INTRODUCTION

1,4-Benzothiazines constitute an important class of heterocyclic organic compounds containing 1,4-thiazine ring fused to a benzene ring. 1,4-Benzothiazines derivatives play an important role in the synthetic chemistry because of their unique chemical, physical and biological properties [1-6]. In particular, the synthesis of 1,4-benzothiazines and their sulfone derivatives has attracted tremendous interest evidenced by a large number of publications [7-9]. The oxidation of disulfide linkage in 1,4-benzothiazines to disulfone leads to an important class of heterocyclic sulfones not only from the medicinal and industrial point of view but also from structural aspects. Conversion of benzothiazine into sulfone has provided an opportunity to study the changes in infrared and nuclear magnetic resonance spectra caused by the conversion of the sulfide linkage to sulfones.

In the worldwide as well as in the developing countries, the most human death occurs due to infectious bacterial disease [10]. Drug resistance in human pathogenic microbes has developed due to the indiscriminate use of the commercial antimicrobial drugs for the treatment of the infectious disease. Drug resistance is the major hurdle of this era which is leading towards mortality and morbidity [11]. This condition has forced to the researcher to search for the new anti-microbial substance which is more effective and having less side effect with improved physical properties. With the aim of developing a new class of effective antimicrobial drugs, several 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazones and hydrazines, antimicrobial activity which whereas compounds 5c and 5e showed moderate antimicrobial activity.

Conclusion: Result obtained in this research work clearly indicated that the compound 5f having methyl at 2 position and nitro groups at 2′ and 4′ position showed the most potent antimicrobial activity.

Keywords: 1,4-Benzothiazines, Sulfones, Hydrazines, Anti-microbial activity

SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF 1-(6-NITRO-2H-BENZO[b][1,4]THIAZINE-3(4H)-YLIDENE)HYDRAZONE-1,1-DIOXIDE DERIVATIVES

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ABSTRACT

Objective: The objective of this research was to synthesize and evaluate anti-microbial properties of 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives.

Methods: These new compounds were synthesized by reaction of 2H-benzo[b][1,4]thiazine-3(4H)-one with hydrazine derivatives and oxidized at the sulfur atom by 30% hydrogen peroxide to obtain sulfones. All the synthesized compounds were evaluated for antimicrobial activity using the disc diffusion method.

Results: The FTIR, 1HNMR, 13CNMR and Mass studies confirms the synthesis of some new 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives. Compound 5f showed potent antimicrobial activity whereas compounds 5c and 5e showed moderate antimicrobial activity.

Conclusion: Result obtained in this research work clearly indicated that the compound 5f having methyl at 2 position and nitro groups at 2′ and 4′ position showed the most potent antimicrobial activity.

Keywords: 1,4-Benzothiazines, Sulfones, Hydrazines, Anti-microbial activity

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MATERIALS AND METHODS

Chemistry

All the chemicals used in the study were of analytical grade. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by thin layer chromatography on silica gel plates with a 0.2 mm thickness. Compounds were powdered, mixed with KBr at 1% concentration and pressed into pellets before IR spectra be recorded on Bruker FT/IR Vertex spectrometer. H-and 13NMR spectra were recorded on a Bruker Avance II 400-NMR spectrometer using DMSO-d as a solvent, TMS as an internal standard and the chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The Mass spectra were recorded on a Waters, Q-TOF MS-ES spectrometer. Elemental analysis was done on Carlo Erba 1108 Elemental analyzer.

The synthesis of 5a-f started from the treatment of 2-chloro-5-nitro-aniline 1 with sodium sulfide and sulfur gave Sodium-2-amino-4-nitrobenzenethiol 2, which was cyclized with β-haloesters in ethanolic solution [13,14] to yield 6-nitro-2H-benzo[b][1,4]thiazin-3-ylidene)hydrazine-1,1-dioxide derivatives 3a-b. Compounds 3a-b were refluxed with some nitrogen containing nuclophilic hydrazines in methanol [15] to yield 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazines 4a-f. The further step, i.e. the oxidation of the sulfur, was usually performed with 30% hydrogen peroxide in glacial acetic acid [7] to produce their sulfones 5a-f. The synthesis, physical and analytical properties of compound 2 and 3a have been previously described in references [14, 16].

Synthesis of 2-methyl-6-nitro-2H-benzo[b][1,4]thiazin-3-one (3b)

Sodium-2-amino-4-nitrobenzenethiol (1.2 gm, 0.01 mol) (2) and methyl-2-chloropropionate (1.1 gm, 0.01 mol) was dissolved in 30 ml ethanol. 5 ml of 10% NaOH was added and refluxed for 3 h. Product was poured in ice, washed with water and recrystallized from ethanol to obtain compound 3b. Light yellow crystal, yield, 93%; m. p. 174-175 °C; Rf, 0.89 (toluene-ethyl acetate, 7:5); UV (H2O) λmax (log ε): 266 (4.18); IR (υ cm−1): 3360, 2924, 1671, 1578, 1392, 650; 1H-NMR (DMSO-d6): 1.5 (d, 3H, J=7 Hz, CH3), 2.2 Hz, H-7), 8.1(d, 1H, J=2. 4 Hz, H-5), 10.95 (s, 1H, NH) ; 13CNMR (ppm, DMSO-d6): 19.9 (CH3), 50.5 (CH, C-2), 115.7 (CH, C-5), 116.9 (CH, C-7), 127.9 (CH, C-8), 131.4 (C, C-9), 143.3 (C, C-10), 145.0 (C, C-6), 169.4 (C, C-3); ISMS m/z (%): 224 (55), 195 (30), 181 (100), 143 (24), 95 (12). Anal. calcd for C16H11N2O3S: C, 48.21; H, 3.60; N, 12.49; S, 14.30. Found: C, 48.23; H, 3.58; N, 12.53; S, 14.31.
The title compound was prepared from 6-nitro-2-benzof[b][1,4]thiazin-3(4H)-one (3a) and hydrazine hydrate. Product was extracted with cyclohexane (3×50 ml) and purified by column chromatography on silica gel with a mixture of toluene-ethyl acetate (8:2) as eluent. Light yellow oil; yield, 86%; R<sub>c</sub> 0.66 (benzene-acetone: 1:3); UV (DMSO) λ<sub>max</sub> (log ε) 220 (4.13) nm; IR (υ cm<sup>-1</sup>): 3148, 2980, 1700, 1523, 1434, 1118, 644. δ<sub>H</sub>-NMR (5 ppm, DMSO-d<sub>6</sub>, 400 MHz): 1.19 (d, 3H, J=7 Hz, CHCH<sub>3</sub>), 2.77 (q, 1H, H-2), 7.19 (1H, d, J=2.5 Hz, H-5). 7.29 (1H, d, J=8.6 Hz, H-8), 7.45 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.51 (s, 1H, NH). 13C-NMR (85 ppm, DMSO-d<sub>6</sub>, 100 MHz): 13.2 (CH, C-5'), 31.8 (C, C-5), 110.6 (CH-C-2'), 123.6 (C, C-9), 121.1 (CH, C-6'), 125.0 (C, C-4), 147.5 (C, C-1), 149.6 (C, C-6), 154.7 (C-C3), ESMS m/z (%): 224 (11), 185 (39), 150 (24), 124 (100), 88 (11), 74 (5). Anal. calcd for C<sub>15</sub>H<sub>12</sub>N<sub>6</sub>O<sub>6</sub>, C: 42.85; H: 3.64; N: 24.96, S: 13.42. 

The title compound was prepared from 6-nitro-2-benzof[b][1,4]thiazin-3(4H)-one (3a) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Light brown crystals; yield, 71%; m. p. 148-150 °C; R<sub>f</sub>, 0.23 (benzene-acetone, 1:3); UV (DMSO) λ<sub>max</sub> (log ε) 220 (4.14) nm; IR (υ cm<sup>-1</sup>): 3148, 2980, 1700, 1523, 1434, 1118, 644. δ<sub>H</sub>-NMR (5 ppm, DMSO-d<sub>6</sub>, 400 MHz): 1.19 (d, 3H, J=7 Hz, CHCH<sub>3</sub>), 2.77 (q, 1H, H-2), 7.19 (1H, d, J=2.5 Hz, H-5). 7.29 (1H, d, J=8.6 Hz, H-8), 7.45 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.51 (s, 1H, NH). 13C-NMR (85 ppm, DMSO-d<sub>6</sub>, 100 MHz): 33.5 (CH, C-2), 110.6 (CH-C-5), 111.8 (CH-C-7), 126.3 (C-C-9), 126.8 (CH-C-8), 145.7 (C-C-10), 149.6 (C-C-6), 154.7 (C-C3), ESMS m/z (%): 224 (11), 185 (39), 150 (24), 124 (100), 88 (11), 74 (5). Anal. calcd for C<sub>15</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub>, C: 42.85; H: 3.64; N: 24.96, S: 13.42. 

The title compound was prepared from 6-nitro-2-benzof[b][1,4]thiazin-3(4H)-one (3a) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Light yellow oil; yield, 86%; m. p. 108-110 °C; R<sub>c</sub> 0.86 (toluene-ethylacetate 3:2); UV (DMSO) λ<sub>max</sub> (log ε) 204 (4.25) nm; IR (υ cm<sup>-1</sup>): 3326, 2941, 1692, 1523, 1359, 1245, 1062, 624. δ<sub>H</sub>-NMR (5 ppm, DMSO-d<sub>6</sub>, 400 MHz): 1.20 (3H, d, J=7 Hz, CH-3'), 32.4 (4.19) nm; IR (υ cm<sup>-1</sup>): 3148, 2980, 1700, 1523, 1434, 1118, 644. δ<sub>H</sub>-NMR (5 ppm, DMSO-d<sub>6</sub>, 400 MHz): 13.2 (CH, C-5'), 31.8 (C, C-5), 110.6 (CH-C-2'), 123.6 (C, C-9), 121.1 (CH, C-6'), 125.0 (C, C-4), 147.5 (C, C-1), 149.6 (C, C-6), 154.7 (C-C3), ESMS m/z (%): 314 (8), 275 (14), 226 (40), 200 (7), 164 (24), 136 (100), 122 (12). Anal. calcd for C<sub>15</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub>, C: 45.73; H: 4.49; N: 17.82, S: 10.20. Found: C: 45.73; H: 4.46; N: 17.80; S: 10.22.

The title compound was prepared from 2-methyl-6-nitro-2-benzof[b][1,4]thiazin-3(4H)-one (3b) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Orange crystals; yield, 85%; m. p. 108-110 °C; R<sub>c</sub> 0.86 (toluene-ethylacetate 3:2); UV (DMSO) λ<sub>max</sub> (log ε) 234 (4.19) nm; IR (υ cm<sup>-1</sup>): 3294, 2924, 1687, 1585, 1422, 1044, 907, δ<sub>H</sub>-NMR (5 ppm, DMSO-d<sub>6</sub>, 400 MHz): 1.21 (3H, d, J=7 Hz, CHCH<sub>3</sub>), 29.7 (1H, d, J=2.5 Hz, H-5), 2.79 (1H, d, J=2.5 Hz, H-8), 2.94 (1H, d, J=2.5 Hz, H-7), 3.73 (3H, s, CH<sub>3</sub>-N), 6.58 -7.07 (3H, m, H -3, 4, 5). Anal. calcd for C<sub>15</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub>, C: 45.73; H: 4.49; N: 17.76, S: 10.22. Found: C: 45.73; H: 4.46; N: 17.80; S: 10.22.
The title compound was prepared by oxidation of 1-(2-methyl-6-nitro-2-
benzo[b][1,4]thiazin-3(4H)-ylidene)-2-(2′,4′-dinitrophenyl)hydrazine (5f).

The title compound was prepared by oxidation of 1-[(2-methyl-6-
nitro-2-
benz[b][1,4]thiazin-3(4H)-ylidene)-2-(2′,4′-dinitrophenyl)hydrazine (5f) and
recrystallized from ethanol. Light brown crystals; yield, 84%; m. p.
207 °C; Rf, 0.73 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ
max (log ε) 270 (4.38) nm; IR (ν cm⁻¹): 3116, 1670, 1586, 1449,
1381, 1331, 1244, 1162, 1066; ¹H-NMR (5 ppm, DMSO-d₆, 400 MHz):
3.51 (s, 2H, H-2), 6.45 (2H, dd, J=1.6, J=8.5 Hz, H-6), 6.65-
7.19 (3H, m, H-3', H-4', H-5'), 7.31 (1H, J=2.5 Hz, H-5), 7.74 (1H, dd, 
J=2.5, 8.6 Hz, H-7), 7.52 (1H, d, J=8.6 Hz, H-8). 10.64 
(s, 1H, NH); 13C-NMR (δ ppm, DMSO-d₆, 100 MHz): 136.1 (CH, C-
3'), 123.2 (C, C-9), 121.1 (C, C-3'), 120.7 (CH, C-2), 127.7 
(CH, C-5), 127.7 (CH, C-6), 127.7 (CH, C-8), 133.3 (C, C-9), 142.3 
(C, C-10), 147.4 (C, C-1'), 153.6 (C, C-3), 153.1 (C, C-6), ESMS 
m/z (%): 332 (12), 261 (6), 212 (52), 186 (38), 150 (23), 136 (100), 122 (12).

In vitro antifungal activity was performed with ketoconazole as a positive control.

Relative percentage inhibition = 100 × (a– b) ÷ (c + b).

Where,
a: total area of inhibition of the test compounds
b: total area of inhibition of the solvent
c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using

Inhibitory zone area = (r² - r₀²)π

Where, r is radius of zone of inhibition

Statistical analysis

The results of the antifungal activity of compounds are expressed as mean±SEM of triplicate samples. Statistically, significant differences between groups were measured using one-way analysis.

In vitro antifungal activity

The relative percentage of inhibition of the compounds with respect to positive control was calculated by the following formula [11].

Relative percentage inhibition = 100 × (a - b) ÷ (c - b)

Where,
a: total area of inhibition of the test compounds
b: total area of inhibition of the solvent
c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using
Area of inhibitory zone = mr²

Where, r is radius of zone of inhibition
of variance (ANOVA) followed by two sample t-test of all groups versus their respective control group and *p<0.05 was considered statistically significant, p>0.05 was considered as non-significant and **p<0.01 was considered highly significant.

RESULT AND DISCUSSION

Chemistry

These compounds were synthesized by a conventional method, and their structures have been elucidated on the basis of spectral analysis. In IR spectra of all compounds, the bands occur in the region 600-2000 cm-1 due to the symmetric and asymmetric stretching vibration of the SO2 group. In 1HNMR spectra, a broad peak observed at δ 2.9-3.5 can be assigned to –CH proton. The sharp peak observed at δ 2.8-3.0 can be assigned to –NH protons. The strong C=N stretching absorption band in the region of 1660-1702 cm-1 region depicted of table 2. After statistical analysis, P value was determined and depicted of table 2. After statistical analysis, P value was determined.

Table 1: Antibacterial and antifungal activity

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli (Mean ±SD)</th>
<th>S. aureus (Mean ±SD)</th>
<th>B. subtilis (Mean ±SD)</th>
<th>C. albicans (Mean ±SD)</th>
<th>A. niger (Mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>17.33±2.52</td>
<td>16.67±0.58</td>
<td>15.33±1.52</td>
<td>11.33±0.58</td>
<td>12.67±1.00</td>
</tr>
<tr>
<td>5b</td>
<td>18.67±0.58</td>
<td>16.66±2.30</td>
<td>16.33±1.16</td>
<td>12.33±1.16</td>
<td>13.33±1.16</td>
</tr>
<tr>
<td>5c</td>
<td>19.67±0.58</td>
<td>18.33±1.53</td>
<td>17.66±1.52</td>
<td>14.67±0.58</td>
<td>15.67±1.16</td>
</tr>
<tr>
<td>5d</td>
<td>17.67±1.16</td>
<td>16.33±1.16</td>
<td>16.33±1.53</td>
<td>12.67±0.58</td>
<td>13.67±1.16</td>
</tr>
<tr>
<td>5e</td>
<td>19.66±0.57</td>
<td>18.66±1.52</td>
<td>18.33±2.52</td>
<td>13.67±0.58</td>
<td>14.33±1.16</td>
</tr>
<tr>
<td>5f</td>
<td>20.66±1.52</td>
<td>20.33±1.53</td>
<td>19.33±2.52</td>
<td>15.67±0.58</td>
<td>16.67±1.16</td>
</tr>
<tr>
<td>Control</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>21.67±0.58</td>
<td>22.3±0.58</td>
<td>20.3±1.53</td>
<td>-</td>
<td>20.3±1.58</td>
</tr>
</tbody>
</table>

Values are expressed as mean±standard deviation (SD) of the three replicates, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, C. albicans: Candida albicans, A. niger: Aspergillus niger, n. a: no activity.

The synthesis of 3-aryldihydrazino-2-methyl-(1H)-1,4-benzothiazines has been previously reported [15]. In the present paper, we describe the synthesis of 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives.

In vitro evaluation of the newly synthesized compounds for the antimicrobial activity is the first step toward achieving the goal of developing a new drug for the infectious disease. Earlier, the synthesis of many 1,4-benzothiazine derivatives [2,11,12,14] and their sulphones [9] have been reported to exhibit antimicrobial activity for pharmacological applications. Various hydrazine derivatives have been previously reported to possessing a broad spectrum antimicrobial activity [17]. In this research, some new class of sulphones of 1,4-benzothiazines containing different hydrazine derivatives in the 3-position was screened for antimicrobial properties. The present study through light on the anti-microbial efficacy of these novel compounds. The result indicated that these synthesized compounds showed more activity towards bacteria as compared to the fungi.
further study of novel 1,4-benzothiazine derivatives is in progress to evaluate a more potent antimicrobial agent with lesser side effects.

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CONFLICTS OF INTERESTS

Declared none

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