Asian Journal of Chemistry Vol. 20, No. 1 (2008), 629-641

Antimicrobial and Antiinflammatory Studies on Some 1,2,4-Triazolo[3,4-b][1,3,4]thiadiazines and 1,2,4-Triazolo[3,4-b][1,3,4]thiadiazoles Containing Quinoxaline

SHIVANANDA WAGLE†, AIRODY VASUDEVA ADHIKARI* and NALILU SUCHETHA KUMARI‡
Department of Chemistry, National Institute of Technology Karnataka Surathkal-575 025, India
E-mail: avchem@nitk.ac.in; avadhikari123@yahoo.co.in

The reaction of 2-(3-methyl-2-oxo quinoxalin-1(2H)-yl) acetohydrazide (**1**), with carbon disulphide in presence of methanolic potassium hydroxide at 0 ºC affords potassium dithiocarbazate (**2**), which on treatment with 70 % hydrazine hydrate at 100 ºC undergoes smooth cyclization to give 1-[(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-3-methyl quinoxalin-2(1H)-one (**3**), in good yield. This triazole has been conveniently converted into title compounds, 3-methyl-1-[(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) methyl]quinoxalin-2(1H)-one (**4a-j**), by condensing it with phenacyl bromides in ethanol at reflux temperature. In another reaction, the triazole **3** has been treated with aromatic carboxylic acids in presence of phosphorus oxychloride at 100 ºC to afford title compounds, 3-methyl-1-[(6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-methyl]quinoxalin-2(1H)-one (**5a-j**). The prepared compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data, followed by elemental analysis. These compounds were screened for their *in vitro* antibacterial activity against five pathogenic strains and antifungal activity against four fungi. Further, selected compounds were subjected to in vivo antiinflammatory activity. Few of them exhibited promising activity.

Key Words: 1,2,4-Triazoles, 1,2,4-Triazolo[3,4-b][1,3,4]-thiadiazines, Triazolo, 1,2,4-Triazolo[3,4-b][1,3,4]thiadiazoles, Quinoxaline, Antibacterial, Antifungal, Antiinflammatory.

INTRODUCTION

Various 1,2,4-triazole derivatives have aroused considerable interest of chemistry due to their versatile practical applications as well as their wide range of biological properties[1-5]. Literature survey reveals that a large number of triazolo thiadiazoles and triazolo thiadiazines, particularly substituted at positions 3 and 6 by aryl, alkyl or heterocyclic moieties have

†Strides Research and Specialty Chemicals Ltd., New Mangalore-575 011, India.
‡Department of Biochemistry, Justice K.S. Hegde Academy, Deralakatte, Mangalore-575 005, India.

been reported to possess CNS depressant, antibacterial, antifungal, anti-HIV-1, antitumor, antiinflammatory activities, in addition to herbicidal, pesticidal and insecticidal properties[6-12]. Also, a number of quinoxaline derivatives have been shown to possess a variety of pharmacological properties like antibacterial, antifungal, antituberculosis, analgesic and anti-inflammatory activities and hence it is found to be an important structural feature in some synthetic drugs[13-18]. Therefore it was thought to combine quinoxaline moiety with 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole or 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine rings together in a molecular frame work to see the additive effects of these rings towards biological activities. In continuation of our research program on the synthesis of novel heterocyclic compounds exhibiting biological activity, we herein report the synthesis of 3-methyl-1-[(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl] quinoxalin-2(1H)-ones (**4a-j**) and 3-methyl-1-[(6-phenyl[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]quinoxalin-2(1H)-ones (**5a-j**) and evaluation of their antibacterial, antifungal and antiinflammatory activities.

EXPERIMENTAL

Melting points were determined by open capillary and are uncorrected. The IR spectra (KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on Perkin-Elmer EM-390 (300 MHz) and Bruker WH-200 (400 MHz) spectrometers using TMS as an internal standard. ^{13}C NMR spectra were obtained on a Perkin-Elmer (Model RB-12, 100 MHz) spectrometer. All chemical shifts are reported in ppm downfield from tetramethylsilane. The mass spectra were recorded on a Jeol JMS-D 300 mass spectrometer operating at 70 eV. Elemental analysis was performed on Perkin-Elmer-CHN analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated sheets using hexane and ethyl acetate 3:2, v/v.

1-[(4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-3-methyl-quinoxalin-2(1H)-one (3): 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl) acetohydrazide (1.0 mol, 15 g), dissolved in methanol (25 mL) and potassium hydroxide (1.05 mol, 4.34 g) dissolved in methanol (50 mL) were mixed and the resulting mixture was cooled to 0 °C. To this mixture carbon disulfide (1.5 mol, l5.9 g) was added slowly while stirring and stirring was continued at ambient temperature for 5 h. Progress of the reaction was evidenced by the formation of thick solid and was monitored by TLC (ethyl acetate/hexane, 2:3, v/v). After the completion of the reaction, the solid product separated was filtered, washed with methanol (10 mL) and dried at 40 ºC. This solid (15 g) was dissolved in water (75 mL), mixed with 70 % hydrazine hydrate (10 mL) and refluxed for 5 h. The reaction

mixture was then cooled to 0 °C and acidified with acetic acid. The precipitated solid was filtered, washed with water, dried and finally recrystallized with methanol (12 g, 64.5 %), m.p. 261-262 °C.

IR (KBr, ν_{max}, cm^{-1}): 3303 (NH_2), 3131 (SH/NH), 1650 (C=N), 1600, 1461, 1378 (Ar-H). MS (m/z, %): 289 (M+1, 100), 288 (M^+, 30), 257(10), 176(10), 163(20), 107(20). ^{1}H NMR (DMSO-d_6) δ: 14.1 (s, 1H, SH), 7.69-7.67 (d, 1H, phenyl, *J* = 8.0 Hz), 7.56-7.54 (d, 1H, phenyl, *J* = 8.0 Hz), 7.48-7.44 (t, 1H, phenyl, *J* = 8.0 Hz), 7.28-7.25 (t, 1H, phenyl, *J* = 8.0 Hz), 5.71 (s, 2H, CH_2), 5.50 (s, 2H, NH_2), 2.49(s, 3H, CH_3). ^{13}C NMR (DMSO): 21.58 (-$\underline{C}H_3$), 37.33 (-$\underline{C}H_2$-), 115.29, 124.12, 129.27, 130.19, 132.53, 132.79, 147.66, 154.53, 157.95 (Aromatic- $\underline{C}$), 167.17 (-$\underline{C}$=O).

General procedure for the preparation of 6-substituted-3-(quinoxalin-2(1H)-one)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (4a-j): A mixture of equimolar quantity of compound **3** (1.0 mol, 0.5 g) and substituted phenacyl bromide in anhydrous ethanol (25 mL) was refluxed for 8 h. The completion of the reaction was monitored by TLC. It was then cooled to room temperature and diluted with water. The reaction mixture was neutralized with dilute sodium bicarbonate solution. The solid separated was filtered, washed with water, dried and recrystallized from chloroform or dimethyl formamide.

4a IR (KBr, ν_{max}, cm^{-1}): 3320 (b, OH), 3080, 3055, 3028 (Ar-H), 1598 (C=N), 1568, 1540, 1498, 1444, 1420 (aromatic skeleton), 1270 (N-N=C), 665(C-S-C). MS (m/z, %): 448(M+1, 100), 447 (M^+, 15), 232(10), 431(10), 414(5), 250(10), 176(15), 107(10). ^{1}H NMR (DMSO-d_6) δ: 13.68(s, 1H, OH), 8.58-8.56 (d, 1H, phenyl proton, *J* = 8.0Hz), 8.21 (s, 1H, phenyl protons), 8.09-8.07 (d, 1H, phenyl protons, *J* = 8.0 Hz), 7.79-7.78 (d, 1H, quinoxaline protons, *J* = 4.0 Hz), 7.63-7.61 (d, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.56-7.52 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.38-7.35 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.07 (s, 1H, NH_2), 5.82 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 2.50 (s, 3H, CH_3). ^{13}C NMR (DMSO): 21.68 (-$\underline{C}H_3$), 23.11 (-$\underline{C}H_2$-), 37.06 (-$\underline{C}H_2$-), 114.94, 115.53, 118.95, 123.93, 124.14, 129.21, 129.31, 130.19, 132.63, 132.92, 133.26, 141.61, 148.74, 154.61, 154.90, 157.98 (Aromatic-$\underline{C}$), 164.73 (-$\underline{C}ONH_2$), 171.79 (C=O).

4b IR (KBr, ν_{max}, cm^{-1}): 3078, 3048, 3024 (Ar-H), 1600 (C=N), 1565, 1536, 1482, 1446, 1421 (aromatic skeleton), 1274 (N-N=C), 663 (C-S-C). ^{1}H NMR (DMSO-d_6) δ: 7.97-7.92 (dd, 2H, phenyl, *J* = 6.3 Hz), 7.79-7.77 (d, 1H, quinoxaline proton, *J*= 8.0 Hz), 7.62-7.60 (d, 1H, quinoxaline proton, *J*=8.0 Hz), 7.56-7.52 (t, 1H, quinoxaline proton, *J*=8.0 Hz), 7.38-7.34 (t, 1H, quinoxaline proton, *J*=8.0 Hz), 7.12-7.06 (dd, 2H, phenyl proton, *J*=8.0 Hz), 5.80 (s, 2H, CH_2), 4.36 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3). ^{13}C NMR (DMSO): 21.66 (-O$\underline{C}H_3$), 23.18 (-$\underline{C}H_3$), 56.07 (-$\underline{C}H_2$), 114.90, 115.48, 124.10, 125.75, 129.30, 129.97, 130.18, 132.60,

132.94, 141.85, 148.66, 154.58, 155.34, 158.00 (All aromatic-C), 162.87 (-C=O).

4d IR (KBr, ν_{max}, cm^{-1}): 3084, 3058, 3030 (Ar-H), 1601(C=N), 1570, 1538, 1490, 1448, 1425 (aromatic skeleton), 1277 (N-N=C), 665 (C-S-C). MS (m/z, %): 389 (M+1, 100), 388 (M^+, 20), 232(10), 229(15), 120(10), 107(10). ^{1}H NMR (DMSO-d_6) δ: 7.69-7.68 (d, 1H, quinoxaline protons, *J* = 4.0Hz), 7.55-7.53 (d, 1H, quinoxaline protons, *J* = 8.0Hz), 7.46-7.42 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.40-7.36 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.34-7.25 (m, 5H, phenyl), 5.77 (s, 2H, CH_2), 4.18 (s, 2H, CH_2), 2.06 (s, 3H, CH_3). ^{13}C NMR (DMSO): 21.62 (-CH_3), 23.28 (-CH_2), 56.07, 114.89, 115.30, 124.14, 125.65, 129.30, 129.85, 130.28, 132.60, 132.92, 141.78, 148.60, 154.68, 155.44, 158.21 (All aromatic- C), 162.96 (-C=O).

4e IR (KBr, ν_{max}, cm^{-1}): 3252 (N-H), 3082, 3054, 3026 (Ar-H), 1605 (C=N), 1574, 1535, 1487, 1451, 1426 (aromatic skeleton), 1273 (N-N=C), 661 (C-S-C).

4f IR (KBr, ν_{max}, cm^{-1}): 3080, 3058, 3024 (Ar-H), 1603 (C=N), 1576, 1539, 1485, 1452, 1427(aromatic skeleton), 1272(N-N=C), 664 (C-S-C).

4g IR (KBr, ν_{max}, cm^{-1}): 3078, 3048, 3024 (Ar-H), 1600 (C=N), 1565, 1536, 1482, 1446, 1421(aromatic skeleton), 1274 (N-N=C), 663(C-S-C).

4h IR (KBr, ν_{max}, cm^{-1}): 3076, 3052, 3027(Ar-H), 1605 (C=N), 1561 1531, 1480 1439, 1419 (aromatic skeleton), 1268 (N-N=C), 883 (C-Cl), 667(C-S-C). ^{1}H NMR (DMSO-d_6) δ: 7.89-7.88 (d, 1H, quinoxaline protons, *J* = 4.0 Hz), 7.83-7.81 (d, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.76-7.72 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.68-7.65 (t, 1H, quinoxaline protons, *J* = 8.0 Hz), 7.59 (d, 1H, H_6 phenyl, *J* = 8 Hz), 7.55 (d, 1H, H_3 phenyl, *J* = 6.0 Hz), 7.43 (m, 1H, H_5 phenyl) 5.78 (s, 2H, CH_2), 4.0 (s, 2H, CH_2), 2.55 (s, 3H, CH_3).

General procedure for the preparation of 6-substituted-3-(quinoxalin-2(1H)-one)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (5a-j): An equimolar mixture of **3** (1.0 mol, 0.5 g) and appropriate aromatic acid dissolved in phosphorous oxychloride (10 mL) was refluxed for 8 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and brought to 7-8 pH by adding dilute solution of sodium carbonate while stirring and stirring was continued at room temperature for 2 h. The solid separated was filtered, washed with water, dried and recrystallized from chloroform and dimethyl formamide.

5a IR (KBr, ν_{max}, cm^{-1}): 3082, 3060, 3026 (Ar-H), 1601(C=N), 1572 1542, 1493, 1452, 1427(aromatic skeleton), 1277(N-N=C), 667(C-S-C). MS (m/z, %): 409(M+1, 100), 408 (M^+, 20), 279(10), 249(15), 165(10), 120(15), 107(20). ^{1}H NMR (DMSO-d_6) δ: 7.92-7.90 (d, 1H, quinoxaline proton, *J* = 6.8 Hz), 7.80-7.78 (d, 1H, quinoxaline, *J* = 8.0 Hz), 7.75-7.71 (t, 1H, quinoxaline, *J* = 8.0 Hz), 7.68-7.64 (t, 1H, quinoxaline, *J* = 8.0 Hz),

7.61-7.35 (m, 4H, Ar-H), 5.99 (s, 2H, CH_2), 2. 57 (s, 3H, CH_3). ^{13}C NMR (DMSO): 21.62 (-$\underline{C}H_3$), 37.20 (-$\underline{C}H_2$), 115.49, 124.18, 127.60, 127.69, 128.66, 129.32, 130.19, 131.60, 131.89, 132.16, 132.64, 132.90, 134.22, 143.07, 154.65, 158.03 (All Aromatic- $\underline{C}$), 163.89 (-$\underline{C}$=O).

5b IR (KBr, ν_{max}, cm^{-1}): 3422, 3220(N-H), 1603 (C=N), 1518, 1496, 1470, 1425 (aromatic skeleton), 1276 (N-N=C), 988, 952, 903, 841, 739 (Ar-H bending vibrations), 699(C-S-C). MS (m/z, %): 390 (M+1, 100), 389 (M^+, 30), 311(10), 230(15), 213(40), 181(75), 163(20), 105(50), 91(80). ^1H NMR (DMSO-d_6) δ: 7.92-7.90 (d, 1H, quinoxaline proton, J = 6.8 Hz), 7.84-7.82 (d, 1H, quinoxaline, J = 8.0 Hz), 7.78-7.76 (t, 1H, quinoxaline, J = 8.0 Hz), 7.74-7.72 (t, 1H, quinoxaline, J = 8.0 Hz), 7.68-6.69 (dd, 4H, Ar-H, J = 8.7 Hz), 5.86 (s, 2H, CH_2), 3.80 (bs, 2H, NH_2), 2.55 (s, 3H, CH_3).

5c IR (KBr, ν_{max}, cm^{-1}): 3128, 3063, 3030 (Ar-H), 1624 (C=N), 1588, 1574, 1517, 1473, 1459 (aromatic skeleton), 1249 (N-N=C), 989, 960, 904, 885, 809, 739 (aromatic bending vibrations), 700 (C-S-C). ^1H NMR (DMSO-d_6) δ: 7.89 (t, 2H, phenyl, J = 6.9 Hz), 7.80 (d, 2H, phenyl, J = 6.8 Hz), 7.76-7.50 (m, 4H, pyridine H), 5.36 (s, 2H, CH_2), 2.58 (s, 3H, CH_3).

5d IR (KBr, ν_{max}, cm^{-1}): 3131, 3078, 3024 (Ar-H), 1624 (C=N), 1518, 1469, 1444, 1426 (aromatic skeleton), 1240 (N-N=C), 691(C-S-C). ^1H NMR (DMSO-d_6) δ: 8.04 (d, 1H, quinoxaline proton, J = 6.8 Hz), 7.94-7.92 (d, 1H, quinoxaline, J = 8.0 Hz), 7.80-7.78 (t, 1H, quinoxaline, J = 8.0 Hz), 7.72-7.70 (t, 1H, quinoxaline, J = 8.0 Hz), 7.68-7.52 (m, 5H, Ar- H), 5.86 (s, 2H, CH_2), 2.80 (s, 3H, CH_3).

5e IR (KBr, ν_{max}, cm^{-1}): 3111, 3012 (Ar-H), 1622 (C=N), 1584, 1508, 1462, 1435, 1422 (aromatic skeleton), 1255 (N-N=C), 699(C-S-C). ^1H NMR (DMSO-d_6) δ: 8.07 (t, 2H, phenyl, J = 5.8Hz), 7.98 (d, 2H, phenyl, J = 6.0 Hz), 7.68-7.21 (m, 4H, Ar- H), 5.90 (s, 2H, CH_2), 4.04 (s, 3H, OCH_3), 2.80 (s, 3H, CH_3).

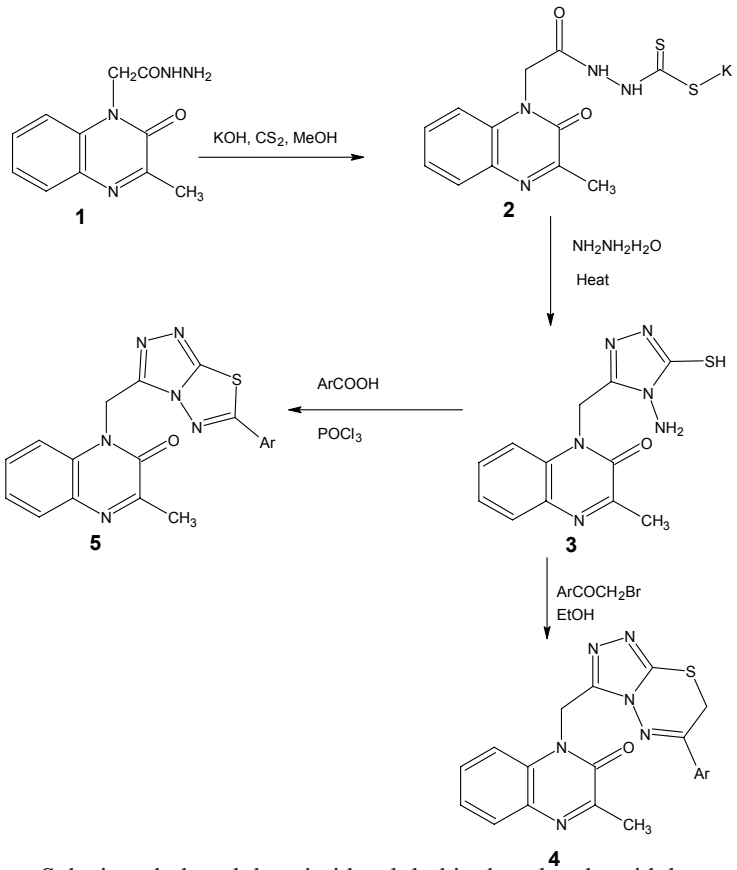
5f IR (KBr, ν_{max}, cm^{-1}): 3118, 3010 (Ar-H), 1610 (C=N), 1521, 1499, 1478, 1432 (aromatic skeleton), 1270 (N-N=C), 993, 954, 908, 847, 745 (Ar-H bending vibrations), 696 (C-S-C). MS (m/z, %): 389 (M+1, 100), 388 (M^+, 30), 217(30), 161(15), 135(40), 109(30), 84(30). ^1H NMR (DMSO-d_6) δ: 7.92-7.80 (m, 4H, phenyl proton), 7.78-7.76 (d, 1H, quinoxaline, J = 8.0 Hz), 7.66-7.62 (t, 1H, quinoxaline, J = 8.0 Hz), 7.54-7.48 (d, 1H, quinoxaline, J = 8.0 Hz), 7.46-7.40 (t, 1H, quinoxaline, J = 8.0Hz), 6.05 (s, 2H, CH_2), 2.60 (s, 3H, CH_3), 2.48 (s, 3H, CH_3).

5g IR (KBr, ν_{max}, cm^{-1}): 3128, 3063, 3030 (Ar-H), 1601(C=N), 1513, 1492, 1460, 1414 (aromatic skeleton), 1271 (N-N=C), 980, 948, 899, 840, 734 (Ar-H bending vibrations), 691 (C-S-C). MS (m/z, %): 443 (M^+, 100), 392(50), 307(15), 279(10), 105(50), 149(80).

5h IR (KBr, ν_{max}, cm^{-1}): 3126, 3061, 3036 (Ar-H), 1608 (C=N), 1511, 1495, 1463, 1411(aromatic skeleton), 1277 (N-N=C), 981, 949, 896, 840, 734 (Ar-H bending vibrations), 690 (C-S-C).

RESULTS AND DISCUSSION

The target compounds were synthesized according to the **Scheme-I**. The required starting material, 2-(3-methyl-2-oxo-quinoxalin-1(2H)-yl) acetohydrazide (**1**) was prepared in good yield according to reported procedure[19]. The reaction of **1** with carbon disulfide in methanolic potassium hydroxide at 0 ºC yielded its potassium dithiocarbazate (**2**). The salt **2**, on reaction with aqueous hydrazine hydrate at 100 ºC, gave the desired product, 1-[(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-3-methylquinoxalin-2(1H)-one (**3**) in good yield. The compound **3** was conveniently converted into 7H-3-aryl-6-phenyl-1,2,4-triazolo [3,4b]-1,3,4-thiadiazenes **4a-j** by condensing it with different phenacyl bromides in boiling ethanol medium. Further, compound **3**, on reaction with various aromatic carboxylic acids in presence of phosphorous oxychloride underwent smooth cyclization to give 3,6-diaryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles **5a-j**.



Ar = Substituted phenyl, benzimidazolyl, thiophenyl and pyridyl group

Scheme-I

The structural assignments to new compounds were based on their elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR and MASS) data. The characterization data of all the newly synthesized compounds are summarized in Table-1.

The formation of 2-(3-methyl-2-oxo-quinoxalin-1(2H)-yl) acetohydrazide (**1**) was confirmed by its IR spectra and elemental analyses. IR spectrum showed absorption bands at 3310, 3224, 1680, 1544 cm^{-1} due to -NH_2, -NH, >C=O and >C=C< groups, respectively. The intermediate potassium dithiocarbazate (**2**) was directly used for the next step without characterization. The formation of cyclized product, 1-[(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-3-methylquinoxalin-2(1H)-one (**3**) from **2** was confirmed by its IR spectrum which showed peaks at 3303, 3131 and 1650 cm^{-1} due to -NH_2, -SH and >C=N, respectively. Further, its ^{1}H NMR spectrum showed four singlets at δ 2.79, 3.46, 5.78 and 14.1 due to -CH_3, -NH_2, -CH_2- and -SH protons, respectively. The appearance of doublet and triplet at δ 7.9 and 7.6 were due to four aromatic protons of quinoxaline ring. In the mass spectrum, it showed molecular ion peak at m/z 289 (100 %), which matches with its molecular formula $C_{12}H_{12}N_6OS$. The cyclization of **3** in presence of phenacyl bromide leading to the formation of title compound **4b** was confirmed by its spectral analysis. IR spectra of **4a-j** showed absorption peaks at 1277 and 665 cm^{-1} due to -N - N=C and C-S-C stretching vibrations, respectively and no absorption peaks at 3303 and 3131 cm^{-1} indicated the smooth cyclization. Further, ^{1}H NMR spectrum of **4d** showed three singlets at δ 2.06, 4.18 and 5.77 due to -CH_3, cyclic -CH_2- and -CH_2-, respectively. The mass spectrum of it showed molecular ion peak at m/z 389 (100 %) which is in agreement with the molecular formula $C_{20}H_{16}N_6OS$. Conversion of **3** to 1,2,4-triazolo[3,4-b][1,3,4] thiadiazole, **5i** in presence of carboxylic acid was confirmed by its spectral studies. IR spectrum of **5a-j** showed absorption bands at 1601, 1277 and 667 cm^{-1} indicating the presence of >C=N, -N-N=C< and >C-S-C< groups, respectively. In ^{1}H NMR spectrum of **5a**, peaks due to -CH_3 and -CH_2- protons appeared at δ 2.57 and 5.56, respectively. Its mass spectrum showed molecular ion peak at m/z 409 (100 %) which is in agreement with the molecular formula $C_{19}H_{13}N_6OSCl$. Physical and elemental analysis data of **5a-5j** are listed in Table-1.

The investigation of antibacterial activity revealed that all the tested compounds **4a-j** and **5a-j** showed moderate to good inhibition at concentration of 10 μg/mL in DMSO. The compounds **4e** and **5j** exhibited good activity and others showed activity below the moderate level against *E. coli*, whereas compounds **4a**, **4d**, **4e** and **5j** showed good activity and other compounds showed low to moderate activity against *S. aureus*. In antifungal screening, the compounds **4a**, **4d**, **4e** and **5d** exhibited good activity

TABLE-1
CHARACTERIZATION DATA OF COMPOUNDS **4a-j** AND **5a-j**

Compd./ Ar	m.f. (m.w.)	m.p. (ºC)/ Yield (%)	Elemental analysis: Found (Calcd.) %		
			C	H	N
4a	$C_{21}H_{17}N_7O_3S$	292-294/	56.10	3.58	21.86
3-($CONH_2$)-4(OH)-C_6H_3	(447.47)	85	(56.31)	(3.79)	(21.90)
4b	$C_{21}H_{18}N_6O_2S$	222-224/	60.15	4.21	20.04
4-(OCH_3)-C_6H_4	(418.47)	80	(60.21)	(4.30)	(20.07)
4c	$C_{21}H_{18}N_6OS$	248-250/	62.45	4.23	20.83
4-(CH_3)-C_6H_4	(402.47)	81	(62.61)	(4.47)	(20.87)
4d	$C_{20}H_{16}N_6OS$	270-272/	61.51	4.01	21.58
C_6H_5	(388.40)	82	(61.79)	(4.11)	(21.62)
4e	$C_{21}H_{16}N_8OS$	236-238/	58.60	3.65	26.12
Benzimidazole	(428.47)	67	(58.81)	(3.73)	(26.13)
4f	$C_{26}H_{20}N_6OS$	220-222/	67.02	4.18	18.07
Biphenyl	(464.54)	80	(67.16)	(4.30)	(18.08)
4g	$C_{18}H_{13}N_6OS_2Br$	196-198/	45.52	2.62	17.71
5-(Br)-Thiophene	(473.37)	83	(45.63)	(2.74)	(17.74)
4h	$C_{20}H_{14}N_6OSCl_2$	268-270/	52.47	3.06	18.36
3,5-($Cl)_2$-C_6H_4	(457.33)	78	(52.45)	(3.07)	(18.38)
4i	$C_{21}H_{18}N_6OS_2$	214-216/	57.86	4.07	19.24
4(SCH_3)-C_6H_4	(434.53)	75	(57.99)	(4.14)	(19.33)
4j	$C_{20}H_{15}N_6OSBr$	230-232/	51.23	3.12	17.94
4-(Br)-C_6H_4	(467.34)	80	(51.35)	(3.20)	(17.97)
5a	$C_{19}H_{13}N_6OSCl$	240-242/	55.70	3.13	20.49
2-(Cl)-C_6H_4	(408.86)	68	(55.76)	(3.17)	(20.54)
5b	$C_{19}H_{15}N_7OS$	238-240/	58.50	3.81	25.12
4-(NH_2)-C_6H_4	(389.43)	78	(58.54)	(3.85)	(25.16)
5c	$C_{18}H_{13}N_7OS$	190-192/	57.40	3.37	26.07
C_5H_4N	(375.40)	70	(57.53)	(3.46)	(26.10)
5d	$C_{19}H_{14}N_6OS$	160-162/	60.81	3.67	22.42
C_6H_5	(374.42)	68	(60.89)	(3.73)	(22.43)
5e	$C_{20}H_{16}N_6O_2S$	234-236/	59.23	3.85	20.68
2-(OCH_3)-C_6H_4	(404.40)	66	(59.30)	(3.92)	(20.72)
5f	$C_{20}H_{16}N_6OS$	260-262/	61.62	4.04	21.61
4-(CH_3)-C_6H_4	(388.44)	71	(61.78)	(4.11)	(21.62)
5g	$C_{19}H_{12}N_6OSCl_2$	276-278/	51.32	2.51	18.88
3,5-($Cl)_2$-C_6H_3	(443.31)	75	(51.43)	(2.70)	(18.90)
5h	$C_{21}H_{18}N_6OS$	296-298/	62.51	4.33	20.85
3,5-($CH_3)_2$-C_6H_3	(402.47)	78	(62.61)	(4.47)	(20.87)
5i	$C_{18}H_{12}N_7OSBr$	206-208/	47.42	2.56	21.54
5-(Br)-C_4H_3N	(454.30)	72	(47.54)	(2.64)	(21.57)
5j	$C_{22}H_{15}N_7OS$	214-216/	62.04	3.50	23.02
Quinoline	(425.46)	74	(62.05)	(3.52)	(23.03)

while remaining compounds showed low to moderate activity against *Trichophyton*. Compounds **4a** and **5j** exhibited good activity against *Penicillium* sp. which is comparable with that of the standard. On the basis of the structure-activity relationship, it has been observed that among the substituents at position 6 of triazolo thiadiazine ring system, the anisole, 5-bromo thiophene, benzimidazole and aniline groups play significant role in antimicrobial activities of title compounds as compared to other substituents.

Amongst the tested compounds for antiinflammatory screening, four compounds, *i.e.*, **4d**, **4e**, **5d** and **5j** (50 mg/mL) showed moderate to good activity in carrageenan induced paw edema model. The antiinflammatory activity of **4d** and **5j** was good and comparable to the standard drug Indomethacin (1.5 mg/mL). The results are based on the findings in a preliminary screening test only. Further studies using larger samples are in progress for obtaining conclusive evidence.

Antibacterial studies: The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* [ATTC-25922], *Staphylococcus aureus* [ATTC-25923], *Pseudomonas aeruginosa* [ATTC-27853] and *Klebsiella pneumoniae* [recultered] bacterial strains by serial plate dilution method[20,21]. Serial dilutions of the drug in Muller-Hilton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth.

A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. 20 µL of agar media was poured into each petri dishes. Excess of suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in DMSO were added into each well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 d. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard[22,23]. The results of such studies are given in the Table-2.

Antifungal studies: The newly synthesized compounds were screened for their antifungal activity against *Aspergilus flavus* (NICM No.524), *Aspergilus fumigatus* (NICM No.902), *Candida albicans* (NICM No.300), *Penicillium marneferei* (recultered) and *Trichophyton mentagrophytes* (recultered) in DMSO by serial plate dilution method[24,25]. Subourands agar

TABLE-2
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **4a-j** AND **5a-j**

Compd.	*E. coli*	*K. pneumoniae*	*S. aureus*	*P. aeruginosa*
4a	12.50(16-20)	25.00(<10)	6.25(16-20)	12.50(11-15)
4b	12.50(16-20)	12.50(16-20)	12.50(16-20)	12.50(11-15)
4c	25.00(<10)	25.00(<10)	12.50(16-20)	12.50(16-20)
4d	12.50(16-20)	6.25(16-20)	6.25(16-20)	12.50(16-20)
4e	6.25(16-20)	12.50(16-20)	6.25(16-20)	12.50(16-20)
4f	25.00(<10)	25.00(<10)	25.00(<10)	12.50(16-20)
4g	12.50(10-15)	12.50(16-20)	12.50(16-20)	25.00(<10)
4h	12.50(16-20)	12.50(16-20)	12.50(16-20)	12.50(16-20)
4i	25.00(<10)	25.00(<10)	25.00(<10)	12.50(16-20)
4j	12.50(11-15)	12.50(16-20)	12.50(16-20)	12.50(16-20)
5a	12.50(16-20)	12.50(16-20)	25.00(<10)	12.50(11-15)
5b	12.50(16-20)	12.50(16-20)	12.50(16-20)	25.00(<10)
5c	12.50(16-20)	12.50(16-20)	12.50(16-20)	12.50(16-20)
5d	6.25(16-20)	12.50(16-20)	25.00(<10)	25.00(<10)
5e	12.50(16-20)	12.50(16-20)	12.50(10-15)	12.50(16-20)
5f	25.00(<10)	25.00(<10)	25.00(<10)	12.50(16-20)
5g	25.00(<10)	12.50(11-15)	12.50(16-20)	6.25(16-20)
5h	12.50(16-20)	12.50(11-15)	25.00(<10)	12.50(16-20)
5i	12.50(11-15)	12.50(11-15)	12.50(16-20)	12.50(16-20)
5j	6.25(16-20)	12.50(16-20)	6.25(16-20))	12.50(16-20)
Standard (Ciprofloxacin)	6.25(30-40)	6.25(23-27)	6.25(25-33)	1.56(22-30)

Note: MIC values were evaluated at concentration range, 1.56-25 µg/mL. The figures in the table show the MIC values and the corresponding zone of inhibition (mm).

media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 µM of agar media was poured into each petridishes. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well is labeled and minimum inhibitory concentrations of the test compounds in DMSO were added into each well. A control was also prepared for the plates in the same way using solvent DMSO. The petridishes were prepared in triplicate and maintained at 37 °C for 3-4 d. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with ciclopirix olamine as standard. The results of such studies are given in the Table-3.

TABLE-3
ANTIFUNGAL ACTIVITY OF COMPOUNDS **4a-j** AND **5a-j**

Compound	*Trichophyton*	*Asp. Fumigatus*	*C. albicans*	*Penicillium*
4a	6.25(16-20)	12.5(16-20)	12.5(16-20)	6.25(16-20)
4b	25(<10)	12.5(16-20)	25(<10)	25(<10)
4c	12.5(11-15)	12.5(16-20)	12.5(16-20)	12.5(16-20)
4d	6.25(16-20)	6.25(16-20)	12.5(16-20)	12.5(16-20)
4e	6.25(16-20)	12.5(16-20)	12.5(16-20)	12.5(16-20)
4f	12.5(11-15)	12.5(16-20)	12.5(16-20)	12.5(11-15)
4g	25(<10)	25(<10)	25(<10)	12.5(16-20)
4h	25(<10)	25(<10)	25(<10)	12.5(16-20)
4i	12.5(16-20)	12.5(16-20)	12.5(16-20)	25(<10)
4j	12.5(16-20)	12.5(16-20)	12.5(16-20)	12.5(16-20)
5a	25(<10)	25(<10)	25(<10)	12.5(16-20)
5b	12.5(16-20)	12.5(11-15)	12.5(11-15)	12.5(16-20)
5c	12.5(16-20)	25(<10)	25(<10)	12.5(16-20)
5d	6.25(16-20)	12.5(16-20)	25(<10)	12.5(16-20)
5e	25(<10)	25(<10)	25(<10)	25(<10)
5f	12.5(16-20)	12.5(16-20)	12.5(16-20)	12.5(16-20)
5g	12.5(16-20)	12.5(16-20)	25(<10)	25(<10)
5h	12.5(16-20)	12.5(16-20)	12.5(16-20)	12.5(16-20)
5i	12.5(16-20)	12.5(16-20)	12.5(16-20)	25(<10)
5j	25(<10)	12.5(16-20)	12.5(16-20)	6.25(16-20))
Standard (Ciclopirix olamine)	6.25(20-27)	3.125(27-33)	3.125(25-30)	6.25(25-30)

Note: MIC values were evaluated at concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values and the corresponding zone of inhibition (mm).

Antiinflammatory activity: Carrageenan induced rat paw edema method was employed for evaluating the antiinflammatory activity of the compounds.

Wister albino rats of either sex weighing 180-250 g were housed in clean polypropylene cages and kept under room-temperature (25 ± 2 °C) relative humidity 60-70 % in a 12 h light-dark cycle. The animals were given standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during experimental hours.

Antiinflammatory study: The animals were divided into groups as shown in the table. Acute inflammation was produced by sub plantar injection of 0.1 mL of 1 % suspension of carrageenan with 2 % gum acacia in normal saline, in the right hind paw of the rats. After 1 h oral administration of the drug. The paw volume was measured plethysmometrically (Ugo

Basile, Italy) at 0 and 3 h after the carrageenan injection. The difference between the two readings was taken as the volume of the edema and the percentage antiinflammatory activity was calculated. Indomethacin 1.5 mg/kg p.o suspended in 25 gum acacia was used as the standard drug.

TABLE-4
ANTIINFLAMMATORY ACTIVITY OF COMPOUNDS

Group	Dose	Increase in paw volume in mL	% Inhibition of paw edema
2 % Gum acacia (control)	10 mL / kg	0.45	
Standard drug	1.5	0.17	62.2
4a	50.0	0.38	15.5
4d	50.0	0.21	53.3
4i	50.0	0.40	11.1
5e	50.0	0.30	33.3
5i	50.0	0.27	40.0
5j	50.0	0.19	57.7

Conclusion

This study reports the successful synthesis of the title compounds in good yields and antimicrobial and antiinflammatory activities of these derivatives containing quinoxaline moiety against wide range of bacterial and fungal stains. The investigation of antimicrobial and antiinflammatory activity revealed that few compounds showed good to moderate antibacterial and antifungal properties. Two compounds, amongst the screened samples showed good antiinflammatory activity which is comparable with the standard. It has been observed that the increased antimicrobial and antiinflammatory activities are due to the presence of pharmacologically active substituents like thiophene, amino, benzimidazole and methoxy substituents in the title compounds.

ACKNOWLEDGEMENTS

The authors are grateful to Head R.S.I.C., Punjab University, Chandigarh, Head, SAIF, CDRI Lucknow and Head, SAIF IISc, Bangalore for providing ^{1}H NMR and mass spectral data. The authors are also thankful to the Head, Department of Chemistry, NITK, Surathkal and Strides Research and Specialty Chemicals Ltd., New Mangalore for providing necessary laboratory facilities.

REFERENCES

1. J.M. Kane, M.A. Staeger, C.R. Dalton, F.P. Miller, M.W. Dudley, A.M. Ogden, J.H. Kehne, H.J. Ketteler, T.C. McCloskey and Y. Senyah, *J. Med. Chem.*, **37**, 125 (1994).
2. O. Matsumoto and T. Uekawa, Japan Kokai Tokkyo Koho, JP 2000239262, (2000); T. Kishimoto, Y. Yamada, T. Iwasa and M. Matsuda, Japan Kokai Tokkyo, Koho, JP 07330742, 19951219 (1995).
3. K.D. Patel, B.D. Mistry and K.R. Desai, *J. Indian Chem. Soc.*, **79**, 964 (2002).
4. P.C Wade, B. Vogt, Richard, T.P Kissick, J.M. Simpkins, D.M. Palmer and R.C. Millonig, *J. Med. Chem.*, **25**, 331 (1982).
5. M.D. Mullican, M.W. Wilson, D.T. Connor and R.D. Dyer, *J. Med. Chem.*, **36**, 1090 (1993).
6. M. Kritsanida, A. Mouroutsou, P. Markos, N. Pouli, S.P. Garoufalias and M. Witvrouw, *IL Farmaco*, **57**, 253 (2002).
7. M.M. Ghorab, A.M. Sh. El-Sharief, Y.A. Ammar and Sh. I. Mohamed, *Phosphorus, Sulfur, Silicon, Rel. Elem.*, **173**, 223 (2001).
8. Z. Wang, H. Shi and H. Shi, *J. Heterocycl. Chem.*, **38**, 355 (2001).
9. L. Labanauskas, V. Kalcas, E. Udrenaite and P. Gaidelis, *Pharmazie*, **56**, 617 (2001).
10. B. Tozkopparan, E. Kupeli, E. Yesilada, S. Islik, M. Ozalp and M. Ertan. *Arzneimittelforschung*, **55**, 533 (2005).
11. R.M. Shaker, A.F. Mahmoud, F.F. Abdel-Latif. *Phosphorus, Sulfur, Silicon Rel. Elem.*, **180**, 2 (2005).
12. M. De La Rosa, E. Kim, H.W. Gunic, C. Jenket, U. Boyle, Y.H. Koh, I. Korboukh, M. Allan, W. Zhang, H. Chen, Z. Hong and Z. Zhang, *Bioorg. Med. Chem. Lett.*, **16**, 4444 (2006).
13. D.A. Vyas, N.A. Chauhan, A.R. Parikh, *J. Indian Chem. Soc.*, **82**, 972 (2005).
14. Z. Michaus and M. Belen, *Spain. Diss. Abstr. Int.*, C, **66**, 148 (2005).
15. J. Dudash, Y. Zhang, B.J. Moore, R. Look, Y. Liang, M.B. Pat, R.B. Conway, Rybczynski, J.P. Demarest and T. Keith, *Bioorg. Med. Chem. Lett.*, **15**, 4790 (2005).
16. C. Obafemi and D. Akinpelu, *Phosphorus, Sulfur, Silicon, Rel. Elem.*, **180**, 1795 (2005).
17. K. Kazuatsu, Japna Kokai Tokkyo Koho, 39 (2004).
18. Y. Sainz, M.E. Montoya, F.J. Martinez-Crespo, M.A. Ortega, A. Lopez de Cerain and A. Monge, *Arzneimittel-Forschung*, **49**, 55 (1999).
19. A.A.F. Wasfy, F.A. Yassin and A.M.F. Eissa, *Indian J. Chem.*, **34B**, 537 (1995).
20. A. Barry, in ed.: Corian, Procedures and Theoretical Considerations for Testing Antimicrobial Agents in Agar Media, Antibiotics in laboratory medicine Williams and Wilkins, Baltimore, edn. 5, (MD 1) (1991).
21. J.D. MacLowry, M.J.J. Jaqua and S.T. Selpak, *Appl. Microbiol.*, **25**, 46 (1970).
22. Christine. H. Fenlon and Michel. H. Cynamon, *Antimicrob. Agents Chemother.*, **29**, 386, (1986).
23. R. Davis, A. Markam and J.A. Balfour, *Drugs*, **51**, 1019 (1996).
24. B.A. Arthington-Skaggs, M. Motley, D.W. Warnock and C.J. Morrison, *J. Clin. Microbiol.*, **38**, 2254 (2000).
25. R.S. Varma, Antifungal Agents, Past, Present and Future Prospects, National Academy and Biology India, Lucknow (1998).

(*Received*: 18 December 2006; *Accepted*: 14 September 2007) AJC-5871