SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL 1,2,4-TRIAZOLES

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ABSTRACT

1,2,4-Triazoles and their derivatives have a distinct place in the field of medicinal and pharmaceutical chemistry. They are utilised as starting materials in the synthesis of various heterocyclic compounds, many of which have important biological functions and diverse biological activities. They serve as the basic building blocks for the design and synthesis of several pharmaceutical molecules. As analgesic, antiseptic, antibacterial, antioxidant, anti-urease, anti-inflammatory, diuretic, anticancer, anticonvulsant, antidiabetic, and antimigraine medicines, 1,2,4-triazole has a wide range of therapeutically attractive pharmacological candidates.Several medications with triazole structures have been created and proven, including fluconazole and ketoconazole. Their structure-activity relationship, biological activity and design strategy of diverse mono and poly substituted triazoles, all work to increase the level of acceptance of this heterocyclic ring in the field of medicinal chemistry. An awareness of the linkage between the chemistry of a certain molecule or combination of compounds and their interaction with the body is the basis of understanding in medicinal chemistry. The mode of action refers to the process through which the chemicals affect the biological system. This is used to maximise the beneficial effects of medications while minimising their negative side effects together with structural research. Hormones, vitamins, and biochemicals were produced as a result of research projects in medicinal chemistry, which helped to pave the way for the creation of medications for veterinary use.

Keywords: 1, 2, 4-Traizole, Structure-activity relationship, Antineoplastic, Veterinary, Fluconazole, Ketoconazole.

INTRODUCTION

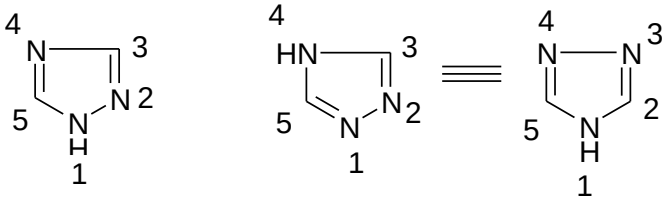
Pharmaceutical chemistry is largely concerned with the investigation of medications and the alteration of structures with known physiologic or pharmacologic effects. The influence of an

organic functional group on a molecule's acid/base characteristics, water solubility, partition coefficient and crystal structure are referred to as a molecule's physico-chemical properties.[1,2]. The word 'drug' is derived from the French word drogue, which means a dry herb. Generally, a drug may be defined as a substance used in prevention, diagnosis, treatment and cure of diseases in men or animals. In many cases, synthetic materials are frequently recommended over natural compounds, for example, synthetic dyes are superior to those derived from natural sources. In other instances, the synthetic materials completely lack any natural counterparts and are used to meet needs that cannot be met by any other source. Ether, glycol, aspirin, and sulpha medicines are few examples. Almost every aspect of life is impacted by synthetic organic chemistry.[3,4,5].

In search of better drugs, the approach employed by the modern synthetic chemists start with the search of leads. This requires the assessment, improvement and extension of the lead. From the practical viewpoint it is the later area where in rational approaches to drug design, have been mostly productive with fruitful results[6,7].

1.3.1. Chemistry of 1,2,4-Triazoles

1,2,4-Triazoles are cyclic hydrazidines with hydrogen atom (or substituent) on either hydrazide nitrogen or amide nitrogen[8,9]. Parent 1,2,4-triazole (1*H*-form) is in tautomeric equilibrium with 1,3,4-triazole (4*H*-form). The inter-conversion of two tautomeric forms occurs rapidly and their separation is difficult, however 1,2,4-triazole tautomer is preferred over 1,3,4-triazole tautomer[10,11,12].

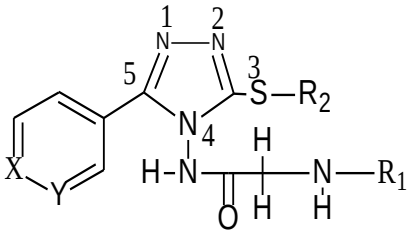


1*H*-1,2,4-triazole 4*H*-1,2,4-triazole 1*H*-1,3,4-triazlole

OBJECTIVES

The objectives of the proposed research work:

►To synthesize newer 4,5-Disubstituted-1,2,4-triazoles and screen them for antibacterial and antifungal activity.

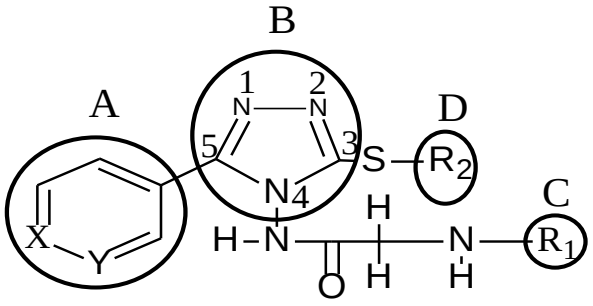


Where: X=C and Y=N- Nicotinic acid, and X=N and Y=C- Isonicotonic acid

►To develop proper SAR around 1,2,4-triazoles to observe optimum antimicrobial activity.

►To understand the significance of heteroaromatic ring placed at 5th position of 1,2,4-triazoles.

►To understand the biological role of heteroaromatic amines positioned at the terminal carbon of fourth position of 1,2,4-triazoles.

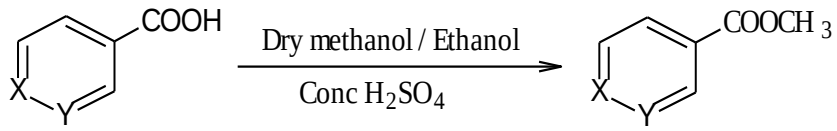


*** Target sites for chemical modification A, B, C, D**

EXPERIMENTAL

Synthesis of compounds were carried out as follows:

Step-I: Synthesis of Ester from acid

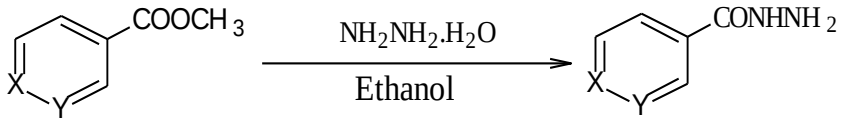


Substitituted benzoic acid Substitituted Ester

Where X=C and Y=N

A mixture of substituted hetero aromatic acid (0.1mol), 130ml of absolute alcohol and 3.3ml of conc. H_2SO_4 was refluxed for 2hrs on water bath. After completion of reaction, excess of ethanol was distilled off and content was transferred into separating funnel containing 310ml distilled water. Carbon-tetrachloride (20ml) was added, aqueous layer and ester layers were separated. Ester layer (lower layer) was taken in another separating funnel and shaken with a strong solution of sodium bicarbonate until all free acid was removed and no further evolution of carbon dioxide occur. Washed with water and dried by pouring into a small conical flask containing 7.5g magnesium sulphate. Corked the flask, shaken for 2 minutes then carbon tetrachloride was distilled off under reduced pressure. The resulting colourless liquid was collected and the completion of reaction was checked by TLC using hexane and ethyl acetate (6:4) and iodinevapours as a detecting reagent[13,14,15].

Step-II: Synthesis of Hydrazide from synthesized Ester

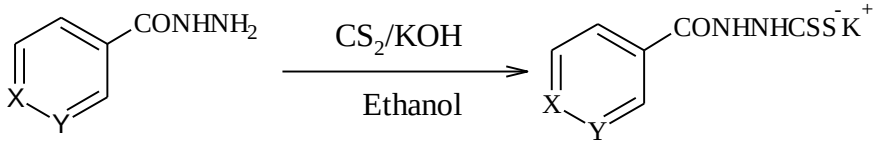


Substitituted Ester — Substituted Acid hydrazide

Where X=C and Y=N

Synthesized heteroaromaticesters (0.1 mol) and 80% hydrazine hydrate (0.1 mol) was refluxed on a water bath for 15 min. Sufficient absolute ethanol was added to obtain a clear solution. Again the contents were refluxed for 2hrs. Then excess alcohol was evaporated and solution was cooled on crushed ice**.** The solidppt obtained was separated and re-crystallized from ethanol to obtain the needle shaped crystals[16,17].

Step-III: Synthesis of Potassium dithiocarbazinate

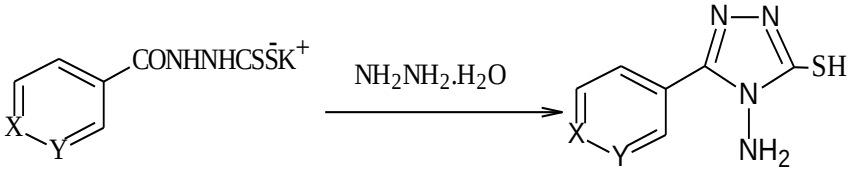


Substituted Acid hydrazide — Substituted Potassium dithiacabazinate

Where- X=C and Y=N; X=N and Y=C

Substituted heteroaromatichydrazides(0.1 mol), KOH (0.012 mol) and CS_2 (0.015 mol) in absolute ethanol (350mL) were stirred for 10 hrs. After the completion of reaction, ether (200mL) was added. The obtained precipitate was filtered, washed and dried. The synthesized dithiocarbazinate was used for the next step without further purification[18,19].

Step-IV: Synthesis of 5-Aryl-4-amino-3-mercapto-4-*H*-1,2,4-triazole

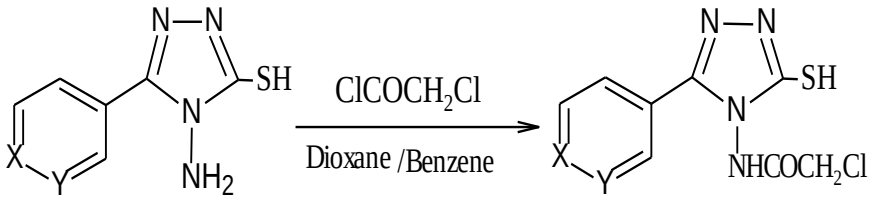


Substituted Potassium dithiocabazinate — Substituted 5-Aryl-4-amino -3-mercapto-4-*H*-1,2,4-triazole

Where-X=C and Y=N; X=N and Y=C

Substituted synthesized dithiocarbazinate (0.079mol), hydrazine hydrate (0.24 mol) and water (50mL) were refluxed for 3hrs, H_2S was evolved during the reaction and clear solution resulted, sufficient cold water was added and then the mixture was cooled to 5°C. Acidified the cold solution with dil. HCl. Obtained precipitate was filtered, washed and re-crystallized from aqueous ethanol (50%)[20,21].

Step-V: Synthesis of 5-Aryl-4-(chloroacetylamino)-3-mercapto-4*H*-1,2,4- triazole



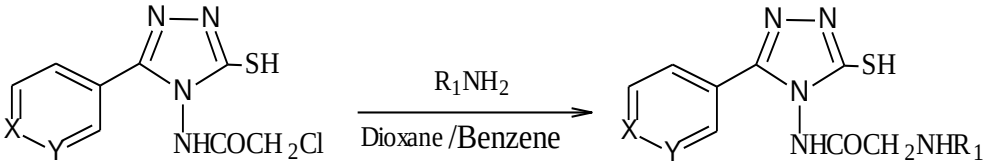
Substituted 5-Aryl-4-amino -3-mercapto-4-*H*-1,2,4-triazole

Substituted 5-Aryl-4-(chloroacetylamino) -3-mercapto-4*H*-1,2,4-triazole

Where - X=C and Y=N
X=N and Y=C

In a two necked flask fitted with reflux condenser containing 100mL benzene and obtained compound (0.1M) and separating funnel containing chloroacetyl chloride in 30mL benzene, the mixture was refluxed and chloroacetyl chloride was added in small portions. After addition of chloroacetyl chloride, solution was again refluxed for 5-6 hrs cooled and thereafter contents were poured on crushed ice. The obtained precipitate was filtered, washed and recrystallized from absolute ethanol[22,23].

Step-VI: Synthesis of Amino derivative of 5-Aryl-4-(chloroacetylamino)-3-mercapto-4*H*-1,2,4-triazole

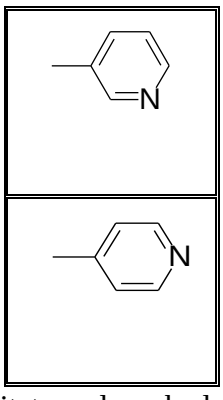


Substituted 5-Aryl-4-(chloroacetylamino) -3-mercapto-4*H*-1,2,4- triazole

Substituted Amino derivative of 5-Aryl-4-(chloroacetylamino) -3-mercapto-4*H*-1,2,4-triazole

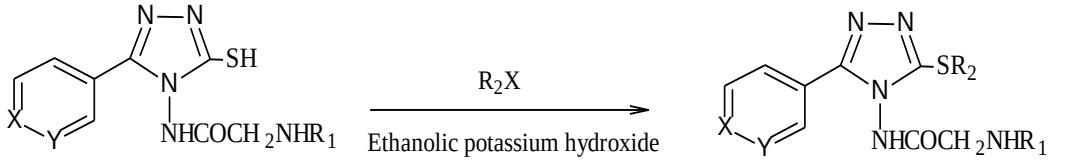
Where- X=C and Y=N
X=N and Y=C

R_1=



Synthesized substituted 5-Aryl-4-(chloroacetylamino)-3-mercapto-1,2,4- triazole (0.03 mol), respective amines (0.03 mol) and 75mL benzene were taken in round bottom flask. The contents were refluxed for 5-6 hrs and cooled. Filtered the precipitate and washed with distilled water several times to remove traces of hydrochloride. Product obtained was recrystallized from appropriate solvent[24,25,26].

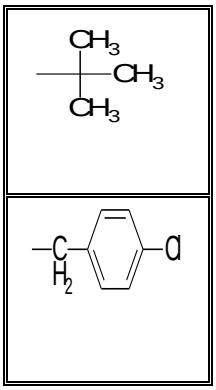
Step-VII: Synthesis of final Alkyl halide derivative of 5-Aryl-4-(chloroacetylamino)-3-mercapto-4*h*-1,2,4-triazole



Substituted Amino derivative of 5-aryl-4-(chloroacetylamino) -3-mercapto-4*H*-1,2,4-triazole

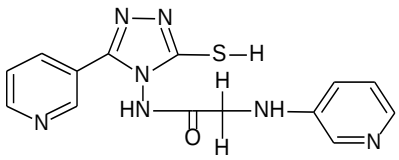
Substituted Alkyl halide derivative of 5-aryl-4-(chloroacetylamino) -3-mercapto-4*H*-1,2,4-triazole

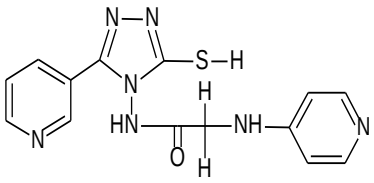
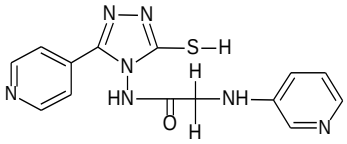
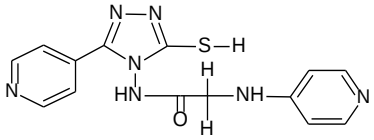
Where-X=C and Y=N; X=N and Y=C



An equimolar mixture of substituted amino derivative of 5-Aryl-4-(chloroacetylamino)-3-mercapto-4H-1,2,4-triazole and Alkyl halide (1.48 × 103 mol,1g) in ethanolic KOH [i.e., 0.08g KOH pellets in 20mL ethanol] was refluxed for 2hrs with continuous stirring which on cooling at 20-25ºC yielded a solid mass. The obtained solid was finally recrystallized with water: ethanol (20:80) to give brownish yellow shiny crystals[27,28].

Table 1: Physico-Chemical Data of SynthesizedAmino derivative of 5-Aryl-4-(chloroacetylamino)-3-mercapto-4*H*-1,2,4-triazole

Compound	**Yield (%)**	**Solvent for Recrystallization**	**Appearance**	**R.F. Value**	**M.P. (°C)**
	52	Absolute Ethanol	Light brown crystal	0.50	219-221

	55	Absolute Ethanol	Dark brown crystal	0.52	234-236
	45	Absolute Ethanol	Light brown crystal	0.54	250-254
	48	Absolute Ethanol	Dark brown crystal	0.56	265-268

Antimicrobial Screening

The antimicrobial activity of synthesized compounds was screened by agar-well diffusion method. One Gram positive bacteria (*Staphylococcus aureus* ATCC-33592) and two Gram negative bacteria (*Pseudomonas aeruginosa* ATCC-15442 and *Escherchia coli* ATCC- DT-01) and one fungal strain (*Aspergillus niger* MTCC-1344) were used for the activity. The test samples of synthesized compounds wereused at 100 µg/mL in DMF. Norfloxacin and Clotrimazole were usedasastandarddrug for antibacterial and antifungal activities respectively.

For the bacterial screening of the synthesized compounds the following bacterial species were taken (Table 2).

Table 2: List of Bacterial Strains (IMTECH, 2000; Pelczar et al., 1993**).**

S. No.	Microbial strains	ATCC No.*	Gram	Incubation period
1.	*Staphylococcus aureus*	33592	+ve	48h
2.	*Pseudomonas aeruginosa*	15442	-ve	24h
3.	*Escherichia coli*	DT-01	-ve	24h

For antifungal screening the following fungal species were used (Table-3).

Table 3: List of Fungal Strains

S.No.	Fungal strain	MTCC* No.	Incubation Temperature (°C)	Incubation period
1.	*Aspergillusniger*	1344	25	5 days

RESULTS AND DISCUSSION

The success of the syntheses was confirmed through physical and spectral characterization. In synthesized compound A, the peaks observed at 3087.78 cm^{-1} for C-H group and 1519.33 cm^{-1} for C=N group in IR spectra matched well with its structure. Chemical shift observed in range of 8.12-8.98 ppm in NMR spectra of synthesized compound has delta value assignable to corresponding protons in its structure. This is supported by mass spectra, where characteristic $(M+H)^+$ peak was observed clearly at 384.2841m/z. Further, in synthesized compound B, the peaks observed at 3269.12 cm^{-1} for C-H group and 1364.60 cm^{-1} for C=N group in IR spectra determined its structure. Chemical shift observed in range of 8.01-8.24 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 384.0458 m/z. Similarly, in synthesized compound C, the peaks observed at 1607.73 cm^{-1} for C=O group and 1518.91 cm^{-1} for C=N group in IR spectra. Chemical shift observed in range of 8.35-8.39 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 450.2210 m/z. In synthesized compound D, the peaks observed at 3177.89 cm^{-1} for C-H group and 1450.59 cm^{-1} for C=N group in IR spectra. Chemical shift observed in range of 8.11-8.62 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 450.3271 m/z. In synthesized compound E, the peaks observed at 1709.21 cm^{-1} for C=O group and 1491.20, 1401.03 cm^{-1} for C=C group in IR spectra.

Chemical shift observed in range of 8.13-8.98 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 384.1432 m/z. In synthesized compound F, the peaks observed at 1618.21 cm^{-1} for C=O group and 1523.06 cm^{-1} for C=N group in IR spectra. Chemical shift observed in range of 8.33-8.73 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 384.0160 m/z. In synthesized compound G, the peaks observed at 3160.57 cm^{-1} for N-H group and 1490.69, 1416.89 cm^{-1} for C=C group in IR spectra. Chemical shift observed in range of 8.11-8.627.04-7.88ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 452.4311 m/z. In synthesized compound H, the peaks observed at 3156.31 cm^{-1} for C-H group and 1522.39, 1491.02, 1449.08 cm^{-1} for C=C group in IR spectra. Chemical shift observed in range of 7.04-7.88 ppm in NMR spectra. In mass spectra, characteristic $(M+H)^+$ peak was observed at 452.1713 m/z. All these spectral analyses expressed the structures of potent synthesized compounds efficiently.

All of these synthesized compounds contain the basic triazole nucleus as well as one additional nitrogen in the heterocyclic aromatic ring attached at the terminal carbon of the chain attached at 5th position of triazole. All the Synthesized compounds of code no A, B, C, D, E, F, G, H resulted in the antifungal activity because of the presence of triazole nucleus.

CONCLUSION

From exhaustive research on antibacterial and antifungal activity of 1,2,4-triazole, it may be concluded that-.

The synthesized compounds are better antibacterial agents as compare to antifungal agent. Almost every compound possessed antibacterial activity whereas antifungal activity was observed with only few compounds. A-9 and A-3 showed significant activity against Gram positive *S. aureus* and *B. subtilis* respectively. C-1 and D-6 observed activity against Gram negative *P. aeruginosa* and *E. coli* respectively. As far as antifungal activity is concerned; only C-3 and D-2 were found to be active.

ACKNOWLEDGEMENT

We express our sincere gratitude to the Hon'ble Vice Chancellor, Prof. Vinay Kumar Pathak for his continuous support and motivation and School of Pharmaceutical Sciences, Chhatrapati Shahu Ji Maharaj University, Kanpur, India, for providing the library facilities and sophisticated analytical instrument facilities.

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