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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME BENZOTHIAZOLE CONTAINING AZETIDINONE DERIVATIVES

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ABSTRACT

Ethyl (6,7-substituted-1,3-benzothiazol-2-yl) carbamate 2.024 was prepared and treated with hydrazine hydrate to yield of N-(6,7-Substituted 1,3-benzothiazol-2-yl) hydrazine carboxamide derivatives 2.025. Reaction of the hydrazine carboxamide derivative 2.025 with substituted benzaldehyde afforded the corresponding Schiff base 2.026 and 2-azetidinones derivatives A6-10 were found in high yield by reaction of 2.026 with chloroacetyl chloride ,triethlyl amine. Characterization of the synthesized compounds was carried out using modern spectroscopic techniques and the purity of the compounds was verified using elemental analysis. The target molecules were evaluated for antitubercular activity.

Key words: Synthesis; azetidinone derivatives; Spectral analysis; Antitubercular activity.

INTRODUCTION

Benzothaizole are an extremely important class of compounds that occur widely as biologically active natural products, as well as marketed drugs or drug candidates. Accordingly, the development of efficient and general synthetic methodology for benzothaizole is a meaningful research challenge having great potential for practical applications in the pharmaceutical industry. The feasible positions for substitution group at C-2 position exhibit various pharmacological activities such as anti tubercular [1], antimicrobial [2], anti-inflammatory[3], anthelmintic[4], Cardiovascular [5], anticancer [6]. The 2-azetidinone ring system, a common structural feature of a number of wide spectrum β-lactam antibiotics, including penicillin, cephalosporins, carbapenems, which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases. The 2-azetidinones, have been reported to possess wide range of biological activities [7].

In our previous studies we confirmed that, benzothiazole with Schiff base and azetidinone nucleus has diverse chemical reactivity and broad spectrum of biological activities such as antimicrobial, anthelmintic activity. Here in as a part of our ongoing research to design and synthesis of new substituted benzothaizole (Figure 1) and based on the above mentioned subject, using 2,3,4(tri substituted benzaldehyde)–*N* - (6,7-substituated-1,3-benzothiazol-2-yl) semicarbazone , Chloroacetyl chloride ,triethlyl amine with DMF, some novel substituted benzothaizole were designed, synthesized (Scheme 1) and evaluated as antitubercular agents.

MATERIALS AND METHODS

Laboratory chemicals were supplied by chemdise chemical Ltd (Rajkot, India). Melting point of synthesized compounds were determined in open capillary and are uncorrected.IR spectra were recorded in Thermo Scientific; NICOLET iS10 spectrophotometer in KBR disc.1HNMR spectra were recorded on 400 MHZ spectrophotometer in DMSO-d6 as a solvent and TMS as an internal stander. The purity of the compounds was checked by TLC. Elemental analyses of all the compounds were in agreement with the calculated values. Antibacterial activity was performed at Micropharm Gandhinagar, Gujarat, India by using Lowenstein Jensen method.

The building blocks 2-amino-6,7 substituted benzothaizole (2.023 a-c) were prepared according to the reported procedures [7].

Method for synthesis of Ethyl (6,7-substituted-1,3-benzothiazol-2-yl) carbamate

2- aminobenzothiazole (0.066 mol) 13.5 g, absolute alcohol 30 ml anhydrous K_2CO_3 (2 g) and ethyl chloroformate (0.0064 mole) 0.7 g, were added under cooled at 0-5^{0}C. The mixture was refluxed for 7- 8 hours at 60-70^{0}C. The solution filtered and the residue was washed with ethanol and the solvent was evaporated under reduced pressure to get the product as solid which was recrystallized with ethanol.

General method for synthesis of preparation of N-(6,7-Substituted 1,3-benzothiazol-2-yl) hydrazine carboxamide

Ethyl (6,7-Substituated-1,3-benzothiazol-2-yl) carbamate (0.021 mole) 5.5 g, treated with 4 ml hydrazine hydrate was dissolved in ethanol (30 ml). The reaction mixture was refluxed for 5 hours and cooled to room temperature. The separated carbamoyl hydrazides were filtered and residue was washed with ethanol and recrystallized with alcohol.

General method for synthesis of preparation of 2, 3, 4 (trisubstituted benzaldehyde)-N-(6, 7-substituted-1,3-benzothiazol-2-yl) semicarbazone

5.21 g of N-(6-fluoro-7-chloro-1,3-benzothiazol-2-yl) hydrazine carboxamide (0.02 mole) was dissolved in absolute ethanol and

substituted benzaldehyde (0.02 mole) 2.40 g were added and refluxed for 3 hours and the solvent was removed under reduced pressure to yield Schiff base.

General method for synthesis of Schiff base to azetidinones

To a solution of Schiff base (0.10 mol) in DMF, chloroacetyl chloride (0.10 mol) and triethlyl amine (0.10 mol) were added and reaction mixture was stirred for 24 hr. The reaction mixture was poured into cooled water and the liberated compound was extracted
with chloroform. Evaporation of the compound afforded the corresponding azetidinones.

1-(3-chloro-2-oxo-4-(p-methoxy) azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea [A6]

IR (KBr)v cm-1: 1675(C=O), 3095(NH), 1605 (C=N), 680 (C-Cl), 720 (C-S-C).^{1}HNMR (DMSO-d6): δ(ppm)= 7.68 (m, 7H, Ar-H) , 2.95 (s, 3H , OCH3) , 6.0 (s, 1H, NH), 4.23 (s,1H, Azetidinone), 3.95 (s, 1H, CH-Cl) , 8.45 (s, 1H, CONH). Anal. Calc. for $C_{18}H_{14}ClFN_4O_3S$: C,51.37; H,3.35; N,13.31. Found:C, 51.35; H, 3.36; N, 13.29%.

1-(3-chloro-2-oxo-4-phenylazetidin -1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea (A7)

Yield 77%; mp 162 IR (KBr) 1650 (C=O), 3010(NH), 1601 (C =N), 1152 (C-F), 725 (C-S), 1680(C=C),**MS** (EI): m/z (%)= 390 (20) M : M+2 (3:1), 195.18 (89), 167.15(15), 195 (8.5), 180.56 (28), 164 (52).^{1}HNMR (DMSO-d6): δ(ppm)= 7.58 (m, 6 H , Ar- H) , 4.3 (S, 3H , -OCH_3) , 11.75 (S, IH , NH), 4.2 (S , IH , Azetidinone), 3.95 (S, IH, CH-Cl) , 8.47 (S , IH ,CONH).Anal.calcd for $C_{17}H_{12}ClFN_4O_2S$: C, 52.24; H, 3.09; N, 14.34. Found: C, 52.23; H, 3.08 ; N, .14.33 %.

1-(3-chloro-2-oxo-4-(m- tolyl) azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea (A8)

Yield 87%; mp 150 ; IR (KBr) 1600 (C=O), 3092 (NH), 1610 (C =N), 1150 (C-F) ;^{1}H NMR(DMSO-d6): δ(ppm)= 7.84 (m, 6 H , Ar- H) , 2.18 (S, IH , CH_3) , 11.34 (S, IH , NH), 3.56 (S , IH , Azetidinone), 5.67 (S, IH, CH-Cl) ,8.72 (S,IH, CONH) , Anal.calcd for $C_{18}H_{14}ClFN_4O_2S$: C, 53.40; H, 3.49; N, 13.84. Found: C, 53.39; H, 3.38; N, 13.83 %.

1 -(3-chloro-2-oxo-4-(o-tolyl) azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea (A9)

Yield 88%; mp 195 ;IR(KBr) 1674 (C=O), 3094(NH), 1604 (C =N), 1157(C-F), 728 (C-S), 1685(C=C); ^{1}HNMR (DMSO-d6): δ(ppm)= 7.85 (m, 6 H , Ar- H) , 2.16 (S, IH , CH_3) , 11.34 (S, IH , NH), 3.57 (S , IH , Azetidinone), 5.64 (S, IH, CH-Cl) ,8.77 (S,IH, CONH) Anal.calcd for $C_{18}H_{14}ClFN_4O_2S$: C, 53.40; H, 3.49; N, 13.84. Found: C, 53.38; H, 3.38; N, 13.82 %.

1-(3-chloro-2-oxo-4-(o-methoxy)azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea(A10)

Yield 85%; mp 180 ;IR(KBr) 1674 (C=O), 3093(NH), 1600 (C =N), 1150(C-F), 725(C-S), 1650(C=C); ^{1}HNMR (DMSO-d6): δ(ppm)= 7.86 (m, 6 H , Ar- H) , 3.89 (S, 3H , -OCH_3) , 9.44(S, IH , NH), 4.25 (S , IH , Azetidinone), 4.15 (S, IH, CH-Cl) , 8.45 (S , IH ,CONH). Anal.calcd for $C_{18}H_{14}ClFN_4O_3S$: C, 51.37; H, 3.35; N, 13.31. Found: C, 51.36; H, 3.34; N, 13.30%.

In-vitro evaluation of anti-mycobacterial activity
Determination of Minimal Inhibition Concentrations

The test compounds were subjected to screening by Lowenstein Jensen Method using H37Rv strain (MTCC – 200) of *Mycobacterium tuberculosis* (L.J. method) (Patel *et al.*, 2009) . All the synthesized compounds were screened for antituberculer activity at Micropharm Laboratory, Gandhinagar.

Preparation of medium

For preparation of medium first eggs were broken aseptically to obtain 500 mL of egg solution. The solution was filtered through a sterile muslin cloth into a sterile conical flask containing glass beads. Sterilized mineral salt solution (consisting of 1.2 g potassium phosphate (anhydrous), 0.12g of magnesium sulfate, 0.3 g magnesium citrate, 1.8g L asparagines, 6 mL of glycerol, distilled water 300 ml) and 4 ml of sterilized malachite green solution 16 ml (2.0%) were integrated to the 500 mL of egg solution. The contents were commixed well to compose a uniform medium.

Compounds 3 mg were dissolved in 1.5 mL of DMSO and were diluted with DMSO make 2000 microgram /ml stock solutions. Two concentrations were acclimated to evaluate antituberculer activity i.c 50 and 25 µg/mL. A 0.5 ml aliquot of each concentration was transferred into different McCartney bottles. To this,5 ml of L.J. medium was integrated and commixed well. The mixture was homogenized in a vortex mixer for a minute and if compulsory the opacity was adjusted by integrating sterile distilled water. Screw-capped Tubes containing 5 ml of medium were inspissated at 85 ^{0} C for 40-45 minutes. This inspissation was repeated 3 times.

INH and rifampicin (3 mg) were culled as the standard drugs for the comparison of antituberculer activity. The drug was dissolved in DMSO and diluted and tested as described above. The bottles were incubated at 75 to 8o^{0}C for 3 days for solidification and sterilization.

Procedure for inoculation

For culture inoculation, dilution of 1 µg/ml of the standard suspension was yare and 0.2 ml of the inoculums was incorporated to each tube. After inoculation the tubes were incubated at 37 0 C in a slanted position with the screw cap scarcely loosened to sanction evaporation of the inoculum. After 24-48 h screw caps were tightened and the tubes were further incubated. The reading of result was carried out at the 28 and 56th day after inoculation. The reading of the results is predicated on counting of magnification on the different slants and calculation of the proportion of bacilli by comparing counts on drug free (control) and drug containing L-J media.

RESULTS

A reaction of 2-amino benzothiazole with anhydrous K_2CO_3 ethyl chloro formate and ethanol gave the Ethyl (6,7 – substituted -1,3-benzothiazol-2-yl) carbamate by the reaction with hydrazine hydrate and ethanol to produce *N*-(6, 7-substituted-1,3-benzothiazol-2-yl)hydrazine carboxamide .*N*-(6, 7 – substituted - 1,3 – benzothiazol – 2 – yl) hydrazine carboxamide again treated with different aromatic aldehyde and produce Schiff base . Obtained Schiff base were treated with chloroaceyl chloride, Triethyl amine in the presence of DMF to get corresponding azetidinones derivatives (**A6-10**) respectively (Figure 1).

Antitubercular activity

The ability of A6-10 to inhibition of mycobacterium tuberculosis growth was determined using in vitro assay. The results are summarized in the table 1. Each compound was dissolved in DMSO. The in vitro screening data (Table 1) indicated that all analogs show a significant anti-tubercular activity in comparison to the reference drug Isoniazid and rifampicin. Comparison of compounds A6-10 with compounds

containing OCH_3 group at C-4 position of phenyl ring and CH_3 group at C-2 position of phenyl ring exhibited maximum and low activity. Based on the observation, it was expected that compound A6 be more active than others may be because of their low partition coefficient and consequently low penetration into the mycobacterium cells.

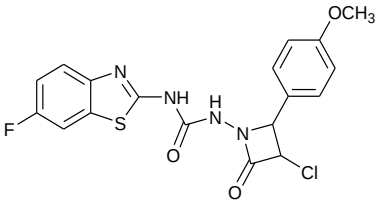
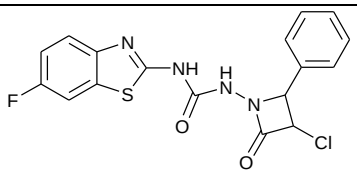
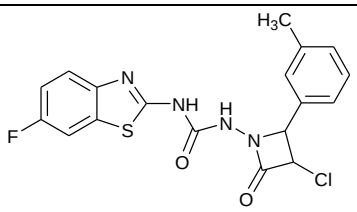
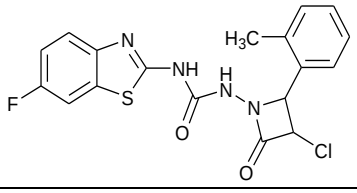
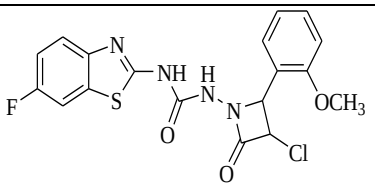
DISCUSSION

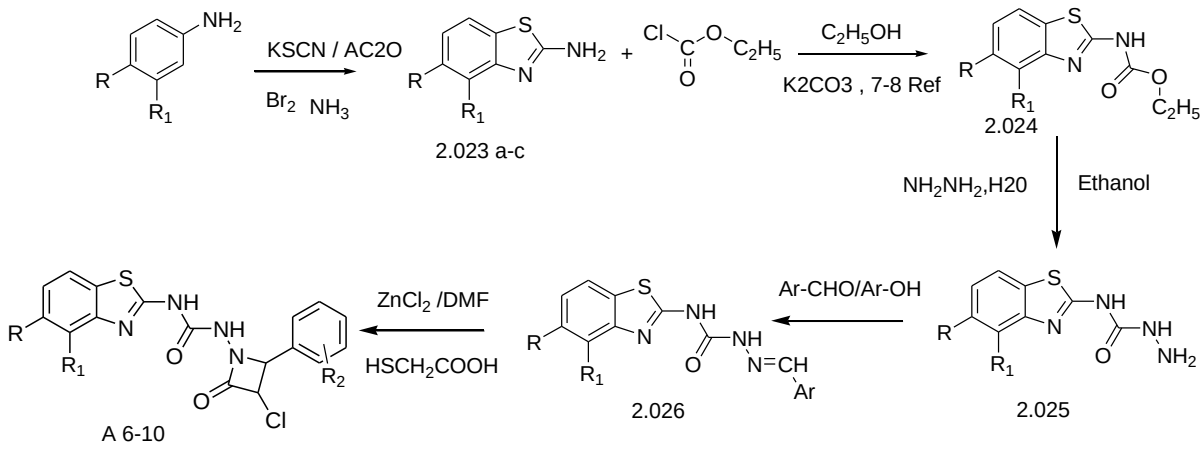
As part of our continuing interest in the construction of novel heterocycles, we now report the results of our studies involving the reactions of 2,3,4 (trisubstituted benzaldehyde) – *N* - (6,7-substituated-1,3-benzothiazol-2-yl) semicarbazone **2.026** and Chloroacetyl chloride ,triethlyl amine which constitutes a synthesis of azetidinones derivatives (scheme 1).

The structures of compounds A6-10 were deduced from their elemental analyses and their IR, ^{1}H NMR and mass spectra. For example, the ^{1}HNMR spectrum of Ethyl (6,7 -Substituated - 1, 3 - benzothiazol - 2 - yl) carbamate (**2.024**) exhibited multiplet at δ 7.20 for aromatic hydrogen. At δ 8.0, 1.30 and 4.12 singlet peak were observe due to presence of one, three, two proton of NH, CH_3CH_2 .For *N*- (6,7- Substituted 1,3 - benzothiazo l - 2 -yl) hydrazine carboxamide (**2.025**) exhibited multiplet at δ 7.95 for presence of aromatic proton. Compound number (**2.025**) also showed singlet at δ 6.0 and 2.0 for two and one proton of NH_2, NH, which confirmed the proposed structure. Similarly compound **2.026** exhibited a broad multiplet at δ 7.98 due to presence of aromatic proton. Singlet at δ 3.80 shows the presence of two protons of CH_2 and singlet at δ 8.50 showed the presence of CONH.

The IR spectrum of For ethyl (6,7-Substituated-1,3-benzothiazol-2-yl) carbamate (**2.024**) characteristic absorption band was at 3085 cm^{-1} for (NH), 1608 cm^{-1}for (C=N), 1157 cm^{-1}for (C-F), 710 cm^{-1}for (C-Cl). Similarly compound no **2.025** exhibited characteristic band at 3080 cm^{-1} for (NH), 1602 cm^{-1} for (C=N), 1158 cm^{-1}for (C-F), 715 cm^{-1}for (C-Cl).

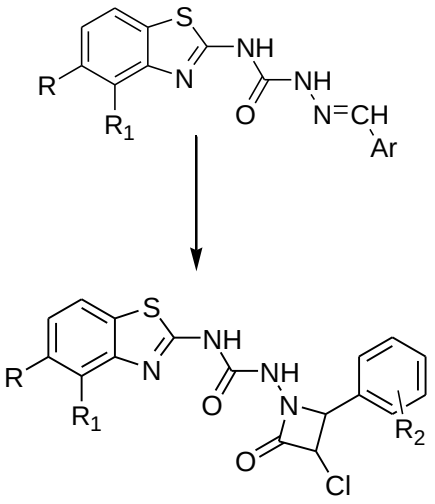
Table 1: In vitro screening (Anti-tubercular activity) of synthesized compounds

Compound no	Chemical structure	Concentration of compound		MIC
		25 µg/Ml (% Growth)	50µg/Ml (%Growth)	
A6		0%	0 %	25 µg/mL
A7		100 %	100 %	50 µg/mL
A8		100 %	0 %	50 µg/mL
A		100 %	100 %	>50 µg/mL
A10		100 %	0 %	50 µg/mL
	Isoniazid	0	0	25 µg/mL
	Rifampicin	0	0	25 µg/mL



Com.	R	R1	R2
A6	F	H	4-OCH3
A7	F	H	H
A8	F	H	3-CH3
A9	F	H	2-CH3
A10	F	H	2-OCH3

Scheme 1- Synthetic protocal for compound A6-10



Com.	R	R1	R2
A6	F	H	4-OCH3
A7	F	H	H
A8	F	H	3-CH3
A9	F	H	2-CH3
A10	F	H	2-OCH3

Fig 1- Graphical abstract of the newly synthesized 1-(3-chloro-2-oxo-4 substituted azetidin-1-yl)-3-(5-fluorobenzo[d]thiazol-2-yl)urea derivatives

CONCLUSION

Ten azetidinone derivatives were synthesized and characterized by TLC, IR and ^{1}HNMR. The elemental analysis has confirmed the purity of products. The in vitro activities of all compounds against mycobacterium were investigated. Based on the in vitro screening data, all the designed and synthesized compounds (A6-10) had good ability to inhibit mycobacterium tuberculosis growth in terms of MIC. The results so far indicated that the activity of these derivatives against the mycobacterium can significantly be influenced by increase in hydrophobicity resulting in better penetration of the *Mycobacterium tuberculosis* cell wall.

REFERENCES

1. Bhusari K P, Khedekar P B .Synthesis and anti-tubercular activity of some substituted 2-(4-aminophenyl sulphonamide) Benzothiazoles. Indian Journal Of Heterocyclic Chemistry 2000; 9 : 213-13.
2. Sarkar S. Synthesis and Evaluation of Antibacterial and Anti-inflammatory Activity of 7- Alkyl /Aryl amino -6-fluoro -2-Phenyl Carboxamido(1,3) .Asian Journal Of Chemistry 2008 ; 20 (4) : 3227-3230 .
3. Sarkar S . Synthesis and biological activity of novel azetidinone and thiazolidinone starting from benzothiazole. Indian Journal Of Heterocyclic Chemistry 2013; **23** : 75-80.
4. Sarkar S, Dwivedi J, Chauhan R. Synthesis of 1-[2 (substituted phenyl)-4-oxothiazolidin-3-yl]-3-(6-fluro-7-chloro-1,3-benzothiazol-2-yl)-ureas as anthelmintic agent. Journal of Pharmy Research 2013; **7**: 439 -442.
5. Jagtap V A, Jaychandran E. Synthesis of some fluoro substituted sulphonamide benzothiazole comprising thiazole for anti-microbial screening. International Journal Of Pharma and Bio science 2010; 1 (4) : 484-490.
6. Kini S , Swain S P . Synthesis and evaluation of novel benzothiazole derivatives against human cervical cancer cell lines. Indian Journal of Pharmaceutical Science 2007; 69 (1): 46-50.
7. Sarkar S . Synthesis and Evaluation of Antibacterial and Antiinflammatory Activity of 7-Alkyl/Aryl amino-6-fluoro-2- phenyl Carboxamido-1,3-benzothiazoles. Asian Journal of Chemistry 2008; 20 (4) : 3227-3230.

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