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An Scalable Approach Towards Synthesis of Biologically Active Fused Benzo[4ˈ,5ˈ]Thiazolo[3ˈ,2ˈ:1,2]Pyrimido[4,5-d][1,3]Thiazines with their Antimicrobial and Antioxidant Activity

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ABSTRACT

A straight forward method has been developed for the synthesis of novel heterocycles such as benzo[4,5]thiazolo[3ˈ,2ˈ:1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i) by condensation of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3) with 2-amino 1/2/3/4-substituted benzothiazoles (4a-i) in DMF by using anhydrous potassium carbonate as catalyst. Compound (3) was prepared by reaction of benzothioamide (1) and bis(methylthio)methylene malononitrile (2) with same reaction condition which is used for title compounds. The chemical structures of newly constructed derivatives were corroborated by IR, ^{1}H-NMR ^{13}C-NMR and Mass spectral analysis. Synthesized compounds were screened for antimicrobial as well as antioxidant activity.

Keywords: Benzothioamide, Antioxidant activity, DMF and Potassium carbonate.

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INTRODUCTION

The growing incidence of drug-resistant of infectious diseases has stimulated the need for the development of new drugs. Synthetic chemistry play an important role in drug designing because it involves identification, synthesis and development of new chemical entities which are suitable for therapeutic use. It also includes the study of existing drugs, their biological properties and their quantitative structure-activity relationship (QSAR). Since in last few decades synthesis of fused heterocyclic rings containing nitrogen atom is an important task for chemist. Heterocyclic system linked to pyrimidine ring system are of great interest because they constitute an important class of natural products and has been recognised as to be rich source of bioactive metabolites with varied biological and pharmacological activities[1-3].

Pyrimido-thiazines are fused heterocyclic scaffold of biological interest as they are thia analogues of isoalloxazines and pteridines which constitute physiologically important substances such as flavins, biopterin and folic acid. Pyrimido-thiazine with various chemically active substituents such as imino groups is an important structural feature and resulting molecule would exhibit promising pharmacological activities. Bozsing *et al* have reported the synthesis of pyrimido-thiazines which inhibited carrageenin edema and decrease yeast induced pyrexia in rats[4]. It has been found in studies of pyrimido[4,5-*b*][1,4]thiazine and their derivatives inhibit the folic acid metabolising enzyme dihydrofolate reductase (DFR) and exhibit antitumor activity[5-6]. Further they also exhibit anti-inflammatory[7], antipyretics and antimicrobial activities[8]. Zarrouk A *et al* investigated 4-amino-2,6-dihydro-2-(4-nitro phenyl)-6-oxo-8-p-tolylpyrimido[2,1-*b*][1,3]thiazine-3,7-dicarbonitrile (AOPT) is an effective inhibitor for corrosion of carbon steel in phosphoric acid 2.0 M H_3PO_4[9]. Robert C Reynolds *et al* reported pyridopyrazine and pyrimidothiazine derivatives as FtsZ (filament temperature sensitive protein Z) inhibitors[10]. In literature many synthetic methods are reported for synthesis of fused pyrimido-thiazine and its derivatives [11-15].

Another important class of heterocyclic compounds is of benzothiazole which drawing interest due to its wide range biological activities such as antimicrobial[16], anti-inflammatory[17], antidiabetic[18], antiviral[19], anticancer[20], anticonvulsant[21]. Literature survey shows that few references are available on synthesis and biological activity of heterocycles containing benzothiazole fused with pyrimidine ring[22-24]. Recently Sambhaji P. Vartale *et al* 2016 has reported synthesis and antioxidant activity of fused benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine derivatives[25].

Biological importance of both benzothiazole and pyrimido-thiazine stimulated us to develop new versatile heteroaromatic ring system in which benzothiazole ring is fused through the nitrogen

atom with another biologically potent ring system such as pyrimido-thiazine. Hence in present investigation we develop a efficient method for the synthesis of benzo [4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives via nucleophilic substitution-cyclization reaction in between 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile and 2-amino 1/2/3/4-substituted benzothiazoles with evaluation of their antimicrobial and antioxidant activity.

MATERIALS AND METHOD

All compounds were purchased from SD-Fine, Spectrochem and Avra chemical companies and used without any additional purification. Melting points of synthesized compounds were determined by Electrothermal IA 9000 SERIES digital melting point apparatus and were uncorrected. Purity of all the products were routinely checked by thin layer chromatography (TLC) on pre-coated sheets of silica gel-C plates of 0.25 mm thickness. FT-IR spectra were recorded in Nujol or as KBr pallets on infrared spectrophotometer. Brukner advance spectrophotometer 400 MHz was used to record ^{1}H-NMR and ^{13}C-NMR spectra using tetramethylsilane (TMS) as internal standard, Mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV.

General procedure:

Synthesis of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3).

A mixture benzothioamide (1) (0.01mol) and bis(methylthio)methylene malononitrile (2) (0.01mol) in 10 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 6-8 hours. The reaction progress was monitored by thin layer chromatography (TLC) by using ethyl acetate:hexane (3:7) as irrigant. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from ethanol to give pure compound (3).

Synthesis of benzo[4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i).

As per scheme-2, a mixture of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3) (0.001mol) and 2-amino 1/2/3/4 substituted benzothiazoles (4a-i) (0.001mol) were independently refluxed in 10 ml of DMF and anhydrous K_2CO_3 (10mg) for 5-6 hours. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from ethanol to give pure compound (5a-i).

Table 1: Physicochemical data.

Com.No.	Molecular Formula	Mol. Wt.	Physical State	Yield %	M.P °C
3	$C_{12}H_9N_3S_2$	259	Faint Red Solid	78.24	166-67
5a	$C_{18}H_{11}N_5S_2$	361	Faint Brown Solid	62.33	142-43
5b	$C_{19}H_{13}N_5S_2$	375	Pale Yellow Solid	71.96	171-72
5c	$C_{19}H_{13}N_5OS_2$	391	Faint Red Solid	58.03	162-64
5d	$C_{18}H_{10}N_6O_2S_2$	406	Faint Red Solid	66.48	244-45
5e	$C_{18}H_{10}N_5S_2Cl$	395	Faint Brown Solid	74.11	215-17
5f	$C_{18}H_{10}N_5S_2Br$	438	Pale Yellow Solid	68.35	191-93
5g	$C_{20}H_{15}N_5S_2$	389	Faint Red Solid	72.56	185-86
5h	$C_{18}H_9N_5S_2Cl_2$	428	Faint Red Solid	67.99	209-10
5i	$C_{18}H_9N_5S_2FCl$	413	Faint Brown Solid	65.81	176-78

SPECTRAL ANALYSIS

6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3).

IR (KBr/cm^{-1}) 1639 (C=N), 2206 (CN), 3359 (=NH): ^{1}H-NMR (400 MHz,DMSO-d$_6$): δ 2.76 (s, 3H, SCH_3), 7.54-7.70 (m, 5H, Ar-H), 8.53 (s, 1H, =NH): ^{13}C-NMR (DMSO-d$_6$): δ 13.12 (S<u>C</u>H$_3$), 78.51 (<u>C</u>-CN), 114.34 (<u>C</u>N), 128.74-131.58 (<u>C</u>=<u>C</u>-Ar), 163.63 (<u>C</u>=NH), 165.13 (<u>C</u>-SCH$_3$), 172.12 (<u>C</u>=N), EI-MS(m/z: RA%): 259 (M^+).

2-phenyl-4a,12a-dihydro-4*H*,5*H*-benzo[4',5']thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine-4,5-diimine (5a).

IR (KBr/cm^{-1}) 1640 (C=N), 3384 (=NH): ^{1}H-NMR (400 MHz,DMSO-d$_6$): δ 7.27-7.55 (m, 5H, Ar-H), 7.71-7.96 (m, 4H, Ar-H), 9.10 (s, 1H, =NH), 10.48 (s, 1H, =NH):EI-MS(m/z:RA%):361 (M^+).

9-methyl-2-phenyl-4a,12a-dihydro-4*H*,5*H*-benzo[4',5']thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine-4,5-diimine (5b)

IR (KBr/cm^{-1}) 1650 (C=N), 3277 (=NH): ^{1}H-NMR (400 MHz,DMSO-d$_6$): δ 2.54 (s, 3H, CH_3), 7.41-7.79 (m, 5H, Ar-H), 7.84-8.1 (m, 3H, Ar-H), 8.34 (s, 1H, =NH), 8.36 (s, 1H, =NH): EI-MS (m/z: RA%): 375 (M^+).

9-methoxy-2-phenyl-4a,12a-dihydro-4*H*,5*H*-benzo[4',5']thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine-4,5-diimine (5c).

IR (KBr/cm^{-1}) 1685 (C=N), 3290 (=NH): ^{1}H-NMR (400 MHz,DMSO-d$_6$): δ 3.80 (s, 3H, OCH_3), 7.01-7.05 (m, 3H, Ar-H), 7.5-7.6 (m, 5H, Ar-H), 8.53 (s, 1H, =NH), 11.37 (s, 1H, =NH): EI-MS (m/z: RA%): 391 (M^+).

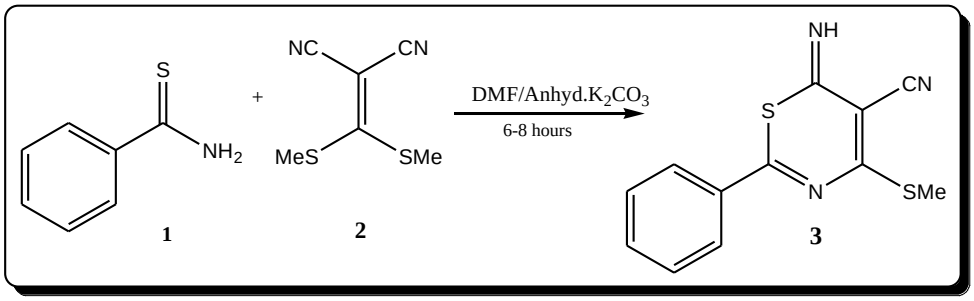
7,9-di-methyl-2-phenyl-4a,12a-dihydro-4*H*,5*H*-benzo[4',5']thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine-4,5-diimine (5g)

IR (KBr/cm^{-1}) 1642 (C=N), 3318 (=NH): ^{1}H-NMR (400 MHz,DMSO-d$_6$): δ 2.23 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.20-7.56 (m, 5H, Ar-H), 7.75-7.81 (dd, 2H, Ar-H), 9.22 (s, 1H, =NH), 9.91 (s,

1H, =NH): EI-MS (m/z: RA%): 389 (M^+).

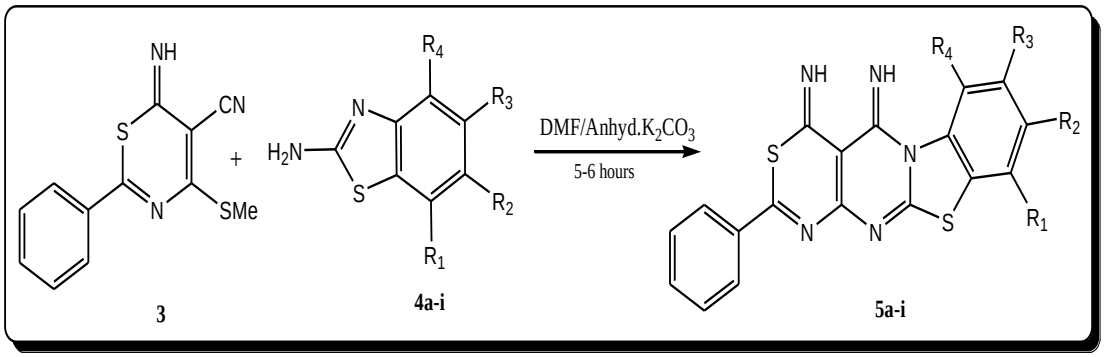
RESULTS AND DISSCUSSION

During the course of our ongoing interest to the synthesis of various heterocyclic compounds using 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3), we observed that compound (3) is key intermediate for the synthesis of benzothiazolopyrimidothiazines. Thus in present view we have synthesized a series of benzo[4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i). The key intermediate (3) was prepared by condensation of benzothioamide (1) and bis(methylthio)methylene malononitrile (2) in DMF and catalytic amount of anhydrous K_2CO_3 to afford (3) **Scheme-1**.



Scheme-1: Formation of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3).

The presence of tautomerisable 1,3 nucleophilic positions of benzothioamide made itself more facile for attack on bis(methylthio)methylene malononitrile resulting in the building of thiazine ring via elimination of simple thiomethyl group. The compound (3) posseses replaceable active thiomethyl group at 4-position and electron withdrawing nature of cyano group at 5-position. Due to presence of thiomethyl group and cyano group on compound (3) which has susceptibility for nucleophilic substitution-cyclization. When compound (3) was condensed independently with 2-amino 1/2/3/4-substituted benzothiazoles (4a-i) under similar experimental condition to afford benzo[4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i) **Scheme-2.**



Scheme-2: Formation of benzo[4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i).

The cyclisation was confirmed by disappearance of IR absorption band in the region of 2206 cm^{-1} for cyano group which was present in compound (3). The final compounds (5a-i) were characterized on the basis of physical and spectral (IR, ^{1}H-NMR and MS) data. Spectral analysis of these compounds are in agreement of the proposed structures.

Table 2. Compound numbers and substituent's position.

Comp. No.	R_1	R_2	R_3	R_4
5a	-H	-H	-H	-H
5b	-H	$-CH_3$	-H	-H
5c	-H	$-OCH_3$	-H	-H
5d	-H	$-NO_2$	-H	-H
5e	-H	- Cl	-H	-H
5f	-H	- Br	-H	-H
5g	-H	$-CH_3$	-H	$-CH_3$
5h	-H	-Cl	-H	-Cl
5i	-H	-Cl	-H	-F

The result of DPPH radical scavenging activity of newly synthesized benzo[4,5]thiazolo [3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives are summarized in table-4. DPPH is relatively stable nitrogen centred free radical that easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl hydrazyl, a stable free radical to decolourise in the presence of antioxidants. When DPPH accepts an electron from antioxidant compound, the DPPH is decolourise which can be quantitatively measured from change in absorbance. Such reactivity has been widely used to test the ability of compounds to act as free radical scavengers. The overall DPPH radical scavenging activity of tested benzo[4,5]thiazolo[3',2':1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i) were in a range of 3.83-26.21 % as compared to the standard ascorbic acid (90.15%). The highest DPPH radical scavenging activity was exhibited by **5e** whereas **5c** demonstrate lowest activity. From the result of present work, it can be concluded that benzo[4,5]thiazolo[3',2':1,2] pyrimido[4,5-*d*][1,3]thiazine derivatives are essential to boost the antioxidant activity.

Antimicrobial activity:

Antimicrobial activity of all synthesized compounds were determined by paper disk diffusion method using nutrient agar medium. Out of all synthesized compounds some derivatives shows considerable antimicrobial activity. From the given data it is found that all derivatives shows moderate antibacterial activity against *Escherichia coli* whereas compound 5b, 5f, 5g and 5i have significant antibacterial activity against all target microorganisms.

Table 3. Antimicrobial activity of compound (5a-i)

Sr. No.	Compounds	Gram positive		Gram negative	
		S.aureus	***B.subtilis***	***E.Coli***	***S.typhi***
1	5a	**12**	-	09	06
2	5b	**14**	11	**13**	10
3	5c	09	-	-	07
4	5d	06	08	11	-
5	5e	-	07	06	09
6	5f	**13**	16	**17**	**14**
7	5g	10	10	09	08
8	5h	-	-	**15**	**13**
9	5i	**16**	08	**12**	11
10	Streptomycin	**19**	**22**	-	-
11	Penicillin	-	-	**16**	**18**

Antioxidant activity:

DPPH radical scavenging assay :

The DPPH radical scavenging assay has been used for preliminary screening of the sample for antioxidant activity. The proton radical scavenging action is known as an important mechanism of antioxidants. The odd electron in DPPH radical gives a strong absorption maximum at 517 nm and is purple in colour.

Table 4: Antioxidant activity of selected compounds.

Sr. No.	Compounds	Antioxidant activity
		DPPH radical scavenging activity (%)
1	5a	22.68±0.55
2	5b	09.22±0.14
3	5c	03.83±0.61
4	5e	26.21±0.08
5	5f	17.08±0.43
6	Ascorbic acid	90.15±0.53

The colour turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from free radicals scavenging antioxidants to form reduced DPPH:H. 1ml (1 mM) of test sample was added in to equal quantity of 0.1 mM solution of DPPH in ethanol. After 10 minutes of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid was taken as standard reference. The result of DPPH reduction is summarized in table-4.

CONCLUSION

This Protocol provide convenient strategy to design novel heterocyclic compounds such as benzo[4,5]thiazolo[3ˈ,2ˈ:1,2]pyrimido[4,5-*d*][1,3]thiazine derivatives (5a-i) by simple rout with excellent product yield. Investigation of biological activity of synthesized compounds we can

conclude that this class of compounds certainly holds great promise towards the pursuit to discover novel class of antimicrobial agents.

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