SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF 1-(4-METHYL-6-NITRO-2H-BENZO[\textit{B}]1,4)THIAZINE-3(4H)-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

ABSTRACT

Objective: The objective of this work was to synthesize and evaluate antimicrobial properties of 1-(4-methyl-6-nitro-2H-benzo[\textit{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), 13CNMR, and mass studies confirm the synthesis of some new 1-(4-methyl-6-nitro-2H-benzo[\textit{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[\textit{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 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; [\textit{\delta}H-NMR (log Ɛ)] 346 (4.66) nm; IR (υ cm\textsuperscript{-1}) 1750; 1H-NMR (d ppm, DMSO-d\textsubscript{6}) 1.5 (d, 3H, J=7 Hz, CH-CH\textsubscript{2}); 3.7 (q, 1H, H-2), 7.6 (d ppm, DMSO-d\textsubscript{6}) 10.95 (s, 1H, NH); 13CNMR (d ppm, DMSO-d\textsubscript{6}) 19.9 (CH, C-N-M); 176.0 (C-N-O), 192.0 (C=O).

The synthesis, physical, and analytical properties of compounds 2, 3a, and 4a has been previously described [10,11].

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 AvanceII 400 NMR spectrometer using DMSO-d\textsubscript{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-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; R\textsubscript{p} 0.89 [toluene-ethyl acetate, 7:5]; ultraviolet (UV) (DMSO)\lambda\textsubscript{max} (log Ɛ) 346 (4.66) nm; IR (υ cm\textsuperscript{-1}) 1750; 1H-NMR (d ppm, DMSO-d\textsubscript{6}) 1.5 (d, 3H, J=7 Hz, CH-CH\textsubscript{2}); 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); 13C-NMR (d ppm, DMSO-d\textsubscript{6}) 19.9 (CH, C-N-M); 176.0 (C-N-O), 192.0 (C=O).
CH-CH₂), 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 C₁₁H₁₀O₂S: C, 55.88; H, 4.26; S, 17.64. Found: C, 55.86; H, 4.31; S, 17.62.

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

2-Methyl-6-nitro-2H-benz[b][1,4]thiazin-3(4H)-one 3b (2.4 g, 0.01 mol) and potassium hydroxide (1.1 g, 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 (3x50 ml) and purified by column chromatography on silica gel with a mixture of toluene-ethyl acetate (3:2) as eluent. Yellow oil; yield, 10%; Rf 0.33 (toluene-ethyl acetate 3:2); UV (DMSO) λ max (log Ɛ) 211 (4.84) nm; IR (υ cm⁻¹) 3393, 2828, 1701, 1593, 1525, 1249, 1093; 13C-NMR (d ppm, DMSO-d₆, 100 MHz): 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 (11), 195 (22), 181 (100), 143 (24), 95 (12). Analysis calculated for C₁₁H₁₀O₂S: C, 55.88; H, 4.26; S, 17.64. Found: C, 55.86; H, 4.31; S, 17.62.

1-(4-Methyl-6-nitro-2H-benz[b][1,4]thiazin-3(4H)-ylidene)-2-(4-dimethylamino)phenol (5c)

The title compound was prepared from 4-methyl-6-nitro-2H-benz[b][1,4]thiazin-3(4H)-one (4a) and 4,4'-methylenebis(phenol) hydrochloride and recrystallized from ethanol. Orange crystals; yield, 78%; m.p. 140-142°C; Rf value: 0.54 (benzene-acetone, 1:3); UV (DMSO) λ max (log Ɛ) 211 (4.84) nm; IR (υ cm⁻¹) 3393, 2924, 1645, 1577, 1404, 1249, 1093; 1H-NMR (d ppm, DMSO-d₄, 100 MHz): 272 (2H, 3H, C-2'), 169.4 (C, C-3); ESMS m/z (%): 305 (100), 297 (40). Analysis calculated for C₁₉H₁₄N₂O₂S: C, 57.65; H, 3.91; N, 13.65. Found: C, 57.63; H, 3.93; N, 13.64.

1-(2,4-Dimethyl-6-nitro-2H-benz[b][1,4]thiazin-3(4H)-ylidene)-2-(4-dimethylamino)phenol (5d)

The title compound was prepared from 4-methyl-6-nitro-2H-benz[b][1,4]thiazin-3(4H)-one (4b) and phenyl hydrazine hydrochloride and recrystallized from ethanol. Brown crystals; yield, 68%; m.p. 95-97°C; Rf 0.45 (toluene-ethyl acetate 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; 1H-NMR (d ppm, DMSO-d₄, 400 MHz): 1.50 (3H, C-2), 10.71 (1H, C-7), 12.75 (1H, C-8), 14.50 (1H, C-9), 142.5 (C, C-3); ESMS m/z (%): 293 (216), 213 (169), 100 (138), 118 (101), 55 (55), 74 (54). Analysis calculated for C₁₉H₁₄N₂O₂S: C, 47.61; H, 4.79; N, 22.21; S, 12.74. Found: C, 47.58; H, 4.76; N, 22.24; S, 12.74.
1-(4-Methyl-6-nitro-2′-benzo[b][1,4]thiazin-3(4H)-ylidene)hydrazone derivative (5a) was obtained by treating 1-(4-methyl-6-nitro-2′-benzo[b][1,4]thiazin-3(4H)-ylidene)hydrazone (5a) with sodium nitrite, followed by recrystallization from ethanol. Yellow oil yielded, 36%; m.p. >300°C; Rf 0.83 (tolueno-ethylacetate-ethanol 3:1:3). UV (DMSO) λmax (log ε) 216 (4.55), 233, 284 (4.40), 289 (4.30), 301 (4.18), 310 (4.20), 320 (4.23), 352, 375 (4.20), 381 (4.20), 450 (3.97). H-NMR (d ppm, DMSO-d6) 7.45 (9H, m, H-7), 8.62 (1H, d, J=8.6 Hz, H-8), 8.85 (1H, d, J=8.6 Hz, H-9). Calculated for C_{41}H_{39}N_{6}O_{5}S: C, 70.14; H, 5.37; N, 19.26; S, 7.21. Found: C, 70.07; H, 5.42; N, 19.25; S, 7.20.

1-(4-Methyl-6-nitro-2′-benzo[b][1,4]thiazin-1,1-dioxide-3(4H)-ylidene)hydrazone (6a) is the title compound prepared by oxidation of 1-(4-methyl-6-nitro-2′-benzo[b][1,4]thiazin-3(4H)-ylidene)hydrazone (5a) and recrystallized from ethanol. Yellow oil yielded, 46%; m.p. >300°C; Rf 0.37 (tolueno-ethylacetate-ethanol 3:1:3). UV (DMSO) λmax (log ε) 238 (4.39) nm; IR (υ cm⁻¹): 3358, 2924, 1641, 1577, 1403, 1325, 1248, 1144, 1051, 655; H-NMR (d ppm, DMSO-d6) 400 MHz: 2.73 (3H, 3-H, CH3), 3.51 (2H, H-2, H-3), 4.62 (2H, H-15, H-16), 6.67-7.22 (m, 6H, H-3′, H-4′, H-5′, H-8). 8.21 (1H, dd, J=2.5, 8.6 Hz, H-7), 8.69 (1H, d, J=2.5 Hz, H-5). 10.64 (1H, NH). H-NMR (d ppm, DMSO-d6, 100 MHz) 30.9 (C-H, C-6, C-8), 38.2 (C, C-10, C-1′), 90.7 (C-H, C-3′), 116.3 (C-H, C-2′, C-6′), 118.9 (C, C-4′), 114.7 (C-H, C-7, C-9, C-11′), 129.7 (C, C-2′, C-5′), 127.9 (C-H, C-8), 133.2 (C, C-9), 147.2 (C, C-10, C-1′), 153.5 (C, C-3′, C-6′). MS m/z (%): 346 (12), 275 (6), 226 (52), 200 (38), 164 (23), 150 (100), 136 (12). Analysis calculated for C_{41}H_{39}O_{5}S_C: C, 50.02; H, 4.07; N, 16.18; S, 9.26. Found: C, 50.04; H, 4.09; N, 16.14; S, 9.29.

1-(2,4-Dimethyl-6-nitro-2′-benzo[b][1,4]thiazin-1,1-dioxide-3(4H)-ylidene)-2′-(2′,4′-dinitrophenyl)hydrazone (6c) is the title compound prepared by oxidation of 1-(4-methyl-6-nitro-2′-benzo[b][1,4]thiazin-1,1-dioxide-3(4H)-ylidene)-2′-(2,4-dinitrophenyl)hydrazine (5c) and recrystallized from ethanol. Orange crystal yielded, yield 42%; m.p. >300°C; Rf 0.27 (tolueno-ethylacetate-ethanol 3:1:3); UV (DMSO) λmax (log ε) 235 (4.25) nm; IR (υ cm⁻¹): 3358, 2924, 1641, 1577, 1404, 1325, 1248, 1144, 1051, 655; H-NMR (d ppm, DMSO-d6, 400 MHz): 2.70 (3H, 3-H, CH3), 3.51 (2H, H-2, H-3), 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=2.5 Hz, H-5). 11.37 (br, 1H, NH). H-NMR (d ppm, DMSO-d6, 100 MHz) 30.5 (CH, C-6, C-8), 50.8 (CH, C-2), 109.6 (CH, C-5), 119.6 (CH, C-6′, C-7′), 121.2 (CH, C-3′, C-5′), 127.9 (CH, C-8), 133.3 (C, C-10, C-1′), 142.9 (C, C-4′), 147.6 (C, C-10, C-1′), 153.7 (C, C-3′, C-6′). 1.24 (d, 1H, J=2.5 Hz, H-7). 8.41 (1H, d, J=2.5 Hz, H-5). 8.89 (1H, d, J=2.5 Hz, H-3). 10.61 (1H, NH); 1.24 (d, 1H, J=2.5 Hz, H-7). 8.41 (1H, d, J=2.5 Hz, H-5). 8.89 (1H, d, J=2.5 Hz, H-3). 1130 (1H, NH). H-NMR (d ppm, DMSO-d6, 100 MHz): 30.5 (CH, C-6, C-8), 50.8 (CH, C-2), 109.6 (CH, C-5), 119.6 (CH, C-6′, C-7′), 121.2 (CH, C-3′, C-5′), 127.9 (CH, C-8), 133.3 (C, C-10, C-1′), 142.9 (C, C-4′), 147.6 (C, C-10, C-1′), 153.7 (C, C-3′, C-6′). MS m/z (%): 436 (11), 365 (12), 316 (52), 290 (150), 254 (100), 136 (10). Analysis calculated for C_{34}H_{32}N_{6}O_{5}S_C: C, 41.31; H, 2.73; N, 19.22; S, 7.38. Found: C, 41.31; H, 2.73; N, 19.22; S, 7.38.
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-thiazine-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 8.5-9.11 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 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. \(^1\)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 bacteria, 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...
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 6e and 6c 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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REFERENCES

1. Parai MK, Panda G. A covenient synthesis of chiral amino acid derived 3,4-dihydro-2H-benzo[b][1,4]thiazines and antibiotics levofloxacin. Tetrahedron Lett 2009;50:4703-5.
2. Fringuelli R, Schiaffella F, Urilla Navaarro MP, Milanesi L, Santini C, Rapucci M, et al. 1,4-benzothiazine analogues and apoptosis: Structure-activity relationship. Bioorg Med Chem 2003;11(15):3245-54.
3. Hori M, Kataoka T, Shmizu H, Imai Y. Ring expansion reactions of benzothiazolines to benzo thiazine. Chem Pharm Bull 1973;5:27.
4. Barange DK, Batchu VR, Gorja D, Patatbroaman VR, Tatini LK, Babu JM, et al. Rigioselective construction of six-membered fused heterocyclic rings via Pd/C-mediated C-C coupling followed by iodocyclization strategy: A new entry to 2H-1,2-benzothiazine-1,1-dioxides. Tetrahedron 2007;63:1775-89.
5. Bakavoli M, Nikpour M, Rahimiezadeh M, Saberi MR, Sadeghian H. Design and synthesis of pyrimido[4,5-b][1,4]benzothiazoline derivatives, as potent 15-lipoxgenase inhibitors. Bioorg Med Chem 2007;15(5):2120-6.
6. Dabholkar VV, Gavande RP. Synthesis of pyrazolyl 1,4-benzothiazine derivatives. Heteroletters 2011;1 Suppl:3-255.
7. Saadouni M, Gailane T, Baukhrs S, Hassiokou A, Habbabi N, Gailane T, et al. Regioslective synthesis of new variety of 1,4-benzothiazines. Org Chem Commun 2014;7(2):77-84.
8. Montis SD, Fattuoni C, Cadoni E, Cabiddu MG, Usai M, Cabiddu S. High yield synthesis of 4H-1,4-benzothiazine-1,1-dioxide derivatives. J Heterocycl Chem 2008;45:1445-9.
9. Schou SC, Hansen HC, Tangmose TM, Boonen HC, Worsaae A, Drabowski M, et al. Synthesis and pharmacological evaluation of 4H-1,4-benzothiazine-2-carbonitrile-1,1-dioxide and N-(2-cyanomethyl) sulfonylethinyljacylamide derivatives as potential activators of ATP sensitive potassium channels. Bioorg Med Chem 2005;13:141-55.
10. Guarda VL, Perrissin M, Thomsasson F, Ximenes EA, Galdino SL, Pitta IR, et al. Synthesis and microbical activity of some 2H-benzo[b][1,4]thiazin-3-one derivatives. Heterocycles 2000;6:49-54.
11. Souza AM, Guarda VL, Leite LF, Filho JM, Lima MC, Galdino SL, et al. On application of knoevenagel condensation for the synthesis of benzo thiazine compounds and structural study. Quim Nov 2006;29:1106-9.
12. Deshmuk MB, Mulik AR, Desal SD. Synthesis of some new 2-methyl-1,4-benzothiazine-3(1H)-one derivatives as potential vasodilators. Eur J Chem 2004;1:206-10.
13. Hamadi MY, Gupta R, Gupta RR. Synthesis and spectral investigations of fluorinated 4H-1,4-benzothiazines and their conversion into sulfones. J Fluorine Chem 1999;94:164-74.
14. Rathore BS, Kumar M. Synthesis of 7-chloro-5-trifluoromethyl/7-trifluoromethyl-4H-1,4-benzothiazines as antimicrobial agents. Bioorg Med Chem 2006;14:5678-82.
15. Maheshwari M, Goyal A. A review: Synthesis and medicinal importance of 1,4-benzothiazine analogous. Asian J Pharm Clin Res 2015;8:41-6.
16. Kartikas C, Mohamed RK, Manivannan S. Physcochemical analysis and evaluation of anti-microbial potential of Semna alata Linn. Leaves extract. Asian J Pharm Clin Res 2016;9:253-7.
17. Maheshwari M, Goyal A. Synthesis and anti-microbial activity of 1-(6-nitro-2H-benzo[b][1,4]thiazine-3(4H)-ylidene)hydrazine-1,1-dioxide derivatives. Int J Pharm Sci 2016;8(10):178-82.
18. Gajbhiye A, Goel KK. Synthesis and anticonvulsant screening of N-aryl-2-(3-oxo-1,4-benzothiazin-2-ylacetamide derivative. Int J Pharm Sci 2013;5(1):220-2.

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              | 14.3±1.53 | 16.3±1.16 | 16.6±0.58 | 12.6±0.58 | 13.3±1.36 |
| 6b              | 15.3±1.16 | 17.6±0.58 | 17.6±1.53 | 15.0±0.00 | 14.0±1.73 |
| 6c              | 16.6±1.53 | 16.6±0.58 | 17.3±1.53 | 15.3±0.58 | 15.3±0.58 |
| 6d              | 15.3±1.53 | 17.6±1.16 | 17.6±1.53 | 15.3±1.16 | 14.0±1.58 |
| 6e              | 17.3±2.52 | 19.6±0.58 | 17.7±1.53 | 17.5±0.58 | 16.7±0.58 |
| 6f              | 18.3±2.52 | 19.6±0.58 | 19.3±1.53 | 16.7±0.58 | 18.3±1.16 |
| Control         | n. a.   | n. a.     | n. a.     | n. a.      | n. a.    |
| Kefloxacline    | 21.6±0.58 | 22.3±0.58 | 17.6±1.53 | 20.3±0.58 | 21.6±0.58 |

Graph 1: Antimicrobial activity at dose 50 μg/ml

Values are expressed as means±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.