SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF 1-(4-METHYL-6-NITRO-2H-BENZO[B][1,4]THIAZINE-3(4H)-YLIDENE)HYDRAZINE-1,1-DIOXIDE DERIVATIVES

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Received: 11 April 2017, Revised and Accepted: 09 June 2017

ABSTRACT

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

Methods: These new compounds were synthesized by methylation in 4-N and reacted 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 Fourier transform infrared, 1H nuclear magnetic resonance (NMR), 13C-NMR, and mass studies confirm the synthesis of some new 1-(4-methyl-6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives. Compound 6f showed the potent antimicrobial activity.

Conclusion: Result obtained in this research work clearly indicated that the compound 6f 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, Antimicrobial activity.

INTRODUCTION

Research in the synthetic chemistry of 1,4-benzothiazine derivatives during the past few decades was mainly attributed to their unique chemical, physical, and biological properties [1-7]. The synthesis of the sulfone system, many sulfones have been shown to exhibit biological activity for the industrial and pharmacological applications [8,9]. The oxidation of sulfide linkage in 1,4-benzothiazines to dioxide leads to a significant class of heterocyclic sulfones from medicinal and structural aspects. Alteration of benzothiazine into sulfone has provided an opportunity to study the changes in infrared and nuclear magnetic resonance (NMR) 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. 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 obstacle of this era which is leading toward mortality and morbidity. This condition has been enforced to the researcher to investigate for the new antimicrobial substance which is more efficient and having lesser side effect with improved physical properties. The alkylation of 4-N position of 2H-benzo[1,4]thiazine’s affords bactericidal and antifungal derivatives. With the aim to investigate more potent antimicrobial activity of structurally related compounds, several 1-(4-methyl-6-nitro-2H-benzo[b][1,4]thiazin-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives 6a-f were synthesized (Fig. 1). The synthesis of compounds 6a-f started from the treatment of 2-chloro-5-nitroaniline 1 with sodium sulfide and sulfur gave sodium-2-amino-4-nitrobenzenethiol 2, which was cyclised with N-heterocycles in ethanolic solution [10] to yield 2H-benzo[b][1,4]thiazin-3(4H)-one derivatives 3a-b. Compounds 4a-b were synthesized by methylation of compounds 3a-b by methyl iodide in dimethyl sulfoxide (DMSO)/ethanol [11], which was refluxed with some nitrogen containing nucleophilic hydrazines in methanol [12] to yield 1-(4-methyl-6-nitro-2H-benzo[b][1,4]thiazine-3-yl)hydrazines 5a-f. The further step, that is, the oxidation of the sulfur was usually performed with 30% hydrogen peroxide in glacial acetic acid [13] to produce their sulfones 6a-f. The newly synthesized compounds have been screened for antibacterial and antifungal activity by disc diffusion method [14].

METHODS

Chemistry

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 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. 1H- and 13C-NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer using DMSO-d6 as 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). Mass spectra were recorded on a Waters, Q-TOF MS-ES spectrometer. Elemental analysis was done on Carlo Erba 1108 elemental analyzer. The synthesis, physical, and analytical properties of compounds 2, 3a, and 4a has been previously described [10,11].

Synthesis of 2-methyl-6-nitro-2H-benzo[b][1,4]thiazin-3-one (3b)
Sodium-2-amino-4-nitrobenzenethiol (1.2 g, 0.01 mol) [2] and methyl-2-chloropropionate (1.1 g, 0.01 mol) were dissolved in 30 ml ethanol. About 5 ml of 10% NaOH was added and refluxed for 3 hrs. 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); ultraviolet (UV) (DMSO) λmax (logΕ) 346 (4.66) nm; IR (υ cm-1): 3360, 2924, 1671, 1578, 1392, 650; 1H-NMR (d ppm, DMSO-d6): 1.5 (d, 3H, J=7 Hz, CH-CH3), 3.7 (q, 1H, H-2), 7.6 (d, 1H, J=8.4 Hz, H-5), 10.95 (s, 1H, NH); 13C-NMR (d ppm, DMSO-d6): 19.9 (CH3...
Synthesis of 2-(4-methyl-6-nitro-2H-benzof[b][1,4]thiazin-3(4H)-yliden)-2-(4-dimethylaminopyridine) hydrazone (5c)

The title compound was prepared from 4-methyl-6-nitro-2H-benzof[b][1,4]thiazin-3(4H)-one (4a) and 2,4-dimethylamino pyridine and recrystallized from ethanol. Orange crystals; yield: 78%; m.p. 140-142°C; Rf value: 0.54 (benzene-acetone, 1:3); UV (DMSO) λmax (log E) 211 (4.84) nm; IR (cm⁻¹): 3310, 2923, 1645, 1577, 1404, 1249, 1093, 134.6; 1H-NMR (d ppm, DMSO-d₆, 400 MHz): 2.72 (s, 3H, N-CH₃), 2.91 (s, 2H-2), 3.65 (2H, J=6.2 Hz, H-8, H-7), 7.41 (1H, J=2.5 Hz, H-5), 7.41 (1H, J=2.5 Hz, H-5), 10.50 (s, 1H, NH); 13C-NMR (d ppm, DMSO-d₆, 100 MHz): 153.0 (CH₂-O, C-3), 137.9 (CH₂-O, C-6), 159.8 (CH₂-O, C-2), 169.4 (C, C-3); ESMS m/z (%): 406 (100), 302 (43), 278 (14), 253 (15), 225 (15), 158 (100), 139 (100), 116 (100), 99 (65), 98 (100), 57 (100), 56 (100), 55 (100), 54 (100), 53 (100), 52 (100), 51 (100), 49 (100), 48 (100), 47 (100), 46 (100), 45 (100), 44 (100), 43 (100), 42 (100), 41 (100), 40 (100), 39 (100), 38 (100), 37 (100), 36 (100), 35 (100), 34 (100), 33 (100), 32 (100), 31 (100), 30 (100), 29 (100), 28 (100), 27 (100), 26 (100), 25 (100), 24 (100), 23 (100), 22 (100), 21 (100), 20 (100), 19 (100), 18 (100), 17 (100), 16 (100), 15 (100), 14 (100), 13 (100), 12 (100), 11 (100), 10 (100), 9 (100), 8 (100), 7 (100), 6 (100), 5 (100), 4 (100), 3 (100), 2 (100), 1 (100). Analysis calculated for C₉H₈N₂O₂S; C: 45.52; H: 4.56; N: 17.28; S: 13.14.
1-(2,4-Dimethyl-6-nitro-2-benzox[b][1,4]thiazin-3(4H)-yldiene)hydrazide (6d)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2-benzox[b][1,4]thiazin-3(4H)-yldiene)hydrazine (5d). 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. Yellow oil; yield, 31%; Rf 0.28 (toluene-ethylacetate ethanol 3:1:3); UV (DMSO) λmax (log E) 221 (4.04) nm; IR (υ cm−1): 3362, 2828, 1692, 1523, 1424, 1348, 1266, 1162, 1062, 660; 1H-NMR (d ppm, DMSO-d6, 400 MHz): 1.24 (d, 3H, J=7.5 Hz, CH3_2), 2.63 (s, 3H, NHCH3), 2.93 (q, 1H, H-5'), 8.15 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.34 (1H, d, J=8.6 Hz, H-8), 7.69 (1H, d, J=2.5 Hz, H-5), 8.59 (s, 2H, NH); 13C-NMR (d ppm, DMSO-d6, 100 MHz): 7.3 (CH3_2), 30.7 (CH3), 49.6 (CH-2), 109.7 (CH-5), 116.8 (CH-7), 128.1 (CH-8), 132.9 (CH-9), 146.5 (C-1), 153.6 (C-3, C-6); ESMS m/z (%): 284 (12), 212 (17), 164 (100), 138 (102), 99 (7), 74 (5). Analysis calculated for C15H10N5O5; C 42.25, H: 4.25; N: 19.17; S: 11.28. Found: C 42.21, H: 4.30; N: 19.66; S: 11.22.

1-(2,4-Dimethyl-6-nitro-2-benzox[b][1,4]thiazin-1,1-dioxide-3(4H)-yldiene)hydrazide (6e)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2-benzox[b][1,4]thiazin-3(4H)-yldiene)hydrazine (5e) and recrystallized from ethanol. Brown crystals; yield, 43%; mp 242–244°C; Rf 0.70 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λmax (log E) 244 (4.25) nm; IR (υ cm−1): 3393, 2927, 1690, 1523, 1449, 1381, 1283, 1214, 1162; 1H-NMR (d ppm, DMSO-d6, 400 MHz): 1.23 (d, 3H, J=7.5 Hz, CH3_2), 2.64 (s, 3H, NHCH3), 2.94 (q, 1H, H-5), 6.46 (2H, dd, J=1.1, J=8.5 Hz, H-2',H-6'), 6.63-7.01 (3H, m, H-3, H-4', H-5'); 8.17 (1H, d, J=2.5 Hz, H-3'), 8.39 (1H, d, J=8.6 Hz, H-8), 8.69 (1H, d, J=2.5 Hz, H-5), 10.61 (s, 1H, NH); 13C-NMR (d ppm, DMSO-d6, 100 MHz): 7.3 (CH3_2), 30.8 (CH3), 49.7 (CH-2), 109.5 (CH-5), 116.2 (CH-2', C-2'), 118.8 (CH-4'), 116.9 (CH-7, C-8), 129.8 (CH-7, C-8), 129.6 (CH-3, C-5'), 131.1 (C-9), 147.1 (C-10, C-1'), 153.4 (C-3, C-6); ESMS m/z (%): 360 (8), 289 (14), 240 (49), 214 (35), 178 (12), 150 (100), 136 (15). Analysis calculated for C15H10N5O5; C 53.2; H: 4.4; N: 15.55; S: 4.90. Found: C 53.36; H: 4.42; N: 15.59; S: 5.86.

1-(2,4-Dimethyl-6-nitro-2-benzox[b][1,4]thiazin-1,1-dioxide-3(4H)-yldiene)-2-(2′,4′-dinitrophthalhydrazine (6f)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2-benzox[b][1,4]thiazin-3(4H)-yldiene)hydrazine (5f) and recrystallized from ethanol. Light brown crystals; yield, 56%; mp 274–276°C; Rf 0.78 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λmax (log E) 252 (4.20) nm; IR (υ cm−1): 3295, 2927, 1690, 1590, 1426, 1339, 1248, 1139, 1059, 647; 1H-NMR (d ppm, DMSO-d6, 400 MHz): 1.24 (d, 3H, J=7.5 Hz, CH3_2), 2.95 (s, 3H, NHCH3), 3.00 (q, 1H, H-2), 2.78 (J=14.4 Hz, H-2, H-5), 3.04 (J=14.4 Hz, H-2, H-5), 8.17 (1H, d, J=2.5 Hz, H-3), 8.39 (1H, d, J=8.6 Hz, H-8), 8.49 (1H, dd, J=2.6, 8.8 Hz, H-7), 8.69 (1H, d, J=2.5 Hz, H-5), 8.89 (1H, d, J=8.6 Hz, H-8), 11.20 (s, 1H, NH); 13C-NMR (d ppm, DMSO-d6, 100 MHz): 7.3 (CH3_2), 30.8 (CH3), 49.7 (CH-2), 109.5 (CH-5), 116.2 (CH-2', C-2'), 118.8 (CH-4'), 116.9 (CH-7, C-8), 127.8 (CH-7, C-8), 129.6 (CH-3, C-5'), 131.1 (C-9), 147.1 (C-10, C-1'), 153.4 (C-3, C-6); ESMS m/z (%): 450 (13), 379 (12), 330 (52), 304 (8), 268 (13), 254 (100), 136 (10). Analysis calculated for C15H10N5O5; C 42.67, H: 3.31; N: 18.66; S: 7.12. Found: C 42.61, H: 3.32; N: 18.82; S: 7.16.

Pharmacology

Test organism and standard drug

All standard drugs (ofloxacin and ketoconazole) were purchased from K K Pharmaceuticals, Udaipur, Rajasthan, whereas all the microorganisms (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger, and Candida albicans) were collected from pathology laboratory of RNT Medical College, Udaipur, Rajasthan. All microbes were cultured overnight in nutrient agar medium.

In vitro antimicrobial potential assessment

In vitro antimicrobial assessment was performed by adopting the disc diffusion method. Representative compounds 5a-f were evaluated for...
their antibacterial against Gram-negative bacteria, *E. coli*, and Gram-positive bacteria, *S. aureus, B. subtilis*, and antifungal activity against *A. niger, C. albicans* at a concentration of 50 μg/ml using DMSO as a solvent by disc diffusion method. The antibacterial activity was performed with standard drug ofloxacin as positive control and DMSO as negative control after 24 hrs of incubation at 37°C. The antifungal activity was performed with ketoconazole as positive control and DMSO was used as negative control after 48 hrs of incubation at 25°C.

Statistical analysis
The results of the antimicrobial activity of compounds are expressed as mean ± SD of triplicate samples. Statistically significant differences between groups were measured using one-way analysis of variance 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 statistically non-significant, and **p<0.01** was considered highly significant.

RESULT AND DISCUSSION

Chemistry
In IR spectra of all compounds, the bands occur in the region 1404-1379 cm\(^{-1}\) and 1577-1578 cm\(^{-1}\) due to the symmetric and asymmetric stretching vibration of the nitro group. The synthesized 4-methyl-2\(H\)-benzo[b][1,4]thiazin-3(4\(H\))-one derivatives 4a-b exhibit a sharp absorption band in the region 2851-2855 due to the CH\(_3\) stretching and 1-(4-methyl-6-nitro-2\(H\)-benzo[b][1,4]thiazine-3-yl)hydrazines 5a-f exhibit absorption bands in the region 3360-3100 cm\(^{-1}\) due to the stretching vibration of the secondary amino group. A weak N-N stretching absorption band in the region of 1106-1052 cm\(^{-1}\) and a strong C=N stretching absorption band in the 1640-1690 cm\(^{-1}\) region are observed.

1\(^{1}\)HNMR spectra of compounds 5a-f exhibit a multiplet in the region δ 8.5-6.8 ppm due to aromatic protons. The broad signal observed in the region δ 9-11 is attributed to –NH protons. Aromatic protons show multiplet in the region δ 6.8-8.9 ppm. The sharp peak observed at δ 3.2-3.4 can be assigned to –CH proton. In compounds 6a, d, a broad peak is observed in the region δ 8.2-8.5 due to –NH\(_2\) protons. In compounds 6d, e, f, a doublet peak is observed in the region δ 1.2-1.3 due to CH\(_3\) protons at C-2. 

13\(^{13}\)C-NMR spectra of compounds 6a-f have been recorded. In mass spectra of 1-(4-methyl-6-nitro-2\(H\)-benzo[b][1,4]thiazine-3(4\(H\))-ylidene)hydrazine-1,1-dioxides 6a-f, the molecular ion peak is in accordance to their molecular weight.

Biological activity
Newly synthesized compounds 6a-f exhibited broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacterial, and fungal cultures. Antimicrobial activity was measured as the zone of inhibition and represented as mean ± standard deviation (n=3) in Table 1. Zone of inhibition is depicted in Table 1. After statistical analysis, p value was determined which was significant, that is, *p<0.05*. It has been noted that compound 6f having methyl at 2 position and nitro groups at 2′ and 4′ position showed the most potent antibacterial activity, whereas compounds 6c having nitro groups at 2′ and 4′ position and 6e having methyl at 2 position showed moderate antibacterial activity as compared to reference.

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 infectious disease. Earlier, the synthesis of many 1,4-benzothiazine derivatives and their sulfones has been reported to exhibit antimicrobial activity for pharmacological applications. Various hydrazine derivatives [15-17] have been previously reported possessing a broad-spectrum antimicrobial activity. In this research, some new class of sulfones of 1,4-benzothiazines containing different hydrazine derivatives in the 3-position was screened for antimicrobial properties. The present study through light on the antimicrobial efficacy
of these novel compounds. Result indicated that these synthesized compounds showed more activity toward bacteria as compared to the fungi. Results are collected in Table 1 and Graph 1 [18].

CONCLUSION

We have reported an easy method to prepare 1-(4-methyl-6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives, using inexpensive reagents and allowing to introduce different hydrazine derivative in the 3-position. It has been noted that compound 6f showed the most potent antimicrobial activity, whereas compounds and 6e showed moderate antimicrobial activity as compared to the reference. This study may be helpful for researchers to further development of a new potent antimicrobial drug.

ACKNOWLEDGMENTS

The authors are thankful to Sophisticated Analytical Instrumentation Facility, Central Instrumentation Laboratory, Punjab University, Chandigarh, for providing spectral and analytical data. The authors are also thankful to the management of B N Institute of Pharmaceutical Sciences, Udaipur, for providing necessary facilities.

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Table 1: Antimicrobial activity of compounds

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Antibacterial and antifungal activity at 50 μg/ml (zone of inhibition in mm±SD)</th>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
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<td>6a</td>
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<tr>
<td>6b</td>
<td>15.3±1.16</td>
</tr>
<tr>
<td>6c</td>
<td>16.6±1.53</td>
</tr>
<tr>
<td>6d</td>
<td>15.3±1.53</td>
</tr>
<tr>
<td>6e</td>
<td>17.3±2.52</td>
</tr>
<tr>
<td>6f</td>
<td>18.3±2.52</td>
</tr>
<tr>
<td>Control</td>
<td>n. a.</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>21.6±0.58</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>-</td>
</tr>
</tbody>
</table>

Graph 1: Antimicrobial activity at dose 50 μg/ml

Values are expressed as mean±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; SD: Standard deviation