ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 1 (5) 2013, 29-36

RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF MICROBIAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS CONTAINING BENZOTHIAZOLE MOIETIES

[*]Rekha.S[1], Prateek bisht[1], Chandrashekhara.S[2], Shantharam. U[3], Vineeth chandy[1], Prof. Satyanand Tyagi[4]

[1]Dept. of Pharmaceutical chemistry, T. John College of Pharmacy, Bangalore, Karnataka, India-560042.

[2]Dept. of Pharmaceutics, M.M College of Pharmacy, Belgaum, Karnataka, India-590016.

[3]Dept. of Pharmaceutical chemistry, Government College of Pharmacy, Bangalore, Karnataka, India-560027.

[4]President & Founder, Tyagi Pharmacy Association (TPA) & Scientific Writer (Pharmacy), New Delhi, India- 110074.

ABSTRACT

New derivatives of five member heterocyclic compounds containing benzothiazole rings are reported. These compounds have been characterized by elemental analysis, FT-IR and ^{1}H NMR spectroscopy, ^{13}C NMR and Mass spectral analysis. This study was designed to show the microbial activity of substituted benzothiazoles. The compounds were screened for antibacterial activity against *Escherichia coli, Staphylococcus aureus and Bacillus subtilis* in nutrient agar medium, and for antifungal activity against *Aspergillus niger and Candida albicans* in Sabouraud's dextrose agar medium. The results show that the derivatives containing benzothiazole moiety are more active.

KEYWORDS: Benzothiazole, *Escherichia coli, Aspergillus niger,* Sabouraud's dextrose agar medium

INTRODUCTION:

Chemistry of heterocyclic compounds is one of the leading lines of investigations in the organic chemistry. Heterocyclic compounds are widely distributed in nature and are essential for life. They play a vital role in the metabolism of all living cells. There are vast numbers of pharmacologically active heterocyclic compounds, many of which are a regular clinical use. Nitrogen, sulphur and oxygen containing five member heterocyclic compounds have occupied enormous significance in the field of drug discovery process. We report herein the synthesis of five membered heterocyclic derivative benzothiazole.[1] On the other hand, benzothiazoles are heterocyclic compounds with multiple applications and, although they have been known from long ago to be biologically active.[2] It is bicyclic ring system with diverse chemical reactivity and broad spectrum of biological activities such as antimicrobial, antitumor, anti-inflammatory, antilieshmanial and antifungal.[3] The synthesized compounds were screened for their antibacterial in nutrient agar medium, and for antifungal activity in Sabouraud's dextrose agar medium. Among the synthesized compounds, **6b** showed good antibacterial activity but less potent as compared to standard reference drug sulfacetamide. **6c** showed anti-fungal activity but less potent as compared to standard reference drug clotrimazole. Structures of the newly synthesized compounds were established by elemental analysis and spectral data.

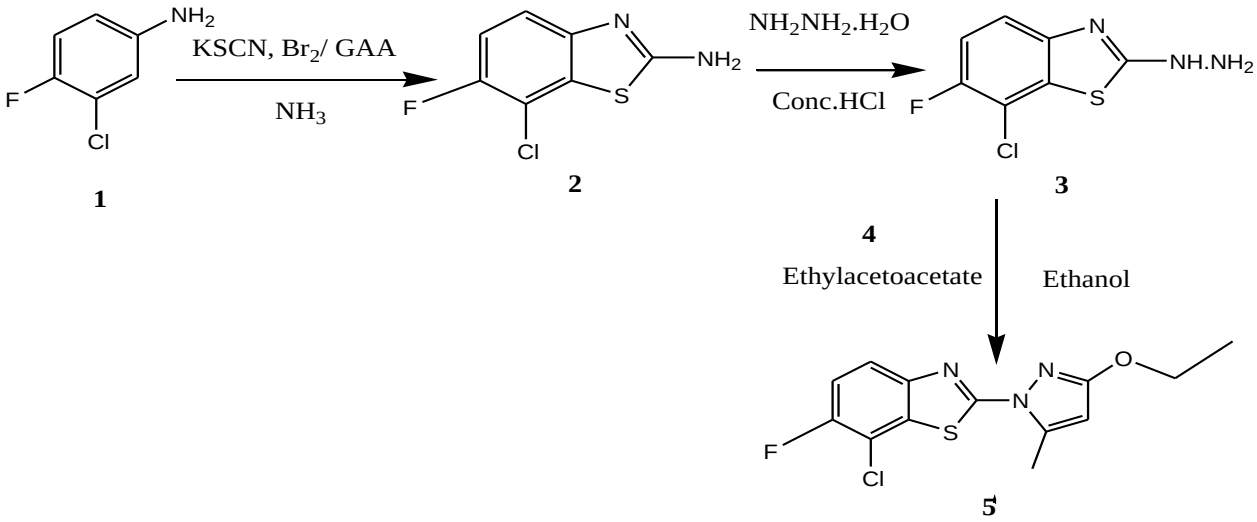
MATERIALS AND METHODS:

The chemicals used in the present project work were purchased from Rankem, Merck and Spectrochem. The melting point of the synthesized compound was determined by open capillary with Thiel's melting point tube (capillary tube method). TLC plates were prepared by using Merck Silica Gel 60 GF 254. Visualization was done in UV light chamber at 254 nm, iodine chamber. The IR spectra of the synthesized compounds were recorded on a Fourier Transform Infra Red spectrometer (model Shimadzu 8400 S) in the range of 400-4000 cm^{-1} as KBr pellets. (^{1}H NMR) data of the compound was carried out in Bruker 200 spectrospin NMR at Astra Zeneca Pharma India Limited, Bangalore and Bruker 400 spectrospin NMR at Indian Institute of Science, Bangalore. The solvent used for NMR was $CDCl_3$.

[*]*Corresponding author:* Rekha.S / *E-mail:* rekha.maheshh@gmail.com

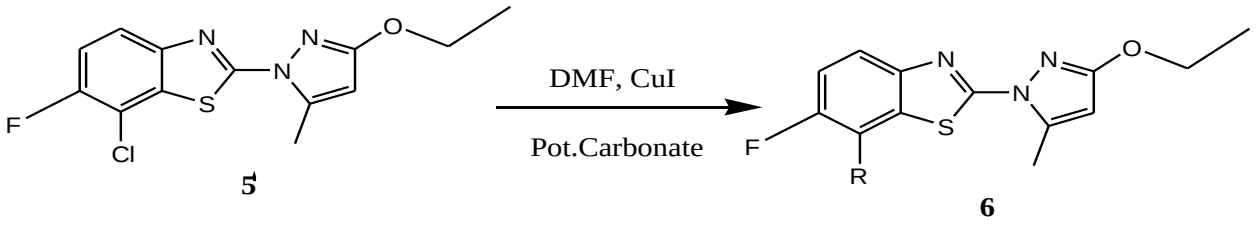
PROTOCOL OF SYNTHESIS:

Scheme 1



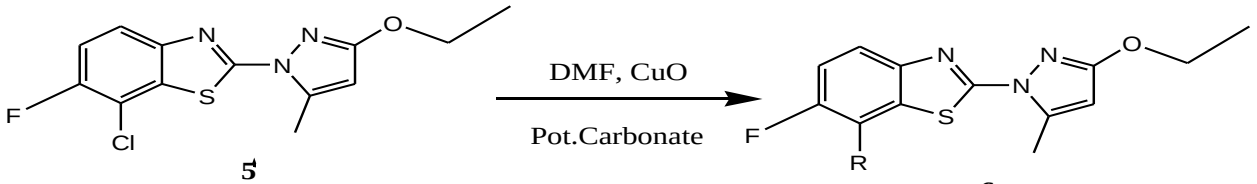
1 **2** **3** **4** **5**

Scheme 2



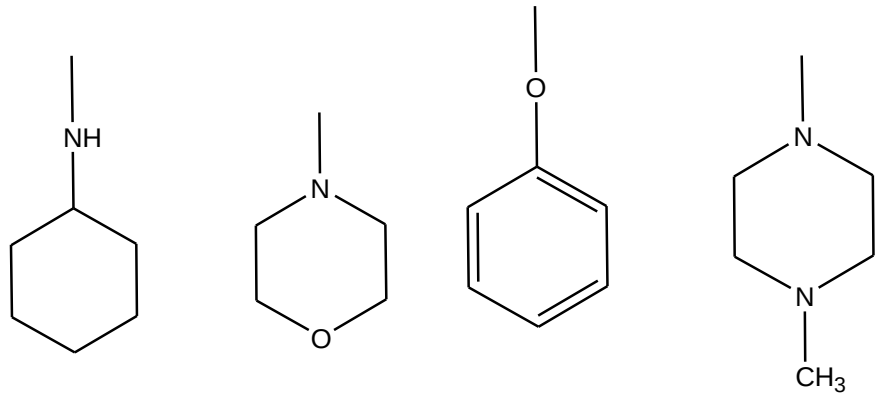
5 **6**

Scheme 3



5 **6**

6 - Cl



6a **6b** **6c** **6d**

Synthesis of 2-amino- 7-chloro-6-fluoro benzothiazole (2):

To the glacial acetic acid (20ml) which is cooled below room temperature, 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01mol) of fluorochloroaniline was added. The mixture was placed in freezing mixture of ice and salt, mechanically stirred while 1.6ml of bromine in 6ml of glacial acetic acid was added, from a dropping funnel at such a rate that the temperature never raised beyond room temperature. After all the bromine was added (105min), the solution was stirred for 2 hours below room temperature and at room temperature for 10- 12 hours, it was then allowed to stand overnight, during which period an orange precipitate settle at the bottom, water (6ml) was added quickly and slurry was heated at 85^0c on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85^0c and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6. A dark yellow precipitate was collected. Recrystalised from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow plates of 2-amino-6-fluoro-7-chloro-(1,3) benzothiazole and it is dried in a oven at 80^0C.

Synthesis of 2- hydrazino-amino- 7-chloro-6-fluoro benzothiazole (3):

Conc. HCl (6 ml) was added drop wise with stirring to hydrazine hydrate (6 ml) at 5-100C continuously with mechanical strirring. To it add ethylene glycol (24 ml) is added drop by drop in dropping funnel at such rate temperature does not exceed 5 -10 ^{o}C. To above add **2-** amino- 7-chloro-6-fluoro benzothiazole (0.03mol) and refluxed for 3-4 hours. On cooling solid separated out which was filtered, washed with wate rand recrystallized from ethanol.

Synthesis of 7-chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazole (4):

N-(7-Chloro-6-fluoro-benzothiazol-2-yl)-hydrazine and ethyl aceto acetate was placed in RBF which was add 50 ml of ethanol and refluxed for 6-10 hrs.The mixture was poured in to crushed ice and solid filter reaction mixture and then monitered by TLC. The crude product recrystallized from ethanol.

Removal of chlorine by different groups (5a-b):

To 7-Chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazole (0.1mol) and corresponding amine/ phenol/ alcohol (0.1mole) was placed in RBF along with 25ml of DMF/NMF. Anhydrous Potassium carbonate (3eq.) and either of cuprous iodide (2.5 mol %) [a] or cuprous oxide (2.5 mol %) [b] were added. The mixture heated for 16-24 hrs under nitrogen atmosphere and is monitered under TLC. The mixture poured in to crush ice and extracted with successive portion of ethyl acetate or ether. The organic layer separated and solvent removed in vaccum to obtain desired product.

***IN VITRO* SCREENING FOR ANTI BACTERIAL ACTIVITY [5-9]:**

In our current study, evaluation of antimicrobial activity was carried out by using the methods mentioned below. Here responses of microorganisms to the synthesized compounds were measured with that of the standard reference drug. The standard reference drug used was sulfacetamide.

Antibacterial Activity

The microbiological assay was based upon a comparison of inhibition of growth of microorganisms by measured concentrations of test compounds with that produced by known concentration of a standard antibiotic. Two methods generally employed were turbidometric (tube dilution) method and cylinder plate (cup-plate) method. In the turbidometric method inhibition of growth of microbial culture in a uniform dilution of antibiotic in a fluid medium was measured. It was compared with the synthesized compounds. Here the presence or absence of growth was measured. The cylinder plate method depends upon diffusion of antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate to an extent such that growth of added micro-organisms was prevented entirely in a zone around the cylinder containing solution of the antibiotics. The cup-plate method was simple and measurement of inhibition of microorganisms was also easy. Here this method was used for antibacterial screening of the test compounds.

Preparation of medium:-

- Nutrient agar 2%
- Peptone 1%
- Beef extract 1%
- Sodium chloride 0.5%
- Distilled water up to 100ml

All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it becomes clear. This nutrient media was sterilized by autoclave.

Apparatus:-

All the apparatus like petri dishes, pipettes, glass rods, test-tubes were properly wrapped with papers and sterilized in hot air oven.

Name of Microorganism

Gram +Ve microorganisms

- ✓ *Staphylococcus aureus* **(MTCC No. 96)**
- ✓ *Bacillus subtilis* **(MTCC No. 121).**

Gram -Ve microorganisms

- ✓ *Escherichia coli* **(MTCC No. 521).**

Agar plate disc diffusion method:-

- ➢ The antibacterial activity was assayed by agar plate disc diffusion method at the concentration of 50 µg per disc.
- ➢ All the synthesized compounds were tested in vitro for their antibacterial activity against Micro organisms such as Staphylococcus aureus, Bacillus subtilis (gram positive), and Escherichia coli (gram negative) strains.
- ➢ Each test compounds were dissolved in dimethylsulphoxide (DMSO) to get a concentration of 10 mg/mL.
- ➢ The disc (6 mm in diameter) was impregnated with 5 µL of each test solution to get 50 µg/disc, air dried and placed on the agar medium, previously seeded with 0.2 mL of broth culture of each organism for 18 hours.
- ➢ The plates were incubated at 37 °C for 24 hours and the minimum inhibitiory concentration measured in mg/l.
- ➢ Discs impregnated with DMSO were used as a control and amoxicillin and ciprofloxacin discs as antibacterial reference standard.

ANTIFUNGAL ACTIVITY: [5-9]

Principle

The antifungal activity of all newly synthesized benzotriazole derivatives were examined against *Candida albicans.* Antifungal screening of all the derivatives was done by using filter disc method. Clotrimazole was used as a standard drug. Activity of the compounds was recorded by measuring the zone of inhibition in mm, and compared with the standard zone of inhibition produced by clotrimazole. This determination indicates whether the organism was sensitive or resistant to the compound.

Materials used:

- ➢ **Test organisms:** *Candida albicans* was used for the determination of the activity.
- ➢ **Growth Media:** The activity was conducted on the Sabouraud dextrose agar media.

Composition:

- ➢ Enzymatic digest of Casein 5g
- ➢ Enzymatic digest of Animal Tissue 5g
- ➢ Dextrose 20g
- ➢ Final pH 5.6 ±0.2 at 25 °C
- ➢ Purified water 1000ml
- ➢

Apparatus:

- ❖ Petri plate: Glass plate, which was previousl sterilized by Dry Heat Sterilization was used.
- ❖ Pipette: Micropipette was used for adding the required concentration of the analogues to the plates.
- ❖ Glass wares: 500ml conical flask and test tubes were used.

Compounds screened: all the synthesized benzothiazole derivatives.

- ❖ Solvent used: Dimethyl sulfoxide
- ❖ Standard used: Clotrimazole

Preparation of standard solution:

The standard drug clotrimazole was dissolved in appropriate quantity of ethanol to obtain the Concentration range of 500, 750 and 1000µg/ml and the zone of inhibition were checked.

Preparation of test solution:

Specified quantity (100mg) of the compound was accurately weighed and dissolved in 100ml of DMSO to get the 1000µg/ml stock solution. Further dilution was made to obtain the concentration in the range 500µg/ml, 750µg/ml and 1000µg/ml.

Procedure:

- o 30g of the medium was suspended in 1000ml of purified water. The mixture was allowed to boil till it forms a homogeneous solution. The medium was autoclaved at 121°C for 15 minutes at 15psi.
- o Media was cooled to the temperature of approximately 40°C temperature and microorganisms were inoculated to the media. 150ml was transferred to a petri plates aseptically. Two such plates were prepared for each organism.
- o Plates were allowed to cool for 20 minutes.
- o Compounds were dissolved in DMSO and diluted in same to get concentration of 1000µg/ml.
- o Both high and low strength discs were applied for each compound to be tested.
- o The organism is reported as being sensitive if clear zone appears around both discs.

Table 1: Screening of Antibacterial activity of synthesized compounds

Compound	Zone of inhibition (mm)			
	Antibacterial activity			
	S.aureus	***B.subtilis***	***E.coli***	***P.vulgaris***
6	11	10	7	12
6a	24	31	21	14
6b	13	13	16	27
6c	8	9	10	16
6d	10	14	11	17
Sulfacetamide	25	28	27	31

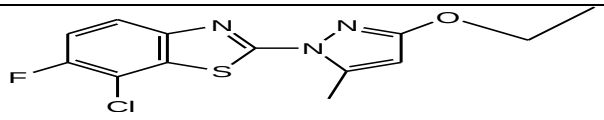
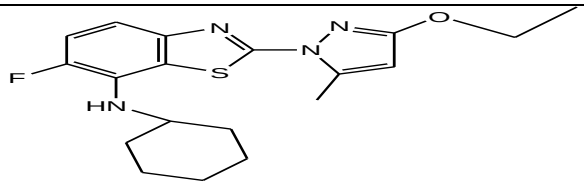
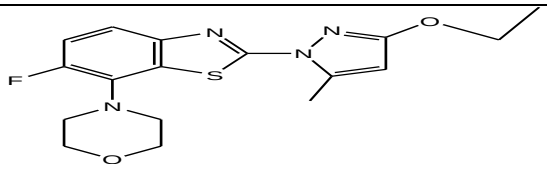
Table 2: Screening of Antifungal activity of synthesized compounds

Compound code	Conc (µgm/ml)	Zone of inhibition (mm), *C.albicans*
6	**1000**	11
6a	**1000**	15
6b	**1000**	13
6c	**1000**	12
6d	**1000**	17
Clotrimaxazole	**1000**	22

RESULTS AND DISCUSSION:

All the synthesized compounds were screened for their anti-bacterial activities against *S.aureus,B.subtilis* and *E.coli* and for anti-fungal activities against *Candida albicans*. Compounds **6a, 6b** and **6d** showed anti-fungal activity but less potent as compared to standard reference drug clotrimazole. Compounds **6b, 6c** and **6d** showed good antibacterial activity but less potent as compared to standard reference drug sulfacetamide. Compounds 6c-d was found to be very less potent towards anti-fungal activity. Rest all the synthesized compounds were found to be very less potent towards anti-bacterial activity as compared to standard reference drug sulfacetamide.

Table 3: List of compounds synthesized

6		7-Chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazole
6a		Cyclohexyl-[2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazol-7-yl]-amine
6b		2-(3-Ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-7-morpholin-4-yl-benzothiazole

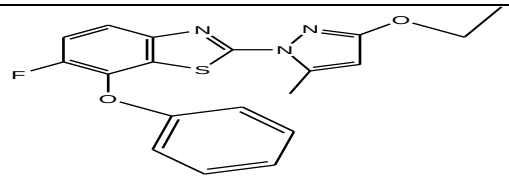
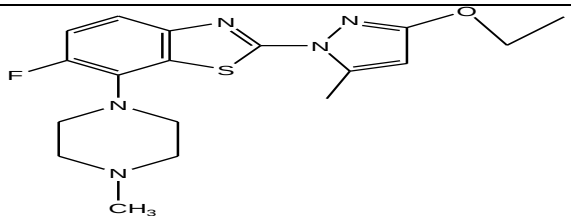
6c		(*E*)-4-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl)-2-methoxyphenol.
6d		2-(3-Ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-7-(4-methyl-piperazin-1-yl)-benzothiazole

Table 4: Physicochemical properties of synthesized compounds

Sr. No.	Compound code	Molecular Formula	Mol. Weight	Melting Point (°C)	% yield	R_f value
01	**6**	$C_{13}H_{11}ClFN_3OS$	312	167	57%	0.75*
02	**6a**	$C_{19}H_{23}FN_4OS$	374	150	58%	0.73*
03	**6b**	$C_{17}H_{19}FN_4O_2S$	362	155	51%	0.80**
04	**6c**	$C_{19}H_{16}FN_3O_2S$	369	175	55%	0.73*
05	**6d**	$C_{18}H_{22}FN_4OS$	375	152	48%	0.82**

*Mobile phase- n-Hexane: Ethyl acetate (3:1)
**Mobile phase- n-Hexane: Ethyl acetate: methanol (3:1:0.25)

Table 5: Predicted mole inspiration data of synthesized compounds

Sr. No	Comp. Code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	miLogP	TPSA(total polar surface area)
01	**6**	-0.75	-1.01	-0.70	-1.52	4.165	39.952
02	**6a**	-0.33	-0.52	-0.53	-1.22	3.048	50.435
03	**6b**	-0.38	-0.56	-0.62	-0.96	3.332	49.435
04	**6c**	-0.51	-0.67	-0.59	-0.97	5.892	38.195
05	**6d**	-0.42	-0.71	-0.51	-1.00	5.113	46.547

Table 6: Predicted data of synthesized compounds

Sr. No.	Compound code	Calculated % of element (C, H, f,0, N)	Clog p	Drug likeness	Drug score
01	**6**	50.08 , 3.56, , 6.09, 13.48 5.13, 10.29, 11.37	5.91	-4.12	0.28
02	**6a**	56.34, 5.28 , 5.24, 15.46, 8.83, 8.85	4.69	-0.81	0.32
03	**6b**	61.77 , 4.37 , 5.14, 11.37, 8.66, 8.68	4.61	-1.69	0.39
04	**6c**	61.77, 4.37, 5.14, 11.37, 8.66, 8.68	6.71	-3.93	0.19
05	**6d**	57.58, 5.91, 5.06, 18.65 , 4.26, 8.54	4.81	-3.92	0.67

Table 7: Infra red spectral study of the synthesized compounds

Sr. No.	Compound Code	Molecular nature and Spectral peaks (cm^{-1})
01	**6**	(Ar str C=C) 1535, (C-H) 2924,(C-F) 1253, (C-Cl) 736, (NH) 3163,(C=O) 1656, (CH_3)1350
02	**6a**	Ar(C=C)1529.80, (C-H)2951.79, (C-F)1276.70, (NH)3447.52, (C=O) 1679.12 ((C=S)1492.89, (CH_3) 1454.11
03	**6b**	Ar(C=C)1507.67,(C-H)2994.12,.49, (NH) str 3317.97, (C=S)1392.81, (CH_3) 1340.22 (C-F)1241
04	**6c**	Ar(C=C)1597.80,(C-H)2951.19,(C-F)1276.70, (NH_2)3447.52, (C=O) 1679.12(C=S) 1492.89, (CH_3) 1454.80
05	**6d**	A(C=C)1597.80,(C-H)2951.19,(C- F)1276.70,(NH)str3298,(C=S) 1492.89, (CH_3) 1394.80 , (C-F) 1240.27

CONCLUSION:

The preliminary in-vitro antibacterial screening of novel benzothiazole substituents were reported. The compounds did not show promising antibacterial activity. The synthetic procedure was optimized for all steps and can easily be carried out on a multigram scale. Further structural optimization studies might thus represent a rationale for further investigation.

REFERENCES:

1. Tomi IHR, Tomma JH, Al-Daraji AHR, Al-Dujaili AH, Synthesis, characterization and comparative study the microbial activity of some heterocyclic compounds containing oxazole and benzothiazole moieties, Journal of Saudi chemical society, 9 may 2012.
2. Prabhu PP, Pande S, Shastry CS, Synthesis and Biological Evaluation of Schiff's Bases of Some New Benzothiazole Derivatives as Antimicrobial Agents, IJCTR, 2011, 3, 185.
3. Latrofa A, Franco MLA, Rosato A, Carone D, Vitali C, Structural modifications and antimicrobial activity of *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles II , Framaco, 2005, 60, 291.
4. Pelczar MJ, Chan ECS, Krieg NR. Microbiology. 5th edition New York: McGraw-Hill Book Company; 1986, 687-688, 73-98.

5. Furniss BS. Vogel's textbook of practical organic chemistry. 5th edition: an imprint of Addison Wesley Longman; 1998, 1166-1168.

6. Chakraborty PA. Text Book of Microbiology. 2nd edition: New Central book agency (P) Ltd; 2005, 9-24, 57-64.

7. Microbiological Assay. Indian Pharmacopeia; 1996, II, 100-103.

8. Kokare CR. Pharmaceutical Experiments and Techniques. 2nd edition: Career Publications; 2007, 153-56.

9. Kaspady M, Narayanaswamy VK, Raju M, Rao GK, Synthesis, antibacterial activity of 2, 4-disubstituted oxazoles and thiazoles as bioisosteres, LDDD,2009, 6 , 21.

10. Mishra S, Srivastava SK, Srivastava SD, Synthesis of 5-arylidene-2-aryl-3-phenothiazinobenzotriazolo-acetamidyl)-1, 3-thiazolidine-4-ones (IX) as anti-inflammatory, anticonvulsant, analgesic and antimicrobial agent, JIC, 1997, 36B, 826–830.

11. Rashid N, Jones PG, Ali M, Hussain R, Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents, EJMC, 2010, 45(4),1323–1331.

12. Gajdos P, Magdolen P, Zahradnik P, Foltinova P, New conjugated benzothiazole-N-oxides: Synthesis and biological activity, Molecules, 2009, 14, 5382.

13. Pattan SR, Babu SNN, Angadi J, Synthesis and biological activity of 2-amino [5'-(4'-sulphonylbenzylidene)-2, 4-thiazolidine dione]-7-(substituted)-6-fluoro benzoth-iazoles, IJHC, 2002, 11, 333.