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Synthesis and *in vitro* anticancer activity of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole and its 2-substituted derivatives

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ABSTRACT

The one pot synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole (3) and its 2-substituted derivatives is convenient over traditional route of synthesis of this compound. This has been prepared by the reaction of 2-amino-7chloro-6-fluoro benzothiazole (1) with ethyl-2-cyano-3, 3-bismethyl thioacrylate (2) in the presence of dimethyl formamide and anhydrous potassium carbonate. Susceptibility of compound (3), towards condensation with different reagents like aryl amines, heteryl amines and compounds containing active methylene groups has been investigated. These newly synthesized compounds were evaluated for their *in-vitro* anticancer activity towards human cancer cell lines derived from various cancer types.

Key words: Benzothiazole, Heterocycles, one pot synthesis, Human cancer cell lines, anticancer activity.

INTRODUCTION

Pyrimidine, iminopyrimidine [1-5] and fused benzothiazole heterocycles [5-6] are reported to be effective pharmacophores. M. F. G. Stevens et. al [7-10] reported that benzothiazoles display antitumor properties that are modulated by substitutes at specific positions on the benzothiazole pharmacophore.

Biological activities and various applications of benzothiazole compounds and compounds containing pyrimidine ring [11-17] have stimulated considerable interest to explore the synthesis of new potential compounds in which pyrimidine ring is fused with another biologically active nucleus such as benzothiazole through nitrogen atom.

A literature survey reveals that very few references are available on the synthesis of pyrimido benzothiazole compounds [18, 21]. Wade *et al.* [19, 20] reported synthesis of acidic derivatives of 4H-pyrimido [2, 1-*b*] benzazole-4-ones by the condensation of 2-aminobenzothiazole, 2-amino benzoxazole and 2-amino-1-methyl benzimidazole independently with 2-aminofumarate and diethyl ethoxy methylene malonate and their anti-allergic activity. Methods of preparation of these compounds reported by these workers [19-20] are cumbersome, since these methods require the presence of steam of nitrogen gas and gave either 2 or 3-substituted derivatives. Hence it was considered appropriate to devise a convenient route to synthesize 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole. It is surmised that pyrimido benzothiazole and its 2-substituted derivatives would exhibit interesting properties and pharmacological applications.

In the present work, we report one pot synthesis of new heterocyclic compound, 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole and its 2-substituted derivatives. Anticancer activity of the compounds was also evaluated and discussed.

EXPERIMENTAL SECTION

All melting points were determined in open capillary tube and were uncorrected. IR spectra were recorded in Potassium Bromide pellets on Thermo-Nicolet, NEXUS 670 FTIR, ¹H-NMR Spectra were recorded on FT-Gemini 200 MHz Spectrometer TMS as internal Standard. Mass Spectra were recorded on FT VG-7070 H Mass spectrometer using EI technique at 70 eV. All the reactions were monitored by Thin layer chromatography carried out on 0.25 mm thick silica gel-G plate using iodine vapour for detection.

Synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3]benzothiazole (3):

A mixture of 2-Amino-7-chloro-6-fluoro-benzothiazole (2.02g 0.01mole) and ethyl-2-cyano-3, 3-bismethyl thioacrylate (2.17g 0.01 mole) was refluxed in the presence of dimethyl formamide and pinch of anhydrous potassium carbonate for 4 hours. The reaction mixture was cooled to room temperature and poured in ice cold water. The separated solid product was filtered, washed with water and recrystallised from DMF-ethanol mixture to give of crystalline solid of **3**.

IR (KBr): 2218 cm⁻¹(CN str.), 1680 cm⁻¹ (C=O str.)

¹H-NMR in DMSO: δ 2.6 (s, 3H, SCH₃), δ 8.2 (d, 2H, Ar-H)

MS (m/e): 327 (M+2, 33%), 325 (M+ 100%), 250, 224, 186, 160

General Procedure for the synthesis of 2-Substituted derivatives of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole:

A mixture of **3** (0.001 mol) and various aromatic amines, heteryl amines and compounds containing active methylene groups (0.001 mol) in 10mL of DMF and pinch of anhydrous potassium carbonate independently was refluxed for 1.5 to 5h. The reaction mixture was cooled

to room temperature and poured in ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give pure respective compounds.

9-Chloro-3-cyano-8-fluoro-2-(p-nitroanilino)-4-oxo-4H-pyrimido [2,1-*b*] [1,3] benzothiazole (4a):

Yield: 65%, m.p. 195⁰ C, IR (KBr) : 1302 cm⁻¹ (-NO₂ symm.), 1474 cm⁻¹, (-NO₂ asym.), 1665 cm⁻¹ (C=O), 2220 cm⁻¹ (CN), 3364 cm⁻¹ (-NH), MS(m/e) : 415, (M⁺ 20%). Anal. Calcd. For: C₁₇H₇N₅O₃SClF, Mol.Weight:415, C, 49.16; H, 1.69; N, 16.87. Found C, 49.34; H, 1.67; N, 16.80.

9-Chloro-3-cyano-8-fluoro-2-(p-toluidino)-4-oxo-4H-pyrimido[2,1-*b*] [1,3] benzothiazole (4b):

Yield: 63%, m. p. 198⁰C, IR (KBr) : 2209 cm⁻¹ (CN), 1673 cm⁻¹ (C=O), 3248 cm⁻¹ (NH), MS (m/e) : 387 (M+2, 23%), 385 (M⁺, 68%). Anal. Calcd. For: C₁₈H₁₀N₄O₂SClF, Mol.Weight:384. C, 56.25, H, 2.60, N, 14.58. Found : C, 56.20, H, 2.58, N, 14.56

9-Chloro-3-cyano-8-fluoro-2-(piperidino)-4-oxo-4H-pyrimido[2,1-*b*] [1,3] benzothiazole (5a):

Yield: 72%, m. p. 272⁰C, IR (KBr) : 2195 cm⁻¹ (CN), 1666 cm⁻¹ (C=O) MS (m/e) : 362 (M⁺, 20%), Anal. Calcd. For: C₁₆H₁₂N₄O₂SClF, Mol.Weight: 362. C, 53.04, H, 3.31, N, 15.47. Found : C, 53.10; H, 3.29; N, 15.44.

9-Chloro-3-cyano-8-fluoro-2-(morpholino)-4-oxo-4H-pyrimido[2,1-*b*] [1,3] benzothiazole (5b):

Yield: 68%, m.p. 204⁰C, IR(KBr) : 2202 cm⁻¹ (CN), 1662 cm⁻¹ (C=O), ¹H-NMR in CDCl₃ : 3.8 (t,4H,two-N-CH₂), 4.1 (t, 4H, two OCH₂) 7.3-7.7 (d, 2H, Ar-H), MS (m/e) : 364 (M⁺, 30%). Anal. Calcd. For: C₁₅H₁₀N₄O₂SClF, Mol.Weight: 364, C, 49.45, H, 2.75, N, 15.38, Found: C, 49.40, H, 2.72, N, 15.35.

9-Chloro-3-cyano-8-fluoro-2-(α -ethyl ceto acetyl)-4-oxo-4H-pyrimido[2, 1-*b*][1, 3]benzothiazole (6a):

Yield: 62%, m. p. 289⁰ C, IR (KBr) : 3422 cm⁻¹ (OH), 2216 cm⁻¹ (CN), 1733 (C=O of ester), 1676 cm⁻¹ (C=O), MS (m/e) : 407 (M⁺, 30%). Anal. Calcd. For: C₁₇H₁₁N₃O₄SClF, Mol.Weight: 407, C, 50.12; H, 2.70; N, 10.32. Found : C, 50.05; H, 2.62; N, 10.30.

9-Chloro-3-cyano-8-fluoro-2-(α -ethyl cyano acetyl)-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole (6b):

Yield: 58%, m. p. 300⁰ C, IR (KBr) : 2212 cm⁻¹ (CN), 1645 cm⁻¹ (C=O) MS (m/e) : 391 (M⁺, 55%). Anal. Calcd For: C₁₆H₈N₄O₃SClF, Mol.Weight:390, C, 49.23, H, 2.05, N, 14.36, Found: C, 49.19, H, 2.00, N, 14.30.

9-Chloro-3-cyano-8-fluoro-2-(aceto acetyl)-4-oxo-4H-pyrimido [2,1-*b*] [1,3] benzothiazole (6c):

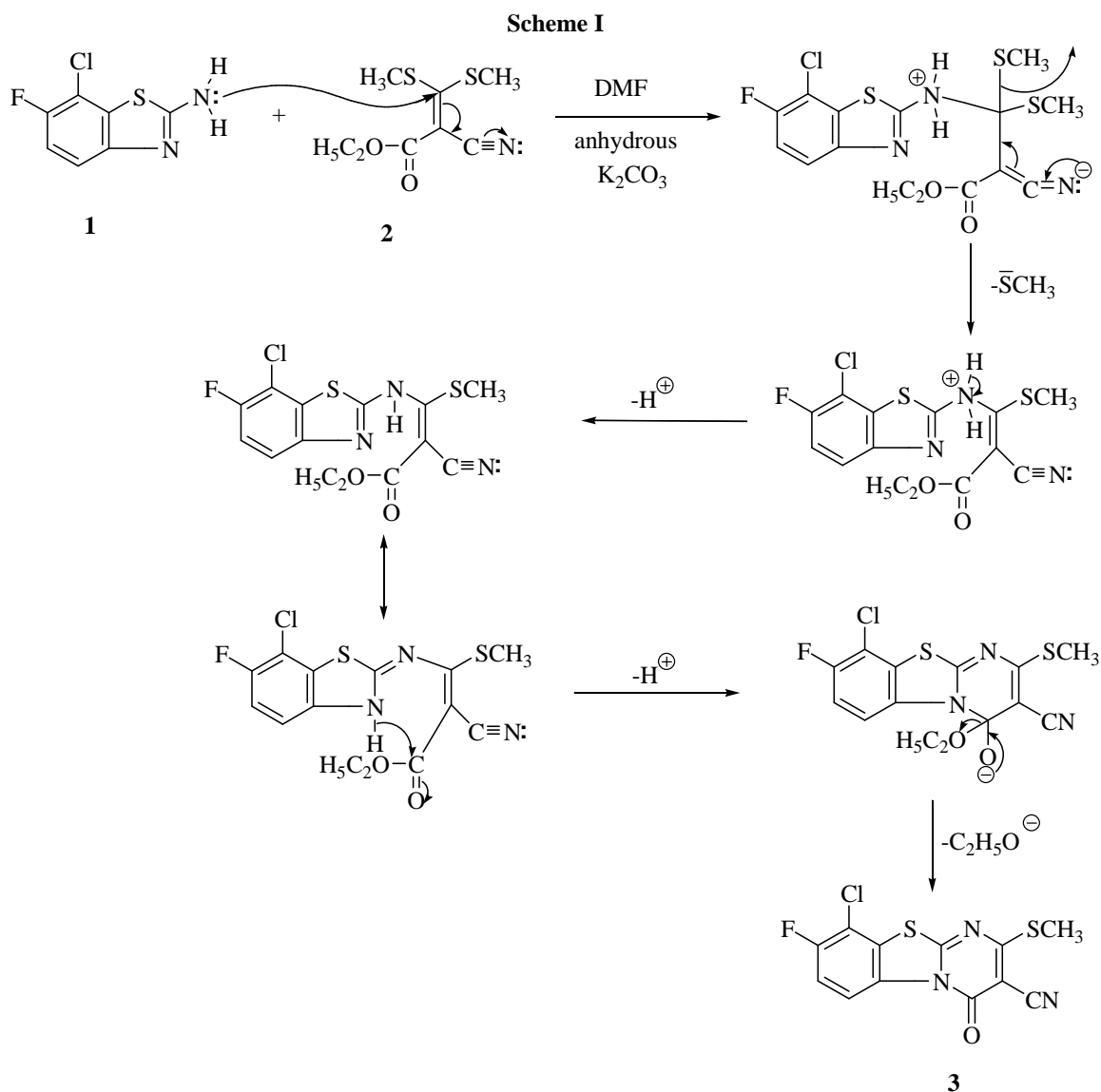
Yield: 62%, m. p. 197⁰ C, IR(KBr) : 2213 cm⁻¹ (CN), 1643 cm⁻¹ (C=O), ¹H-NMR in DMSO : 4.1 (s, 1H, CH), 7.2 (d, 2H, Ar-H), MS(m/e) : 377 (M⁺ 10%). Anal. Calcd. For: C₁₆H₉N₃O₃SClF, Mol.Weight:377, C, 50.93, H, 2.39, N, 11.14. Found: C, 50.90; H, 2.36; N, 11.12.

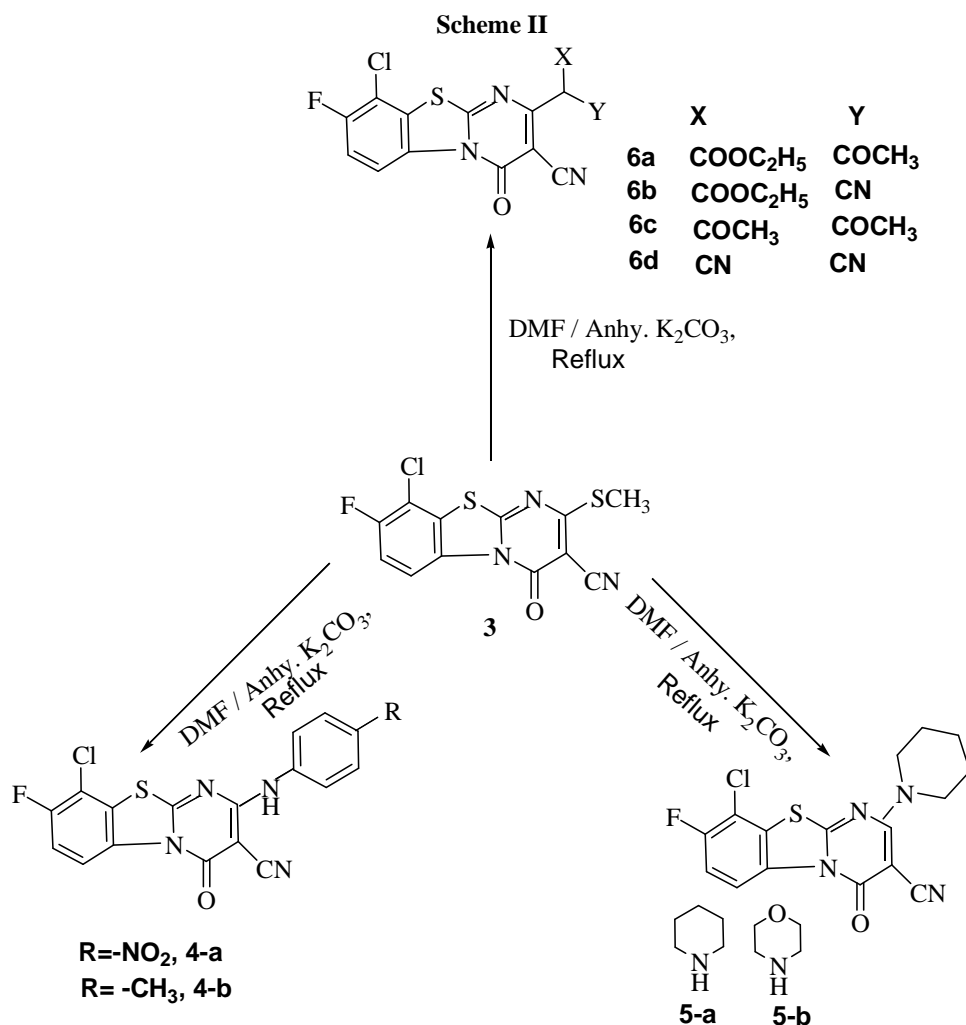
9-Chloro-3-cyano-8-fluoro-2-(α -malononitrile)-4-oxo-4H-pyrimido [2,1-*b*] [1,3] benzothiazole (6d):
 Yield: 63%, m. p. 210⁰ C, IR(KBr) : 2213 cm⁻¹ (CN), 1651 cm⁻¹ (C=O), MS(m/e) :343 (M⁺ 10%). Anal. Calcd. For: C₁₄H₃N₅OSCIF, Mol.Weight:343, C, 48.98; H, 0.87; N, 20.41. Found : C,48.95; H, 0.85; N, 20.39.

RESULTS AND DISCUSSION

The parent compound (**3**) was prepared by the reaction of 2-amino-7-chloro-6-fluoro benzothiazole (**1**) with ethyl-2-cyano-3, 3-bismethyl thioacrylate (**2**) in the presence of dimethyl formamide and anhydrous potassium carbonate. The structure of this compound was assigned on the basis of analytical and spectral data [Mass: M⁺ at *m/z* 325; IR (KBr): 2218cm⁻¹ (CN), 1680cm⁻¹ (C=O); ¹H-NMR (DMSO): δ 2.6 (s, 3H, SCH₃) δ 8.2 (d, 2H, Ar-H)]

The mechanism for the formation of parent compound (**3**) can be adduced as shown in Scheme I.





Since compound (**3**) possesses replaceable active thiomethyl group at 2-position, the susceptibility of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2,1-*b*] [1,3] benzothiazole towards condensation with different reagents like aryl amines, heteryl amines and compounds containing active methylene group has been investigated. These reactions resulted in the formation of 2-substituted derivatives of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2,1-*b*] [1,3] benzothiazole. According to this method, compound (**3**) on reaction with *p*-nitroaniline in the presence of dimethyl formamide and anhydrous potassium carbonate afforded compound (**4a**) to which on the basis of elemental analysis and spectral data was assigned the structure 9-Chloro-3-cyano-8-fluoro-2-(*p*-nitroanilino)-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole.

Similarly, compound (**3**) on heating independently under experimental conditions with *p*-toluidine / piperidine / morpholine / ethylaceto acetate / ethyl cyano acetate / acetyl acetone / melanonitrile afforded respective 9-chloro-3-cyano-8-fluoro-2-(*p*-toluidino (**4b**) / 2-piperidino (**5a**) / morpholino (**5b**) / α -ethyl acetoacetyl (**6a**) / α -ethyl cyano acetyl (**6b**) / acetoacetyl (**6c**) / α - malanonitrile (**6d**)-4-oxo-4-H-pyrimido [2, 1-*b*] [1, 3] benzothiazole (**Scheme II**).

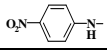
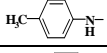






IR spectra of compounds (**4a-b**, **5a-b** and **6a-d**) showed absorption bands in the range of 2200-2226 cm^{-1} . which can be assigned to CN stretch and absorption bands in the range of 1665-1675 cm^{-1} are due to C=O stretch. Mass spectra of compounds showed molecular ion peaks which correspond to their molecular weights. $^1\text{H-NMR}$ spectral data is also in agreement with structures assigned to compounds

Anticancer activity studies:

All the compounds synthesized were screened for in-vitro anticancer activity against MCF-7 (Human Breast cancer cell line), Hepg2 (Human Liver cancer cell line), B16 (Mouse Melanoma cells), A-549 (Human Lung cancer cell line) and HeLa (Human Epithelial cervix cancer cell line) cell lines by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasolium bromide] assay method along with standard Doxorubicin. The percentage inhibition of each compound and also standard compound was calculated.

Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrasolium dye to a highly colored blue formazan product. 1×10^4 Cells (counted by trypan blue exclusion dye method) in 96- well plates were incubated with compounds with series of concentrations tested for 48 hrs at 37°C in DMEM [Dulbecco's Modified Eagles Medium] / MEM [Minimum Essential Medium] with 10% FBS [Fetal Bovine Serum] medium. Then the above media was replaced with 90 μl of fresh serum free media and 10 μl of MTT reagent (5mg/ml) and plates were incubated at 37°C for 10mins. The absorbance at 570nm was measured on a spectrophotometer (spectra max, Molecular devices) IC-50 values were determined and results are summarized in the Table 1.

Table 1: Anticancer activity of newly synthesized compounds

Comp. no.	R	IC ₅₀ values ($\mu\text{g/ml}$)				
		MCF-7	HeLa	A549	B16	Hepg2
3	-S-CH ₃	122.0	129.48	117.48	118.6	42.2
4a		NA	NA	NA	NA	100.0
4b		NA	NA	NA	NA	NA
5a		NA	NA	NA	NA	110.1
5b		NA	NA	NA	NA	142.76
6a		134.22	NA	NA	188.70	204.3
6b		84.82	NA	204.17	NA	164.26
6c		201.47	102.54	NA	NA	61.18
6d		3.04	14.63	2.39	1.37	15.06
Doxorubicin		2.55	5.6	3.36	2.8	1.24

NA = Not active

The parent compound 9-Chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole (**3**) exhibited anticancer activity against MCF-7 (Breast cancer), HeLa (Epithelial cervix cancer), A549 (Lung cancer), B16 (Mouse melanoma), Hepg2 (Liver cancer)

and U937 (Leukemic monocyte lymphoma) cell lines. Compounds with substituents of active methylene groups also exhibited cytotoxic effect against different human cancer cell lines. These includes replacement of methylthio group at 2-position by ethyl acetoacetate group (**6a**) exhibited anticancer activity against MCF-7 (Breast cancer), B16 (Mouse melanoma), Hepg2 (Liver cancer). Compounds with substituents ethyl cyano acetate (**6b**) has shown inhibitory effect against MCF-7 (Breast cancer), A549 (Lung cancer), Hepg2 (Liver cancer) and U937 (Leukemic monocyte lymphoma). Compounds with substituents of acetyl acetone (**6c**), malononitrile (**6d**) have also exhibited cytotoxic effect against MCF-7 (Breast cancer), HeLa (Epithelial cervix cancer), A549 (lung cancer), B16 (Mouse melanoma) and Hepg2 (Liver cancer). Among all the compounds studied in this series, compound (**6d**) was more active against all the cell lines and comparable with standard Doxorubicin activity.

CONCLUSION

A simple, one pot synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-*b*] [1, 3] benzothiazole (**3**) and its 2-substituted derivatives has been presented. Among compounds synthesized compound (**6-d**) exhibited remarkable inhibitory effects against all the cell lines studied, MCF-7, HeLa, A549, B16 and Hepg2. The parent compound (**3**) and (**6c**) were also exhibited anticancer activity against Hepg2. Fused benzothiazoles demonstrated cytotoxic properties justifying further investigation as the potential anticancer agents and may be used as a basis for the design of new anticancer drugs.

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