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Synthesis, characterization and biological activity of triazolothiadiazines bearing 2*H*-1,4-benzothiazin-3(4*H*)-one moiety

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ABSTRACT

*A new series of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**7**) of 4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-2H-1,4-benzothiazin-3(4H)-one (**4**), were synthesized from (3-oxo-2,3-dihydro-4H-1,4-benzothiazin-4-yl)acetic acid (**3**). The structures of all newly synthesized compounds were elucidated by elemental analysis, FT-IR, ^{1}H-NMR and mass spectral data. Synthesized compounds were screened for their antioxidant and antibacterial activity and a few of them exhibited significant activity. Among the tested compounds, (3-oxo-2,3-dihydro-4H-1,4-benzothiazin-4-yl)acetic acid (**3**) posssess antibacterial activity comparable to that of streptomycin. However the activity decreased on derivatization of the acid group.*

Keywords: 1,24-triazole, 1,4-benzothiazine, Antioxidant, Antibacterial

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INTRODUCTION

The advances in science and the availability of literature have helped the research and as a result the drug synthesis has gained pace. On the other hand there is a genuine need to search for safer and ideal moiety which will be able to overcome drug resistance experienced by patients and able to fulfill the demands; so also to be the ideal replacement for limited natural sources. In our quest for such moiety, it was observed that 1,4-benzothiazine-3-one, 1,2,4-triazolothiadiazine and 1,2,4-triazole moieties played prominent role due to their excellent biological activities.

It is well documented that 1,4-benzothiazin-3-one derivatives possess important pharmacological properties and play vital role in neurodegenerative diseases, such as Parkinson's disease and Alzheimer disease [1-4], *in vivo* antitumor, cytotoxic activity [5,6], vasodilators [7], antifungal [8], calcium channel blockers [9] phosphodiesterase-7 inhibitors[10], anticataract agents [11], dopamine D_4, Na^+/H^+ exchange inhibitors [12], matrix metalloproteinase inhibitors [13]. According to the numerous examples, 4*H*-benzo[1,4]thiazin-3-one fragment can be considered as a typical "privileged" substructure [14].

Along with the 1,4-benzothiazine-3-one, the prominence of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives was observed because of enormous importance in the field of medicine and agriculture. Studies have shown that they have herbicidal [15], insecticidal [16], antibacterial, anti-fungal, CNS depressant, diuretic and anthelmintic activities [17-21], anti-inflammatory [22,23], antituberculosis activity [24], analgesic [25], cytotoxic [26], anticancer [27], Urease Inhibition and antioxidant properties [24] and so on. A large number of ring systems containing 1,2,4-triazoles have been incorporated into a wide variety of therapeutically interesting drug candidates such as

Fluconazole, Itraconazole and Voriconazole. Also FDA approved drugs like Triazolam, Alprazalam, Etizolam, Furacylin, Ribavirin, Hexaconazole, Triadimefon , Mycobutanil, Rizatriptan, Propiconazole, and Fluotrimazole etc. contain 1,2,4-triazole core [29].

Enthused by the enormous pharmacological importance of 1,4-benzothiazine-3-one, thiadiazines and 1,2,4-triazoles and in continuation of our research work [30-33], a plan was drawn to incorporate them in a single moiety. Hence novel triazolo[3,4-*b*][1,3,4]-thiadiazines were synthesized from 4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one and screened for their antibacterial and antioxidant activities.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. The purity of synthesized compounds was checked by TLC on Merck silica gel 60 F254 coated alumina plates. The IR spectra (cm^{-1}) were recorded on a Shimadzu-FTIR 177 Spectrophotometer in KBr pellets. The ^{1}H-NMR spectra were recorded on a Brucker AMX-400 (400 MHz) spectrometer using $CDCl_3$/DMSO-d_6 as solvent and TMS as an internal standard. All the chemical shift values are reported in δ scale downfield from TMS. The Mass spectra were recorded on Perkin-Elmer 018444 -Y, Triple Quadrupole LC/MS Spectrometer. The elemental analysis (C, H and N) was carried out on a Elementar Vario EL III analyzer.

General procedure for the preparation of (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid (4).

Ethyl (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetate (39 mmol) was added to a stirred solution of KOH (58.5 mol) in water. The resulting reaction mixture was refluxed for 1 h. After cooling, the reaction mixture was acidified with 4M HCl to get a solid product (3), collected by filtration and dried. The crude product was recrystallized from dichloromethane. This compound was characterized by its melting point with reference to the literature [34]. Yield 60%; m.p. 64-65 ^{o}C (lit. 65 ^{o}C).

Procedure for the preparation of 4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (5).

4-[(4-Amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one was prepared according to the procedure described in the literature [35, 36].

Procedure for the synthesis of 4-[(4-amino-5-{[2-(4-substituted phenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (6).

Equimolar quantities of 4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (**5**) (0.01 mol), different substituted phenacyl bromides (0.01 mol) and anhydrous sodium acetate (0.01 mol) in ethanol–DMF mixture were stirred at room temperature for 4h. The precipitated solid was filtered, washed with water, dried and recrystallized from ethanol-DMF mixture.

4-({4-amino-5-[(2-oxo-2-phenylethyl)sulfanyl]-4*H*-1,2,4-triazol-3-yl}methyl)-2*H*-1,4-benzothiazin-3(4*H*)-one (6a).

IR (KBr) ν cm^{-1}: 3328 (NH_2), 3061 (Ar C-H stretch), 2928 (Aliph C-H stretch) 1672 (C=O), 1602 (C=N). ^{1}H NMR ($CDCl_3$) δ ppm: 3.39 (s, 2H, benzothiazine S-$\underline{CH_2}$), 4.78 (s, 2H, S-$\underline{CH_2}$), 5.19 (s, 2H, N-NH_2), 5.58 (s, 2H, benzothiazine N-$\underline{CH_2}$), 7.01-8.08 (m, 9H, Ar-H). LC-MS (m/z): 412 (M^++1).

4-[(4-amino-5-{[2-(4-methoxyphenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (6c).

IR (KBr) ν cm^{-1}: 3313 (-NH_2), 3053 (Ar C-H stretch), 2934 (Aliph C-H stretch), 2841 (-OCH_3 stretch), 1675 (C=O), 1591 (C=N); ^{1}H NMR ($CDCl_3$) δ ppm : 3.39 (s, 2H, benzothiazine S-$\underline{CH_2}$), 3.90 (s, 3H, -O$\underline{CH_3}$), 4.77 (s, 2H, S-CH_2), 5.19 (s, 2H, N-NH_2), 5.52 (s, 2H, benzothiazine N-CH_2), 6.98-8.04 (m, 8H, Ar-H). LC-MS (m/z): 442 (M^++1).

4-[(4-amino-5-{[2-(4-chlorophenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (6d)

IR (KBr) ν cm^{-1}: 3325, 3246 (-NH_2), 3055 (Ar C-H stretch), 2916 (Aliph C-H stretch), 1670 (C=O), 1594 (C=N), 801 (C-Cl). ^{1}H NMR ($CDCl_3$) δ ppm : 3.41 (s, 2H, benzothiazine S-$\underline{CH_2}$), 4.74 (s, 2H, S-$\underline{CH_2}$), 5.25 (s, 2H, N-NH_2),

5.52 (s, 2H, N-$\underline{CH_2}$), 7.05-7.09 (t, 1H, *J* = 7.4 Hz, benzothiazine), 7.28-7.30 (m, 1H, benzothiazine), 7.37-7.39 (d, 1H, *J* = 7.6 Hz, benzothiazine), 7.63-7.65 (d, 2H, *J* = 8.4 Hz, 4-bromophenyl), 7.86-7.89 (d, 2H, *J* = 8.4 Hz, 4-bromophenyl), 7.97-7.99 (d, 1H, *J* = 8 Hz, benzothiazine). LC-MS (m/z): 445 (M^++1), 447 (M^++3).

4-[(4-amino-5-{[2-(4-bromophenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (6e).
IR (KBr) ν cm^{-1}: 3330, 3250 (-NH_2), 3032 (Ar C-H stretch), 2910 (Aliph C-H stretch) 1666 (C=O), 1581 (C=N), 752 (C-Br). ^{1}H-NMR ($CDCl_3$) δ ppm: 3.39 (2H, s, benzothiazine S-CH_2), 4.70 (s, 2H, S-CH_2), 5.17 (s, 2H, benzothiazine N-CH_2), 5.52 (s, 2H, N-NH_2), 7.03-7.07 (t, 1H, *J* = 7.4 Hz, benzothiazine), 7.26-7.28 (m, 1H, benzothiazine), 7.33-7.35 (d, 1H, *J* = 7.6 Hz, benzothiazine), 7.60-7.62 (d, 2H, *J* = 8.4 Hz, 4-bromophenyl), 7.84-7.86 (d, 2H, *J* = 8.4 Hz, 4-bromophenyl), 7.97-7.99 (d, 1H, *J* = 8 Hz, benzothiazine). LC-MS (m/z): 490 (M^++1), 492 (M^++3).

4-[(4-amino-5-{[2-(4n)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (6f).
IR (KBr) ν cm^{-1}: 3318 (NH_2), 3057 (Ar C-H stretch), 2920 (Aliph C-H stretch), 1685 (C=O), 1597 (C=N), 1519 (NO_2 asymmetric), 1344 (NO_2 symmetric). ^{1}H NMR ($CDCl_3$) δ ppm : 3.60 (s, 2H, benzothiazine S-$\underline{CH_2}$), 4.82 (s, 2H, S-$\underline{CH_2}$), 5.35 (s, 2H, N-NH_2), 5.63 (s, 2H, benzothiazine N-$\underline{CH_2}$), 7.03-7.06 (t, 1H, *J* = 7.2 Hz, benzothiazine 6-H), 7.23-7.26 (t, 1H, *J* = 7.2 Hz, benzothiazine 7-H), 7.35-7.37 (d, 2H, *J* = 7.4 Hz, benzothiazine 5-H and 8-H), 8.17-8.19 (d, 2H, *J* = 7.76 Hz, protons meta to the nitro group), 8.36-8.38 (d, 2H, *J* = 8 Hz, protons ortho to the nitro group). LC-MS (m/z): 457 (M^++1).

Procedure for the synthesis of 4-{[6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (7).
4-[(4-Amino-5-{[2-(4-substituted phenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (**6**) (0.01 mol) in phosphorous oxychloride (25 mL) was refluxed for 2-3h. The excess of phosphorous oxychloride was removed under reduced pressure and poured in to crushed ice and neutralized. The solid triazolothiadiazine (**7)** thus separated was filtered, washed with water and recrystallised from ethanol.

4-[(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (7a).
IR (KBr) ν cm^{-1}: 3017 (Ar C-H stretch), 2904 (Aliph C-H stretch), 1670 (C=O), 1603 (C=N), 749 (C-S-C); ^{1}H-NMR ($CDCl_3$) δ: ^{1}H-NMR ($CDCl_3$) δ: 3.39 (s, 2H, S-$\underline{CH_2}$), 3.92 (s, 2H, benzothiazine S-$\underline{CH_2}$), 5.41 (s, 2H, benzothiazine N-$\underline{CH_2}$),), 6.97-7.02 (t, 1H, *J* = 7.6 Hz, benzothiazine 6-H), 7.21-7.24 (t, 1H, J=7.8 Hz benzothiazine 7-H), 7.29-7.31 (d, 1H, *J* = 7.6 Hz, benzothiazine 5-H), 7.35-7.39 (d, 1H, *J* = 8.4 Hz, benzothiazine 8-H), 7.41-7.73 (m, 5H, Ar-H). ^{13}C-NMR ($CDCl_3$): 23.17 (thiadiazine C-6), 30.48 (benzothiazine, C-2), 38.80 (C between triazole and benzothiazine), 118.71, 123.41, 123.68, 128.07, 129.25, 131.75 (6C atoms, benzothiazine), 128.21, 129.05, 132.78, 138.42 (4C atoms, phenyl), 141.57 (C=N thiadiazine ring), 149.62 (triazole C-3), 154.352 (triazole C-5) and 165.48 (C=O). LC-MS (m/z): 394 (M^++1).

4-{[6-(4-Methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (7c).
IR (KBr) ν cm^{-1}: 3064 (Ar C-H stretch), 2927 (Aliph C-H stretch), 2835 (-OCH_3 stretch) 1674 (C=O), 1603 (C=N), 748 (C-S-C); ^{1}H-NMR ($CDCl_3$) δ ppm : 3.43 (s, 2H, S-$\underline{CH_2}$), 3.87 (s, 3H, -O$\underline{CH_3}$), 3.89 (s, 2H, benzothiazine S-$\underline{CH_2}$), 5.49 (s, 2H, benzothiazine N-$\underline{CH_2}$), 6.97-7.01 (m, 3H, 1H of benzothiazine 6-H & 2H of 4-methoxyphenyl), 7.18-7.22 (t, 1H, J=7.8 Hz, benzothiazine 7-H), 7.29-7.31 (d, 1H, J=7.6 Hz, benzothiazine 8-H), 7.35-7.37 (d, 1H, J=8.4 Hz, benzothiazine 5-H), 7.85-7.88 (d, 2H, J=7.0 Hz, 4-methoxyphenyl). ^{13}C NMR ($CDCl_3$): 23.31 (thiadiazine C-6), 31.54 (benzothiazine, C-2), 39.56 (C between triazole and benzothiazine), 55.56 (-OCH_3), 114.5 & 129.24 (4C-atoms of Ar-OCH_3), 118.64, 123.57, 123.91, 127.51, 128.25, 131.89 (6C atoms, benzothiazine), 139.00 (phenyl C-4), 141.32 (C=N thiadiazine ring), 149.55 (triazole C-3), 153.3 (triazole C-5), 162.84 (phenyl C-1) and 165.41 (C=O). LC-MS (m/z): 424 (M^++1).

4-{[6-(4-Chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (7d).
IR (KBr) ν cm^{-1}: 3061(Ar C-H stretch), 2918 (Aliph C-H stretch), 1667 (C=O), 1583 (C=N), 752 (C-S-C); ^{1}H-NMR ($CDCl_3$) δ ppm : 3.43 (s, 2H, S-$\underline{CH_2}$), 3.92 (s, 2H, benzothiazine S-$\underline{CH_2}$), 5.51(s, 2H, benzothiazine N-$\underline{CH_2}$), 6.99-7.03 (t, 1H, *J* = 7.6 Hz, benzothiazine 6-H), 7.20-7.24 (t, 1H, *J* = 7.8 Hz, benzothiazine 7-H), 7.30-7.32 (d, 1H, *J* =

7.6 Hz, benzothiazine 5-H), 7.38-7.40 (d, 1H, *J* = 8.4 Hz, benzothiazine 8-H), 7.47-7.49 (d, 2H, *J* = 7.0 Hz, 4-chlorophenyl), 7.84-7.86 (d, 2H, *J* = 6.8 Hz, 4- Chlorophenyl); ^{13}C NMR ($CDCl_3$): δ ppm : 23.39 (thiadiazine C-6), 31.58 (benzothiazine, C-2), 39.43 (C between triazole and benzothiazine), 118.68 (benzothiazine C-6), 123.64 (benzothiazine C-7), 124.03 (benzothiazine C-5), 127.58 (benzothiazine C-8), 128.33 (benzothiazine C-10), 128.72 (phenyl C-2 and C-6), 129.43 (phenyl C-3 and C-5), 131.73 (benzothiazine C-9), 138.57 (phenyl C-4), 138.89 (phenyl C-1), 141.18 (C=N thiadiazine ring), 149.78 (triazole C-3), 152.66 (triazole C-5) and 165.45 (C=O). LC-MS (m/z): 473 (M^++1), 475 (M^++3).

4-{[6-(4-Nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (7g).

IR (KBr) ν cm^{-1}: 3011 (Ar C-H stretch), 2904 (Aliph C-H stretch), 1673 (C=O), 1581 (C=N), 1527 (NO_2 asymmetric), 1343 (NO_2 symmetric), 749 (C-S-C); ^{1}H-NMR ($CDCl_3$) δ: 3.51 (s, 2H, S-$\underline{CH_2}$), 3.94 (s, 2H, benzothiazine S-CH_2), 5.55 (s, 2H, benzothiazine N-$\underline{CH_2}$), 7.01-7.04 (t, 1H, J=7.2 Hz, benzothiazine 6-H), 7.23-7.26 (t, 1H, *J* = 7.2 Hz, benzothiazine 7-H), 7.29-7.31 (d, 1H, *J* = 7.26 Hz, benzothiazine 5-H), 8.17-8.19 (m, 2H, protons meta to the nitro group), 8.35-8.37 (d, 2H, *J* = 8.16 protons ortho to the nitro group). ^{13}C NMR ($CDCl_3$): 24.01 (thiadiazine C-6), 31.87 (benzothiazine, C-2), 38.80 (C between triazole and benzothiazine), 118.84 (benzothiazine C-6), 123.46 (benzothiazine C-7), 124.08 (benzothiazine C-8), 127.45 (benzothiazine C-5), 129.85 (benzothiazine C-10), 131.1 (benzothiazine C-9), 123.64 (nitrophenyl C-2 & C-6), 129.84 (nitrophenyl C-3 & C-5), 137.99 (nitrophenyl C-4), 149.50 (nitrophenyl C-1), 141.87 (C=N thiadiazine ring), 149.78 (triazole C-3), 154.41 (triazole C-5) and 165.18 (C=O). LC-MS (m/z): 439 (M^++1).

4-{[6-(Biphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (7h).

IR (KBr) ν cm^{-1}: 3017 (Ar C-H stretch), 2904 (Aliph C-H stretch), 1667 (C=O), 1583 (C=N), 759 (C-S-C); ^{1}H-NMR ($CDCl_3$) δ ppm: 3.30 (s, 2H, S-$\underline{CH_2}$), 3.54 (s, s, 2H, benzothiazine S-$\underline{CH_2}$), 5.20 (s, 2H, benzothiazine N-$\underline{CH_2}$), 7.03-7.06 (t, 1H, *J* = 7.2 Hz, benzothiazine 6-H), 7.22-7.26 (t, 1H, J=7.36 Hz, benzothiazine 7-H), 7.31-7.33 (d, 1H, *J* = 6.4 Hz, benzothiazine 8-H), 7.39-7.44 (m, 2H, 1H of benzothiazine 5H & 1H of biphenyl), 7.48-7.52 (t, 2H, *J* = 7.4 Hz, biphenyl), 7.74-7.77 (d, 2H, *J* = 6.4 Hz, biphenyl), 7.83-7.85 (d, 2H, *J* = 8.4 Hz, biphenyl), 8.07-8.10 (d, 2H, *J* = 8.4 Hz, biphenyl). ^{13}C-NMR ($CDCl_3$): 23.32 (thiadiazine C-6), 31.54 (benzothiazine, C-2), 39.51 (C between triazole and benzothiazine), 118.68 (benzothiazine C-6), 123.64 (benzothiazine C-7), 124.03 (benzothiazine C-5), 127.58 (benzothiazine C-8), 128.33 (benzothiazine C-10), 131.73 (benzothiazine C-9), 129.7, 128.0, 127.9, 127.7, 129.3, 132.9, 136.5, 138.8 (8C-atoms of phenyl), 141.58 (C=N thiadiazine ring), 149.47 (triazole C-3), 154.1 (triazole C-5) and 165.13 (C=O). LC-MS (m/z): 470 (M^++1).

Biological Evaluation

Antibacterial Activity

The *in vitro* antibacterial activity was carried out against two Gram positive bacterial strains; *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (ATCC-6633) and two Gram negative bacterial strains *Pseudomonas aeruginosa* (NCIM-2200) *Klebsiella pneumonia* (NCIM -2957). The antibacterial activity of the newly synthesized compounds in the present investigation was assessed by Zone of Inhibiton (ZOI) by disc diffusion method [37]. The bacterial strains were inoculated on Nutrient Agar (NA) and incubated for 24 h at 37 ^{o}C. The test compounds were dissolved in 5 mL of DMSO taken as the solvent; from the stock solution 100 µL of respective compound in the selected concentration (200 µg/disc) was loaded on the disc individually and aseptically, dried and were used for screening the antibacterial assay.

Sterile discs were saturated with 100 µL of the test solution, dried under laminar air flow and placed on the Nutrient Agar (NA) plate for bacteria, which was inoculated with a lawn of the test microorganisms. Plates were incubated at 37 ^{o}C, for 18-24 h for bacteria. The compound that produced distinct circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of antibacterial activity. Streptomycin was the reference standard and results are tabulated in Table I. The antibacterial data revealed that (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid (**4**) possesses activity comparable to that of streptomycin and its antibacterial activity decreased by derivatization of the carboxylic acid group. Among the four bacterial strains used in the study, the compounds are more susceptible towards *S. aureus* and *K. pneumonia* compared to the other two strains *B. subtilis* and *P. aeruginosa*. The compounds **7a, 7b and 7c,** carrying electron releasing substituent on the phenyl ring at the 6th position of the heterocyclic compound, showed better antibacterial activity and all other compounds did not show any significant activity.

Table I: Antibacterial activity data of (3) and derivatives (7a-7h)

Compound	Zone of Inhibition			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumonia*
4	21.4±0.23	20.54±0.00	18.07±0.32	18.4±0.2
7a	18.06±0.11	13.88±0.76	14.43±0.37	17±0.52
7b	16.16±0.28	11.26±0.25	14.13±0.23	14.9±0.36
7c	12.16±0.28	12.4±0.4	-	13.2±0.2
7d	10.16±0.15	-	10±0.00	-
7e	9.06±0.11	-	8±0.00	14.23±0.2
7f	10.36±0.32	8±0.00	-	9±0.00
7g	9±0.00	-	-	-
7h	12±0.00	-	-	8±0.00
Streptomycin	24.3±0.30	22.16±0.37	21.6±0.52	20.6±0.52

Antioxidant activity by DPPH radical scavenging assay

The assay method is based on the ability of DPPH, a stable free radical to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron which is responsible for the absorbance at 517nm and also for the deep purple colour. This purple colour generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecues can quench DPPH free radicals by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule, and convert them into colourless product. The degree of decolouration indicates the scavenging potential of the antioxidant compounds.

The DPPH assay was based on the reported method [38]. The newly synthesized compounds were dissolved in DMSO at 100, 300 and 500 µg/mL concentrations and 4 mL of 0.1 mM methanolic solution of DPPH was added. The mixed solution was incubated at room temperature for 30 minutes and the absorbance was measured at 517 nm using UV-Visible spectrophotometer. Ascorbic acid was the reference standard. The percentage of scavenging activity was calculated as follows.

$$\text{DPPH scavenging activity (\%)} = \frac{[\text{Ac-As}]}{[\text{Ac-Ab}]} \times 100$$

Where Ac was the absorbance of the control, As for the sample and Ab for the blank (MeOH + DMSO). The results revealed that (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid (**4**) showed mild antioxidant activity and its antioxidant activity increased by derivatization of the carboxylic acid group (Table II). Compound **7c** displayed the highest activity. This probably could be attributed to the methoxy group attached to the phenyl ring in the molecule. Compounds **7b** ($-CH_3$), **7g** ($-NO_2$) and **7c**(OCH_3) were the other few compounds which showed moderate activity whereas **7d** (-Cl) and **7h** (-Ph) showed slight activity in comparison with the standard drug ascorbic acid.

Table II: Antioxidant activity data of (3) and derivatives (7a-7h)

Compound	Concentration (µg/mL)		
	100	200	300
	% inhibition	% inhibition	% inhibition
3	43.59±1.42	58.82±0.08	63.00±2.98
7a	23.53±0.50	52.75±1.38	79.19±0.17
7b	84.63±2.99	87.72±0.66	93.81±0.46
7c	84.87±0.35	88.78±0.37	94.19±1.76
7d	21.16±1.51	51.86±0.65	73.22±1.57
7e	72.04±0.94	84.93±0.12	85.19±0.40
7f	68.11±1.16	86.10±0.18	92.62±0.93
7g	55.68±1.49	59.37±0.91	64.66±0.82
7h	23.47±0.48	53.71±2.06	65.06±0.88
Ascorbic acid	89.63±0.41	97.63±2.34	98.56±0.95

RESULTS AND DISCUSSION

Chemistry

The key intermediate, (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid **(4)** was prepared by the hydrolysis of corresponding ethyl ester **(3),** which in turn was prepared by treating 2*H*-1,4-benzothiazin-3(4*H*)-one with ethyl chloroacetate in DMF in the presence of anhydrous potassium carbonate. The triazole employed in these reactions

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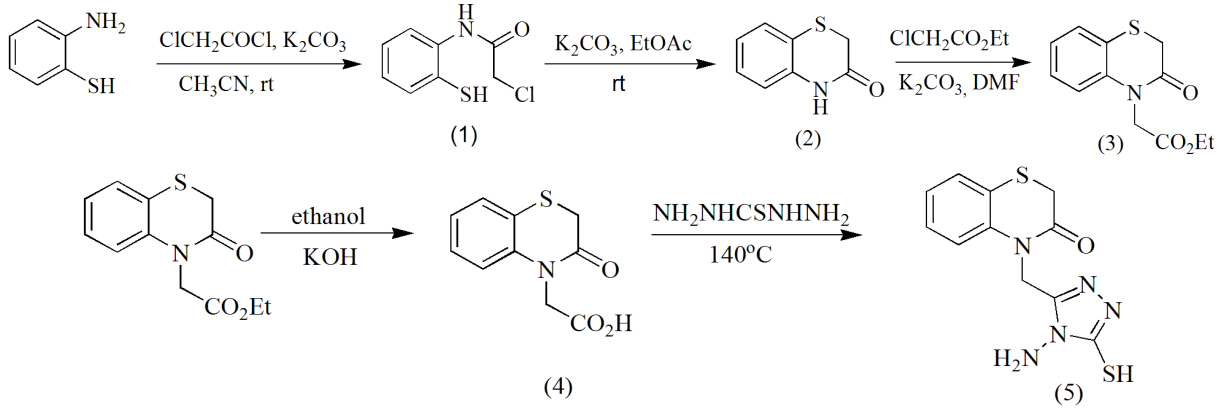
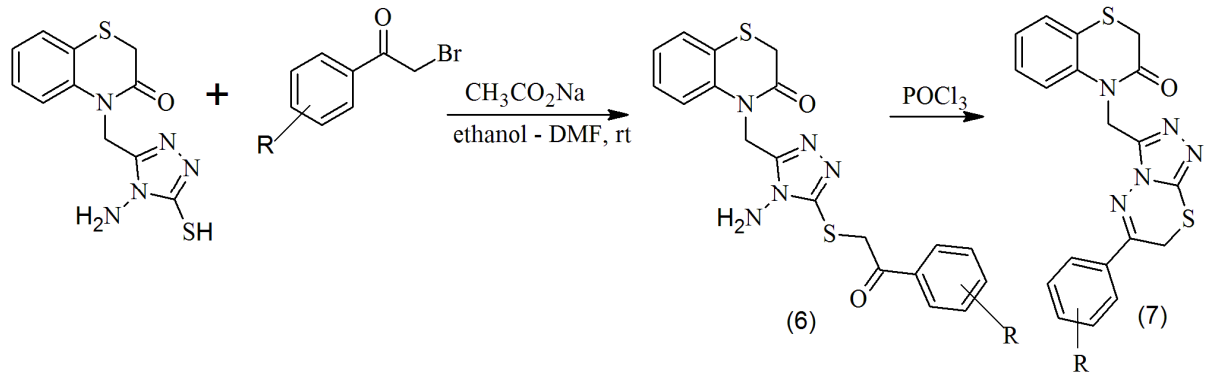
was substituted at 3-position by 2*H*-1,4-benzothiazin-3(4*H*)-one moiety and it was prepared by the fusion of (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid **(4)** with thiocarbohydrazide under solvent free condition [35,36] (**Scheme 1.1**).

Reaction of equimolar mixture of triazole (**5**) and α-halo ketones in anhydrous sodium acetate initially gave 4-[(4-amino-5-{[2-(4-substituted phenyl)-2-oxoethyl]sulfanyl}-4*H*-1,2,4-triazol-3-yl)methyl]-2*H*-1,4-benzothiazin-3(4*H*)-one (**6**) instead of the expected cyclised triazolothiadiazines (**7**). However, (**6**) on further reaction with $POCl_3$ underwent intramolecular ring closure resulting in the formation of triazolo[3,4-*b*][1,3,4]thiadiazines in good yields (**Scheme 1.2**). S-substitution is confirmed by the presence of a strong peak at ~ 1680 cm^{-1} characteristic of carbonyl group. Further evidence is seen in ^{1}H-NMR spectra, with the disappearance of the characteristic peak belonging to NH/SH tautomeric proton and also the appearance of sharp signal integrating to two protons at δ ~ 4.7 ppm for S-CH_2 group. Transformation of S-substituted 1,2,4 triazoles (**6)** into triazolothiadiazines (**7)** was proved by the disappearance of the C=O absorption bands in the IR spectra and by the remarkable up-field shift of the S-C**H**$_2$ protons in the ^{1}H-NMR spectra from ~ 4.75 ppm in **6** to ~ 3.5 ppm in **7**. This shift was attributed to the absence of C=O group next to S-C**H**$_2$ in thiadiazine ring**.** In a typical example, the ^{1}H-NMR spectra of 4-{[6-(4-bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methyl}-2*H*-1,4-benzothiazin-3(4*H*)-one (**7f**), two sharp singlets resonated at δ 3.42 and 3.91 ppm integrating for two protons of S-CH_2 of thiadiazine and the two protons of benzothiazine S-CH_2. Another singlet at δ 5.51 ppm integrated for two protons was due to benzothiazine N-CH_2. The ortho and meta protons of the p-bromo phenyl appeared as two doublets centered at δ 7.63 ppm and δ 7.78 ppm, each integrating for two protons with a coupling constant of J = 8.8 Hz. The four benzothiazine protons appeared as multiplets in the region δ 6.98-7.41 ppm. The physico-chemical and characterisation data of new 7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives is presented in TableIII.



Scheme 1.1



R = H, 4-CH_3, 4-OCH_3, 4-Cl, 4-F, 4-Br, 4-NO_2, 4-Ph,

Scheme 1.2

Table III: Characterization data of 7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (7a-7h)

Compd	R	m.p. (°C) (Yield %)	Mol. Formula (Mol. wt)	% Composition, Found (Calcd)		
				C	H	N
7a	H	149-150	$C_{19}H_{15}N_5OS_2$ (393.48)	58.08 (58.00)	3.81 (3.84)	17.75 (17.80)
7b	4-CH_3	231-33	$C_{20}H_{17}N_5OS_2$ (407.51)	58.86 (58.95)	4.24 (4.20)	17.26 (17.19)
7c	4-OCH_3	97-98	$C_{20}H_{17}N_5O_2S_2$ (423.51)	56.64 (56.72)	4.13 (4.05)	16.51 (16.54)
7d	4-Cl	167-168	$C_{19}H_{14}ClN_5OS_2$ (427.93)	53.26 (53.33)	3.33 (3.30)	16.28 (16.37)
7e	4-F	167-69	$C_{19}H_{14}FN_5OS_2$ (411.47)	55.44 (55.46)	3.47 (3.43)	16.99 (17.02)
7f	4-Br	159-160	$C_{19}H_{14}BrN_5OS_2$ (472.38)	48.27 (48.31)	3.04 (2.99)	14.80 (14.83)
7g	4-NO_2	121-122	$C_{19}H_{14}N_6O_3S_2$ (438.48)	51.96 (52.04)	3.25 (3.22)	19.2 (19.17)
7h	4-Ph	142-143	$C_{25}H_{19}N_5OS_2$ (469.58)	63.86 (63.94)	4.11 (4.08)	14.88 (14.91)

CONCLUSION

2*H*-1,4-benzothiazin-3(4*H*)-one containing triazolothiadiazines (7a-h) were synthesized by initial condensation of triazole **(5)** with α-halo ketones and further cyclization using $POCl_3$. The series of compounds were examined for antibacterial and antioxidant properties. These results indicated that (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid as the interesting lead molecules for more synthetic and biological evaluation. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic (3-oxo-2,3-dihydro-4*H*-1,4-benzothiazin-4-yl)acetic acid moiety.

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