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Synthesis, Antimicrobial and Anti-inflammatory Activity of Some 5-Substituted-3-pyridine-1, 2, 4-Triazoles

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Abstract: In our present study we were synthesized 5-substituted-3-pyridine-1, 2, 4-triazole and tested for antibacterial, antifungal and anti-inflammatory activity. In this we used isoniazid treated with different aromatic and aliphatic acid which undergoes oxidative cycalisation and obtained different oxadiazoles derivatives, these oxadiazoles undergoes condensation reaction in presence of hydrazine hydrate and the synthesized compound were characterized using IR, NMR and Mass spectrum.

Keywords: 5-substituted-3-pyridine-1,2,4-triazole, antibacterial, antifungal, anti-inflammatory, aromatic and aliphatic acid, hydrazine hydrate.

INTRODUTION:

The development of resistance of current antibacterial therapy continues to search for more effective agent. In addition, primary and opportunistic fungal infection continues to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several review have appeared by today's infectious disease clinicians.[1]

Non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used for reducing pain and swelling associated with inflammation, represent a research area of continuous and ever growing development. Aryl and heteroarylacetic acids are well established class of non-steroidal anti-inflammatory agent therapeutically useful in the treatment of acute and chronic inflammatory condition. Furthermore, it had been reported that many compounds having a 1, 2, 4-triazole skeleton possessed significant anti-inflammatory activity. [2]

1, 2, 4-triazoles and its derivatives represent one of the most biologically active classes of compound, possessing a wide spectrum of activities. The 1,2,4-triazole nucleus is associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic.[3]

Pyridine, a heterocyclic nucleus, played a vital role in the development of different medicinal agents and in the field of agrochemicals. This nucleus is present in many products such as drugs, vitamins, food. It is seen from current literature that pyridine congeners are associated with different biological properties like pesticidal, insecticidal and fungicidal. These reports encouraged us to modify 1,2,4-triazole scaffold into various bioactive structures and their subsequent evaluation for antibacterial, antifungal and anti-inflammatory activities as reported in the present communication.[4]

EXPERIMENTAL:

The melting point of synthesized on a Buchi 510 melting point apparatus and are uncorrected. The IR

spectra were recorded in 4000-400cm^{-1} range using KBr disks on Shimandzu FTIR Spectrophotometer. The ^{1} H-NMR spectra were recorded on Brukner Avance 300 spectrometer in DMSO-d_6 with TMS as an internal standard. Mass spectra were recorded on Jeol GC mate inlet direct probe. TLC using silicagel-G checked the purity of the compounds.

Synthesis of aromatic/ aliphatic substitued 1,3,4 oxadiazole A(I-X)

In a round-bottom flask a mixture of taken Isoniazid (0.01 mol) and phosphorus oxychloride (5 ml) in Ethanol (50 ml) and different substituted aromatic and aliphatic acids (0.01mol) were added. The reaction mixture was refluxed for 5-8hr at 90^0 C and then cooled to room temperature and poured into crushed ice. A solid mass separated out which was filtered and recrystallized from appropriate solvent system. [5]

Synthesis of substituted 5-substitued-3pyridine-1, 2, 4 triazole B (I-X)

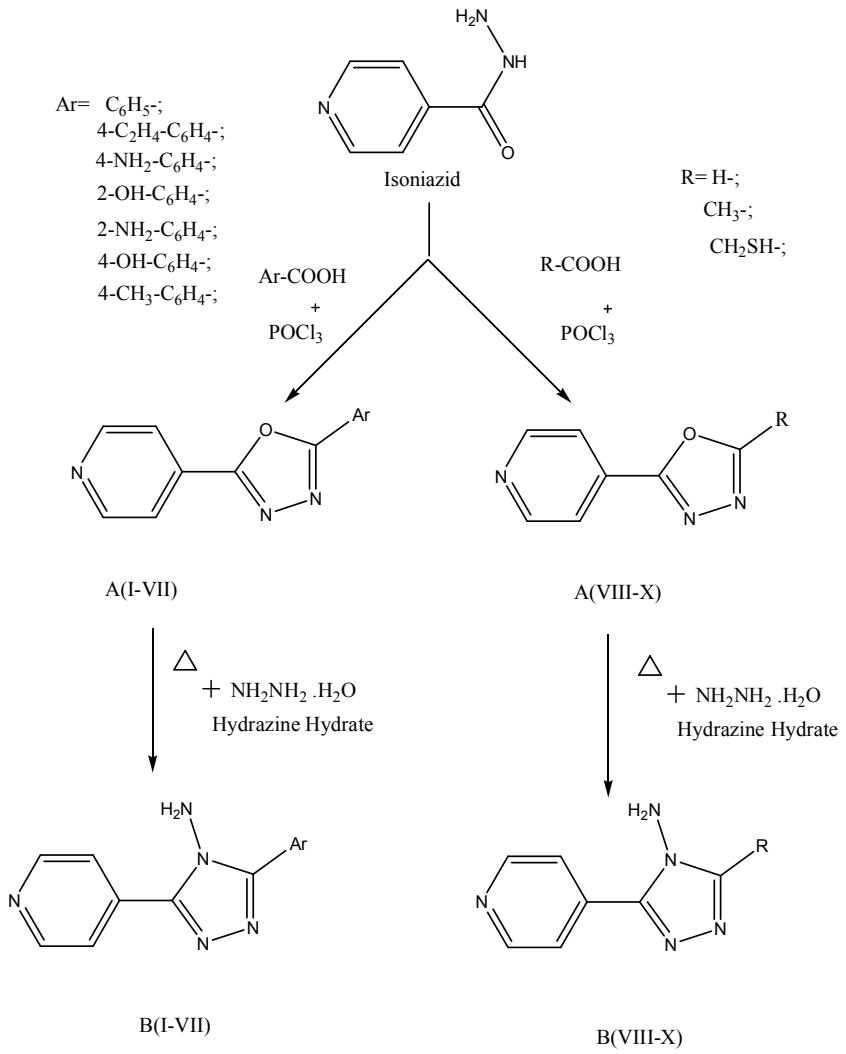
A mixture containing compound **A (I-X)** (0.1mol) and hydrazine hydrate (0.2mol) was taken in Round Bottom Flask and refluxed on water bath for 2-3 Hr at 70-80^0 C. Then it was cooled to room temperature and the contents were poured into water (100ml). On acidification with conc. HCl, white thick solid mass separated. It was collected washed with water, and recrystallized from appropriate solvent system.[6]

The melting point , yield and elemental analyes of compound **B(I-X)** are given in table no.1

Table1: **Physical and analytical data of compounds B(I-X)**

Comp.	Ar/R	Mol. Formula.	Mol. weight	Melting point	% Yield (w/w)	Elemental Analysis Calc. (Found) (%)		
						C	H	N
I	C_6H_5-	$C_{13}H_{11}N_5$	237	95-8	59.66	65.82	4.64	29.53
II	4-C_2H_4-C_6H_4-	$C_{15}H_{13}N_5$	263	180-3	66.67	68.44	4.94	26.61
III	4-NH_2-C_6H_4-	$C_{13}H_{12}N_6$	252	116-9	62.67	61.90	4.76	33.33
IV	2-OH-C_6H_4-	$C_{13}H_{11}N_5O$	253	181-4	64.89	61.67	4.34	27.66
V	2-NH_2-C_6H_4-	$C_{13}H_{12}N_6$	252	155-8	64.36	61.90	4.76	33.33
VI	4-OH- C_6H_4-	$C_{13}H_{11}N_5O$	253	185-8	65.65	61.67	4.34	27.67
VII	4-CH_3- C_6H_4-	$C_{14}H_{13}N_5$	251	167-9	64.78	66.93	4.38	27.89
VIII	H-	$C_7H_7N_5$	161	190-3	64.74	52.17	4.34	43.47
IX	CH_3-	$C_8H_9N_5$	175	135-8	60.89	54.85	5.14	40.01
X	CH_2SH-	$C_8H_9N_5S$	207	195-98	66.35	46.37	4.34	33.81

Scheme



B (I): IR (cm^{-1}): 3443.05(-NH_2 str.), 3068.85, (Pyr. str.), 2955.04 (–CH Ar-str.), 1602.90 (>C=N-str.), 1070.53 (triazole str.); [1] H-NMR: 7.65-8.65 (4H, Pyridine), 7.22 and 7.48 (5H, Ar-), 2.0 (2H, -NH_2-Triazole).MS(*m/z*) : M^+ calculated 237, found 236.

B(II): IR (cm^{-1}): 3323.46 (-NH_2 str), 3263.66 (Pyridine str), 2806.52 (–CH Ar- str), 1618.33 (>C=C< str.), 1491.02 (>C=N- str.), 1099.46 (triazole str); [1] H-NMR: 7.65-8.65 (d. 4H, -Pyridine), 7.36 and 7.6 (d. 4H, -Ar), 5.31-5.78 (d. 3H, -CH=CH_2), 2.0 (s. 2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 263, found 262.

B(III): IR (cm^{-1}): 3319.60 (-NH_2 str), 3234.73 (Pyridine str), 3149.46 (–CH Ar- str), 1508.38 (>C=N-str.), 1101.39 (triazole str); [1] H-NMR: 6.52-7.23 (d. 4H, -Ar), 7.65-8.65 (d. 4H, -Pyridine), 4.0 (d. 2H, NH_2-Ar), 2.1 (d. 2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 252, found 251.

B(IV): IR (cm^{-1}): 3688.02 (–OH Ar- str), 3242.45 (-NH_2 str), 3009.05 (Pyridine str), 3070.78 (H- Ar str.), 1612.54 (>C=N- str.), 1089.82 (triazole str); [1] H-NMR: 7.4-8.6 (d. 4H, -Pyridine), 6.6-7.2 (d. 4H, -Ar), 5.0 (s. 1H, OH-Ar-), 2.0 (s.2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 253, found 252.

B(V): IR (cm^{-1}): 3662.94 (-NH_2 str), 3140.10 (Pyridine str), 2953.12 (–CH Ar- str), 1483.31 (>C=N- str.), 1095.60 (triazole str); [1] H-NMR: 7.5-8.7 (d. 4H, -Pyridine), 6.4-7.13 (d. 4H, -Ar), 4.0 (2H, NH_2-Ar.), 2.0 (s. 2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 252, found 251 .

B(VI): IR (cm^{-1}): 3263.68 (-NH_2 str), 3223.46 (Pyridine str), 2405.21 (S-H str.), 1521.89 (>C=N-str.), 1099.46 (triazole str); [1] H-NMR: 7.65-8.65 (d. 4H, -Pyridine), 3.82 (s. 2H, -CH_2-Triazole), 2.2 (s. 2H, NH_2-Triazole), 1.51 (s. 1H, SH). MS(*m/z*) : M^+ calculated 253, found 252.

B(VII): IR (cm^{-1}): 3672.59 (Ar-OH str.), 3242.45 (-NH_2 str), 3070.78 (>CH Ar- str), 3009.05 (Pyridine str), 1483.31 (>C=N- str.); 1089.82 (triazole str); [1] H-NMR: 7.65-8.65 (d. 4H, -Pyridine), 5.1 (s. Ar-OH), 2.0 (d. 2H, -NH_2-Triazole). MS(*m/z*) : M^+ calculated 251, found 250

B(VIII): IR (cm^{-1}): 3425.69 (-NH_2 str.), 3072.71 (Pyridine str), 3036.06 (C-H Ar- str.), 1496.81 (>C=N- str.), 1327.07 (–CH_3 Ar- str), 1072.46 (triazole str); [1] H-NMR: 7.65-8.65 (4H, -Pyridine), 7.32-7.61 (4H, -Ar), 2.35 (3H, CH_3-Ar), 2.0 (2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 161, found 160.

B(IX): IR (cm^{-1}): 3308.03 (-NH_2 str), 3010.98 (Pyridine str), 1481.38 (>C=N- str.), 1093.67 (triazole str); [1] H-NMR: 8.65 (s. 1H, -Triazole), 7.65-8.35 (d. 4H, -Pyridine), 2.1 (s. 2H, NH_2-Triazole). MS(*m/z*) : M^+ calculated 175, found 174.

B(X): IR (cm^{-1}): 3574.21 (-NH_2 str), 3037.90 (Pyridine str), 1483.31 (>C=N- str.), 1332.86 (>C-CH_3 str.), 1003.02 (triazole str); [1] H-NMR: 7.65-8.65 (d. 4H, -Pyridine), 2.35 (d. 3H, -CH_3 Triazole), 2.0 (s. 2H, NH_2Triazole). MS(*m/z*) : M^+ calculated 207, found 206.

In vitro Antimicrobial Study [7]

A cup plate method was employed for the *in vitro* study of antibacterial and antifungal effect against *Bacillus subtilis, Staphylococcus aureus, Proteus mirabilis, Salmonella typhi, Candida albicans* and *Aspergillus niger*. The inhibitory effect of compound **B(I-X)** against these organisms are given in table 2.

Table No. 2: Antibacterial and antifungal Activity of Synthesized Compounds.

Comp.	Conc. (µg/ml)	Zone of Inhibition (mm)					
		Bacillus subtilis	***Staphylococus aureus***	***Proteus mirabilis***	***Salmonella typhi***	***Candida albicans***	***Aspergillus niger***
I	1000	25	24	27	26	18	17
	500	20	20	22	21	14	12
	250	17	16	18	17	9	0
	Std. 1000	28	28	28	28	25	22
II	1000	25	23	25	24	18	17
	500	22	20	21	20	12	10
	250	19	17	19	17	4	4
	Std. 1000	28	28	28	28	25	22
III	1000	26	25	27	25	20	18
	500	24	23	22	21	14	12
	250	19	18	19	17	9	0
	Std. 1000	28	28	28	28	25	22
IV	1000	25	24	27	23	20	19
	500	21	20	24	20	14	12
	250	18	17	21	17	9	0
	Std. 1000	28	28	28	28	25	22
V	1000	24	25	25	23	20	18
	500	21	23	22	20	14	12
	250	18	20	19	17	9	0
	Std. 1000	28	28	28	28	25	22
VI	1000	27	27	27	26	20	19
	500	23	24	25	24	14	12
	250	21	19	20	20	9	0
	Std. 1000	28	28	28	28	25	22
VII	1000	25	26	25	24	20	18
	500	22	24	22	21	14	12
	250	19	18	19	17	9	0
	Std. 1000	28	28	28	28	25	22
	1000	26	25	24	21	18	17
	500	23	23	21	16	14	12

VIII	250	19	18	18	15	9	0
	Std. 1000	28	28	28	28	25	22
IX	1000	26	25	27	25	18	17
	500	24	23	22	21	12	10
	250	19	18	19	17	4	4
	Std. 1000	28	28	28	28	25	22
X	1000	26	25	27	25	20	18
	500	24	23	22	21	14	12
	250	19	18	19	17	9	0
	Std. 1000	28	28	28	28	25	22

Std. = Levofloxacin for antibacterial activity
Std. = Amphotericin B for antifungal activity

In vivo Anti-inflammatory Study [8]

Carrageenan- induced hind paw odema model in rat was used in order to screen the *in vivo* anti-inflammatory profile of synthesized compounds. We selected **B (II, III, IV, XI and XII)** compounds for their anti-inflammatory activity given in table 3.

Table No. 3. Anti-inflammatory Action of Synthesized compounds in Carrageenan-induced rat paw Oedema:-

Sr. No.	Treatment	Paw Thickness measured by digital Vernier Calipers at			Percentage of Inhibition
		1 hr	2 hr	3 hr	
1	Solvent Control (0.5% CMC) (1 ml/kg)	0.82±0.04	0.85±0.06	0.87±0.08	-
2	Indomethacin (20 mg/kg, s.c.)	0.31±0.02**	0.28±0.01**	0.24±0.01**	72.41
II	Test Compound (100 mg/kg, p.o.)	0.59±0.03	0.54±0.02	0.48±0.02*	44.76
	Test Compound (200 mg/kg, p.o.)	0.53±0.03	0.48±0.03*	0.43±0.02**	50.57
III	Test Compound (100 mg/kg, p.o.)	0.61±0.03	0.85±0.06	0.48±0.03*	44.18
	Test Compound (200 mg/kg, p.o.)	0.56±0.03	0.28±0.01**	0.41±0.02**	52.32
IV	Test Compound (100 mg/kg, p.o.)	0.66±0.04	0.60±0.03	0.53±0.03*	38.37
	Test Compound (200 mg/kg, p.o.)	0.63±0.03	0.57±0.03**	0.48±0.02**	44.18
VI	Test Compound (100 mg/kg, p.o.)	0.62±0.04	0.59±0.03	0.54 ± 0.03*	37.93
	Test Compound (200 mg/kg, p.o.)	0.58±0.04	0.53±0.02**	0.49 ±0.02**	43.67
VII	Test Compound (100mg/kg, p.o)	0.61±0.03	0.55±0.05	0.52±0.04*	35.65
	Test Compound (200mg/kg, p.o)	0.54±0.04	0.52±0.05**	0.47±0.06**	41.56

Values are mean ± SEM,
No. of animals in each group are 6 (n=6)
*P value < 0.05, **P value < 0.01 compared with the corresponding control.

RESULT AND DISCUSSION:

The aim of this work was synthesis of 5-substituted-3-pyridine-1, 2, 4 triazoles. In order to achieve this aim it was neccesary to first synthesize oxadiazole i.e **A(I-VII)** and **A(VIII-X)** by using some substituted aromatic and aliphatic acid repectively . Oxadiazole were prepared by reaction of isoniazid and different aromatic and aliphatic acid in presence of phophorous oxychloride . The next step was conversion of the derivative **A** into 5-substituted-3-pyridine-1,2,4 triazole . The strcuture of compound **B(I-X)** were established by their IR,^{1}H-NMR and Mass Spectra. The synthesized compounds contains C=N group which give absorption in IR-spectra study in range (1400-1650 cm^{-1}), Pyridine stretching in the range of (3000-3250 cm^{-1}), >NH group absorbs in the range of (3250-3650 cm^{-1}), triazole stretching in the range of (1000-1100 cm^{-1}). In the ^{1} H-NMR spectra all the protons of the synthesized compound of pyridine given the δ (ppm) value in the range of (7.65-8.65) it confirms that the synthesized compounds contains Pyridine ring. The synthesized compounds were confirmed by the Mass spectra studies which have given the M+ peak of the all the synthesized compounds. All the compounds obtained in the form of moderate to high melting solid. The spectral data are in good agreement with the proposed structures.

The screening result indicates that all compound exhibited moderate to good antibacterial and antifungal activities. It can be noted that compounds with free NH_2 in 4^{th} position **C (I-X)** showed the greatest inhibitory effect against one or more type of bacteria. Also due to presence of triazole ring system in synthesized compounds, that exhibited antimicrobial activity. Compounds **II, III, IV** showed moderate antibacterial activity. Among the synthesized compounds, compound **VI** showed good antibacterial activity. From antifungal activity it is cleared that, compound **II, III and IV** showed mild activity. Among the synthesized compounds, compound **VII** showed moderate antifungal activity. From result, we can see the compound showed lower fungicidal effect compared with their bactericidal effect. In *in vivo* antiinflammatory activity, among the selected synthesized compounds ,compound **II and III** showed good activity. We also screened all selected compounds for ulcerogenic adverse effect at 200mg/kg dose level. After microscopic examination, no ulceration risk was seen in selected compounds which have triazole moiety in their structure.

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