified with conc. HCl. Solid product was obtained was separated by vacuum filtration and washed with cold water. Crude product was recystallized with 95% ethanol to give **7a-d** (Scheme 1). Reaction was monitored by TLC (Mobile phase: Toluene: Ethyl acetate 5:5 and Chloroform: methanol 9.5:0.5).



(a) TEA, DCM, stirred overnight, rt; (b) PCl_5, 120°C, 2 h; (c) CH_3COONa, NaN_3, stirred overnight, ice bath; (d) $ClSO_3H$, 60 °C, 2h; (e) NH_3, reflux, 2h

Scheme 1: Reagents and reaction conditions

2.2. Biological Screening

2.2.1. *Pharmacology*

The synthesized compounds were tested for their anti-inflammatory, analgesic, ulcerogenic properties in experimental animals. Wistar rat of either sex was procured from Zydus Research Centre, Ahmedabad were used in the present study. The animals were kept at controlled conditions (temperature 23±2°C, humidity 60±10%) and a 12/12-h light/dark cycle with access to food and water. The institutional animal ethics committee approved the protocol adopted for the experimentation of animals (Project Number: IPS/PCHEM/MPH10/001; CPCSEA Registration No.:1667/GO/a/12/CPCSEA) Utmost care was taken to ensure that the animals were treated and kept in well-maintained hygienic and environmental conditions. All the synthesized compounds and standard drugs (diclofenac sodium and nimisulide) were administered orally and intra-peritoneal in the form of suspension prepared using 0.5% w/v carboxymethyl cellulose (CMC) solution. Results of biological activity are presented by mean (percentage protection) ± SEM.

2.2.2. *Anti-inflammatory activity*

The newly synthesized compounds 5a-d, and 7a-d were screened for their anti-inflammatory activity in Waster albino rats for carrageenan induced rat paw edema as per Winter *et al* method [32]. The animals were divided into eleven groups. Group 1 was labelled as a control and administered only vehicles. Diclofenac sodium (STD-1) at a dose of 20 mg/kg of body weight was administered to group 2 and nimisulide (STD-2) as dose 20 mg/kg of body weight was administered orally to group 3 as a reference for drug comparison. While groups 4-11 were administered with synthesized test compounds (20 mg/kg body weight) in the form of suspension in aqueous solution of 0.5%W/V sodium carboxymethyl cellulose (CMC) as a vehicle by the oral route (*p.o*). All the animals were injected subcutaneously with 0.1 ml. of 1% freshly prepared Carrageenan suspension in 0.9% w/v normal saline, into the sub-plantar region of left hind paw to induce inflammation. Percentage inhibition of inflammation with time is determined for all test and standard compounds by measuring the hind paw volume using

plethysmometer of experimental animal after 1 h, 2 h, 3 h and 4 h time intervals and percentage inhibition in the inflammation was calculated (mean ± SEM).

2.2.3. Analgesic activity

Wistar rat weighing 130-180 g were used. Eight compounds (5a-d, and 7a-d) were further evaluated for analgesic activity by Eddy's hot plates method [33, 34]. Eleven groups of Wister rat having 6 animals in each were formed. Group 1 was kept as a control, group 2 were administered the standard drug-1 diclofenac sodium (STD-1) at a dose of 20 mg/kg body weight i.p., and group 3 nimesulide (at a dose of 20 mg/kg body weight .p.o.) (STD-2). The tested compounds from groups 4-11 were administrated per orally at a dose 20 mg/kg body weight. Analgesia produced by the compounds was measured at different time intervals including 30, 60, and 90 min by placing the rats on a hot plate maintained at 55±0.5°C.

2.2.4. Ulcerogenic potential

To evaluate and compare ulcerogenic activity caused by the administration of positive controls and two synthesized molecules 2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine (5a) and 6-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide (7a), Wister rats of either sex (130-180 kg) and method by Cioli *et al*. were adopted [35]. The single dose of 20 mg/kg body weight of therapeutic dose of selected test compounds and two standard drugs diclofenac sodium and nimesulide were administered to the experimental animals to assess their toxic effects on the gastric mucosa. Ulcerogenic potential was determined by removing the stomach from the sacrificed animal after 7 hrs of the time of administration in each case and placed on saline-soak filter paper until inspection. A longitudinal incision along the greater curvature is made with fine scissors. The stomach was inverted over the index finger and the presence or absence of gastric irritation was determined. The presence of a single or of multiple lesions (erosion, patches, ulcer or perforation) was considered positive.

2.2.5. Anti-oxidant activity

The synthesized compounds were evaluated for *in vitro* antioxidant activities against 2,2-diphenyl-1-picryl-hydrazyl (DPPH). 1ml of 0.1mM of DPPH (2, 2 diphenyl-2-picrylhydrazyl) in methanol was added in 1ml of references standard (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml) and test solution of different concentrations (10µg/ml, 50µg/ml, 100µg/ml, 250µg/ml, and 500µg/ml). It was kept in dark for 30 minutes to protect from light and the absorbance was measured at λ-max 517nm in UV Visible spectrophotometer (SHIMADZU). The assay was performed in triplicate. Ascorbic acid was used as a reference standard. All solutions were freshly prepared. The test solution was prepared by diluting methanol from stock solution of 10mg/ml [36, 37]. The DPPH scavenging was measured by the following equation:

$$\% \text{ inhibition} = A_0\text{-}A_t/A_0 \times 100.$$

Where, A_0 = absorbance of control (containing all measurement except test), A_t = absorbance of test solution. The percentage inhibition after 30 min was plotted against concentration, and equation for the line was used to get the IC_{50} value, calculated data for antioxidant potential are presented as means ± SD of triplicate.

2.2.6. Antibacterial Activity

In Agar well diffusion method (Cup plate method) nutrient broth Agar was employed to study the preliminary *in vitro* antibacterial activity of the newly synthesized compounds against one gram-positive *Staphylococcus aureus* (MTCC 737) and one gram-negative *Escherichia coli* (MTCC 1687) bacterial species [38]. Bacterial strains used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), and Chandigarh. Zone of inhibition in both of the microorganism was determined and compared with standard ofloxacin (ZydusCadila Pvt. Ltd) 50 µg/0.1ml.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The general strategy used for synthesis of title compounds is shown in Scheme 1. Firstly, 2-aminopyridine **1** reacted with 2, 4-disubstituted benzoyl chloride **2** using triethylamine (TEA) and dichloro-methane (DCM) as a reaction medium to give the 2, 4-disubstituted-N-(pyridine-2-yl) benzamide **3**. Resulting compound **3** chlorinated with phosphorus pentachloride (PCl_5) to give precursors **4** and reacted with reagent sodium azide (NaN_3) in aqueous acetone to generate the first general structure of 2-(5-(2,4 -disubstituted phenyl)-1*H*-tetrazol-1-yl) pyridine **5a-d,** which further reacted with chlorosulfonic acid ($ClSO_3H$) to give precursors **6** and subsequently with ammonia to obtain it's sulphonamide analogue **7a-d**. All the synthesized compounds were separated as the organic phase using

separating funnel and recrystallized using methanol and ethanol as applicable. Subsequently, physicochemical parameters of synthesized compounds 5a-d and 7a-d, is summarized in Table 1.

Table 1: Physicochemical parameters of synthesized compounds 5a-d and 7a-d

Compounds	R_1	R_2	% Yields	m.p. (°C)	R_f Values
5a	Cl	H	41%	240-241	0.80
5b	Cl	Cl	42%	320-322	0.75
5c	H	OCH_3	57%	140-142	0.60
5d	Br	H	30%	130-132	0.72
7a	Cl	H	20%	340-342	0.22
7b	Cl	Cl	18%	318-320	0.61
7c	H	OCH_3	10%	260-262	0.88
7d	Br	H	45%	190-192	0.53

*3.1.1. 2-(5-(2-Chlorophenyl)-1H-tetrazol-1-yl) pyridine (***5a)**

Yield (41%), R_f (0.80), mp 240-242°C; FTIR (KBr) cm^{-1}: 3041.74 (C-H stretch), 1292.31 (-C-N stretch), 1438.90 (C=C stretch), 1589.90 (N=N stretch), 1232.51(N-CH_2 stretch), 748.38(C-Cl stretch); ^{1}H NMR ($CDCl_3$): **δ** 8.56 (s, 1H), 8.25-8.27 (d, 1H, *J* =6.4 Hz) CH, 7.42-7.44 (t, 2H, *J* = 5.2 Hz) CH, 7.31-7.34 (t, 2H, *J* =6.8 Hz) CH, 7.05-7.08 (d, 2H, *J*= 6Hz) CH; MS; m/z: 259.9 $(M+H)^+$.

*3.1.2. 2-(5-(2,4-dichlorophenyl)-1H-tetrazol-1-yl)pyridine (***5b)**

Yield (42%), R_f (0.75), mp. 320-322°C; FTIR (KBr) cm^{-1}: 3050.73 (C-H stretch), 1588.12 (N=N stretch), 1532.21 (C=C stretch), 1283.34 (C-N stretch), 1231.15 (N-CH_2 stretch), 731.42 (C-Cl stretch); ^{1}H NMR ($CDCl_3$): **δ** 8.59 (s, 1H), 7.90 (2H, ddd, *J* = 8.2, 1.3 Hz), 7.60 (2H, dd, *J* = 1.4 Hz), 7.67 (1H, t, *J*= 7.8 Hz), 7.36 (1H, ddd, *J* = 5.1, 1.3 Hz); MS; m/z: 293$(M+H)^+$.

*3.1.3. 2-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl) pyridine (***5c)**

Yield (57%), R_f (0.60), mp 140-142°C; FTIR (KBr) cm^{-1} ; 3061.03 (C-H aromatic stretch), 2993.73 (C-H aliphatic stretch), 1311.59 (-C-N stretch), 1273.02 (C-O stretch), 1406.11 (C=C stretch), 1591.27 (N=N stretch), 1240.23(N-CH_2 stretch); ^{1}H NMR ($CDCl_3$): **δ** 8.6 (s, 1H), 7.82 (ddd,2H, *J* =6.4, 3.4 Hz), 7.40 (t, 2H, *J*=7.7, 1.2 Hz), 7.37 (ddd, *J*=4.7, 1.4 Hz), 6.83 (d, 1H, *J*= 8.3 Hz), 3.73 (s, 3H, OCH_3); MS; m/z: 254 $(M+H)^+$.

*3.1.4. 2-(5-(4-bromophenyl)-1H-tetrazol-1-yl) pyridine (***5d)**

Yield (30%), R_f (0.72), mp 130-132°C; FTIR (KBr) $cm^{-1;}$ 3032.12 (C-H aromatic stretch), 1590.25 (N=N stretch), 1452.93 (C=C stretch), 1291.23 (-C-N stretch), 1232.45(N-CH_2 stretch), 724.56 (C-Br stretch); ^{1}H NMR ($CDCl_3$): **δ** 8.59 (s, 1H), 7.9 (ddd, 1H, *J*= 1.5, 0.4 Hz), 7.8 (ddd, 2H, *J*= 8.2, 1.3 Hz), 7.53 (1H, ddd, *J*= 7.7, 1.5 Hz), 7.4 (t, 2H, *J*= 4.7, 1.3 Hz), 7.30-7.44 (2H, *J*= 7.5, 4.7, 1.3 Hz), 7.11 (d, 1H, *J*= 7.5 Hz); MS; m/z: 302 $(M+H)^+$.

*3.1.5. 6-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide (***7a)**

Yield (20%), R_f (0.22), mp 340-342°C; FTIR (KBr) cm^{-1}: 3259.70 (primary amine), 3047.53 (C-H stretch), 1332.81 (-C-N stretch), 1390.68 (C=C stretch), 1589.34 (N=N stretch), 1228.66(N-CH_2stretch), 734.88(C-Clstrech), 1163(S=O_2 stretch); ^{1}H NMR ($CDCl_3$): δ 9.025 (s, 1H), 8.48 (d, 1H, *J*= 7.2 Hz), 7.91 (dd, 1H, *J*= 7.2, 1.2 Hz), 7.82 (d, 1H, *J*= 7.2 Hz,), 7.42-7.31 (ddd, 3H, *J*=6.8, 5.6, 1.2 Hz); MS; m/z: 336.1$(M+H)^+$.

*3.1.6. 6-(5-(2,4-dichlorophenyl)-1H-tetrazol-1-yl)pyridine-3-sulfonamide***(7b)**

Yield (18%), R_f (0.61), mp 318-320°C; FTIR (KBr) cm^{-1} 3180.70 (primary amine), 3037.89 (C-H stretch), 1315.45 (-C-N stretch), 1436.97 (C=C stretch), 1589.34 (N=N stretch), 1240.23(N-CH_2 stretch), 734.80/ 777.31(C-Clstrech), 1151(S=O_2 stretch); ^{1}H NMR ($CDCl_3$): δ9.03 (s, 1H), 8.4 (d, 1H, *J*= 8.7 Hz), 7.7 (dd, 1H, *J*= 8.7, 1.5 Hz), 7.7 (t, 1H, *J*= 7.9 Hz), 7.36 (dd, 2H, *J*= 1.9, 0.5 Hz); MS; m/z: 371 $(M+H)^+$.

3.1.7. 6-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide (7c)

Yield (10%), R_f (0.88), mp 260-262°C; FTIR (KBr) cm^{-1} 3262.80 (primary amine), 3036.62 (C-H stretch), 1322.16 (-C-N stretch), 1435.77 (C=C stretch), 1588.43 (N=N stretch), 1227.32(N-CH_2 stretch), 1161(S=O_2 stretch); ^{1}H NMR ($CDCl_3$): δ 9.0 (s, H), 8.4 (dd, 1H, *J*= 7.6, 1.7 Hz), 7.75 (ddd, 1H, *J*= 8.1, 7.6, 1.8 Hz), 7.5 (td, 1H, *J*= 7.6, 1.0 Hz), 7.37 (d, 1H, *J*= 8.1 Hz), 7.2 (ddd, 1H, *J*= 8.1, 1.0, 0.4 Hz), 3.73 (s, 3H, OCH_3) ; MS; m/z: 333.07$(M+H)^+$.

3.1.8. 6-(5-(2-bromophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide (7d)

Yield (45%), R_f (0.53), mp 190-192°C; FTIR (KBr) cm^{-1} 3210.16 (primary amine), 3032.70 (C-H stretch), 1315.50 (-C-N stretch), 1390.70 (C=C stretch), 1586.34 (N=N stretch), 1242.50(N-CH_2 stretch), 1162(S=O_2 stretch), 724.12 (C-Br strecth); ^{1}H NMR ($CDCl_3$): δ 9.03 (d, 1H, *J*= 2.0 Hz), 8.4 (d, 1H, *J*= 1.5 Hz), 7.7 (ddd, 1H, *J*= 8.1, 1.5, 0.5 Hz), 7.5 (dd, 1H, *J*= 7.6, 1.5 Hz), 7.43-7.61 (ddd, 3H, *J*= 8.1, 7.7, 1.6 Hz); MS; m/z: 381 $(M+H)^+$.

3.2. Biological assays

3.2.1. Anti-inflammatory activity

Compounds **5a-d**, and **7a-d** were evaluated against anti-inflammatory activity in an experimental model of carrageenan induced edema in rat paw (Table 2). The synthesized compounds at 0 h, 1 h, 2 h, and 4 h were compared with the standard drug diclofenac sodium and parent drug nimesulide. All the tested compounds exhibited degrees of inflammatory activity but better activity was observed at 4 h ranging from 20.6% to 37.32%. Among all the tested compounds, 2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine **5a** showed the highest anti-inflammatory activity with 37.32% inhibition. The other compounds namely 2-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl) pyridine **5c**, 6-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide **7a**, showed good activity with 28.08% and 31.47% inhibition respectively. The rest of the compounds showed the moderate inhibition (<30%).

Table 2: Effect of synthesized compounds (5a-d and 7a-d) on the carrageenan-induced edema test, in rat paw (n=6)

Drugs	% Inhibition (1 h)	% Inhibition (2 h)	% Inhibition (3 h)	% Inhibition (4 h)
Control	0±0.067	0±0.095	0±0.139	0±0.112
STD-1	3.51±0.098	3.52±0.230	26.37±0.107*	47.26±0.185*
STD-2	8.5±0.132	3.52±0.05	22.34±0.046*	42.8±0.056*
5a	13.5±0.074	9.01±0.0814	25.82±0.0625*	37.32±0.052*
5b	9.54±0.081	12.94±0.094	19.78±0.041*	26.02±0.026*
5c	18.29±0.073*	11.37±0.116	18.68±0.102*	28.08±0.074*
5d	10.05±0.107	20.39±0.119*	22.23±0.015*	25.68±0.091*
7a	12.02±0.071	19.6±0.074*	25.27±0.052*	31.47±0.044*
7b	9.7±1.11	11.9±0.082	12.1±0.63*	20.6±0.1.19*
7c	15.1±1.34*	14.5±0.93	19.3±0.61*	24.8±1.38*
7d	10.2±1.03	13.1±0.85	17.1±1.89*	24.2±1.65*

*The values were expressed as mean ± SEM of difference between paws, *p < 0.05*

3.2.2. Analgesic activity

All the compounds were further investigated for their analgesic activity using hot plate method. The tested compounds showed considerable variation in analgesic activity (Table 3) ranging from 12.85% to 18.8% in comparison to positive controls diclofenac sodium (20.07%) and nimesulide (21.93%). Among all the screened compounds, 2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine **5a** showed the highest analgesic activity with 18.8% protection. Compound, 6-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide **7a** also showed good activity with 18.255% protection. Rest of the compounds showed moderate analgesic activity in the range of 12.85% to 17.3 % inhibition.

3.2.3. Ulcerogenic potential

The acute ulcerogenic activity was done for the compounds which showed good anti-inflammatory activity (>30% inhibition of edema). The two tested

compounds showed severity index in the range of 35.55-36.1 (Table 4). Compound **5a** namely 2-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine showed the severity index of 35.55 followed by 6-(5-(2-chlorophenyl)-1H-tetrazol-1-yl) pyridine-3-sulfonamide **7a** with a severity index of 36.1. Compounds **5a** and **7a** showed better GI safety profiles than diclofenac sodium (42.85).

Table 3: Analgesic activity of synthesized compounds (5a-d and 7a-d)

Drugs	Mean latency		
	30 minute	60 minute	90 minute
Control	7.29±0.054	7.6±0.458	7.77±0.418
STD-1	8.9±0.173*	17.34±0.689*	20.07±0.586*
STD-2	11.53±0.682*	18.83±0.707*	21.93±1.36*
5a	9.75±0.346*	17.85±0.867*	18.8±0.96*
5b	9.24±0.349*	15.67±0.615*	15.79±0.723*
5c	9.37±0.4078*	13.78±0.73*	17.02±1.02*
5d	8.013±0.412	13.106±0.789*	15.595±0.914*
7a	1.135±0.412	15.11±0.735	18.255±0.455*
7b	11.04±0.73*	12.12±1.00*	12.85±0.94*
7c	10.50±0.7638*	12.75±0.73*	17.33±0.78*
7d	8.04±0.73*	13.66±0.50*	16.67±0.7149*

*Each value represents the mean ± SEM (n = 6); *p < 0.05 significant*

Table 4: Ulcerogenic potential of synthesized compounds 5a and 7a

DRUGS	Patches	Area(mm^2)±SEM
Control	48	9.6±3.72
STD-1	284	42.85±2.98*
STD-2	169	33.8±3.38*
5a	177.75	35.55±4.6*
7a	181	36.1±9.68*

*Each value represents the mean ± SEM; *p < 0.05 significant*

3.2.4. Anti-oxidant activity

The antioxidant activities of the synthesized compounds were determined and are represented in Table 5. The results revealed that all compounds were found to be potent. Moreover, the results showed that nearly five compounds **5b, 5c, 5d, 7b** and **7c** were found to be the most potent levels of activity. Additionally, compounds **5d** and **7d** were found to have moderate activity. While compound **5a** and **7a** showed least anti-oxidant activity. The results were compared between the compounds **5b, 5c, 5d, 7b** and **7c,** it was noticed that compound **7b** indicating the presence of SO_2NH_2 group was more effective than the **5b**. On the other hand when R_1=Cl, and R_2= Cl convert into R_1=H, and R_2=OCH_3 in compound **5c**, and **7c** antioxidant activity decreases. **5a** and **7a** is less active than **5d** and **7d**. **7b** displayed most potent antioxidant activity when compared to **5b, 5c, 5d**, and **7c**.

3.2.5. Antibacterial Activity

Compounds **5a-d** and **7a-d** were evaluated against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria strains. The individual minimum inhibitory concentration (MIC, µg/mL) obtained for compounds **5a-d** and **7a-d** are presented in tables 6 & 7. It is observed that compounds **5b** (R_1=Cl, R_2=Cl), **5d** (R_1=Br, R_2=H), and **7b** (R_1=Cl, R_2=Cl) are the most active compounds. When we replaced hydrogen by 4-Cl group in **5a**, it will lead to compound **5b** and is possessed very good activity against *S. aureus*. Introducing 3-SO_2NH_2 group in **5a-d**, it will lead to **7a-d**. The compound **7b** enhanced the activity and it possessed potent activity against *S. aureus* as compared to other synthesized compounds **5a, 5b, 5c, 5d, 7a, 7c** and **7d**. Compounds **5a, 5b, 5c, 7a, 7b, 7c** and **7d** possessed very least activity, while compound **5d** (R_1=Br, R_2=H)) possessed good activity against *E. coli*. The minimum inhibitory concentration (MIC) values were determined by comparison with ofloxacin as reference drugs. Each value represents the mean ± SEM (n = 6); *p < 0.05 significant.

3.2.6. Structure-activity relationship

Structure-activity relationship study suggested that this kind of skeleton is responsible for activity because its pharmacophoric region inhibit both COX-I & COX-II. Tetrazole rings having an extreme value of acidity and it are responsible for the anti-inflammatory activity.

Pyridine ring having basic nature and as a result overall the acidic nature of ring was decreased and cut down it GIT toxicity. Substitution of R_1 in phenyl ring electron withdrawing group shows good anti-inflammatory and analgesic activities. Substitution of R_2 position in phenyl ring electron donating group-OCH_3 has shown good antioxidant activity. Electron withdrawing group at R_1 and R_2 position show good antioxidant activity as well as anti-bacterial activity against *S. aureus*. Substitution of SO_2NH_2 at R_3 position and electron withdrawing at R_1 and R_2having good anti-inflammatory, analgesic, antioxidant and antibacterial activity, as well as very low ulcerogenic potential as shown below in fig. 1.

Table 5: Antioxidant activities of test compounds were recorded in terms of % scavenging shown by each compounds (5a-d and 7a-d) at different concentrations.

DRUGS	10µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml
CONTROL	0	0	0	0	0
STD	81.43±0.04*	84.46±0.003*	85.981±0.034*	84.857±0.037*	91.518±0.020*
5a	7.345±0.0023	5.538±0.0017	1.246±0.0035	6.225±0.0044	9.854±0.0017*
5b	4.265±0.0046	7.153±0.002*	27.414±0.0048*	52.574±0.011*	59.289±0.008*
5c	8.846±0.007*	23.384±0.007*	19.548±0.0022*	45.196±0.0121*	43.537±0.007*
5d	6.872±0.006*	1.615±0.0021	14.563±0.0073*	17.371±0.0077*	31.340±0.0072*
7a	0.552±0.0034	1.769±0.0014	3.738±0.0043	6.302±0.0035	4.604±0.0059
7b	3.396±0.0093	21.923±0.015*	43.068±0.011*	63.643±0.0146*	82.633±0.0067*
7c	2.211±0.0023	24.307±0.010*	24.844±0.0131*	41.890±0.0063*	37.156±0.0179*
7d	5.37±1.06*	2.63±0.73	16.8±0.2*	16.6±0.7*	28.4±0.4*

*Each value represents the mean ± SEM (n = 6); *p < 0.05 significant*

Table 6: Zone of Inhibition (mm) in *S. aureus* of synthesized compounds (5a-d and 7a-d)

Compound codes	Concentration (µg/0.1 ml)	Zone of Inhibition (mm)			
		E1*	E2*	E3*	Mean ± SEM
5a	50	4	2	4	3.33±0.67
	100	6	6	4	5.33±0.67
	250	6	5	5	5.33±0.33
7a	50	5	3	2	3.33±0.88
	100	6	5	6	5.66±0.33
	250	8	6	6	6.66±0,67
5b	50	6	5	3	4.66±0.88
	100	6	6	5	5.66±0.33
	250	8	6	8	7.33±0.66
7b	50	8	7	7	7.33±0.33
	100	10	7	10	9±1.001
	250	10	9	11	10±0.57
5c	50	2	0	2	1.33±0.67
	100	2	2	3	2.33±0.33
	250	4	4	3	3.66±0.33
7c	50	0	0	0	0±0
	100	2	2	3	2.33±0.33
	250	3	4	4	3.66±0.33
5d	50	6	6	4	5.33±0.67
	100	6	6	6	6±0
	250	8	9	8	8.33±0.33
7d	50	4	2	4	3.33±0.67
	100	4	4	3	3.66±0.33
	250	4	3	4	3.66±0.33
STD	50	22	24	25	23.66±0.88

*Each value represents the mean ± SEM (n = 6); *p < 0.05 significant*

Table 7: Zone of Inhibition (mm) in *E. coli* of synthesized compounds (5a-d and 7a-d)

Compound code	Concentration (µg/0.1 ml)	Zone of Inhibition (mm)			
		E1*	E2*	E3*	Mean ± SEM
	50	0	0	0	0±0
5a	100	0	0	0	0±0
	250	0	3	2	1.66±0.88
	50	0	0	0	0±0
7a	100	2	2	0	1.33±0.67
	250	2	2	2	2±0
	50	0	0	0	0±0
5b	100	0	2	0	0.66±0.66
	250	2	2	2	2±0
	50	1	0	0	0.33±0.33
7b	100	2	2	2	2±0
	250	4	2	2	2.66±0.67
	50	0	0	0	0±0
5c	100	0	0	0	0±0
	250	0	0	0	0±0
	50	0	0	0	0±0
7c	100	0	2	2	1.33±0.67
	250	2	3	2	2.33±0.33
	50	2	3	3	2.66±0.33
5d	100	6	6	7	6.33±0.33
	250	12	11	12	11.66±0.33
	50	0	0	0	0±0
7d	100	3	3	2	2.66±0.33
	250	4	3	4	3.66±0.33
STD	50	23	22	23	22.66±0.33

*Each value represents the mean ± SEM (n = 6); *p < 0.05 significant*

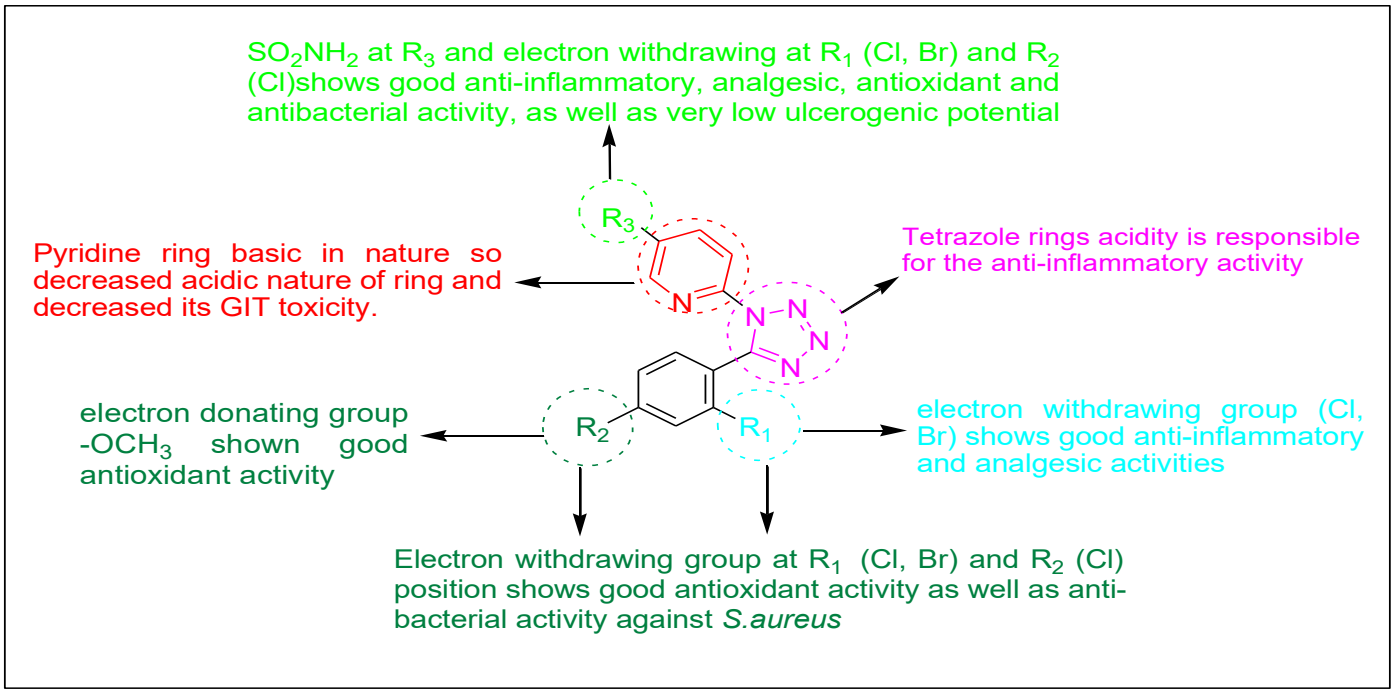


Fig. 1: Structure-activity relationship of the synthesized compounds

4. CONCLUSIONS

In summary, the results of the current study demonstrated that compounds **5a**, **5b** and **7a** showed significant anti-inflammatory, and analgesic activities. Compounds **5a** and **7a** showed low ulcerogenic potential and showed better GI safety profiles. Compounds **5b**, **5c**, **5d, 7b** and **7c** exhibited good anti-oxidant activity among other derivatives but still they are somewhat less potent than the standard. Compounds **5b**, **5d,** and **7b** showed significant MIC values against *S.aureus,* whereas compound **5d** showed significant MIC values against *E.coli,* and other synthesized compounds exhibited moderate to low antibacterial activity. The newly synthesized compounds will be useful in generating new hybrid molecules in future showing anti-inflammatory without ulcerogenic effects.

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Conflict of interest

The authors declare no conflict of interest.

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