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Research Article

ANTI-*HELICOBACTER PYLORI* ACTIVITY OF SUBSTITUTED BENZOTHIAZOLES: *IN VITRO* STUDY

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ABSTRACT

Objective: *Helicobacter pylori*, originally classified as *Campylobacter pylori*, is a Gram negative, micro aerophilic, spiral-shaped, motile bacterium associated with gastritis, peptic ulcer, duodenal ulcer and chronic gastritis. *Helicobacter pylori* have been a major cause of peptic ulcer, gastric ulcer, duodenal ulcer disease and are an early risk factor for gastric carcinoma. This study has been undertaken for isolation of *Helicobacter pylori* form clinical specimens using culture technique and detection the role of this technique in the investigation of *H. pylori* infection. New series substituted benzothiazoles have been synthesized. Substituted benzothiazole has an antibacterial activity against a wide range of bacteria. However, in the present work its activity against *Helicobacter pylori* has been reported.

Methods: The structures of the synthesized compounds were confirmed by FTIR, ^{1}H NMR,^{13}C NMR and Mass spectral analysis. The antimicrobial potential of substituted benzothiazoles was evaluated by agar plate disc diffusion method.

Results: The newly synthesized compounds 6(a-d) were tested for their *in vitro* antibacterial activity against *H. pylori* by using the agar disc diffusion method. All of the synthesized compounds showed good antibacterial activity. However the antibacterial activity of the synthesized compounds against the tested organisms was found to be less than that of respective standard drug at tested dose level.

Conclusion: Many of the compounds from the series have emerged as potent antibacterial agents endowed with moderate to good activity. The antimicrobial activity of the synthesized compounds against the tested organisms was found to be less than that of respective standard drug at tested dose level. In future study the activity of the compounds may be manipulated

Keywords: Benzothiazole, *Helicobacter pylori*, duodenal ulcer, Gastric carcinoma,

INTRODUCTION

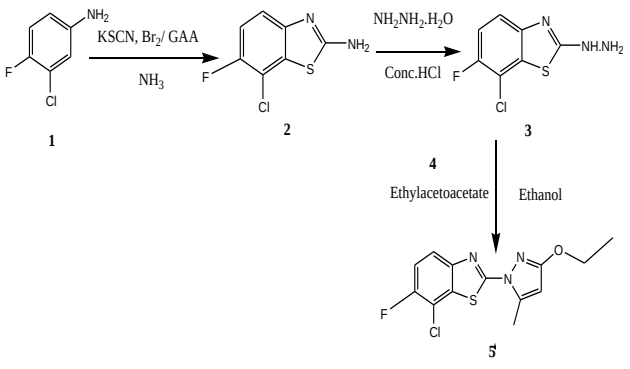
Helicobacter pylori are a Gram negative, motile bacterium that has been implicated in the etiology of most gastritis, duodenal ulcers and is associated with lympho proliferative disorders as well as gastric carcinoma [1]. *H.pylori* fastidious bacterium that resides on the human gastric epithelium. It grows under micro aerophilic environment.Subsequent to the first isolation of *H.pylori* in 1982, its association with gastritis, peptic ulcer (PU) and gastric cancer [2]. The diagnosis of *H. pylori* infection is an important issue. Recently, there are at least seven diagnostic assays for *H. pylori*: bacterial culture, urease test, urea breath test, histology, PCR, serology, and a stool antigen test culture, urease test, urea breath test [3]. The chemical and pharmacology of benzothiazoles have been of great interest to medicinal chemistry because its derivatives possessed various bio activities suh as antimicrobial, antifungal, neuroleptic, anti-HIV, anthelmintic, antihistaminic, antiulcer, cardio tonic & antihypertensives, anti hepatitis B virus, analgesic, anticonvulsant, antineoplastic [4]. Almost all benzothiazoles derivatives with their two ring system bear different functional substituents and this leads to essential modification of the physicochemical, metabolic, pharmacokinetic properties of these drugs. In the past decades, benzothiazoles and its derivatives have received much attention due to their chemotherapeutic value. The structures of the various synthesized compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. These compounds were also screened for their *in vitro* antibacterial activity.

MATERIALS AND METHODS

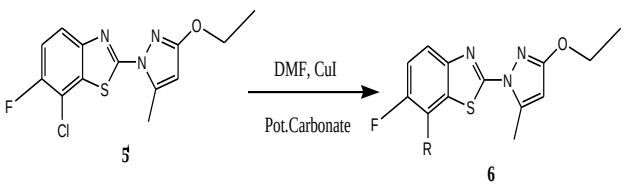
The chemicals used in the present project work were purchased from Rankem, Merck and Spectrochem. The melting point of the synthesized compound was determined by open capillary with Thiel's melting point tube (capillary tube method). TLC plates were prepared by using Merck Silica Gel 60 GF 254. Visualization 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 $CDCl_3$.

PROTOCOL OF SYNTHESIS

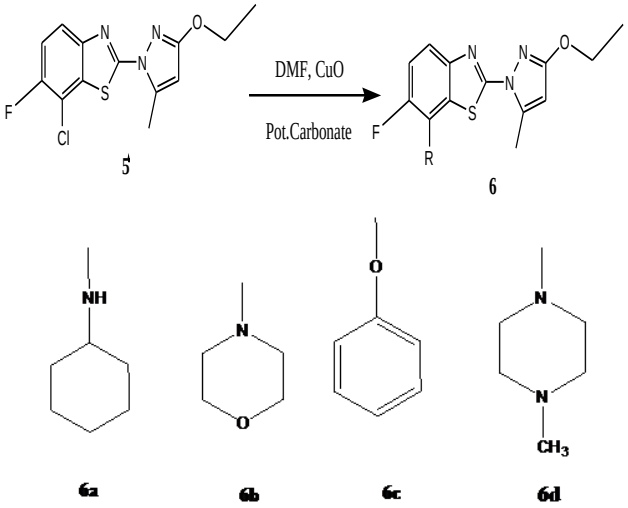
Scheme 1



Scheme 2



Scheme 3



6-Cl

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 (0.01 mol) 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 stirring. 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 (0.03 mol) 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)

To7-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**

Enumeration and identification of *H. pylori*

Aliquots of 250 mL from each sample were filtered by the membrane filtration technique using 47 mm cellulose acetate filters with a nominal pore size of 0.22 µm (Sartorius). The filter papers were cultured on modified Columbia urea agar medium [13] consisting of Columbia agar supplemented with 1% haemin, 5% urea solution, 4 µg of vancomycin and 0.12 mg of phenol red and incubated at 37 ºC for 5–7 days under microaerophilic conditions (5% CO2, 10% H2, 85% N2) for the isolation of *H. pylori*. *H. pylori* was identified using biochemical tests which included: the catalase, oxidase and urease tests, tests for hydrogen sulphide (H2S) production, nitrate reduction, growth with 3.5% NaCl, growth with 1% glycine, growth at varying temperatures (25 °C and 42 °C), growth on peptone-starchdextrose agar and sensitivity to ampicillin and ciprofloxacin. *H. pylori* isolates were tested for their antibiotic susceptibility as shown in **Table-5**

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.

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 organism such as *H.Pylori.*
- 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.

RESULTS AND DISCUSSION

The starting material flour-chloro-aniline (**1**) was treated with potassiumthiocyanate and glacial acetic acid to obtain 2-amino- 7-chloro-6-fluoro benzothiazole (**2**) which was confirmed by the change in R_f value and appearance of NH_2 peak at 3120 cm^{-1}. 2-amino- 7-chloro-6-fluoro benzothiazole (**2**) was stirred with hydrazine hydrate and Conc HCl using ethylene glycol as solvent to get 2- hydrazino-amino- 7-chloro-6-fluorobenzothiazole **(3)**. 2-hydrazino-amino- 7-chloro-6-fluoro benzothiazole **(3)** obtained is then treated with ethylacetoacetate using ethanol as solvent to get 7-chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazole **(4)**,which was confirmed by the appearance of C=O peak in the IR spectra at 1656cm^{-1}. 7-chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazole **(4)** is treated with various amine/ phenol/ alcohol using DMF as solvent. Anhydrous potassium carbonate and cuprous iodide are also used for replacement of chlorine (**6a-d**) which was confirmed by the difference in the R_f value, physical and spectroscopical data described in **Table 1-3**. The compounds **6a-d** was screened for anti-bacterial activity using *H.pylori* as shown in **Table 4.** The compounds did not show the promising results.

Table 1: List of compounds synthesized

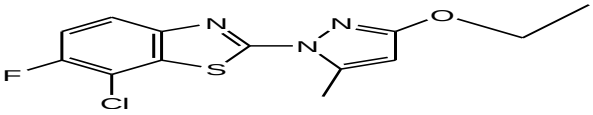
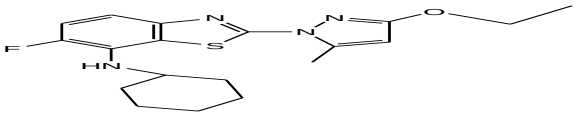
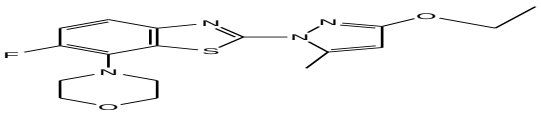
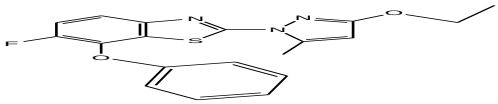
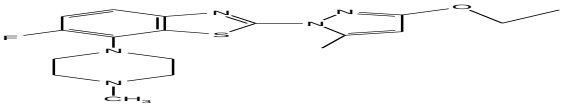
6		**7-Chloro-2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazol**
6a		**Cyclohexyl-[2-(3-ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-benzothiazol-7-yl]-amine**
6b		**2-(3-Ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-7-morpholin-4-yl-benzothiazole**
6c		**(*E*)-4-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl)-2-methoxyphenol.**
6d		**2-(3-Ethoxy-5-methyl-pyrazol-1-yl)-6-fluoro-7-(4-methyl-piperazin-1-yl)-benzothiazole**

Table 2: Physicochemical properties of synthesized compounds

Sl. 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 3: Predicted moleinspiration data of synthesized compounds

Sl. 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 4: Predicted data of synthesized compounds

Sl 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 5: Infra red spectral study of the synthesized compounds

Sl 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

Table 6: Criteria for identification of helicobacter pylori

Gram Stain	Gram Negative Ox Bow, U Shaped Spiral Rods
Motility	Sluggishly Motile
Catalase	Positive
Oxidase	Positive
Urease	Very Strongly Positive Within 1-5 Minutes
Nalidixic Acid Sensitivity	Resistant
Cephalothin	Sensitive
Growth On BHI + HRBC + 40mg/L Triphenyl Tetrazolium Chloride	Golden Colonies

Table 7: Antimicrobial activity of synthesized compounds

Sl.no	Compound code	MIC (mg/L) Anti bacterial activity
1	6a	0.0015
2	6b	0.0009
3	6c	0.0010
4	6d	0.0004
6	Amoxicillin	0.002–2
7	Ciprofloxacin	0.03–32

CONCLUSION

The preliminary in-vitro antibacterial screening of novel benzothiazole substituents on *H Pylori* were reported. Many of the compounds from the series have emerged as potent antibacterial agents endowed with moderate to good activity. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic benzothiazole nucleus. 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.

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