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Synthesis, Characterization and Antifungal Activity of 3-phenyl-2H-benzo [1, 4] thiazines

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ABSTRACT
Here in we report the convenient preparation of 3-phenyl-2H-benzo[1, 4]thiazine derivatives (**5-8**) after reacting acetophenone and its derivatives (**1-4**) with 2-aminothiophenol and iodine in one-pot synthesis in absolute ethanol. The characterization of compounds (**4-6**) was done by spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) as well as by analytical data. The new compounds (**5-8**) were screened for in vitro antifungal activity by Disk Diffusion Method during which compounds depicted potential in vitro antifungal activity in comparison with Nystatin.

KEYWORDS: Antifungal activity; Benzo[1,4]thiazine; 2-aminothiophenol, Acetophenone

Highlights

1. Benzo[1, 4]thiazine have been proved to be attractive moieties in drug discovery
2. Four novel Benzo[1, 4]thiazine derivatives were successfully synthesized in better yields
3. The new compounds showed potential in vitro antifungal activity with respect to a standard drug Nystatin
4. The presence of a fold along the nitrogen-sulphur axis in benzothiazines is one of the features responsible for their antifungal activity
5. The structure of these new compounds was successfully characterized by spectral and analytical data.

I. INTRODUCTION

Heterocyclic chemistry has often attracted important attention because of being an essential class of biochemically nodding molecules. In addition to their significant and profound clinical importance [1], they have the ability to be urbanized as drugs for the handling of a large number of disorders like breast and prostate cancer, cardiovascular, autoimmune, brain tumours and osteoarthritis [2-4]. Most of the heterocyclic based clinical drugs are semi-synthetic compounds prepared by connecting a new moiety or group of atoms with distinct functionality to the core or basic structure [5]. Most important of such functionalities are the heterocyclic systems because of their potent receptor binding properties. The advantage of employing hydrophobic heterocyclic units is their capacities to intermingle with cell membranes and interact with receptors hence cover the approach for biological behaviour of such complex and fused molecules [4].

Nitrogen containing heterocyclic compounds has the ability to normalize a variety of biological processes and thus have capacity to cure number of disorders including breast cancer [6], prostate cancer [7], leukaemia [8], autoimmune diseases [9] and osteoporosis [10]. So is the case with the nitrogen containing derivative, benzothiazines in which the presence of a fold along the nitrogen-sulphur axis is one of the features responsible to impart their biological activity [11], hence they show broad spectrum of biological activities such as antagonists [12], anticancer [11], vasorelaxant [13], antidiabetic [14], antihypertensive [15] and antimicrobial activity [16]. 1, 4-Benzothiazines are the molecules of interest to biologists and chemists as they are the precursors in many synthetic transformations leading to the biologically active heterocyclic molecules [12-15] and are therefore the molecules of prime interest to synthetic chemists as well. Intrigued by the above observation and in continuation of previous work [17, 18], we represent synthesis of phenyl benzothiazines and investigate their antimicrobial behaviour.

II. EXPERIMENTAL

2.1 General remarks

Chemicals were purchased from Merck and Sigma-Aldrich as ‘synthesis grade’ and used without further purification. Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr

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pellets with Pye Unicam SP3-100 spectrophotometer and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in ppm (δ). Mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapours to check the homogeneity as well as the progress of reaction. Sodium sulphate (anhydrous) was used as a drying agent.

2.2. General procedure of synthesis of 3-phenyl-2H-benzo[1, 4]thiazine derivatives (5-8)

To a solution of acetophenone and its derivatives (**1-4**) (1 mmol) in absolute ethanol (10 mL) was added 2-aminothiophenol (1 mmol) and iodine (2 mmol) in the same solvent (25 mL) and reaction mixture was refluxed for 6 h. The progress of the reaction was monitored by TLC. After completion of reaction, the excess solvent was reduced to three fourths of the original volume under reduced pressure. Then it was cooled to room temperature, diluted with $Na_2S_2O_7$ solution and subsequently with water. The mixture was extracted with ether, washed with water and finally dried over anhydrous sodium sulfate. Evaporation of solvents and crystallization of the oily residue from methanol afforded corresponding products (**5-8**).

2.2.1. 3-Phenyl-2H-benzo[1, 4]thiazine (5)

Yield 83%; m.p. 152 ^oC; Analysis found: C 74.59, H 9.35, N 6.18%. $C_{14}H_{11}NS$ requires: C 74.63, H 9.42 N 6.22%; IR (KBr): ν $_{max}$ 3062, 1600 (aromatic), 1628 (C=N), 1385 (C-N), 711 (C-S); ^1H NMR ($CDCl_3$): δ 6.43-6.24 (m, 4H, aromatic), δ 5.9-6.3 (m, 5H, aromatic), 2.3 (s, 2H, CH_3); ^{13}C NMR ($CDCl_3$): δ 163 (C=N), 149, 136, 134, 132.8, 131, 130 (6 aromatic carbons), 129, 125, 124, 122.8, 122, 120 (6 aromatic carbons); MS: m/z 225 [$M^{+\cdot}$].

2.2.2.3-(3′-Hydroxy)phenyl-2H-benzo[1, 4]thiazine (6)

Yield 76%; m.p. 163 ^oC; Analysis found: C 69.54, H 4.39, N 6.68%. $C_{14}H_{11}NOS$ requires: C 69.68, H 4.59 N 5.80%; IR (KBr): ν $_{max}$ 3410 (OH), 3060, 1610 (aromatic), 1650 (C=N), 1080 (C-O), 1388 (C-N), 750 (C-S); ^1H NMR ($CDCl_3$): δ 7.3 (s, 1H, OH, exchangeable with D_2O), 6.4-6.7 (m, 4H, aromatic), δ 5.7-6.0 (m, 4H, aromatic), 2.3 (s, 2H, CH_3); ^{13}C NMR ($CDCl_3$): δ 163 (C=N), 149, 136, 134, 132.8, 131, 130 (6 aromatic carbons), 129, 128, 127, 126, 125, 124 (6 aromatic carbons); MS: m/z 241 [$M^{+\cdot}$].

2.2.3. 3-(4′-Hydroxy)phenyl-2H-benzo[1, 4]thiazine (7)

Yield 70%; m.p. 141 ^oC; Analysis found: C 69.54, H 4.39, N 6.68%. $C_{14}H_{11}NOS$ requires: 69.68, H 4.59 N 5.80%; IR (KBr): ν $_{max}$ 3409 (OH), 3060, 1610 (aromatic), 1645 (C=N), 1080 (C-O), 1380 (C-N), 735 (C-S); ^1H NMR ($CDCl_3$): δ 7.0 (s, 1H, OH, exchangeable with D_2O), 6.4-6.7 (m, 4H, aromatic), δ 5.7-6.0 (m, 4H, aromatic), 2.3 (s, 2H, CH_3); ^{13}C NMR ($CDCl_3$): δ 163 (C=N), 139, 136, 134, 133, 131, 130 (6 aromatic carbons), 129, 128, 127, 126.5, 126, 124 (6 aromatic carbons); MS: m/z 241 [$M^{+\cdot}$].

2.2.4.3-(2′,4′-Dihydroxy)phenyl-2H-benzo[1, 4]thiazine (8)

Yield 70%; m.p. 141 ^oC; Analysis found: C 65.24, H 4.27, N 5.36%. $C_{14}H_{11}NO_2S$ requires: 65.35, H 4.31, N 5.44%; IR (KBr): ν $_{max}$ 3419 (OH), 3409 (OH), 3060, 1610 (aromatic), 1645 (C=N), 1080 (C-O), 1380 (C-N), 735 (C-S); ^1H NMR ($CDCl_3$): δ 7.7 (s, 1H, OH, exchangeable with D_2O), 7.0 (s, 1H, OH, exchangeable with D_2O), 6.2-6.5 (m, 4H, aromatic), δ 5.8-6.1 (m, 3H, aromatic), 2.4 (s, 2H, CH_3); ^{13}C NMR ($CDCl_3$): δ 164 (C=N), 149, 136, 134, 133, 131, 130 (6 aromatic carbons), 129, 128, 127, 126.5, 126, 124 (6 aromatic carbons); MS: m/z 257 [$M^{+\cdot}$].

2.2 In vitro antifungal activity

Antifungal screening of these substituted 1, 4-benzothiazines (**5-8**) was done against the cultures of Candida albicans (ATCC 10231), Aspergillus fumigatus (ATCC 1022), Trichophyton mentagrophytes (ATCC 9533) and Pencillium marneffei (recultured) in DMSO by agar diffusion method [19, 20]. Sabouraud agar medium was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 ℃ for 1 h. using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37 ℃ for 3-4 days. Minimum inhibitory concentration (MIC) was determined by

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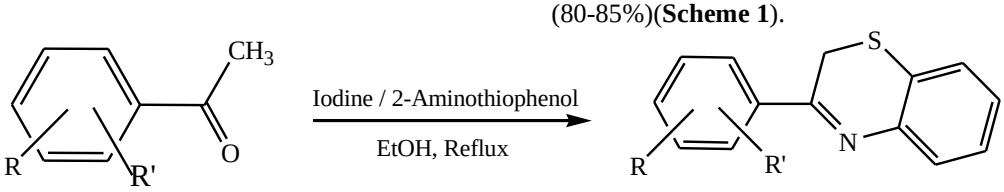
broth dilution technique as in antibacterial activity. The Inhibition zones of compounds (**5-8**) were compared with Nystatin used as standard drug. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4 - 6×10^4c.f.u. mL^{-1}. The cultures were incubated at 35 ℃ for 48 h and the growth was monitored.

III. RESULTS AND DISCUSSION

Chemistry

Development of highly functional molecules from simple building blocks has always attracted the curiosity of synthetic chemists. The reaction does not demand rigorously dried solvents, reagents or inert atmosphere but is actually one-pot synthesis of new benzothiazines from acetophenone, iodine & 2-aminothiophenol under reflux conditions; on the completion of the reaction, the products were obtained in better yields (80-85%)(**Scheme 1**).

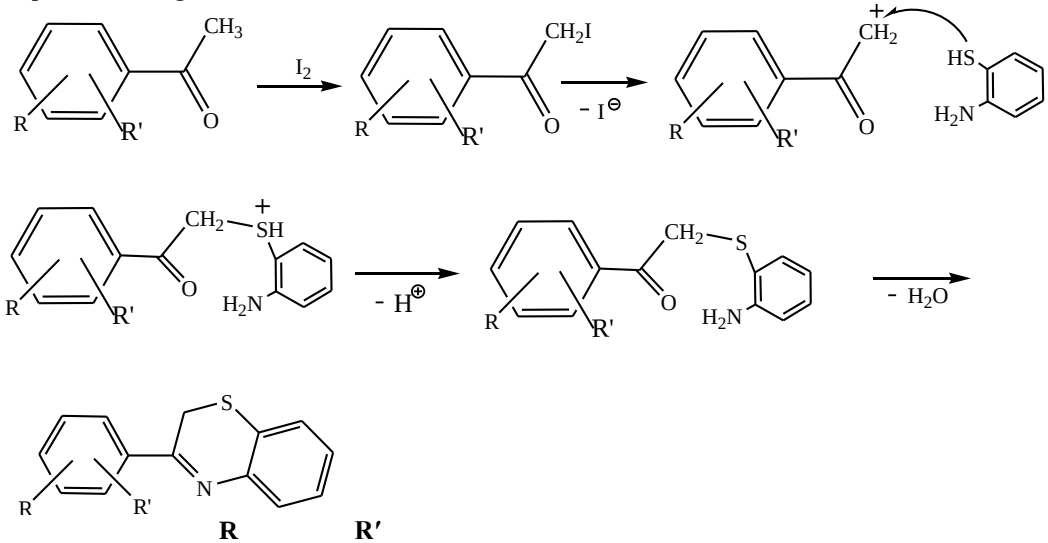


R	**R′**
(**1**) H	H
(**2**) H	3-OH
(**3**) H	4-OH
(**4**) 2-OH	4-OH

R	**R′**
(**5**) H	H
(**6**) H	3-OH
(**7**) H	4-OH
(**8**) 2-OH	4-OH

Scheme 1. Showing the formation of benzothiazines **4-6**

Formation of these benzothiazines (**5-8**) may be shown according to the proposed mechanism [21-23] (**Scheme 2**). The mechanism for the formation of these benzothiazines involves formation of iodoacetophenone in situ as an intermediate, which on further reaction with 2-aminothiophenol undergoes SN^1 reaction at C_1 and condensation at C_2 resulting in cyclization that leads to the formation of corresponding product. The remarkable feature of the reaction is the formation of 2-iodoacetophenone in situ as an intermediate which might be obtained separately by the reaction of ketones with iodine.



R	**R′**
(5) HH	
(6) H	3-OH
(7) H	4-OH
(8) 2-OH	4-OH

Scheme 2. Mechanism for the formation of 3-phenyl-2H-benzo[1,4]thiazine derivatives (**5-8**)

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In vitro antifungal activity

It is clear from the antifungal screening data **Table 1** that compounds (**5-8**) showed better in vitro antifungal activity. During antifungal screening, compounds **5**, **6** and **7** showed potential inhibition zones against C. albicans, A. fumigates and T. mentagrophytes strains. But the inhibition zone of compound **6** is larger than compound **5** against C. albicans in comparison with the standard drug, Nystatin. As shown in **Table 1**, compound **6** showed potential zones of inhibition i.e. 21.5, 18.4, 19.4 and 17.1 mm against C. albicans, A. fumigatus, T. mentagrophytes and P. marneffei, respectively in comparison with the Nystatin.

Compounds	Corresponding effect on microorganism			
	CA	AF	TM	PM
5	18.4 ± 0.5	17.2 ± 0.5	18.3 ± 0.5	15.4 ± 0.2
6	21.5 ± 0.2	18.4 ± 0.3	19.4 ± 0.3	17.1 ± 0.4
7	19.5 ± 0.5	18.3 ± 0.4	17.2 ± 0.2	17.2 ± 0.5
8	20.5 ± 0.5	19.4 ± 0.5	19.4 ± 0.1	16.3 ± 0.5
Nystatin	24.1 ± 0.3	21.5 ± 0.5	22.2 ± 0.5	20.2 ± 0.5
DMSO	-	-	-	-

Table 3. Antifungal activity of 1,4-benzothiazine derivatives measured by Halo Zone Test (Unit, mm)

The MIC's of compounds (**5-8**) with different fungal strains are given in **Table 2** and it is clear from the data that MIC's of the compounds (**5-8**) are active against different fungal strains at lower concentrations. From **Table 2** it is also clear that the lowest concentration at which compounds **5** and **6** inhibited the visible growth of fungi is 16 µg/mL and 32 µg/mL while compound **7** inhibited the growth of fungi at lower concentration i.e. 64 µg/mL against the different fungal strains. From the data, it is quite clear that the compounds are showing lower MIC values and are therefore having efficacious zone of inhibition and are therefore active in showing antifungal activity.

MIC (µg/ml)	Compounds				
Strains	**5**	**6**	**7**	8	**Fluconazole**
Candida albicans	16	32	32	64	16.0
Aspergillus fumigatus	32	16	32	64	16.0
Trichophyton mentagrophytes	64	32	32	64	16.0
Pencillium marneffei	128	32	32	128	16.0

Table 4. Showing minimum inhibition concentration (MIC) of 1, 4-benzothiazine derivatives

The reason for this enhanced antifungal activity in the synthesized compounds (**5-8**) may be due to the presence of a fold along the nitrogen-sulphur axis which is one of the features responsible to impart their antifungal activity.

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IV. CONCLUSION

In summary, the development a facile and convenient approach for the synthesis of new 1, 4-benzothiazine derivatives was successful. During the in vitro screening, the new compounds depicted potential to better antifungal activities against different fungal strains in comparison with the standard drug, Nystatin. It is clear from the literature that the possible reason of the antifungal activity of 1, 4-benzothiazine moiety may be due to the presence of fold along the nitrogen and sulphur axis in them. Moreover the aromatic moiety may be also enhancing the biological activity because most of the heterocyclic compounds adjoined with the aromatic group, often show potential antimicrobial activity.

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