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Synthesis, antimicrobial and anti-inflammatory activities of 2-N[aryl-methylidene]-6-fluoro-5-piperazinyl[1, 3]benzothiazole-2-amines

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ABSTRACT

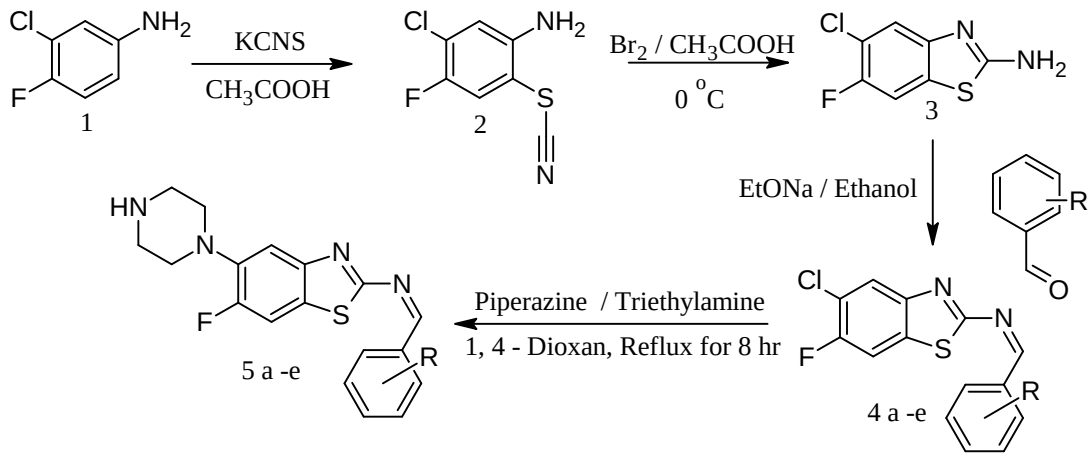
*3-Chloro-4-fluoroaniline **1** on reaction with potassium thiocyanate in glacial acetic acid yielded corresponding thiocyanate derivative **2,** which upon oxidative cyclization with bromine in acetic acid yielded 2-amino-5-chloro-6-fluoro[2,1-b][1,3]benzothiazole **3**. Compound **3** on reaction with various aromatic aldehydes yielded corresponding aryleneimines **4a-j**, which upon reaction with piperazine in the presence of triethylamine gave the title compounds **5a-j**. Thus synthesized compounds were characterized compounds by UV, IR, NMR and MASS spectral data. The title compounds were then evaluated for antibacterial, antifungal and anti-inflammatory activities.*

Key words: Fluorobenzothiazoles, antibacterial, antifungal, anti-inflammatory activities.

INTRODUCTION

Benzothiazoles are well known for their cytotoxic[1,2] activity. Apart from their this, they have shown variety of potent biological activities[3-7] as reflected in the literature. Benzothiazoles when combined with other aromatic and hetero-aromatics have shown a great diversity in their biological properties [8-12]. Enhancement of the biological activity by the presence of fluorine evidenced by a good number of examples from the marketed products and was further supported by the literature. Gaining impetus by the above observations, synthesis of 2-aryleneimino-6-fluoro-5-piperazinyl[2,1 –*b*][1, 3]benzothiazoles was under taken for the purpose of biological evaluation.

Scheme



R = H, 4-OCH_3, 4-NMe_2, 2-CHO, 2-Cl

MATERIALS AND METHODS

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Perkin Elmer and Nicolet FTIR spectrphotometers. NMR spectra were recorded on AMX and Brucker 400MHz.

Synthesis of 2-Amino-5-chloro-6-fluoro[1,3]benzothiazole (3)

A mixture of 0.01mol (1.45g) of 3-chloro 4-flouro aniline and 8g of potassium thiocyanate were added to 20mL of glacial acetic acid pre-cooled to 0^0 c. A solution of 1.6mL of bromine in 6mL of acetic acid was added slowly with constant stirring. The temperature was maintained at 0^0c through out the addition. After all the bromine has been added (105min) the solution was stirred for an additional 2hrs at 0^0c and at room temperature for 10hrs. It was then allowed to stand over night during which an orange residue settled at the bottom, water (6mL) was added quickly and the slurry was heated to 85^0C on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10mL glacial acetic acid, heated again to 85^0C and filtered hot. The filtrates were combined, cooled and neutralized with conc. ammonia solution to pH6. The dark yellow precipitate was collected re-crystallized (twice) from benzene. After treating with activated charcoal gave colorless plaques of 2-amino-5-chloro-6-fluro[1,3] benzothiozole. The dry material ($59^0/_0$ yield) melted at 207 to 209^0C.

Synthesis of 2-N[phenylmethylidene]-5-chloro-6-fluoro-[1, 3]benzothiazole-2-amine (4a)

A mixture of 0.01 mol (2.02 g) of **3** and 1.06 g of benzaldehyde was refluxed in 20ml of absolute ethanol and 2g of sodium metal for 3 hours. The excess alcohol was distilled off under vacuum. The yellow solid separated was filtered and recrystalised from absolute ethanol. The dried material (yield 85 %) melted at 234 – 236 ºC.

Similarly compounds 4b-e were synthesized by reacting 3 wth appropriate aromatic aldehydes.

Synthesis of 2-N[phenylmethylidene]-6-fluoro-5-piperazinyl[1, 3]benzothiazole-2-amine (5a)

An equimolar (0.01) quantities of **4a** and piperazine were stirred in 10ml of 1,4-dioxan 2ml of triethylamine for 10 minutes. The reaction mixture was then refluxed for 8 hours, cooled to room temperature. The solid separated was then filtered and recrystallised from absolute ethanol. The dried material (yield 72 %) melted at 242 -244 ºC.

Physical data the synthesized compounds has been summarized in table 3

RESULTS AND DISCUSSION

3-Chloro-4-fluoroaniline 1 shows λmax at 274 nm and the corresponding 2-aminobenzothiazole absorbs maximum at 309 nm. A bathochromic shift in UV absorption was due to heteroaromatisation of **1** to fused thiazole ring. Formation of **3** was indicated in IR by the absence of primary amino absorption at 3398 cm^{-1} and appearance of secondary amino band at 3475 $cm^{-1.}$ Further evidence for the formation of thiazole ring was based on the appearance of band at 1244 cm^{-1} due to C-S bond. The reaction presumed to happen through the formation of thiocyanate intermediate was supported by the presence of strong absorption band at 2108 cm^{-1} due to –SCN, when recorded by drawing the reaction mixture during the middle of the reaction. ^{1}HNMR, ^{13}CNMR and MASS spectra were in agreement with the proposed structure of **3**. The details of the spectral investigation have been discussed in our earlier publication [13].

The ^{1}HNMR spectrum the parent aniline exhibited a high degree of complexity due to $–NH_2$ mediated long range coupling with fluorine which was conspicuously absent in its N-arylidene derivative. Formation of **4b** was indicated in the IR absorption by the absence –NH2 band at 3475 cm^{-1} followed by the appearance of –N=CH- at 1594 cm^{-1}. The two singlets at δ 8.2 ppm and 7.3 ppm were assigned to the two protons on benzothiazole ring. The two doublets at 6.8 and 7.5 ppm have been assigned to the protons on methoxyphenyl ring. The arylidene proton and –OCH3 protons appeared at 8.8 and 3.9 ppm respectively. The above structure was further supported ^{13}CNMR by the presence of peaks at 58 ppm and 160 ppm due to –OCH3 and C-F respectively. The peak at 164 ppm was assigned to arylidene carbon as it suffers a strong inductive effect from aromatic ring and double bond. The signal at 153 ppm has been conformed to C=N of the thiazole ring.

For the synthesis of title compounds **5a-e,** a number of methods were tried such as refluxing the corresponding N-arylidenes with piperazine in n-butanol analogous to the piperazine substitution in ciprofloxacin. It was found that the most appropriate method was to reflux the arylidenes with piperazine in 1, 4-dioxan in the presence triethylamine. Formation of **5a** was confirmed based on the appearance of two doublets at δ 2.8 and 3.3 ppm due to four methylenes of the piperazine. The peak at 2.1 ppm was assigned to the NH proton on the piperazine. ^{13}CNMR and MASS spectra were consistent with the structure proposed.

Antimicrobial activity [14, 15]

In vitro antibacterial activity was determined by agar well diffusion methodagainst 24hr old cultures of *Staphylococcus aureus ATCC 11632, B. subtilis ATCC 60511 Escheria coli ATCC 10536,* and *Pseudomonas aerugenosa ATCC 10145* using 0.001 mol/mL of ofloxacin and ampicillin as standards. The test samples were prepared in NN-dimethylformamide showing 0.0mm of inhibition zone was taken as control. Compounds **5b, 5c**and **5e** showed a moderate activity against both gram positive and gram negative bacteria. However none of them was comparable to the standards used. Antibacterial activity of the synthesized compounds has been summarized in table -1.

In vitro antifungal activity was determined by agar well diffusion methodagainst *Aspergillus niger* and *Aspergillus flavus* using 0.001mol/mL of fluconazole as standard. The test samples were prepared in NN-dimethylformamide showing no inhibition was taken as control. Compounds **4e** and **5e** showed an excellent activity against *A. niger* and *A. flavus*. Antifungal activity of the synthesized compounds has been summarized in table -1.

Toxicity and dose

LD_{50} of the title compounds was determined according to OECD guidelines 423. The compounds were tolerated to a maximum dose of 30mg / kg body weight when tested against albino rats and Swiss mice. Therefore, 3mg / kg was taken as effective dose for the purpose anti-inflammatory activity evaluation.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by rat paw edema method. Edema represents the early phase of inflammation and carragenin-induced paw edema is the simplest and widely accepted model for studying the anti-inflammatory activity of chemical compounds. This method is based on plethysmographic measurement of carrageennan -induced rat paw edema produced by sub plantar injection of carrageenan in hid paw of the rat. The method described by Willhemi and Domenjoz, later modified by Sirodia and Rao was used for measuring the paw volume. For this study, Wistar rats of either sex, weighing between 100 and 200 g, were used and divided into 10 groups of six animals each. The group 1 served as control and received tween-80 (0.1%, 1 cm3) solution orally. The group 2 received diclofenac sodium in tween-80 (0.1%, 1 cm3) at a dose of 40 mg/kg body weight and served as standard. The groups 3-10 received orally the test compounds mentioned at the dose of 30 mg/kg body weight in tween-80 (0.1%, 1 cm3) solution.

Table – 1 Antibacterial activity`
Zone of inhibition in mm

Compd.	***S. aureus*** G+Anaer	***B. subtilis*** G+Aerobic	***E. coli*** G-Anaero	***P. aerugenosa*** G-Aerobic	**Antifungal activity** ***A. niger***	***A. flavus***
4a	10	12	12	14	19	17
4b	08	12	13	10	20	18
4c	13	15	10	12	17	18
4d	09	13	17	14	17	19
4e	14	13	09	09	20	20
5a	10	12	09	09	14	14
5b	14	14	12	13	17	17
5c	14	12	12	12	14	14
5d	10	12	10	09	15	15
5e	18	17	17	18	20	21
Standard 1	33	39	28	36	-	-
Standard 2	28	36	24	30	-	-
Standard 3	-	-	-	-	18	18
Minimum inhibitory concentration (micrograms per ml)						
4e	-	-	-	-	25	20
5e	100	100	100	50	25	25
Std. 1	50	50	50	50	-	-
Std. 2	50	50	50	50	-	-
Standard 3	-	-	-	-	25	25

Standard1- Ofloxacin, Standard 2 - Ampicillin, Standard 3-Fluconazole, Control - DMF
Sample concentration: 0.001mol/mL. Sample volume: 1mL in each well

These compounds were administered 1 hr before injection of an irritant, carrageenan. After 1 hr all the animals were injected subcutaneously with a suspension of carrageenan in tween-80 (0.1%, 0.5 cm3) solution to the left hind paw in the sub plantar region and the paw volume was

measured immediately. After 3 hr the paw volume was measured in control, in standard and in test groups. Percent inhibition of paw volume was calculated by using formula, % inhibition = (1-Vt/Vc) X 100, where Vt = mean increase in the paw in test animals, Vc = mean increase in the paw volume in control group. Statistical analysis was carried out to determine % protection and the results are presented in table-2.

For carrying out experiments with animals, approval from Institutional Animal Ethics Committee in accordance with "Principles of Laboratory Animal Care" was obtained as per certificate No. 3, 2003-04, issued to Sree Siddaganga College of Pharmacy, Tumkur, Karnataka.

Table -2.Anti-inflammatory activity of the synthesized compounds (n=6, t = 60 min)

Compd.	Mean Paw volume-in mL ±SEM	% Protection
Control	1.62±0.03	
Standard (D)	0.85±0.08	48
4a	0.87±0.06	46
4b	0.75±.0.10	54
4c	0.36±0.09	78
4d	0.57±0.06	65
4e	0.78±0.04	52
5a	1.00±0.02	38
5b	0.82±0.01	50
5c	0.97±0.06	40
5d	0.49±0.11	70
5e	0.78±0.12	52

Table -3. Physical characterization data of the synthesized compounds

Compd	R	Mol. Formula	M.P in °C	Mass
4a	H	$C_{14}H_8ClFN_2S$	234	290
4b	4-OCH_3	C15H10ClFN2OS	237	320
4c	4-NMe_2	C17H16ClFN3S	238	348
4d	2-CHO	C15H8ClFN2OS	231	318
4e	2-Cl	C14H7Cl2FN2S	236	325
5a	H	C18H17FN4S	242	340
5b	4-OCH_3	C19H19FN4OS	248	370
5c	4-NMe_2	C20H22FN5S	240	383
5d	2-CHO	C19H17FN4OS	243	368
5e	2-Cl	C18H16ClFN4S	246	374

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