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Research Article

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

MONIKA MAHESHWARI*, ANJU GOYAL

Department of Pharmaceutical Chemistry, B N Institute of Pharmaceutical Sciences, Sevashram, Udaipur, Rajasthan, India.
Email: mona30mph@gmail.com

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ABSTRACT

Objective: The objective of this work was to synthesize and evaluate antimicrobial properties of 1-(4-methyl-6-nitro-2*H*-benzo[*b*][1,4]thiazine-3(4*H*)-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, ^{1}H nuclear magnetic resonance (NMR), ^{13}CNMR, and mass studies confirm the synthesis of some new 1-(4-methyl-6-nitro-2*H*-benzo[*b*][1,4]thiazine-3(4*H*)-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.

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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 sulfoxide 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-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-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 β-heloesters in ethanolic solution [10] to yield 2*H*-benzo[b][1,4]thiazin-3(4*H*)-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-2*H*-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. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer using DMSO-d_6 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-2-*H*-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; R_f, 0.89 (toluene-ethyl acetate, 7:5); ultraviolet (UV) (DMSO) λ_{max} (log Ɛ) 346 (4.66) nm; IR (υ cm^{-1}): 3360, 2924, 1671, 1578, 1392, 650; ^{1}H-NMR (d ppm, DMSO-d_6): 1.5 (d, 3H, J=7 Hz, CH-CH_3), 3.7 (q, 1H, H-2), 7.6 (d, 1H, J=8.4 Hz, H-8), 7.9 (dd, 1H, J=8.6 and 2.2 Hz, H-7), 8.1(d, 1H, J=2.4 Hz, H-5), 10.95 (s, 1H, NH); ^{13}C-NMR(d ppm, DMSO-d_6): 19.9 (CH_3,

CH-CH_3), 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); ESMS m/z (%): 224 (55), 195 (38), 181 (100), 143 (24), 95 (12). Analysis calculated for $C_9H_8N_2O_3S$: 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.

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

2-Methyl-6-nitro-2-*H*-benzo[b][1,4]thiazin-3(4*H*)-one 3b (2.4 g, 0.01 mol) and potassium hydroxide (1.1g, 0.02 mol) were dissolved in DMSO (20 ml) and ethanol (25 ml). The mixture was stirred for 10 minutes before methyl iodide (1.2 ml, 0.02 mol) was added. Solution was heated at 50°C with stirring for 15 hrs. After cooling, water was added and the organic phase 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, 80%; R_f, 0.52 (toluene-ethyl acetate, 7:5); UV (DMSO) λ_{max} (log Ɛ) 211 (4.84) nm; IR (υ cm^{-1}): 2924, 1670, 1591, 1443, 1245, 640; ^{1}H-NMR (d ppm, DMSO-d_6): 1.5 (d, 3H, J=7 Hz, CH-CH_3), 3.4 (s, 3H, N-CH_3), 3.7 (q, 1H, H-2), 7.7 (d, 1H, J=8.5 Hz, H-8), 7.9 (dd, 1H, J=8.5 and 2.2 Hz, H-7), 8.0 (d, 1H, J=2.2 Hz, H-5); ^{13}C-NMR(d ppm, DMSO-d_6): 19.9 (CH_3, CH-CH_3), 32.1 (CH_3, N-CH_3), 40.5 (CH, C-2), 116.4 (CH, C-5), 117.5 (CH, C-7), 128.5 (CH, C-8), 131.9 (C, C-9), 140.4 (C, C-10), 146.6 (C, C-6), 165.1 (C, C-3); ESMS m/z (%): 238 (24), 195 (100), 166 (13), 154 (54), 95 (12). Analysis calculated for $C_9H_8N_2O_3S$: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.37; H, 4.27; N, 11.73; S, 14.00.

General method for the synthesis of compounds 5a-f

2-substituted-4-methyl-6-nitro-benzo[b][1,4]thiazin-3(4*H*)-one (4a-b) (0.01 mol) and hydrazine derivative (0.01 mol) were dissolved in 15 ml methanol. About 10 ml concentration, HCl was added into reaction mixture and heated on a steam bath at 70-80°C for 2 hrs. The reaction mixture was concentrated and cooled in an ice bath.

1-(4-Methyl-6-nitro-2*H*-benzo [b][1,4]thiazin-3(4*H*)-ylidene) hydrazine (5a)

The title compound was prepared from 4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4a) and hydrazine hydrate. Product was extracted with cyclohexane (3x50 ml) and purified by column chromatography on silica gel with a mixture of toluene-ethyl acetate (8:2) as eluent. Light yellow oil; yield, 52; R_f, 0.52 (benzene-acetone, 1:3); UV (DMSO) λ_{max} (log Ɛ) 217 (4.77) nm; IR (υ cm^{-1}): 3315, 2899, 1688, 1590, 646; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.77 (s, 3H, N-CH_3), 2.83 (s, 2H, H-2), 7.36 (1H, d, J=8.6 Hz, H-8), 7.59 (1H, d, J=2.5 Hz, H-5), 7.91 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.23 (s, 2H, NH_2); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 30.0 (CH_3, N-CH_3), 31.1 (CH_2, C-2), 107.6 (CH, C-5), 109.8 (CH, C-7), 123.3 (C, C-9), 128.6 (CH, C-8), 140.7 (C, C-10), 145.6 (C, C-6), 153.7 (C, C-3); ESMS m/z (%): 238 (11), 199 (39), 164 (24), 138 (100), 102 (16), 88 (11), 74(5). Analysis calculated for $C_9H_{11}N_3O_2S$: C, 45.37; H, 4.23; N, 23.51; S, 13.46. Found: C, 45.33; H, 4.26; N, 23.55; S, 13.44.

1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-phenylhydrazine (5b)

The title compound was prepared from 4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4a) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Brown crystals; yield, 66%; m.p. 190-192°C; R_f, 0.50 (benzene-acetone, 1:3); UV (DMSO) λ_{max} (log Ɛ) 211 (4.84) nm; IR (υ cm^{-1}): 3362, 2898, 1685, 1592, 1442, 1245, 639; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.73 (s, 3H, N-CH_3), 2.91 (s, 2H, H-2), 6.45 (2H, dd, J=1.6, J=8.5 Hz, H-2',6'), 6.65-7.19 (3H, m, H-3', H-4', H-5'), 7.22 (1H, d, J=8.6 Hz, H-8), 7.31 (1H, d, J=2.5 Hz, H-5), 7.41 (1H, dd, J=2.5, 8.6 Hz, H-7), 10.45 (s, 1H, NH); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 30.1 (CH_3, N-CH_3), 31.5 (CH_2, C-2), 109.5 (CH, C-5), 116.3 (CH, C-2', C-6'), 118.8 (C, C-4'), 119.7 (CH, C-7), 123.2 (C, C-9), 127.8 (CH, C-8), 129.7 (CH, C-3', C-5'), 140.2 (C, C-10), 147.1 (C, C-1'), 153.3 (C, C-3, C-6); ESMS m/z (%): 314 (14), 275 (7), 226 (11), 200 (39), 164 (24), 150 (100), 136 (12). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 57.31; H, 4.49; N, 17.82; S, 10.20. Found: C, 57.35; H, 4.45; N, 17.86; S, 10.18.

1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-(2,4-dinitrophenyl) hydrazine (5c)

The title compound was prepared from 4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4a) and 2,4-m-dinitrophenyl hydrazine and recrystallized from ethanol. Orange crystals; yield, 78%; m.p. 140-142°C; R_f, value: 0.54 (benzene-acetone, 1:3); UV (DMSO) λ_{max} (log Ɛ) 211 (4.84) nm; IR (υ cm^{-1}): 3310, 2923, 1645, 1577, 1404, 1249, 1093, 656; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.72 (s, 3H, N-CH_3), 2.81 (s, 2H, H-2), 7.22 (1H, d, J=8.6 Hz, H-8), 7.44 (1H, dd, J=2.5, 8.6 Hz, H-7), 7.48 (1H, d, J=2.5 Hz, H-5), 8.03 (1H, d, J=8.7 Hz, H-6'), 8.68 (1H, dd, J=2.6, 8.8 Hz, H-5'), 8.89 (1H, d, J=2.6 Hz, H-3'), 11.34 (br, 1H, NH); ^{13}C-NMR(d ppm, DMSO-d_6, 100 MHz): 30.2 (CH_3, N-CH_3), 31.3 (CH_2, C-2), 109.4 (CH, C-5), 119.4 (CH, C-6', C-7), 121.1 (CH, C-3'), 123.3 (C, C-9, C-2'), 127.0 (CH, C-5'), 127.7 (CH, C-8), 140.3 (C, C-10), 142.7 (C, C-4'), 147.4 (C, C-1'), 153.6 (C, C-3, C-6); ESMS m/z (%): 404 (10), 365 (38), 316 (44), 290 (25), 254 (100), 136 (8). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 44.55; H, 2.99; N, 20.78; S, 7.93. Found: C, 44.57; H, 2.96; N, 20.75; S, 7.96.

1-(2,4-Dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene) hydrazine (5d)

The title compound was prepared from 2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4b) and hydrazine hydrate. Product was extracted with cyclohexane (3x50 ml) and purified by column chromatography on silica gel with a mixture of toluene-ethyl acetate (8:2) as eluent. Light yellow oil; yield, 70%; R_f, 0.42 (toluene-ethylacetate 3:2); UV (DMSO) λ_{max} (log Ɛ) 225 (4.49) nm; IR (υ cm^{-1}): 3362, 2828, 1692, 1523, 1245, 659; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.19 (d, 3H, J=7 Hz, $CHCH_3$), 2.42 (s, 3H, N-CH_3), 2.53 (q, 1H, H-2), 7.21 (1H, d, J=8.6 Hz, H-8), 7.28 (1H, d, J=2.5 Hz, H-5), 7.41 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.51 (s, 2H, NH_2); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 15.0 (CH_3, $CHCH_3$), 30.1 (CH_3, N-CH_3), 34.6 (CH, C-2), 107.7 (CH, C-5), 109.6 (CH, C-7), 123.3 (C, C-9), 128.0 (CH, C-8), 139.3 (C, C-10), 149.5 (C, C-6), 153.6 (C, C-3); ESMS m/z (%): 252 (16), 213 (39), 164 (100), 138 (11), 102 (55), 88 (4), 74(5). Analysis calculated for $C_9H_{11}N_3O_2S$: C, 47.61; H, 4.79; N, 22.21; S, 12.71. Found: C, 47.58; H, 4.76; N, 22.24; S, 12.74.

1-(2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-phenylhydrazine (5e)

The title compound was prepared from 2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4b) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Brown crystals; yield, 68%; m.p. 95-97°C; R_f, 0.45 (toluene-ethylacetate 3:2); UV (DMSO) λ_{max} (log Ɛ) 218 (4.25) nm; IR (υ cm^{-1}): 3393, 2899, 1701, 1593, 1525, 1443, 1245, 828, 637; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.20 (d, 3H, J=7 Hz, $CHCH_3$), 2.54 (s, 3H, N-CH_3), 2.84 (q, 1H, H-2), 6.47 (2H, dd, J=1.6, J=8.5 Hz, H-2',6'), 6.58-7.07 (3H, m, H-3', H-4', H-5'), 7.22 (1H, d, J=8.6 Hz, H-8), 7.31 (1H, d, J=2.5 Hz, H-5), 7.41 (1H, dd, J=2.5, 8.6 Hz, H-7), 10.50 (s, 1H, NH); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 15.3 (CH_3, $CHCH_3$), 30.2 (CH_3, N-CH_3), 35.9 (CH, C-2), 107.5 (CH, C-5), 109.6 (CH, C-7), 116.2 (CH, C-2', C-6'), 118.8 (C, C-4'), 123.2 (C, C-9), 127.8 (CH, C-8), 129.6 (CH, C-3', C-5'), 140.0 (C, C-10), 147.1 (C, C-1'), 150.4 (C, C-6), 153.4 (C, C-3); ESMS m/z (%): 328 (8), 289 (14), 240 (40), 214 (7), 178 (24), 150 (100), 136 (12). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 58.52; H, 4.91; N, 17.06; S, 9.76. Found: C, 58.57; H, 4.95; N, 17.02; S, 9.72.

1-(2,4-Dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-(2',4'-dinitrophenyl)hydrazine (5f)

The title compound was prepared from 2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-one (4b) and 2,4-dinitrophenyl hydrazine and recrystallized from ethanol. Orange crystals; yield, 74%; m.p. 103-105°C; R_f, 0.78 (toluene-ethylacetate 3:2); UV (DMSO) λ_{max} (log Ɛ) 211 (4.84) nm; IR (υ cm^{-1}): 3294, 2924, 1687, 1585, 1422, 1044, 907; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.21 (d, 3H, J=7 Hz, $CHCH_3$), 2.71 (s, 3H, N-CH_3), 2.87 (q, 1H, H-2), 7.29 (1H, d, J=8.6 Hz, H-8), 7.48 (1H, d, J=2.5 Hz, H-5), 7.55 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.03 (1H, d, J=8.8 Hz, H-6'), 8.49 (1H, dd, J=2.6, 8.8 Hz, H-5'), 8.84 (1H, d, J=2.6 Hz, H-3'), 11.27 (br, 1H, NH); ^{13}C-NMR(d ppm, DMSO-d_6, 100 MHz): 15.3 (CH_3, $CHCH_3$),

30.4 (CH_3, N-CH_3), 35.2 (CH, C-2), 107.5 (CH, C-5), 109.6 (CH, C-6′, C-7), 120.7 (CH, C-3′), 126.7 (CH, C-5′), 127.8 (CH, C-8), 133.0 (C, C-9, C-2′), 140.1 (C, C-10), 142.6 (C, C-4′), 147.4 (C, C-1′), 150.1 (C, C-6), 153.1 (C, C-3); ESMS m/z (%): 418 (13), 379 (11), 330 (54), 304 (7), 268 (13), 254 (100), 136 (11). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 45.93; H, 3.37; N, 20.09; S, 7.66. Found: C, 45.91; H, 3.39; N, 20.05; S, 7.68.

General method for the synthesis of compounds 6a-f

1-(4-Methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)hydrazine derivative (5a-f) (0.01 mol) in glacial acetic acid (20 ml) and 30% hydrogen peroxide (5 ml) were added and refluxed for 15 minutes. Heating was stopped and another lot of hydrogen peroxide (5 ml) was added. The reaction mixture was again refluxed for 3-4 hrs. Excess of the solvent was removed by distillation under reduced pressure and poured into crushed ice.

1-(4-Methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)hydrazine (6a)

The title compound was prepared by oxidation of 1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)hydrazine (5a) and recrystallized from ethanol. Yellow oil; yield, 36%; m.p. >300°C; R_f, 0.83 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 216 (4.55) nm; IR (υ cm^{-1}): 3390, 2891, 1655, 1576, 1448, 1373, 1274, 1080, 649; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.73 (s, 3H, N-CH_3), 3.53 (s, 2H, H-2), 8.11 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.36 (1H, d, J=8.6 Hz, H-8), 8.59 (1H, d, J=2.5 Hz, H-5), 8.91 (s, 2H, NH_2); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 30.1 (CH_3, N-CH_3), 50.5 (CH_2, C-2), 109.6 (CH, C-5), 116.9 (CH, C-7), 128.2 (CH, C-8), 132.9 (C, C-9), 146.7 (C, C-10), 153.7 (C, C-3, C-6); ESMS m/z (%): 270 (54), 199 (14), 164 (100), 138 (16), 102 (11), 88 (8), 74(5). Analysis calculated for $C_9H_{11}N_3O_2S$: C, 40.00; H, 3.73; N, 20.73; S, 11.86. Found: C, 40.04; H, 3.71; N, 20.71; S, 11.89.

1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)-2-phenylhydrazine (6b)

The title compound was prepared by oxidation of 1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-phenylhydrazine (5b) and recrystallized from ethanol. Brown crystals; yield, 46%; m.p. >300°C; R_f, 0.37 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 233 (4.39) nm; IR (υ cm^{-1}): 3362, 2884, 1657, 1578, 1419, 1374, 1231, 1162, 1080, 649; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.74 (s, 3H, N-CH_3), 3.51 (s, 2H, H-2), 6.46 (2H, dd, J=1.6, J=8.5 Hz, H-2′,6′), 6.67-7.22 (3H, m, H-3′, H-4′, H-5′), 8.21 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.40 (1H, d, J=8.6 Hz, H-8), 8.69 (1H, d, J=2.5 Hz, H-5), 10.64 (s, 1H, NH); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 30.9 (CH_3, N-CH_3), 50.9 (CH_2, C-2), 109.6 (CH, C-5), 116.3 (CH, C-2′, C-6′), 118.9 (C, C-4′), 119.7 (CH, C-7), 129.7 (CH, C-3′, C-5′), 127.9 (CH, C-8), 133.2 (C, C-9), 147.2 (C, C-10, C-1′), 153.5 (C, C-3, C-6); ESMS m/z (%): 346 (12), 275 (6), 226 (52), 200 (38), 164 (23), 150 (100), 136 (12). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 52.02; H, 4.07; N, 16.18; S, 9.26. Found: C, 52.04; H, 4.03; N, 16.14; S, 9.29.

1-(4-Methyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)-2-(2′,4′-dinitrophenyl)hydrazine (6c)

The title compound was prepared by oxidation of 1-(4-methyl-6-nitro-2*H*-benzo [b][1,4]thiazin-3(4*H*)-ylidene)-2-(2,4-dinitrophenyl) hydrazine (5c) and recrystallized from ethanol. Orange crystals; yield, 42%; m.p. >300°C; R_f, 0.27 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 235 (4.25) nm; IR (υ cm^{-1}): 3358, 2924, 1641, 1577, 1404, 1325, 1248, 1144, 1051, 655; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 2.70 (s, 3H, N-CH_3), 3.51 (s, 2H, H-2), 8.05 (1H, d, J=8.7 Hz, H-6′), 8.21 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.41 (1H, d, J=8.6 Hz, H-8), 8.50 (1H, dd, J=2.6, 8.8 Hz, H-5′), 8.71 (1H, d, J=2.5 Hz, H-5), 8.92 (1H, d, J=2.6 Hz, H-3′), 11.37 (br, 1H, NH); ^{13}C-NMR(d ppm, DMSO-d_6, 100 MHz): 30.5 (CH_3, N-CH_3), 50.8 (CH_2, C-2), 109.6 (CH, C-5), 119.6 (CH, C-6′, C-7), 121.2 (CH, C-3′), 127.1 (CH, C-5′), 127.9 (CH, C-8), 133.3 (C, C-9, C-2′), 142.9 (C, C-4′), 147.6 (C, C-10, C-1′), 153.7 (C, C-3, C-6); ESMS m/z (%): 436 (11), 365 (12), 316 (52), 290 (13), 254 (100), 136 (36). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 41.29; H, 2.77; N, 19.26; S, 7.35. Found: C, 41.31; H, 2.73; N, 19.22; S, 7.38.

1-(2,4-Dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)hydrazine (6d)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)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%; R_f, 0.28 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 221 (4.04) nm; IR (υ cm^{-1}): 3362, 2828, 1692, 1523, 1339, 1245, 1186, 1062, 660; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.24 (d, 3H, J=7 Hz, $CHCH_3$), 2.63 (s, 3H, N-CH_3), 2.93 (q, 1H, H-2), 8.15 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.34 (1H, d, J=8.6 Hz, H-8), 8.60 (1H, d, J=2.5 Hz, H-5), 8.89 (s, 2H, NH_2); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 7.3 (CH_3, $CHCH_3$), 30.7 (CH_3, N-CH_3), 49.6 (CH, C-2), 109.7 (CH, C-5), 116.8 (CH, C-7), 128.1 (CH, C-8), 132.9 (C, C-9), 146.6 (C, C-10), 153.6 (C, C-3, C-6); ESMS m/z (%): 284 (12), 213 (17), 164 (100), 138 (23), 102 (39), 88 (7), 74(5). Analysis calculated for $C_9H_{11}N_3O_2S$: C, 42.25; H, 4.25; N, 19.71; S, 11.28. Found: C, 42.21; H, 4.30; N, 19.66; S, 11.22.

1-(2,4-Dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)-2-phenylhydrazine (6e)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-phenylhydrazine (5e) and recrystallized from ethanol. Brown crystals; yield, 43%; m.p. 242-244°C; R_f, 0.70 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 244 (4.25) nm; IR (υ cm^{-1}): 3393, 2938, 1670, 1586, 1449, 1381.28, 1244, 1162; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.23 (d, 3H, J=7 Hz, $CHCH_3$), 2.64 (s, 3H, N-CH_3), 2.94 (q, 1H, H-2), 6.46 (2H, dd, J=1.6, J=8.5 Hz, H-2′,6′), 6.63-7.01 (3H, m, H-3′, H-4′, H-5′), 8.17 (1H, dd, J=2.5, 8.6 Hz, H-7), 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); ^{13}C-NMR (d ppm, DMSO-d_6, 100 MHz): 7.3 (CH_3, $CHCH_3$), 30.8 (CH_3, N-CH_3), 49.9 (CH, C-2), 109.5 (CH, C-5), 116.2 (CH, C-2′, C-6′), 118.8 (C, C-4′), 119.6 (CH, C-7), 127.8 (CH, C-8), 129.6 (CH, C-3′, C-5′), 133.1 (C, C-9), 147.1 (C, C-10, C-1′), 153.4 (C, C-3, C-6); ESMS m/z (%): 360 (8), 289 (14), 240 (49), 214 (35), 178 (12), 150 (100), 136 (15). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 53.32; H, 4.47; N, 15.55; S, 8.90. Found: C, 53.36; H, 4.42; N, 15.59; S, 8.86.

1-(2,4-Dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-1,1dioxide-3(4*H*)-ylidene)-2-(2′,4′-dinitrophenyl)hydrazine (6f)

The title compound was prepared by oxidation of 1-(2,4-dimethyl-6-nitro-2*H*-benzo[b][1,4]thiazin-3(4*H*)-ylidene)-2-(2′,4′-dinitrophenyl) hydrazine (5f) and recrystallized from ethanol. Light brown crystals; yield, 56%; m.p. 274-276°C; R_f 0.78 (toluene-ethylacetate-ethanol 3:1:3); UV (DMSO) λ_{max} (log Ɛ) 252 (4.20) nm; IR (υ cm^{-1}): 3295, 2927, 1690, 1590, 1426, 1339, 1248, 1139, 1059, 647; ^{1}H-NMR (d ppm, DMSO-d_6, 400 MHz): 1.24 (d, 3H, $CHCH_3$), 2.65 (s, 3H, N-CH_3), 2.95 (q, 1H, H-2), 8.04 (1H, d, J=8.8 Hz, H-6′), 8.17 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.39 (1H, d, J=8.6 Hz, H-8), 8.49 (1H, dd, J=2.6, 8.8 Hz, H-5′), 8.69 (1H, d, J=2.5 Hz, H-5), 8.89 (1H, d, J=2.6 Hz, H-3′), 11.30 (s, 1H, NH); ^{13}C-NMR(d ppm, DMSO-d_6, 100 MHz): 7.3 (CH_3, $CHCH_3$), 30.8 (CH_3, N-CH_3), 49.9 (CH, C-2), 109.5 (CH, C-5), 119.6 (CH, C-6′, C-7), 121.0 (CH, C-3′), 126.9 (CH, C-5′), 127.8 (CH, C-8), 133.1 (C, C-9, C-2′), 142.8 (C, C-4′), 147.5 (C, C-10, C-1′), 153.4 (C, C-3, C-6); ESMS m/z (%): 450 (13), 379 (12), 330 (52), 304 (8), 268 (13), 254 (100), 136 (10). Analysis calculated for $C_{15}H_{13}N_5O_6S$: C, 42.67; H, 3.31; N, 18.66; S, 7.12. Found: C, 42.61; H, 3.32; N, 18.62; 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

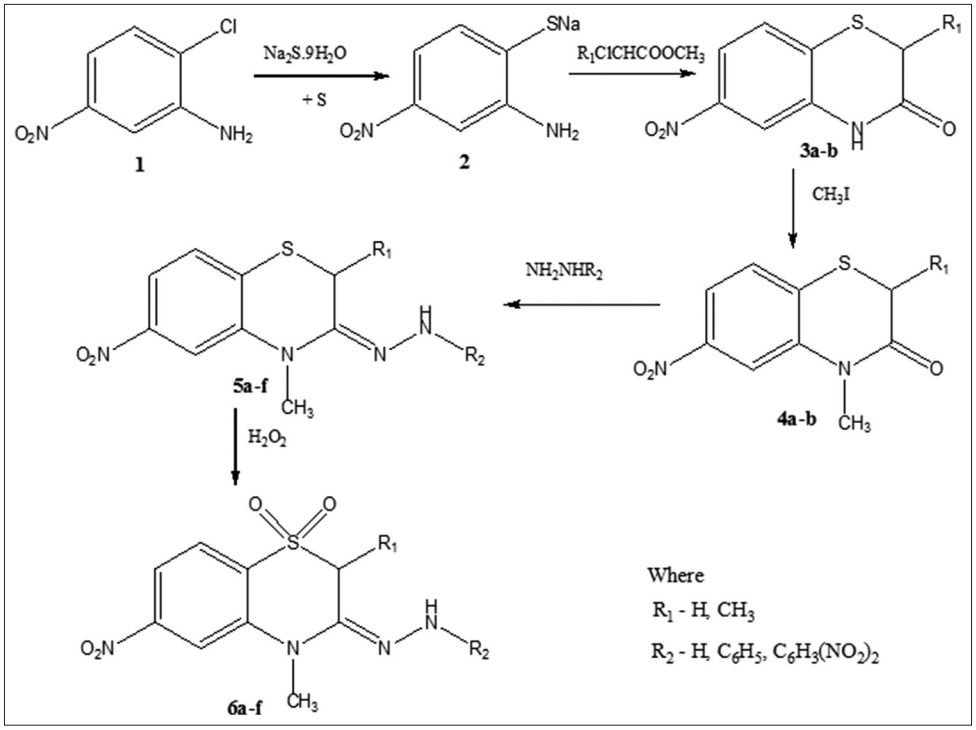


Fig. 1: Synthetic pathway of 1-(4-methyl-6-nitro-2*H*-benzo[*b*][1,4]thiazine-3(4*H*)-ylidene)hydrazine-1,1-dioxide derivatives

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}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. The broad peak observed at δ 2.6-2.8 can be assigned to –CH proton.

In IR spectra, the synthesized 1-(4-methyl-6-nitro-2*H*-benzo[b][1,4] thiazine-3(4*H*)-ylidene)hydrazine-1,1-dioxide derivatives 6a-f exhibit two sharp absorption bands in the region 1195-1135 cm^{-1} and 1380-1335 cm^{-1} due to the symmetric and asymmetric stretching vibration of the SO_2 group.

In^1 HNMR spectra, a broad peak observed in the region δ 8-11 in all 1-(4-methyl-6-nitro-2*H*-benzo[b][1,4]thiazine-3(4*H*)-ylidene) hydrazine-1,1-dioxides 6a-f is due to N-H proton. 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 1.2-1.3 due to CH_3 protons at C-2. ^{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

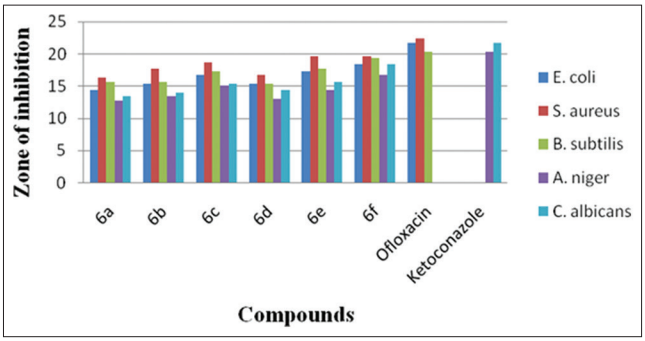
Newly synthesized compounds 6a-f exhibited broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacterial, and fungal cultures. Antimicrobial activity was measured as the zone of inhibition and represented as mean ± standard deviation (n=3) in Table 1. Zone of inhibition is depicted in Table 1. After statistical analysis, p value was determined which was significant, that is, <0.05 (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

Table 1: Antimicrobial activity of compounds

Compound number	Antibacterial and antifungal activity at 50 µg/ml (zone of inhibition in mm±SD)				
	E. coli	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
6a	14.33±1.53	16.33±1.16	15.67±0.58	12.67±0.58	13.33±1.53
6b	15.33±1.16	17.67±0.58	15.66±1.53	13.33±0.58	14.00±1.73
6c	16.66±1.53	18.67±0.58	17.33±1.53	15.00±0.00	15.33±0.58
6d	15.33±1.53	16.67±1.16	15.33±1.16	13.00±1.00	14.33±0.58
6e	17.33±2.52	19.66±0.58	17.66±1.53	14.33±0.58	15.67±0.58
6f	18.33±2.52	19.66±1.53	19.33±1.53	16.67±0.58	18.33±1.16
Control	n. a.	n. a.	n. a.	n. a.	n. a.
Ofloxacin	21.67±0.58	22.33±0.58	20.33±1.53	-	-
Ketoconazole	-	-	-	20.33±0.58	21.67±2.08

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



Graph 1: Antimicrobial activity at dose 50 µg/ml

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-2*H*-benzo[*b*][1,4]thiazine-3(4*H*)-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.

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