Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis of Nitrogen Mustards of fluoro- benzothiazoles of pharmacological interest

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ABSTRACT

We have synthesized 2-amino-7-chloro-6-fluoro Benzothiazole(1) by using 4-fluoro-3-chloroaniline and potassium thiocyanate in presence of bromine in glacial acetic acid. which further treated with hydrazine hydrate and concentrated HCl to get 7-chloro-6-fluoro-2-hydrazino-1,3-benzothiazole (2). Then Schiff's base substituted benzaldehyde (7-chloro-6-fluoro-1,3-benzothiazol-2-yl)hydrazone (3a-e) is prepared by reaction of (2) with different type of aromatic aldehydes. This compound further treated with diethanolamine and triethylamine to get substituted benzaldehyde {7-[bis(2-hydroxyethyl) amino]-6-fluoro-1,3-benzothiazol-2-yl}hydrazone (4a-e). The structures of new compounds were established on the basis of spectral and elemental analysis. The title compound were screened for anti-bacterial (agar diffusion method) and anti-oxidant activity (ferric ion reduction method) and inhibition of denaturation of protein. Most of the compounds have shown promising activity.

Keywords: Fluoro benzothiazole, nitrogen mustards, anti-bacterial activity, anti-oxidant activity.

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INTRODUCTION

Benzothiazoles are an interesting group of compounds and biological activities of this class of compounds that are reported in literature are anti-cancer [1], antitumor[2], amyloid-imaging agents[3], anti-microbial[4], anticonvulsant [5], anti-diabetic [6], anti-tubercular [7], muscarinic receptor agonist [8], antibacterial activity [9].

Fluorine has been incorporated in the drug molecule as a means of increasing therapeutic efficacy, based on considerations such as, its ability to mimic hydrogen with respect to steric requirements, strong electron withdrawing inductive effect which can affect reactivity and stability, inhibition of metabolism because of high C-F bond energy, altered lipid solubility which alters absorption and distribution.

In present work 4-fluoro-3-chloroaniline was treated with potassium thiocyanate in presence of bromine in glacial acetic acid to get 2-amino-7-chloro-6-fluoro Benzothiazole (1), which further treated with hydrazine hydrate and concentrated HCl to get hydrazino compound of 2-amino-7-chloro-6-fluoro-benzothiazole (2). Then Schiff's base (3a-e) is prepared by reaction of hydozino compound of fluoro-benzothiazole (2) with different type of aromatic aldehydes. This compound (3a-e) further treated with diethanolamine and triethylamine to get next compound. (4a-e).

EXPERIMENTAL

The melting point of the compounds was taken in open capillaries and is uncorrected. The infrared spectrum was recorded using KBr as the medium, utilizing SHIMADZU Infrared spectrophotometer. ^{1}H NMR were recorded from Astra-Zeneca Pharma India Ltd. Bangalore. All the reactions were monitored using thin layer chromatography (TLC) using a glass plate coated with Silica Gel G or GF_{254} and spots were visualized either by iodine vapour or by irradiation with ultraviolet light (254 nm).

Synthesis of 2-amino- 7-chloro-6-fluoro benzothiazole(1).

To glacial acetic acid (40ml) precooled to 5^0C were added 40g (2.4 mol) of potassium thiocyanate and 7.25g (0.05mol) of 3-chloro-4-fluoroaniline. The mixture was placed in freezing mixture of ice and salt, mechanically stirred while 6 ml of bromine in 24 ml of glacial acetic acid was added from a drooping funnel at such a rate that the temperature does not rise beyond 0^0C. After all the bromine has been added (105 min), the solution was stirred for an addition 2 hours at 0^0C and at room temperature for 10 hours. It was allowed to stand overnight during which an orange precipitate settled at the bottom, water (30 ml) 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 10 ml 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 when a dark yellow precipitate was collected. Recrystallized from toluene.

Preparation of hydrazino compound of 2-amino-7-chloro-6-fluoro-benzothiazole (2).

To hydrazine hydrate (5 ml) in cold condition (5-10^0C), was added concentrated hydrochloric acid (5 ml) by dropping funnel with continuous mechanically stirring. After that, added ethylene glycol (20 ml) in above solution by dropping funnel at such a rate that the temperature does not rise beyond 5-10^0C with continuous stirring. Added 2-amino-6-fluoro-7-chloro-benzothiazole(**1**) to the above solution and refluxed for 4 hours, cooled, filtered the solution and dried the product. Recrystallized from methanol.

Preparation of Schiff's base (3a-e).

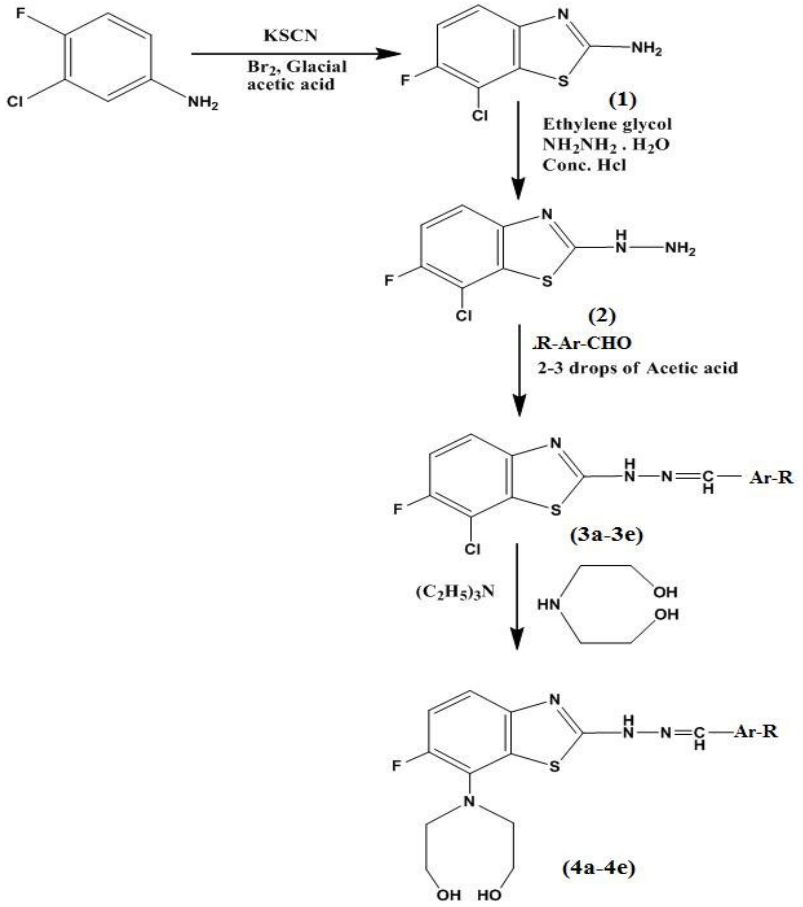
A sample of hydrazino compound of 2-amino-6-fluoro-7-chloro-benzothiazole (1.08 g) was reacted with different type of aromatic aldehydes (0.005mol), added glacial acetic acid (2-3drops) and benzene (as a solvent). Refluxed for 2-12 hours, filtered and dried the product.

Preparation of diethanolamine base (4a-e).

Take Schiff's base (100 mg), added diethanolamine (3-5drops), added alcohol (as a solvent), mixed well and added triethylamine (2 drops). Refluxed for 4 hours, filtered and dried the product.

Compound 4a: **IR spectra in KBr (cm^{-1})**: OH alcoholic – 3196.15, C-H straching aromatic-3076.56, C-H straching aliphatic- 2891.39, C=N straching (Schiff's base)- 1512.12, C-S- straching – 2369.02, C=N straching (benzothiazole) - 1448.99, C-F strachingAromatic-1170.8, C-Cl straching aromatic- 1033.8

^{1}**HNMR data:** 1H singlet at 8.07 δ, N=CH., 3H singlet at 3.81 δ, OCH3. Multiple peaks at 7.00-7.98 δ of aromatic protons, 1H singlet at 12.34 δ, -OH.



R = 2-OH; 4-OH, 3,4,5-OCH_3; 4-OCH_3; 3- OCH_3-4-OH.

Inhibition of denaturation of protein

Bovine serum albumin (Merck Limited), Ibuprofen and all other chemicals are of analytical grade. The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in phosphate buffer and incubated at $(27 \pm 1)^0$C for 15 min. Denaturation was induced by keeping the reaction mixture at $(60 \pm 1)^0$C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage of inhibition is calculated from the following formula,

$$\% \text{ Inhibition} = 100\ (1 - Vt/Vc)$$

Where, Vt – Absorbance of test solution, Vc – Absorbance of control

Anti-bacterial activity

Agar diffusion method

In our current study, the antimicrobial activity was carried out by the agar diffusion method. The micro organisms used were *Staphylococcus aureus* (G +ve) and *Bacillus subtillis* (G -ve). Here responses of organisms to the synthesized compounds were measured and compared with the response of the standard reference drug. Each test compound was dissolved in DMF to get a concentration of 50μg/ml. The standard reference drug used in the present work was Ampicillin.

Antioxidant activity

In Vitro Free Radical Scavenging activity (Reduction of Ferric Ions)

The reaction mixture contaning O-phenanthroline (0.5 m), ferric chloride (0.2 mM), and test compounds (different concentrations) in a final volume of 5 ml was incubated for 15-20 min at ambient temperature. The absorbance at 510 nm was measured. In another set, Sodium dithionite (0.3 mM) was added instead of the test compound and the absorbance was taken as equivalent to 100% reduction of all the ferric ions present. Scavenging activity was expressed as Percentage Ferric ion reduction using the following formula,

$$\% \text{ Anti radical activity} = \frac{\text{Standard Abs.} - \text{Sample Abs.}}{\text{Standard Abs.}} \times 100$$

RESULTS AND DISCUSSION

Biological activity

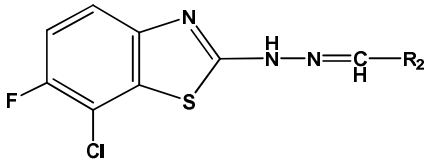
The antibacterial screenings revealed that some of the tested compounds showed good inhibition at 50μg/0.1ml concentration. The antibacterial screening indicated that among the tested compounds 4b and 4e showed excellent activity against the tested bacterial strains namely *S. aureus* and *B. subtilis* (Table 4). The compounds 4c showed least activity in the series. The remaining compounds were found to be moderately active.

Pharmacological Activity

In-vitro inhibition of denaturation of protein screening revealed that some of the tested compounds showed good activity at 100μg/0.1ml concentration. The results indicated that among the tested compounds 4b and 4e showed excellent activity (Table 4). The compounds 4c showed least activity in the series. The remaining compounds were found to be moderately active.

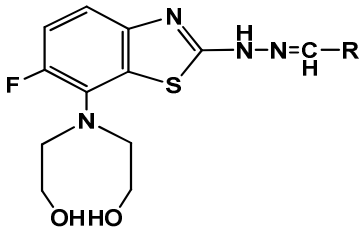
Five compounds were also screened for anti-oxidant activity (reduction of Ferric Ions). Among them compound 4b showed good inhibition at lower concentration (Table 3).

Table-1. Properties of synthesized compounds (3a-e)



S.N.	Compound's code	R=(aromatic benzaldehyde)	% yield	Melting Point(^{o}C)	R_f value
1.	3a	4-methoxy	52%	252	0.40
2.	3b	3,4,5-tri methoxy	67%	240	0.7
3.	3c	2-hydroxy	61%	240	0.52
4.	3d	4-hydroxy	47%	232	0.48
5.	3e	3-methoxy-4-hydroxy	54%	238	0.56

Table-2. Properties of synthesized compounds (4a-e)



S.N.	Compound's code	R= (aromatic benzaldehyde)	% yield	Melting Point(^{o}C)	R_f value
1.	4a	4-methoxy	49%	280	0.48
2.	4b	3,4,5-tri methoxy	60%	278	0.62
3.	4c	2-hydroxy	53%	284	0.57
4.	4d	4-hydroxy	48%	276	0.62
5.	4e	3-methoxy-4-hydroxy	56%	278	0.48

Table-3. Anti-oxidant activity

Conc in µg/ml	4a	4b	4c	4d	4e
50	11.32	13.33	11.45	6.37	8.14
100	17.31	25.76	14.30	11.45	16.24
150	19.47	37.18	23.74	18.95	27.26
200	28.01	45.52	28.45	24.22	36.72
250	37.89	53.77	33.05	28.98	45.12
IC_{50}^{*}	181	98	178	229	127

***IC_{50} value** not detected at the highest concentration tested. So it was determined by extrapolating the graph.

Table-4 Anti-bacterial and Protein denaturation Activity

Sr.No.	Compound Code	Zone of Inhibition (Diameter in mm)				% Inhibition of denaturation of protein
		Staphylococcus aureus (+ve)		*Bacillus subtillis* (-ve)		
		50 µg	100 µg	50 µg	100 µg	
01.	3a	1.2 mm	1.1 mm	1.6 mm	1.1 mm	73.65
02.	3b	1.9 mm	2.3 mm	2.5 mm	2.7 mm	41.39
03.	3c	2.2 mm	1.2 mm	2.1 mm	1.9 mm	54.69
04.	3d	2.3 mm	2.6 mm	1.4 mm	1.7 mm	68.46
05.	3e	2.1 mm	2.9 mm	1.5 mm	2.1 mm	62.98
06.	4a	4.7 mm	5.7 mm	4.3 mm	6.8 mm	46.59
07.	4b	6.9 mm	6.2 mm	7.1 mm	6.6 mm	75.98
08.	4c	4.2 mm	4.6 mm	3.6 mm	4.9 mm	36.24
09.	4d	6.1 mm	5.9 mm	5.1 mm	5.6 mm	54.49
10.	4e	5.5 mm	6.8 mm	6.5 mm	6.9 mm	70.89
11.	Chloroform	3	3.5	1.5	2.2	-
12.	Ampicillin	8.2	9.5	7.4	7.8	-
13	Diclofenac Na	-	-	-	-	**77.83**

CONCLUSION

Two series of substituted fluoro benzothiazole have been synthesized and screened for their inhibition of denaturation of protein, anti-bacterial and anti-oxidant activities. Series of fluoro benzothiazole compound having methoxy shown better activity. The results suggest that among the compounds tested 4b have exhibited higher activity. It can be inferred from the above results that the new synthesized compounds possessing methoxy group exhibit better antibacterial, inhibition of denaturation of protein and anti-oxidant activity.

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