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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 1, 4-BENZOTHIAZINE DERIVATIVES

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ABSTRACT
Equimolar quantity of 2-aminothiophenol and ethylchloroacetate was condensed and the solid mass obtained was refluxed. The compound obtained was refluxed with 4-aminoacetophenone to produce 3(4'-acetylphenylamino) 1,4-benzothiazine. The 3(4'-acetylphenylamino)1,4-benzothiazine was reacted with corresponding aldehyde to give 3[4'-(3"-substituted-2"-propenone-1"-yl) phenylamino]1,4-benzothiazine. The above synthesized compounds and guanidine nitrate were refluxed to afford 3[4'(2"-amino-4"-substituted-phenyl)-pyrimidine-4"yl)phenylamino]1,4-benzothiazine. The structures of the newly synthesized compounds were elucidated on the basis of elemental analysis, FTIR and ^{1}H-NMR and have been screened for antimicrobial activity.

Keywords: *1, 4-benzothiazine; Spectral Analysis; Antibacterial; Antifungal.*

INTRODUCTION

Synthesis of sulphur and nitrogen containing heterocycles has been explored for their therapeutic activity[1]. It has been revealed from the literature that benzothiazine exhibit antibacterial[2], antifungal[3], cardiovascular[4], antihypertensive[5], anthelmintic[6] and cytotoxic activity[7]. In our earlier studies we have reported several heterocyclic compounds having sulphur and nitrogen showing considerable antimicrobial activity[8,9]. In view of these records it appeared of interest to synthesize some new benzothiazine analogues and examine their biological activity.

Our synthetic strategy to prepare 1,4-benzothiazine analogues is based on the utilization of 2,3-dihydro-3-oxo-1,4-benzothiazine(**1**)**.** The compound (**1**) was prepared by the reaction of 2-aminothiophenol with ethylchloroacetate. The compound (**1**) was treated with phosphorous oxychloride to give 3-chloro-1,4-benzothiazine(**2**)**.** The compound (**2**) was reacted with 4-aminoacetophenone to yield 3(4'-acetyl phenylamino) 1,4-benzothiazine(**3**). The compound (**3**) was reacted with various benzaldehyde derivative to produce corresponding 3[4'(3''-substituted-2''-propenone-1''-yl)phenylamino]1,4-benzothiazines. All the newly synthesized compounds have been characterized by elemental analysis, spectroscopic data and have been screened for antimicrobial activity.

EXPERIMENTAL

All the chemicals used were of analytical grade. Melting points were determined in open capillary tubes and are uncorrected. Purity of the compounds was checked by TLC on silica gel and was purified using column chromatography. ^{1}HNMR spectra were recorded on a Jeol FTNMR spectrometer using $CDCl_3$ as solvent, TMS as an internal standard and the chemical shifts are expressed in ä units. IR spectra were recorded by using a JASCO FT/IR-300 E spectrometer in KBr.

Synthesis of 2,3-dihydro-3-oxo-1,4-benzothiazine (1)

2-Aminothiophenol (1.0ml, 0.01mol) and ethylchloroacetate (1.6ml, 0.01mol) was dissolved in 30ml ethanol. 5ml of 10% KOH was added and refluxed for 3 hours. Product obtained was poured in ice and washed with water. The compound is then recrystalised with ethanol to obtain compound (**1**) (2.23gm), m.p. 174-175ºC.

IR (KBr): 3227.71 (N-H stretching), 3116.55 (ArC-H stretching), 1662.52 (C=O stretching), 1583.45 (C=C stretching), 657.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 3.17 (s, 2H, -S-CH_2), 7.27-6.95 (m, 4H, Ar-H), 8.17 ppm (s, 1H, -NH).

Synthesis of 3-chloro-1, 4-benzothiazine (2)

2,3-Dihydro-3-oxo-1,4-benzothiazine (**1**) (1.39gm, 0.01 mol) and 4ml of phosphorous oxychloride was dissolved in 30 ml ethanol and refluxed for 1 hour, the product obtained was cooled in ice bath with constant stirring for 30 minutes. White solid separated out which was recrystalised with hot water to obtain compound (**2**) (5.01gm), m.p. 280-282ºC.

IR (KBr): 3218.85 (ArC-H stretching), 1626.22 (C=N stretching), 1590.70 (C=C stretching), 779.19 (C-Cl stretching), 657.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.87 (s, 2H, -S-CH_2), 6.25 ppm (m, 4H, Ar-H).

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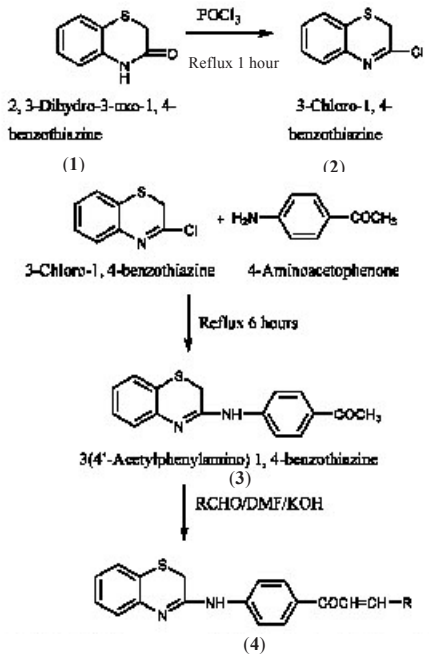
Synthesis of 3(4'-acetyl phenyl amino) 1, 4-benzothiazine (3)

3-Chloro-1,4-benzothiazine(**2**) (1.1gm, 0.01mol) and 4-aminoacetopheneone (1.15gm, 0.01mol) was dissolved in 40 ml acetone and refluxed for 6 hours with continuous addition of 10 ml of 5% sodium carbonate solution. The product obtained was cooled, poured in crushed ice, solid was separated which was filtered and washed with water and product was recrystalised with ethanol (**3**) (0.50gm), m.p. 98-99ºC. IR (KBr): 3231.69 (N-H stretching), 3120.33 (ArC-H stretching), 1679.51 (C=O stretching), 1655.76 (C=N stretching), 1594. 56 (C=C stretching), 682.74 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.40(s, 3H,-$COCH_3$), 2.92 (s, 2H, -S-CH_2), 6.85-7.27 (m, 8H, Ar-H), 8.12 ppm (s, 1H, -NH).

Synthesis of 3[4'-(3''-substituted-2''-propenone-1''-yl)phenylamino]1,4- Benzothiazines (4a-4j)

2.81gm (0.01mol) of 3(4'-Acetylphenylamino)1,4-benzothiazine (**2**) was dissolved in 30ml dimethylformamide and 2-chlorobenzaldehyde (1.1ml, 0.01mol) was added to the reaction mixture with constant stirring at room temperature. 30ml of 10% KOH was added to the reaction mixture with constant stirring at 15-20ºC, after 24 hour of continuous stirring, reaction mixture was poured in crushed ice, the solid separated out, filtered and washed with water and product was recrystalised with ethanol to give compound **4a-4j**.

(Scheme-1)



Scheme-1. *Synthesis of substituted chalcones of 1, 4-benzothiazine from 2, 3-dihydro-3-oxo-1, 4-benzothiazines (4a-4j)*

Derivatives synthesized (Table 1)

4a: IR (KBr): 3235.82 (N-H stretching), 3126.64 (ArC-H stretching), 1669.12 (C=O stretching), 1634.67 (C=C stretching), 1611.77 (C=N stretching), 1590.55 (C=C stretching), 786.62 (C-Cl stretching), 654.69 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.63 (s, 2H, -S-CH_2), 6.92 (s, 1H, -COCH=), 7.31 (s, 1H, =CH-), 7.18-7.81 (m, 12H, Ar-H), 8.92 ppm (s, 1H, -NH).

4b: IR (KBr): 3201.62 (N-H stretching), 3118.72 (ArC-H stretching), 1666.12 (C=O stretching), 1649.11 (C=C stretching), 1634.77 (C=N stretching), 1590.45 (C=C stretching), 785.65 (C-Cl stretching), 667.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.74 (s, 2H, -S-CH_2), 6.27 (s, 1H, -COCH=), 6.62 (s, 1H, =CH-), 6.63-7.50 (m, 12H, Ar-H), 9.09 ppm (s, 1H, -NH).

4c: IR (KBr): 3218.49 (N-H stretching), 3126.75 (ArC-H stretching), 1660.52 (C=O stretching), 1635.52 (C=C stretching), 1612.42 (C=N stretching), 1583.15 (C=C stretching), 1342.15 (N=O stretching), 657.38 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.54 (s, 2H, -S-CH_2), 6.64 (s, 1H, -COCH=), 6.64-7.89 (m, 12H, Ar-H), 6.99 (s, 1H, =CH-), 8.97 ppm (s, 1H, -NH).

4d: IR (KBr): 3209.79 (N-H stretching), 3120.65 (ArC-H stretching), 1642.56 (C=O stretching), 1630.42 (C=C stretching), 1611.44 (C=N stretching), 1583.65 (C=C stretching), 1375.15 (N=O stretching), 657.78 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.60 (s, 2H, -S-CH_2), 6.75 (s, 1H, -COCH=), 6.98 (s, 1H, =CH-), 6.76-8.06 (m, 12H, Ar-H), 8.71 ppm (s, 1H, -NH).

4e: IR (KBr): 3220.79 (N-H stretching), 3136.54 (ArC-H stretching), 1682.52 (C=O stretching), 1656.57 (C=C stretching), 1646.92 (C=N stretching), 1589.41 (C=C stretching), 647.61 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 1.24 -1.95 (m, 7H, Ali-H), 2.60 (s, 2H, -S-CH_2), 6.76 (s, 1H, -COCH=), 6.76-7.51 (m, 8H, Ar-H), 6.96 (s, 1H, =CH-), 8.85 ppm (s, 1H, -NH).

4f: IR (KBr): 3220.56 (N-H stretching), 3134.71 (ArC-H stretching), 1674.81 (C=O stretching), 1638.63 (C=C stretching), 1619.54 (C=N stretching), 1581.63 (C=C stretching), 1035.70 (C-O-C stretching), 649.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.80 (s, 2H, -S-CH_2), 3.85 (s, 3H,-OCH_3), 6.76 (s, 1H, -COCH=), 6.75-8.21 (m, 12H, Ar-H), 6.97 (s, 1H, = CH-), 8.95 ppm (s, 1H, -NH).

4g: IR (KBr): 3219.71 (N-H stretching), 3163.45 (ArC-H stretching), 1672.43 (C=O stretching), 1653.33 (C=C stretching), 1656.43 (C=N stretching), 1583.84 (C=C stretching), 1378.45 (C-N stretching), 687.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.64 (s, 2H, -S-CH_2), 2.77 (s, 6H,-N-$(CH_3)_2$), 6.67 (s, 1H, -COCH=), 6.67-7.69 (m, 12H, Ar-H), 6.99 (s, 1H, =CH-), 8.78 ppm (s, 1H, -NH).

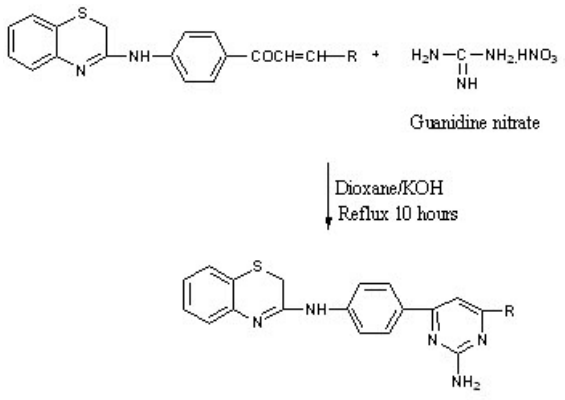
4h: IR (KBr): 3229.76 (N-H stretching), 3121.33 (ArC-H stretching), 1652.21 (C=O stretching), 1656.45 (C=C stretching), 1632.12 (C=N stretching), 1589.45 (C=C stretching), 667.18 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 1.93 (s, 3H,-CH_3), 2.67 (s, 2H, -S-CH_2), 6.77 (s, 1H,-COCH=), 6.77-7.58 (m, 8H, Ar-H), 6.97 (s, 1H, =CH-), 8.81 ppm (s, 1H -NH).

4i: IR (KBr): 3207.79 (N-H stretching), 3110.21 (ArC-H stretching), 1654.42 (C=O stretching), 1637.25 (C=C stretching), 1645.52 (C=N stretching), 1584.45 (C=C stretching), 687.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.60 (s, 2H, -S-CH_2). 6.75 (s, 1H, -COCH=), 6.98 (s, 1H, =CH-), 6.75-7.72 (m, 13H, Ar-H), 8.70 ppm (s, 1H, -NH).

4j: IR (KBr): 3239.29 (N-H stretching), 3126.23 (ArC-H stretching), 1672.82 (C=O stretching), 1645.45 (C=C stretching), 1632.51 (C=N stretching), 1573.42 (C=C stretching), 1385.15 (C-N stretching), 1025.70 (C-O-C stretching), 667.18 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □2.74 (s, 2H, -S-CH_2), 3.82 (s, 6H, $(OCH_3)_2$), 6.72 (s, 1H, -COCH=), 6.72-7.57 (m, 11H, Ar-H), 6.94 (s, 1H, =CH-), 8.07 ppm (s, 1H, -NH).

Synthesis of 3[4'-(2''-amino-4''-(substituted phenyl)-pyrimidine-4''-yl)-phenylamino]1,4-benzothiazines (5a-5d)

A Mixture of Compound (0.01 mol) and guanidine nitrate (1.22 g, 0.01 mol) in 25ml dioxane was mixed with 2ml of 40% KOH solution which was refluxed for 10 hours, then the reaction mixture was cooled and poured into ice, the solid separated out, filtered and washed with water and product was recrystalised with ethanol to give compound **5a-5d (Scheme-2).**



3[4'-(2''-Amino-4''-(substituted phenyl)-pyrimidine-4''-yl)-phenylamino] 1, 4-benzothiazines (5a-5d)

Scheme-2. *Synthesis of aminopyrimidine analogue of 1, 4-benzothiazine (5a-5d)*

Derivatives synthesized (Table 2)

5a: IR (KBr): 3216.19 (N-H stretching), 3076.75 (ArC-H stretching), 1636.32 (C=N stretching), 1593.45 (C=C stretching), 1352.35 (C-N stretching), 667.48 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □2.64 (s, 2H, -S-CH_2), 2.79 (s, 6H, -N-$(CH_3)_2$), 3.97 (s, 2H,-NH_2), 6.45-7.49 (m, 13H, Ar-H), 8.97 ppm (s, 1H, -NH).

5b: IR (KBr): 3224.72 (N-H stretching), 3096.42 (ArC-H stretching), 1631.53 (C=N stretching), 1573.65 (C=C stretching), 1379.19 (N=O stretching), 1345.30 (C-N stretching), 659.88 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 2.64 (s, 2H, -S-CH_2), 4.15 (s, 2H,-NH_2), 6.94-7.94 (m, 13H, Ar-H), 8.97 ppm (s, 1H, -NH).

5c: IR (KBr): 3219.69 (N-H stretching), 3116.75 (ArC-H stretching), 1622.22 (C=N stretching), 1583.45 (C=C stretching), 1335.70 (C-N stretching), 657.68 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □ 1.93 (s, 3H,-CH_3), 2.50 (s, 2H, -S-CH_2), 3.97 (s, 2H,-NH_2), 6.97-7.56 (m, 9H, Ar-H), 8.77 ppm (s, 1H, -NH).

5d: IR (KBr): 3231.43 (N-H stretching), 3117.15 (ArC-H stretching), 1629.12 (C=N stretching), 1589.35 (C=C stretching), 1338.60 (C-N stretching), 1036.40 (C-O-C stretching), 668.78 cm^{-1} (C-S stretching). ^{1}HNMR (DMSO-d_6): □2.74 (s, 2H, -S-CH_2), 3.85 (s, 3H,-OCH_3), 4.07 (s, 2H,-NH_2), 6.67-7.55 (m, 14H, Ar-H), 9.25 ppm (s, 1H, -NH).

Antimicrobial activity

The *in vitro* antimicrobial activity was carried out by disc diffusion method in DMSO-d_6 as a solvent[10]. All the newly synthesized compounds (**4a-4j**) and (**5a-5d**) were screened for antimicrobial activity against *Staphylococcus aureus, Escherischia coli, Basillus subtilis, Candida albicans* and *Aspergillus niger* at a concentration of 100 µg ml^{-1}. The zone of inhibition was compared with ofloxacin (100 µg ml^{-1}) for antibacterial activity after 24 hr of incubation at 25º and ketoconazole (100 µg ml^{-1}) for antifungal activity after 48 hr of incubation at 30º. The compounds showed varying degree of antimicrobial activity. Results are reported in Table 1.

RESULT AND DISCUSSION

A total of 14 [(4a-4j) and (5a-5d)] compounds belonging to 1, 4-benzothiazines were synthesized. TLC confirmed the purity of the title compounds. The structure elucidation was done by interpreting FTIR spectra, 1H-NMR and elemental analysis. Among (4a-4j) and (5a-5d) synthesized compounds of the present series compounds 4e, 4f, 5b, 5c and 5d exhibited maximum activity against *E. coli, S. aureus* and *B. subtilis* while compounds 4b, 4h, 4i and 4j exhibited moderate activity. Compounds 4h, 4i and 4j displayed maximum activity against *C. albicans and A. niger*, while the compounds 4b, 4c, 4d and 5a exhibited moderate activity against *C. albicans*.

Table 1: *Physical data of 1, 4-benzothiazine from 2, 3-dihydro-3-oxo-1, 4-benzothiazines (4a-4j)*

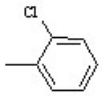
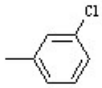
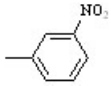
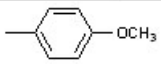
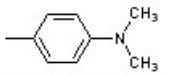
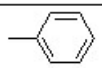
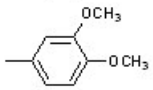
Compound Number	-R	M. P. (°C)	Yield (%)
4a		273-275	62.7
4b		265-267	61.9
4c		258-259	63.2
4d		240-241	62.3
4e	$CH=CH-CH_3$	245-247	75.8
4f		239-240	76.2
4g		245-247	79.5
4h	$-CH_3$	215-216	77.2
4i		265-266	72.6
4j		250-252	70.5

Table 2: *Physical data of Aminopyrimidine analogue (5a-5d)*

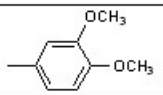
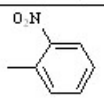
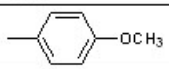
Compound Number	-R	M.P. (°C)	Yield (%)
5a		195-197	64.3
5b		220-222	66.1
5c	$-CH_3$	185-187	71.2
5d		175-176	73.8

Table 3: *Antimicrobial Activity of Synthesized Compound 4a-5d.*

Compd. No.	Antibacterial activity at 100 µg ml[-1]			Antifungal activity at 100 µg ml[-1]	
	E. coli	*S. aureus*	*B. subtilis*	*A. niger*	*C. albicans*
4a	7.67	16.66	8.33	10.33	9.33
4b	12.33	15.66	15.00	25.00	15.33
4c	13.00	11.33	10.67	14.33	15.67
4d	10.67	11.00	9.00	20.67	14.67
4e	27.67	29.32	26.62	14.33	12.00
4f	28.00	27.53	27.33	14.00	15.00
4g	15.67	16.33	15.33	13.33	13.33
4h	10.33	11.33	11.33	28.33	27.33
4i	11.33	11.00	12.67	26.67	28.33
4j	11.00	10.33	10.33	27.33	27.00
5a	22.67	12.67	10.67	10.33	12.33
5b	25.48	27.33	26.00	11.33	10.67
5c	28.00	24.66	28.00	11.00	10.33
5d	24.66	27.00	35.66	17.67	18.33
Std.	36.00	40.67	41.00	34.67	37.00
Blank	-	-	-	-	- **

* *Zone of inhibition are given in triplicate*
*** The negative control disk used for solvent had no zone of inhibition*

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