Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1618-1620

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis and Biological Activity of 2-Amino-4*H*-pyrimido[2,1-b][1,3]benzothiazole-3-carboxylates

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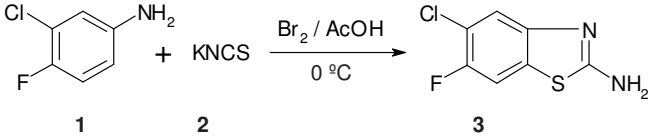
(*Received*: 21 June 2010; *Accepted*: 7 December 2010) AJC-9371

3-Chloro-4-fluoro aniline (**1**) on reaction with potassium thiocyanate (**2**) followed by the addition of bromine in acetic acid medium yielded 2-amino 5-chloro-6-fluro[2,1-b][1,3]benzothiazole (**3**). Compound **3** when treated with β-cyanoester undergoes cycloaddition reaction to produce 2-amino-4*H*-pyrimido[2,1-b][1,3]benzothiazole. Analogous cycloaddition reaction occurs on refluxing **3** with arylidene β-cyanoesters (**6a-j**) in sodium ethoxide and absolute ethanol. The resulting compounds were identified as ethanol 2-amino-4*H*-pyrimido[2,1,-b][1,3]benzothiazole-3-carboxylates (**7a-j**). The arylidenes **6a-j** were conveniently prepared by adopting Knoevenagel method. The newly synthesized compounds were characterized by physical constant, elemental analysis and spectral data. The compounds were then evaluated for antibacterial, antifungal and anthelmintic activities.

Key Words: Fluorinated benzothiazole, Arylidene cyanoacetate, Pyrimidobenzothiazole, Biological activity.

INTRODUCTION

Nitrogen and sulfur containing compounds are known for their potent antimicrobial activity. Benzothiazoles when combined with other biologically active heterocycles have exhibited varied biological and pharmacological activities, *viz.*, antibactrerial[1], antifungal[2], anthelmintic[3], antitumour[4], anti-cancer[5], analgesic[6], antiinflammatory[7], antitubrcular[8] and anti-HIV activities[9]. Therefore, heterocyclic compounds containing benzothiazole moiety have drawn attention of synthetic organic medicinal chemists globally. Guided very much by the observation that the presence of fluorine resulted in improvement of the activity profile of the compounds, synthesis of 2-amino-6-fluoro-4*H*-pyrimido[2,1-b][1,3]benzothiazole-3-carboxylates was undertaken for the purpose of biological activity evaluation.



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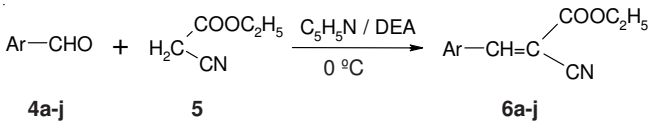
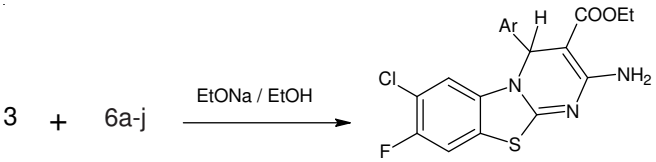


4a-j 5 6a-j



7a-j

whereas Ar is **a** , **b** , **c** , **d** , **e** , **f** , **g** , **h** , **i** , **j**

Scheme

EXPERIMENTAL

Synthesis of 2-amino-5-chloro-6-fluoro benzothiazole (3): A mixture of 0.01 mol (1.45 g) of 3-chloro-4-flouro aniline and 8 g of potassium thiocyanate were added to 20 mL of glacial acetic acid pre-cooled to 0 ºC. A solution of 1.6 mL of bromine in 6 mL of acetic acid was added slowly with constant stirring. The temperature was maintained at 0 ºC through out the addition. After all the bromine has been added (105 min) the solution was stirred for an additional 2 h at 0 ºC and at

room temperature for 10 h. It was then allowed to stand over night during which an orange residue settled at the bottom, water (6 mL) was added quickly and the slurry was heated to 85 ºC on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10 mL glacial acetic acid, heated again to 85 ºC and filtered hot. The filtrates were combined, cooled and neutralized with conc. ammonia solution to pH 6. The dark yellow precipitate was collected recrystallized (twice) from benzene. After treating with activated charcoal gave colourless plaques of 2-amino-5-chloro-6-fluro benzothiozole. Dry material (59 % yield) melting point 207-209 ºC.

UV 269 and 307 nm; IR (KBr, ν_{max}, cm^{-1}): 3475, 1649 (-NH_2) aromatic, 1546 (C=C) aromatic; 1456 (-C=S) thiazole, 1257 (C-F) aromatic, 715 (C-Cl) aromatic; NMR (DMSO-d_6): δ 6.5 to 6.9 3H (aromatic protons), δ 3.6 2H (-NH_2 protons); Mass-44, 69, 81, 93, 101, 113, 139, 148, 167, 175, 202 (M^+), 202 (M+2), base peak -202 (M^+).

Synthesis of arylidene ethyl cyanoacetate (6a-j): Equimolar (0.01) quantities of ethyl cyanoacetate (12.7 g) and benzaldehyde (10.6 g) were treated together in presence of 1 mL of pyridine and few drops of diethyl amine. The reaction mixture was kept in ice-salt bath for 5 h and then overnight at room temperatures, when colourless to pale yellow crystals of arylidene ethyl cyanoacetate separate out. The product is recrystallized from absolute ethanol. Yield is 90 % the pure product was characterized by IR and NMR spectra.

IR-2980 cm^{-1} (alkyl C-H), 3038 cm^{-1} (aromatic C-H), -2252 to 2256 cm^{-1} (-CN), 1709 to 1690 cm^{-1}, (-C=O), 1601 cm^{-1} (C=C), 1542 cm^{-1} (aromatic C=C); ^{1}H NMR δ 1.4 (-CH_3), 4.4 (-CH_2), δ 7.2(-CH=C) and d 8.16 to 8.35 (phenyl).

Ethyl 4-aryl 2-amino-7-chloro-8-fluro-4*H*-pyrimido-[2,1-b][1,3]benzothiozole-3-carbpxylate (7a-j): A mixture of 0.01 mol quantities of arylidene ethyl cyano acetate and 2-amino-5-chloro-6-fluro benzothiozole was dissolved in 10 mL of absolute ethanol and 1 mL of pyridine. The reaction mixture was refluxed for 3 h. the excess solvent was distilled off and then cooled room temperature. The reaction mixture was poured on the crushed ice. The dark coloured product was filtered re crystallized from aqueous ethanol. The yield was 86 %. Thus obtained was characterized by IR and NMR spectra.

IR: 3472 cm^{-1} (-NH_2), 16998 cm^{-1} (-C=O), 1598 cm^{-1} (C=C), ^{1}H NMR ($CDCl_3$) δ 8.0 to 8.2 (aromatic H of benzothiozole), δ 7.0 to 7.55 (aromatic H of aryl group), δ 7.4 (NH_2), δ 5.3 benzylic proton, d 1.4 (-CH_3).

RESULTS AND DISCUSSION

Fluorine containing 2-amino functionality was thought to be appropriate for synthesizing title compounds. Thus the required 2-amino-5-chloro-6-fluro benzothiozole (**3**) was synthesized by the reaction of 3-chloro-4-fluroaniline with potassium thiocyanate followed by addition of bromine in acetic acid medium. Formation of compound **3** was evident from the absence of primary amino absorption peak in IR spectrum with subsequent appearance of 2-amino absorption peak.

Arylidene cyanoacetates were thought to be appropriate building blocks for the construction of pyrimidine ring around benzothiazole (**3**). Thus the required arylidene cyanoacetates **6a-j** were synthesized by employing Knoevenagel method[10] *i.e.*, by the condensation of β-cyanoester with various aromatic aldehydes in the presence of base. Different strategies were adopted to effect the condensation. In the first method, aromatic aldehydes were condensed with β-cyanoesters in the presence of a mixture of pyridine and piperidine at freezing temperature. In the second method, a mixture of β-cyanoester and aromatic aldehyde was refluxed in absolute ethanol in the presence of piperidine.

In the third method, a mixture of ester and aromatic aldehyde was cooled about freezing temperature in the presence of mixture of pyridine and diethylamine. The products obtained in quantitative yields were characterized based on spectral data. Compound **4b** was identified by its infrared absorption at 2226 cm^{-1} due to -CN stretch. Strong absorption peaks at 1724 cm^{-1} and at 1590 cm^{-1} have been attributed to -C=O of the ester and -C=C-, respectively. ^{1}H NMR was consistent with the structure, exhibiting characteristic absorption signals due to the compound. The strong prominent signals at δ 4.1 was obviously assigned to -OCH_3 followed by two doublets in between 7.1 and 8.4 were assigned to four aromatic protons.

It is a well known fact that the combination of benzothiozoles with other biologically active heterocycles brings about dramatic changes in the interactive abilities of the compounds with biomolecules. Such interactions bring about various beneficial changes in the physiology and biochemistry of the organism. Literature provides number of examples where in the presence of fluorine improved the biological efficiency the compound. Guided by these observations, synthesis of fluorinated benzothiozoles containing bridge head nitrogen was undertaken.

Various strategies were adopted for the condensation of arylidenes **6a-j** with compound **3** to yield title compounds. In the first method, arylidenes **6a-j** were heated with compound **3** in the presence of polyphosphoric acid. The compounds were isolated in aqueous medium and then were characterized by spectral analysis. In the second method, arylidenes were refluxed with **3** in the presence of sodium and liquid ammonia in absolute ethanol to yield title compounds. In the third method, benzothiozole **3** was refluxed with **6a-j** in the presence of sodium ethoxide in absolute ethanol. The compounds isolated from aqueous ethanol were identified as ethyl 2-amino-4*H*-pyrimido[2,1-b][1,3]benzohtiozole-3-carboxylates, on the basis of physical constants and spectral data.

Treatment of (**1**) with various arylidene cyanoacetates **6a-j** in presence of sodium ethoxide/absolute ethanol solvent gave ethyl 2-amino-4-aryl-7-chloro-8-fluoro-4*H*-pyrimido[2,1-b][1,3]benzothiazole-3-carboxylate derivatives **7a-j** in quantitative yields. Formation of **7a** was confirmed by IR showing the absence of absorption at 2226 cm^{-1} due to -CN. The structure was supported by ^{1}H NMR showing the triplet at 1.4 (-CH_3), a quartet at 4.4 (-CH_2), a broad singlet at 7.4 (NH_2), two doublets at 7.0-8.0 (aromatic protons on pyrimidine ring), two multiplets at 7.4 and 7.6 exhibiting long range coupling due to aromatic ring of benzothiazole. ^{13}C NMR showed aromatic carbons in between 108.24 and 133.54 and -CO- of ester was recorded at 163.73. Final confirmation was done with mass spectra showing M + 2 and abase peak at m/e 447 and M + 2 449 due to **7b**.

Biological activity: *In vitro* antibacterial activity was determined by agar well diffusion method[11] against 24 h old cultures of *S. aureus, M. luteus E. coli and P. aerugenosa* using 0.001 mol/mL of ofloxacin and ampicillin as standards. The test samples were prepared in NN-dimethylformamide showing 0.0 mm of inhibition zone was taken as control. Compounds **7f-l** showed excellent activity against *E. coli*, **7j** against both *E. coli* and *P. aerugenosa* whereas **7d** showed good activity against *S. aureus* (Table-1).

TABLE-1

Compd.	Antimicrobial activity (zone of inhibition in mm)					
	Antibacterial activity				Antifungal activity	
	SA	ML	EC	PA	AN	AF
7a	06	10	06	12	12	36
7b	09	06	04	18	10	00
7c	14	10	12	16	11	29
7d	26	14	20	20	09	24
7e	15	13	13	25	07	26
7f	19	17	26	22	32	33
7g	12	06	22	23	39	16
7h	20	08	13	23	21	22
7i	20	00	37	23	40	28
7j	00	00	34	39	30	32
Control (DMF)	00	00	00	00	00	00
Ofloxacin (Std. 1)	28	33	24	30	–	–
Ampicillin (Std. 2)	33	36	28	36	–	–
Fluconazole (Std. 3)	–	–	–	–	18	20

SA = *S. aureus* (g+ *anaer*); ML = *M. luteus* (g+ *aer*); EC = *E. coli* (g+ *anaer*); PA = *P. aeru* (g+ *aer*); AN = *A. niger*; AF = *A. flavus*; Sample concentration = 0.001 mol/mL; Sample volume = 1 mL in each well.

In vitro antifungal activity was determined by agar well diffusion method[12] against *Aspergillus niger* and *Aspergillus flavus* using 0.001 mol/mL of fluconazole as standard. The test samples were prepared in NN-dimethylformamide showing no inhibition was taken as control. Compounds **3f-j** showed against *A. niger*. All the compounds except **3b** and **3g** have exhibited excellent activity against *A. flavus* (Table-1).

Anthelmentic activity was carried out against earth warms, *Pheretima posthuma* by a reported method[11,12]. The compounds were tested at a dose of 0.01 mol/mL of suspension in Tween 80. Albendazole suspensions (time of paralysis P, 10 min, time of death D, 15 min) was taken as standard. Compounds **3d-e** have shown good activity but none of them was comparable to albendazole (Table-2).

TABLE-2

Compd.	Anthelmentic activity (time in min)	
	Paralysis (P)	Death (D)
7a	34	45
7b	20	Not observed
7c	33	80
7d	13	19
7e	15	19
7f	06	Not observed
7g	21	29
7h	Not observed	Not observed
7i	25	50
7j	26	28
Albendazole (Std.1)	10	15
Control	Not observed	Not observed

Control = 0.05 mL Tween 80 in 6 % w/v dextrose in normal saline, observed for 90 min.; Standard = 10 mL of the suspension equivalent to 50 mg of albendazole.

ACKNOWLEDGEMENTS

The authors are thankful to The Management, Principal and staff of Siddaganga College of Pharmacy, Tumkur for providing laboratory facilities for the research. The authors profusely thank The Chairman, Department of Organic Chemistry, Indian Institute of Science, Bangalore for providing NMR and Mass spectral analyses.

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