Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):317-322

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Novel synthesis and *in-vitro* anticancer activity of 3-amino-9-chloro-8-fluoro-4-oxo-(2H)/aryl/heteryl-pyrazolo[3',4':4,5]pyrimido [2,1-b] [1,3]benzothiazoles

P. Ravi Prasad

Analytical Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, Telangana State, India

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ABSTRACT

9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4-H-pyrimido[2,1-b] [1,3] benzothiazole(3) on reaction in presence of dimethyl formamide (DMF) and catalytic amount of anhydrous potassium carbonate with hydrazine hydrate / aryl hydrazine/heteryl hydrazine afforded 3- amino -9- chloro-8- fluoro -4- oxo- (2H)/aryl/heteryl- pyrazolo [3', 4' : 4, 5] pyrimido [2,1-b] [1,3] benzothiazoles ***(4a-g)*** *in good yield. These newly synthesized compounds were evaluated for their in-vitro anti-cancer activity towards human cancer cell lines derived from various cancer types.*

Key words: Heterocycles, Anti-cancer activity, Human cancer cell lines

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INTRODUCTION

A survey of literature revealed that very little work has been carried out on the synthesis of pyrimido benzothiazole system condensed with other heterocyclic rings such as pyridine, pipyridine, pyrazole etc.,[1,2]. Pyrimidine, iminopyrimidine and fused benzothiazole heterocycles[3,8] were reported to be effective pharmacophores. Fused pyrimido benzothiazoles were reported to exhibit a wide spectrum of activities like anti-tumer, phosphodiesterase inhibition, anti-allergic, anti-inflammatory and anti-parkinsonism[9,13]. In view of various applications of this system, synthesis of such condensed system has attracted much attention in recent years.

In the present work, we report the synthesis of a novel heterocyclic system, 3-amino-9-chloro-8-fluoro-4-oxo-(2H)-pyrazolo [3', 4': 4,5] pyrimido (2, 1-b) (1,3) benzothiazole and its substituted derivatives. Anticancer activity of the synthesized compounds was also evaluated and discussed.

EXPERIMENTAL SECTION

Materials and Methods

All melting points were determined in capillary tube and are uncorrected. IR spectra were recorded on Thermo Nicolet Nexus 670 FT-IR, ^{1}H-NMR Spectrum on a FT Gemini 60(200MHZ) spectrometer with TMS as internal standard and mass spectra on a FT VG-7070H Mass spectrometer using EI technique at 70 eV. All the reactions were monitored by TLC, carried out on 0.25mm thick gel –G plate using iodine vapour for detection.

Chemistry

2-amino-7-chloro-6-fluoro benzothiazole (0.01mole) and ethyl-2-cyano-3, 3-bismethyl thioacrylate (0.01mole) was refluxed in the presence of dimethyl formamide (DMF) and a pinch of potassium carbonate for 4hr. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered, washed with water and re-crystallized from DMF-ethanol mixture to give crystalline solid 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido (2, 1-b) (1, 3) benzothiazole (3) **(Fig 1)**. The structure of the compound (3) was established based on spectral analysis data.

IR (KBr): 2218 cm^{-1} (CN str.), 1680 cm^{-1} (C=O str.)
^{1}H NMR in DMSO: δ 2.6 (s, 3H, SCH_3), δ 8.2 (d, 2H, Ar H)
MS (m/e): 327(M^{+2}, 33%), 325(M^+. 100%), 250, 224, 186, 160

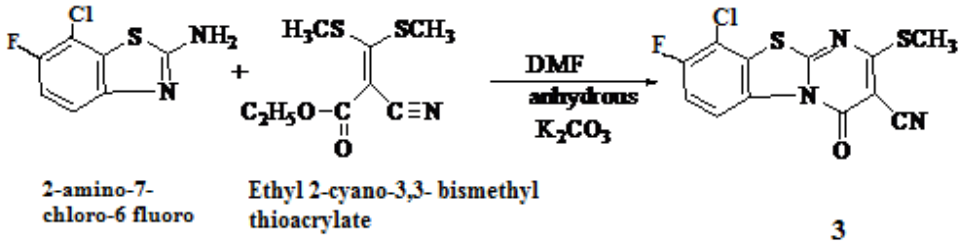


Figure 1: Synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole

Compound (3) possesses reactive methylthio group at 2 position and cyano group at 3 position. Hence, the compound (3) would become best precursor for the synthesis of 3-amino-9-chloro-8-fluro-4-oxo-(2H)-pyrazolo [3', 4': 4, 5] pyrimidine (2,1-b) (1,3) benzothiazole and its substituted derivatives **(4 a-g)**.

General procedure for the synthesis of compounds (4a-g): A mixture of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4 H-pyrimido (2, 1-b) (1,3) benzothiazole (0.01 mole) and hydrazine hydrate/ aryl hydrazine / heteryl hydrazine (0.02mole) was refluxed in the presence of catalytic amount of anhydrous potassium carbonate and 20-25ml of dimethyl formamide (DMF) for 4 hr. The reaction mixture was cooled to room temperature and poured into ice-cold water. The separated solid product was filtered, washed with water and re-crystallized from DMF-ethanol mixture to give pure compounds **(4a-g)**.

3-amino-9-chloro-8-fluoro-4oxo-2(H) pyrazolo [3',4': 4,5] pyrimido (2, 1-b) (1,3) benzothiazole (4-a) : yield: 52%, M.P: 272°C, IR (nujol): 3353 cm^{-1}, 3310 cm^{-1} (NH_2) , 1712 cm^{-1} (C=O) MS (m/e): 309 (M^+, 20%), Anal.calcd.for: $C_{11}H_5N_5OSClF$: C, 42.72 ; H, 1.64; N, 22.60. found: C, 42.70; H, 1.63; N, 22.58.

3-amino-9-chloro-8-fluoro-4-oxo-2(phenyl) pyrazolo [3',4':4,5] pyrimido (2,1-b) (1,3) benzothiazole (4-b) : yield: 51%, M.P : 280°C, IR (nujol) 3386$cm^{-1,}$ 3325 cm^{-1} (NH_2), 1712 cm^{-1} (C=O) MS (m/e): 385 (M^+, 15%), Anal.calcd.for : $C_{17}H_8N_5OSClF$: C, 52.99 ; H, 2.34 ; N, 18.18. Found: C, 52.95; H, 2.33; N, 18.16.

3-amino-9-chloro-8-fluoro-4-oxo-2(4'-nitro phenyl) pyrazolo [3',4': 4,5] pyrimido (2,1-b) (1,3) benzothiazole (4-c) : yield : 45%, M.P : 230°C, IR (nujol) 3380cm^{-1}, 3315cm^{-1} (NH_2), 1658 cm^{-1} (C=O) MS (m/e): 431 (M^+, 10%), Anal.calcd.for : $C_{17}H_8N_6O_3SClF$: C, 47.44 ; H, 1.86 ; N, 19.53. Found: C, 47.40; H, 1.85; N, 19.50.

3-amino-9-chloro-8-fluoro-4-oxo-2(2',4'-dinitrophenyl) pyrazolo [3',4': 4,5] pyrimido (2,1-b) (1,3) benzothiazole (4-d) : yield : 53%, M.P : 290°C, IR (nujol) 3380 cm^{-1}, 3321 cm^{-1} (NH_2), 1711cm^{-1} (C=O) MS (m/e): 475 (M^+, 60%), Anal.calcd.for : $C_{17}H_7N_7O_5SClF$: C, 42.95 ; H, 1.68 ; N, 20.63. Found: C, 42.93; H, 1.67; N, 20.60.

3-amino-9-chloro-8-fluoro-4-oxo-2(2'-benzothiazolyl) pyrazolo [3',4': 4,5] pyrimido [2,1-b] [1,3] benzothiazole (4-e) : yield : 61%, M.P: 300°C, IR(nujol): 3480cm^{-1},3353cm^{-1},1171 cm^{-1}(C=O), MS (m/e): 442 (M^+, 22%), Anal.calcd.for : $C_{18}H_8N_6OS_2ClF$: C, 48.87; H, 1.81; N, 19.00. Found: C, 48.81; H, 1.80; N, 19.01.

3-amino-9-chloro-8-fluoro-4-oxo-2(4',7'-dimethyl)pyrazolo [3',4': 4,5] pyrimido [2,1-b] [1,3] benzothiazole (4-f) : yield : 52%, M.P :>300°C, IR(nujol): 3383 cm^{-1},1172 cm^{-1}(C=O), MS (m/e): 472 (M^{+2}, 33%), 470(M^{+} ,99%) Anal.calcd.for: $C_{20}H_{12}N_6OS_2ClF$: C, 51.06; H, 2.55; N, 17.87. Found: C, 51.00; H, 2.53; N, 17.85.

3-amino-9-chloro-8-fluoro-4-oxo-2(5',6'-dichloro)pyrazolo [3',4': 4,5] pyrimido [2,1-b] [1,3] benzothiazole (4-g) : yield : 59%, M.P 288°C, IR(nujol): 3383 cm^{-1},1171 cm^{-1}(C=O), MS (m/e): 514 (M^{+4}, 16%), 512(M^{+2}, 49%), 510(M^{+}, 50%) Anal.calcd.for: $C_{18}H_6N_6OS_2Cl_3F$: C, 42.35; H, 1.18; N, 16.47. Found: C, 42.30; H, 1.16; N, 16.45.

RESULTS AND DISCUSSION

The fused pyrazolo pyrimido benzothiazoles **(4a-g)** were synthesized from 9-chloro-3-cyano-8-fluoro-2-methylthioacrylate on heating with hydrazine hydrate/aryl hydrazine/ heteryl hydrazine in the presence of dimethyl formamide and a catalytic amount of anhydrous potassium carbonate **(Fig.2)**. The structures of these newly synthesized compounds were confirmed on the basis of elemental analysis, IR, ^{1}H-NMR and mass spectral data.

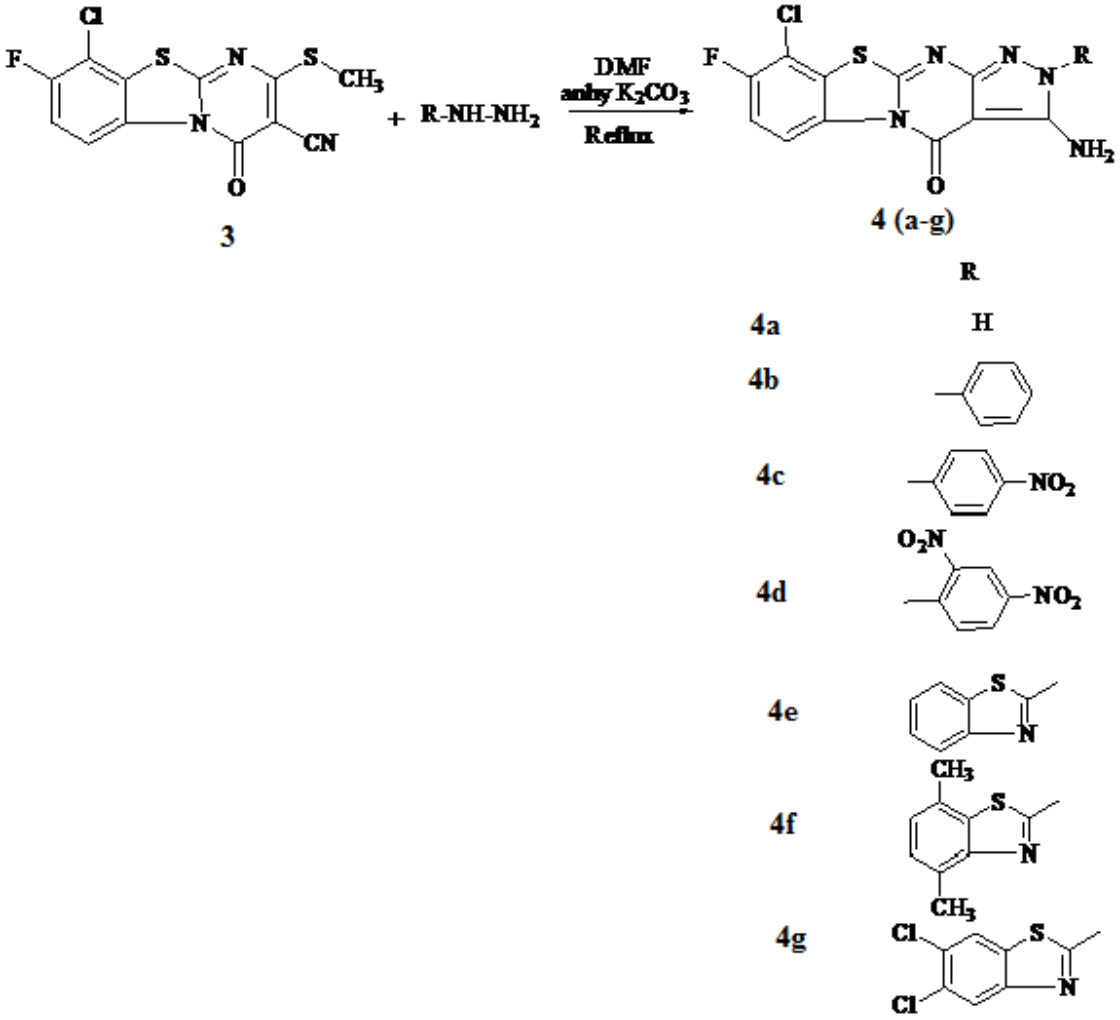


Figure 2: Synthesis of 3-amino-9-chloro-8-fluoro-4-oxo-2(H)/aryl/heteryl - Pyrazolo [3', 4': 4, 5] Pyrimido [2, 1-b] [1, 3] benzothiazoles

The IR spectra of the compounds **(4a-g)** showed the absence of -CN stretching absorption band in the region 2190-2250cm^{-1} and showed the presence of absorption bands in the region 3300-3450cm^{-1} which can be assigned to $-NH_2$ group. The presence of absorption band region 1680-1740cm^{-1} can be assigned to C=O stretching. The NMR spectra exhibited peaks in the region δ 7.3-7.7 and broad peak in the region δ 4.0-5.2 which can be assigned to aromatic protons and $-NH_2$ protons respectively. Mass spectra of compounds **(4a-g)** exhibited a molecular ion peak which

corresponds to respective molecular weights. The mechanism for the formation of compounds **(4a-g)** is shown in **Fig.3**.

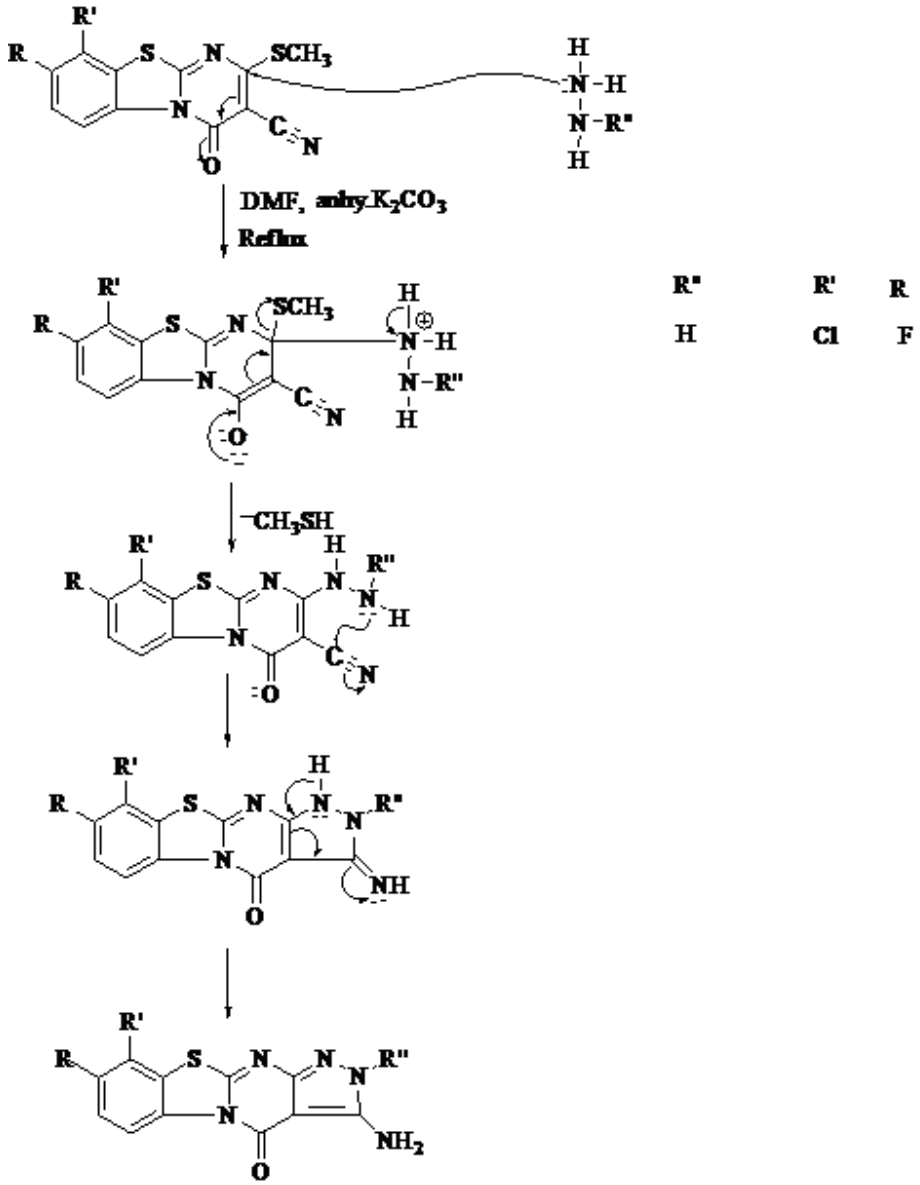


Figure 3: Mechanism for the formation of compounds (4a-g)

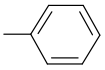
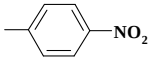
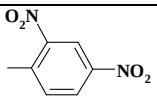
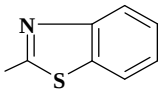
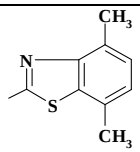
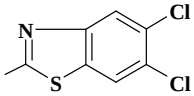
Anticancer activity studies

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 cell line), A-549 (Human Lung Cancer cell line) and HeLa (Human Epithelial Cervix cancer cell line) by MTT [3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrasolium bromide] assay method along with standard Doxorubicin. The percentage inhibition of each compound and standard 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 coloured blue formazan product. 1x104 cells(counted by trypan blue exclusion dye method) in 96-well plates were incubated with compounds with series of concentrations tested for 48hrs at 37°C in DMEM [Dulbecco's modified Eagles medium]/ MEM [Minimum Essential Medium] with 10% FBS [Fatal Bovine

serum] medium. Then the above media was replaced with 90µl of fresh serum free media and 10µl of MTT reagent (5mg/ml) and plates were incubated at 37°Cfor 10mins. The absorbance at 570nm was measured on a spectrophotometer. IC_{50} values were determined and results are summarized in **Table 1**.

Table 1: Anticancer activity of newly synthesized compounds

Comp. No.	R	IC 50 Values (µg/ml)				
		MCF-7	HeLa	A 549	B16	Hepg2
4a	-H	6.67	26.79	38.71	33.65	18.62
4b		NA	88.29	NA	80.23	NA
4c		161.78	NA	NA	NA	154.76
4d		21.37	12.58	30.90	8.43	124.35
4e		85.63	NA	NA	NA	84.66
4f		NA	NA	NA	NA	NA
4g		NA	NA	128.28	NA	NA
STD	Doxirubicin	2.55	5.60	3.36	2.80	1.24

NA = Not Active

The synthesized compounds **(4a-g)** exhibited anticancer activity against the cell lines under study except compound 4-f. Compounds 4-a & 4-d exhibited good anticancer activity against all the cell lines studied. Compound 4-a has shown very good activity against MCF-7 (Breast cancer) and Hepg2 (Liver cancer) cell lines. Compound 4-d has shown very good activity against HeLa (Human Epithelial Cervix cancer) and B16 (Mouse Melanoma) cell lines compared to standard Doxirubicin.

CONCLUSION

New series of heterocyclic compounds synthesized **(4a-g)** exhibited anticancer activity against the human cancer cell lines under study. It is evident from the results that very good activity was shown by compound 4-a & 4-d against MCF-7, HeLa, A549, B16 and Hepg2 cell lines compared to standard Doxirubicin .

Acknowledgements
The author is grateful to the Director CSIR-IICT for providing facilities to carry out the work. The author is also grateful to Prof. S. V. Kuberkar, Yeshwant Mahavidyalaya, Nanded for his discussions and suggestions throughout this work.

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