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SYNTHESIS OF BENZOTHIAZOLE WITH PYRAZOLES AND STUDY ITS ANTIBACTERIAL ACTIVITY AGAINST VARIOUS MICROORGANISMS

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ABSTRACT

Background: Benzothiazole is a five membered thiazole ring fused to a benzene ring attached at 2nd, 3rd position having a wide range of therapeutic efficacy like antibiotic, antitubercular, anticancer, antimycotic, NSAID and antihypertensive effects. Currently, we reported synthesis of Benzothiazole derivatives by using pyrazole and screened for their antibacterial activity. Benzothiazole derivatives are found to be crucial with their great therapeutic effectiveness of in various paths of both bio-chemistry and medicinal chemistry. Pyrazole is an organic heterocyclic compound with the formula $C_3H_3N_2H$. **Methodology:** Chemicals (Glacial Acetic Acid, Bromine, Potassium Thiocyanate, Benzene, Ethanol, Ammonia Solution, Alcohol, Ethylene Glycol) Equipments (Magnetic stirrer and Water bath) Standard drug-Streptomycin and Procaine penicillin and the bacterial activity of synthesized Benzothiazole derivative was evaluated by using Cup-plate method. **Result:** In present work, fluorochloroaniline was treated with Potassium thiocyanate accompanied by bromine, glacial acetic acid, NH_3 to obtain 2- amino–6-fluoro-7-chloro (1,3)- benzothiozole, added in hydrazine hydrate and ethylene glycol followed by concentrated Hydrochloric Acid. It was then refluxed up to 8 hours to obtain 7-chloro-6-fluoro-2-hydrazinyl- 1,3-benzothiazole. Isolated compound was identified by the application of various related standard parameters including solubility test, TLC evaluation, laboratory interpretation findings, Infrared screening, Proton nuclear magnetic resonance and Mass spectral studies, etc. The compounds were then analyzed for

their antibiotic, antitubercular, anticancer, antimycotic, NSAID and antihypertensive effects (*in-vitro*). **Discussion:** Chemical isolation and therapeutically identification of the compound 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl)-3-methyl-1H-pyrazol-5-ol was performed for antibiotic efficacy opposed to various microbial agents and the outcome was found to be gram positive with Staphylococcus aureus and Streptococcus aureus and gram negative with respect to E.coli and pseudomonas respectively.

KEYWORDS: Benzothiazoles, Pyrazole, Antibacterial activity, Cup-plate method, Staphylococcus aureus, E.coli, Dimethyl formamide and fluorochloroaniline.

INTRODUCTION

Pyrazole compounds are generally familiar for their antibiotic as well as therapeutic properties. Pyrazole derivative shows antimicrobial, anticancer and anthelmintic, anticonvulsant activity. Benzothiazoles with pyrazole are described to have numerous therapeutic values in clinical aspects. Pyrazoles are heterocyclic compounds (chemical formula $C_3H_4N_{2)}$ with 3 carbon atoms fused in a 5-membered ring. Pyrazole is a weak base with pKb 11.5. Benzothiazole is a transparent, modest sticky solution, aromatic in nature. Benzothiazole is a prevalent bicycle ring system. It is known to demonstrate a wide range of biological characteristics encompassing antibiotic, antitubercular, anticancer, antimycotic, NSAID and antihypertensive effects.

An effort made to synthesis of fluorobenzothiazoles comprising pyrazole derivatives. Pyrazole nucleus was subjected to study its biological and therapeutically effectiveness, to synthesize substituted Fluorobenzothiazolopyrazole studied for antimicrobial and analgesic (*In-vitro*) activity.[1]

Medicinal chemistry is concerned with a wide range of biological, medicinal, and pharmacological issues. It mainly deals with the development, discovery, design, identification, and synthesis of biologically active compounds, as well as the research of their metabolism, molecular interpretation, and the building of structure-activity connections.

Much like synthetic chemistry, medicinal chemistry is really an art and a science. To collect and synthesize mountains of chemical and biological data, you'll need to have a broad thinking. It takes instinct to choose the proper path to take, as well as skill to

execute the plan the strategy that will lead to the desired outcome. Most importantly, achieving the aim of a successful commercialized product demands a balance of originality and endurance in the face of adversity.[2]

Medicinal chemistry's technologies have evolved substantially in the last several years, and they continue to evolve today. Once they approach the pharmaceuticals industry, majority molecular biologists learn about using these instruments though trial and error, which could also take years. As the industry tries to establish a suitable paradigm to meet the high expectations for producing new medications, medicinal chemists continue to reinterpret their involvement in the process of drug discovery. However, whatever the outcome of this new paradigm, synthetic and medicinal chemistry will continue to play a critical role in drug development. Designing and synthesising new chemicals, as well as evaluating biological testing data to generate a new hypothesis as the basis for future investigations, are all part of pharmaceutical chemistry.

It is the science of drugs' particle and functional characteristics which also helps in designing, formulating, study of therapeutic efficacy of a drug. Study of functional properties is crucial for the creation of novel medications. Design and synthesis of novel compounds employing technologies like structure activity relationships, computational chemistry, and computer-aided targeted therapies are currently essential for progress in the field. Owing to simultaneous breakthroughs in life science and chemistry, particularly the understanding entire genes, rational design of therapies targeted to specific proper positions has become a reality in recent years. Chemists are still working on product development, formulation, evaluation.[3]

A medicinal chemist's level of experience is not only a reward as well as a challenge also. Analyzing such great variety of subjects is not at all easy matter, but there is enough of theoretical inducement in recognizing conflict in case of disorder from microscopic level.

With the discovery of cimetidine and captopril the first attempts at truly constructing a medicine to work near to specific objective occurred in1970. Afterwards the drug design has picked up steam, with sophisticated computational and structural methodologies supporting in the operation.

To assess the molecule formation, novel breakthroughs in organoleptic administration including X-ray crystallography, electrophoresis, micro-raman investigations such as UV-Visible, IR, 1H NMR, and Thermogravimetric analysis have proven to be immensely helpful to drug design.[4]

Pyrazole Compounds AND Their pharmacological interest

Chemistry

Pyrazole is a colourless solid with a melting point of 700° C. Such great merit is caused by inter-molecular hydrogen bonds, causeing the pyrazole to dim. Pyrazole may be deduced from pyrazole derivatives. Pyrazole has aromatic features, such as the ability to be bleeched, nitrated and sulphonated easily at 4^{th} place. Pyrazine can have the resonant formation listed below. Pyrazole is a weak base forming salts with inorganic acids when an acyl group replaces the imino hydrogen. Pyrazole can't be effected by reducing and oxidising chemicals, although it can be catalytically hydrogenated, first to pyrazoline, then to pyrazolidine. Both of these are important.[5]

Pharmacological interest

Pyrazole derivatives are a fascinating group of organic molecules with a wide range of chemical and pharmacological properties. Pyrazolines have gotten a lot of press in the last several yrs. Pyrazoline analogs have special role because of the broad spectrum of biological activities they exhibit.

MATERIALS AND METHOD

Chemicals

- Glacial Acetic Acid
- Bromine
- Potassium Thiocyanate
- Benzene
- Ethanol
- Ammonia Solution
- Alcohol
- Ethylene Glycol

Equipments

- Magnetic Stirrer

- Water Bath

Method

- Cup plate method

Experimental section

Synthesis procedure

- 8 gm/(0.08mol) KSCN and 1.45g/(0.01 mol) fluorochloroaniline were added to 20 ml glacial acetic acid and allowed to be cooled below 25^0 C.
- IT was then positioned in a water bath, being agitated continuously. 1.6 ml bromine was added in 6ml of glacial acetic acid and then introduced with the help of dropping funnel keeping the temperature at 25^0 C.
- On adding bromine, the solution was stirred up to 2 hrs and kept overnight. As a result, an orange precipitate was formed at the base. To it 6 ml water was mixed and allowed to heat at 85^0 C and filtered.
- The orange precipitate was poured in a reaction flask adding it to 10 ml glacial acetic acid and heated at 85^0 C. The hot solution was cooled and pH was dropped to 6 by adding NH_3 solution. The result was a dark yellow precipitate of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole obtained during re-crystallization by ethanol in benzene (1:1). Melting of the final residue 210-212^0 C after drying in an oven at 80^0C.

Discovery and Characterisation

To ensure that all of the produced compounds had distinct chemical natures than their respective parent compounds, the following approach was used to identify and characterise them.

1. Boiling point
2. Dispersibility
3. TLC
4. IR
5. NMR
6. Mass spectroscopy

Boiling point evaluation

The open capillary tube approach was applied for the evaluation of the melting points of organic composition. These have not been corrected. Because a pure crystal has a definite

and sharp boiling point. Purity should be determined by observing the altering boiling point after purification by recrystallisation of the compound. After recrystallization, produced compounds revealed minor altered boiling point.

Dispersibility

The solubility characters were listed in tabular column. Results are represented in the Table 1.

TLC

Chromatography is a useful technique for determining the purity of a substance as well as identifying the production of new compounds. The Rf value is unique to each of the compounds. Findings are mentioned in Table 2.

Composition of chromatogram

Glass plates were cleaned and dried. A uniform silica Gel-G slurry was added into alcohol and placed into the TLC applicator's chamber, which had been spotted with 0.5 mm thick layer. The TLC sheets were allowed to move smoothly along with the applicator to ensure a consistent slurry lamination. The TLC sheets were exposed to normal temperature for drying before being activated in microwave at 110^0C up to 1 hour.

Solvent formulation

The chromatogram development solvent system was meticulously produced on fusing n-Butanol: Ethyl acetate: chloroform [1:2:1]

Sample application

In a tiny drilled capillary tube, the parent chemicals and their target molecule were mixed and marked 2 cm above the plate's bottom. After spotting, plates were allowed to dry at ambient temperature before being transported to a chromatographic cell with a development solvent solution.

Chromatogram formulation

When the solvent front had reached a distance of 10-12 cm, plates were developed by ascending method and dried at room temperature.

Spotting

Exposure to iodine vapours was used to detect the formed spots.

R_F value determination

The given formula was used to compute the Rf values of substances.

$$\text{Rf Value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

The sample run through different length in all cases from the length covered by the parent component identified alongside it. As a result, the fact that the compounds created were completely different from the parent substance was confirmed. Because the sample yielded solitary spot, chemicals were supposed to be pure. Compounds' Rf values were noted.

Study of antimicrobial activity

The selected compounds prepared different screened anti- bacterial characteristics:

- Staphylococcus aureus (Gram+ve) (MTCC727)
- Streptococcus aureus (Gram+ve) (MTCC737)
- E.coli (Gram-ve) (MTCC1687)
- Pseudomonas aeruginosa(Gram -ve) (MTCC1035)
- Candida albicans(MTCC3018)
- Aspergillus fumigates. (MTCC2737)

Antimicrobial efficacy

Cup Plate Method is used to undertake antibacterial activities (Diffusion Technique). Fresh bacteria cultures are prepared by inoculating bacteria into nutrient broth media and incubating for 18–24 hours at 37 2 C.

Following aseptic methods, this culture was combined with nutritional agar media and put onto petridishes. The plates were placed in the refrigerator for 2 hours after the media had solidified.

Using a sterile steel cork borer, four bores are created at similar distances after two hours of cold incubation (8 mm diameter). Different concentrations of conventional medications and synthetic substances are added to these cups. Dimethyl formamide added as a controlling

agent. Adding with standard drugs, synthesized product plates were allowed to settle inside the refrigerator for 2 hrs for proper drug-media diffusion.

2 hrs later, the plate was placed in an incubator and kept at 37^0 degrees Celsius for 18 to 24 hours. Using a vernier scale, the petriplates were inspected for zone of inhibition after the incubation period.

The results were assessed by comparing the synthetic compounds' zone of inhibition to those of conventional medicines. The results represent the average value of two sets of zone of inhibition Millimetres are used to measure distances.

The reference medication was dissolved in the smallest amount of distilled water possible, whereas the compounds were dissolved in the smallest amount of DMF possible. As conventional medications, procaine penicillin is used to treat Staphylococcus aureus, Streptococcus aureus, and Streptomycin is used to treat Escherichia coli and Candida albicans.

RESULT AND DISCUSSION

The compound's chemical isolation and medicinal identification 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl) The antibacterial activity of -3-methyl-1H-pyrazol-5-ol against several microbiological agents was tested, and the results were determined to be gramme positive with Staphylococcus aureus and Streptococcus aureus, and gramme negative with E.coli and pseudomonas, respectively.

R_{15}, R_{12}, R_{14}, R_1, R_5, R_7, R_2, R_6, R_8, R_{11}, R_9, and R_{10} showed good antibacterial activity against Staphylococcus aureus (gramme +ve), whereas R4 and R13 showed considerable antibacterial activity.

The antibacterial activity of compounds R1 to R15 opposed to E.coli (gram–ve) and pseudomonas aeruginosa was promising (gram-ve)

Compounds R_2, R_5, R_7, R_9, R_6, R_{11}, R_{13}, R_1, R_{15}, R_3, R_{12}, R_8, and R_{10} showed promising antibacterial activity against Streptococcus aureus (gram+ve), whereas R14 showed considerable antibacterial activity.

Table 1: Solubility.

Sl. No.	Compound Code	Solubility	Insoluble in
1	R1	DMF, DMSO, Alcohol, chloroform,	water, Benzene
2	R2	DMF, Alcohol, DMSO, chloroform,	water, Benzene
3	R3	DMF, Alcohol, DMSO, Chloroform,	Water, benzene
4	R4	DMF, Alcohol, DMSO, chloroform,	water, Benzene
5	R5	DMF, Alcohol, DMSO, chloroform,	water, Benzene
6	R6	DMF, Alcohol, DMSO, chloroform,	water, Benzene
7	R7	DMF, Alcohol, DMSO, chloroform,	Water, benzene
8	R8	DMF, Alcohol, DMSO, Chloroform,	water, Benzene
9	R9	DMF, Alcohol, DMSO, Chloroform,	Water, benzene
10	R_{10}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene
11	R_{11}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene
12	R_{12}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene
13	R_{13}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene
14	R_{14}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene
15	R_{15}	DMF, DMSO, Alcohol, Chloroform,	Water, Benzene

Table 2: TLC Findings.

Sl. no.	Compound Code	Solvent system	Proportion	Rf Rate
1	R1	Ethyl acetate: n-Butanol:chloroform	2:1:1	0.91
2	R2	Ethyl acetate: n-Butanol:chloroform	2:1:1	0.93
3	R3	Ethyl acetate: n-Butanol:chloroform	2:1:1	0.83
4	R4	Ethyl acetate: n-Butanol:chloroform	2:1:1	0.86
5	R5	Ethyl acetate: n-Butanol:chloroform	2:1:1	0.81

6	R6	Chloroform:n-Butanol:Ethyl acetate	1:2:1	0.53
7	R7	Chloroform:n-Butanol:Ethyl acetate	1:2:1	0.60
8	R8	Chloroform:n-Butanol:Ethyl acetate	1:2:1	0.81
9	R9	Chloroform:n-Butanol:Ethyl acetate	1:2:1	0.75
10	R_{10}	Chloroform:n-Butanol:Ethyl acetate	1:2:1	0.61
11	R_{11}	Ethyl acetate:n-Butanol:Chloroform	1:1:2	0.75
12	R_{12}	Ethyl acetate:n-Butanol:Chloroform	1:1:2	0.76
13	R_{13}	Ethyl acetate:n-Butanol:Chloroform	1:1:2	0.73
14	R_{14}	Ethyl acetate:n-Butanol:Chloroform	1:1:2	0.70
15	R_{15}	Ethyl acetate:n-Butanol:Chloroform	1:1:2	0.64

Table 3: Antibacterial activity.

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)*			
		Staphylococcus aureus		***Streptococcus aureus***	
		1mg/ml	**2mg/ml**	**1mg/ml**	**2mg/ml**
01	Procaine penicillin	20	21	18	20
03	R1	10(0.50)	12(0.60)	10(0.55)	13(0.65)
04	R2	10(0.50)	10(0.5)	9(0.50)	12(0.60)
05	R3	9(0.45)	14(0.7)	10(0.55)	11(0.55)
06	R4	16(0.80)	11(0.55)	14(0.77)	15(0.75)
07	R5	11(0.55)	12(0.60)	14(0.77)	15(0.75)
08	R6	14(0.70)	10(0.5)	13(0.72)	15(0.75)
09	R7	12(0.60)	14(0.7)	11(0.61)	14(0.70)
10	R8	12(0.60)	13(0.65)	10(0.55)	13(0.65)
11	R9	12(0.60)	10(0.5)	12(0.66)	15(0.75)
12	R_{10}	9(0.45)	13(0.65)	12(0.66)	14(0.70)
13	R_{11}	10(0.50)	14(0.7)	11(0.61)	13(0.65)
14	R_{12}	9(0.45)	12(0.60)	10(0.55)	11(0.55)
15	R_{13}	10(0.50)	16(0.80)	12(0.66)	13(0.65)
16	R_{14}	11(0.55)	12(0.60)	15(0.83)	16(0.80)
17	R_{15}	10(0.50)	9(0.45)	13(0.72)	15(0.75)

Table 4: Antimicrobial activity.

Sl. No	Name of the compounds	Mean inhibition zone (mm)*			
		E.coli		*Pseudomonas*	
		1mg/ml	2mg/ml	1mg/ml	2mg/ml
01	Streptomycin	23	24	20	21
03	R1	13(0.56)	16(0.66)	10(0.50)	12(0.54)
s04	R2	11(0.47)	14(0.58)	15(0.75)	17(0.77)
05	R3	10(0.43)	11(0.45)	12(0.60)	14(0.63)
06	R4	14(0.60)	16(0.66)	11(0.55)	12(0.54)
07	R5	12(0.52)	15(0.62)	11(0.55)	13(0.59)
08	R6	12(0.52)	15(0.62)	11(0.55)	14(0.63)
09	R7	10(0.43)	14(0.58)	11(0.55)	12(0.54)
10	R8	14(0.60)	15(0.62)	11(0.55)	12(0.54)
11	R9	13(0.56)	15(0.62)	12(0.60)	14(0.63)
12	R10	11(0.47)	12(0.50)	10(0.50)	11(0.55)
13	R11	10(0.43)	13(0.54)	10(0.50)	12(0.54)
14	R12	10(0.43)	14(0.58)	11(0.55)	13(0.59)
15	R13	10(0.43)	13(0.54)	11(0.55)	12(0.54)
16	R14	11(0.47)	13(0.54)	14(0.70)	15(0.71)
17	R15	13(0.56)	14(0.58)	13(0.65)	16(0.76)

GRAPHS

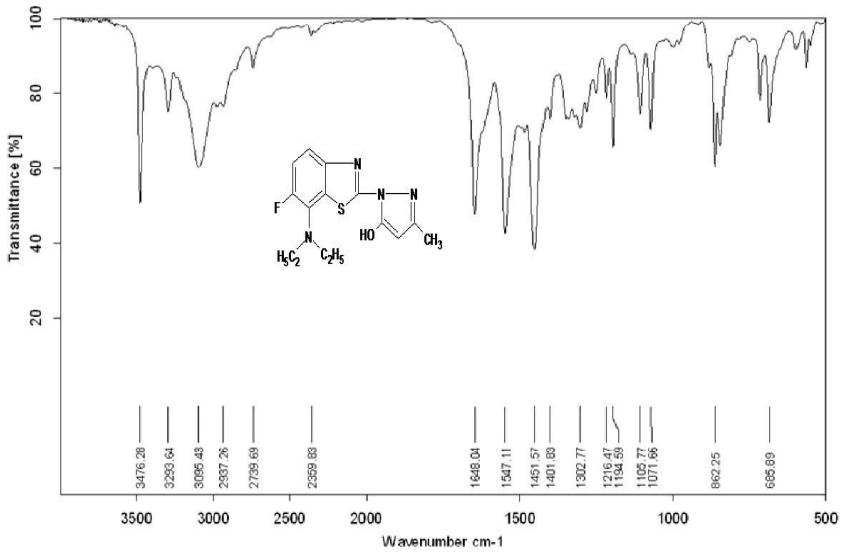

Fig. 1: Antibacterial activity of streptomycin.

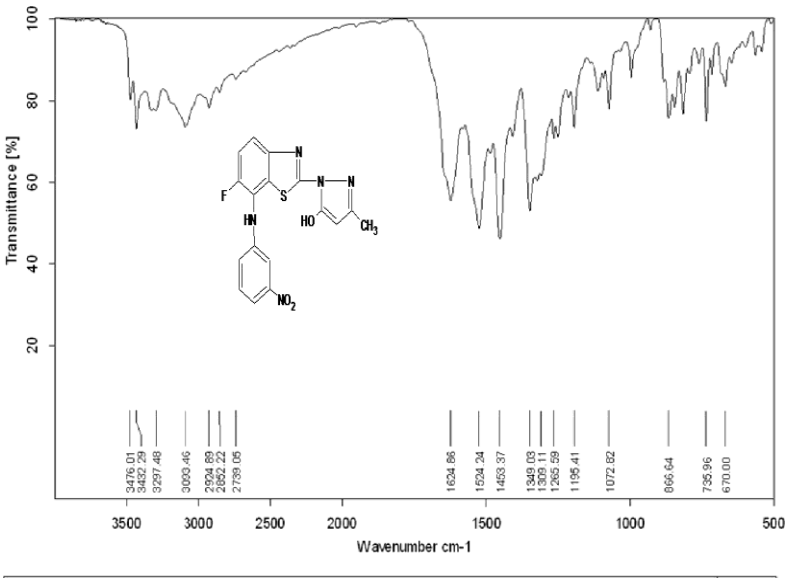

Fig. 2: Antibacterial activity of procaine penicillin.

CONCLUSION

In the current study, fluorochloroaniline was treated with KSCN and bromine along with acetic acid & ammonia to produce 2-amino–6-fluoro-7-chloro(1,3)-benzothiozole, later managed by hydrazine hydrate & ethylene glycol following conc. HCl, refluxed up to 8 hours to produce 7-chloro-6-fluoro-2-hydrazinyl-1. The synthesized compounds were recognized on the basis of dispersibility, chromatography, analytical evaluation, IR, 1HNMR, and Mass spectral, analyses. Subsequently compounds were evaluated for antibacterial, antifungal, and anti-inflammatory (in-vitro) properties (7-chloro-6-fluoro-1,3-benzothiazole-2-yl) -3-methyl-1H-pyrazol-5-ol. Different aromatic amines, N-methyl piperzine, and morpholine in the presence of DMF were treated with the aforesaid product to obtain advanced product by restoring chlorine at the point-7.

ABBRIVIATIONS

Conc: Concentration**, E. Coli:** Eshcherichia Coli, **MF:** Molecular Formula, **M. Wt:** Molecular Weight, **Min:** Minute, **BW:** Body Weight, **PO:** Per Oral.

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