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ORIGINAL ARTICLE

Synthesis and antimicrobial activities of some novel 1,2,4-triazole derivatives

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Abstract In the present investigation, a series of new Schiff bases **4a–f** were synthesized by the condensation of *N*-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamides **3a–b** with various substituted aromatic aldehydes in ethanol–dioxane mixture using catalytic amount of sulfuric acid. The starting materials **3a–b** were in turn synthesized by the fusion of benzoyl glycine/substituted benzoylglycine with thiocarbohydrazide. Newly synthesized compounds were characterized by IR, NMR, mass spectra and elemental analyses. All the compounds were evaluated for their antibacterial and antifungal activity using the Minimum Inhibition Concentration (MIC) method by serial dilution technique. Few of the compounds were found to be biologically active.
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1. Introduction

Since last few decades, there is tremendous growth of research in the synthesis of nitrogen containing heterocyclic derivatives because of their utility in various applications, such as pharmaceuticals, propellants, explosives, pyrotechnics and especially in chemotherapy (Chavez and Parrish, 2009). A large number of ring systems containing 1,2,4-triazoles have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, anti-anxiety, antimicrobial agents (Nadkarni et al., 2001; Isloor et al., 2009), antimycotic agents such as Fluconazole, Itraconazole and Voriconazole (The Merck Index, 1996; Haber, 2001). Also there are some known drugs containing 1,2,4-triazole moiety, e.g. Triazolam (Brucato et al., 1978),

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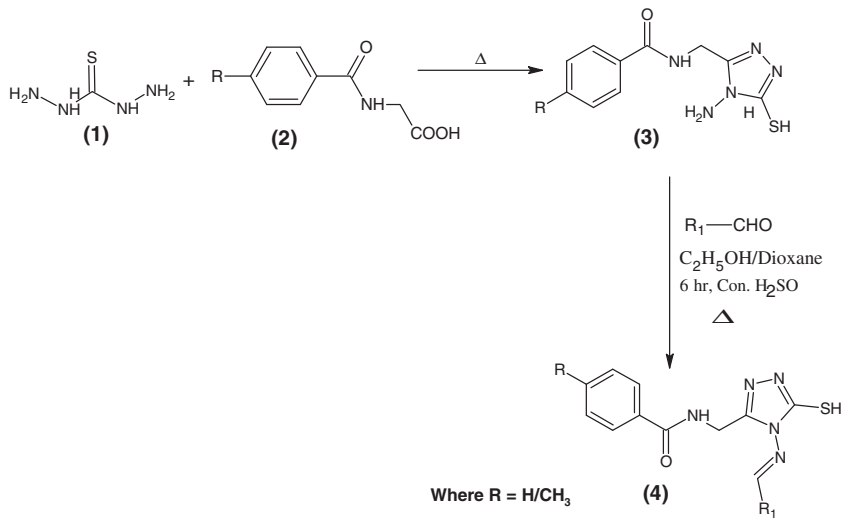

4a: R=H, R^1= p-NO_2-C_6H_5; **4b:** R=H, R^1= p-OCH_3-C_6H_5; **4c:** R=CH_3, R^1= p-NO_2-C_6H_5;
4d: R=CH_3, R^1= p- OCH_3-C_6H_5; **4e:** R=CH_3, R^1= p- Cl-C_6H_5; **4f:** R=CH_3, R^1= 3,4- OCH_3-C_6H_5.

Scheme 1 Synthetic route for the Schiff bases **4a**–**f**.

Alprazalam (Coffen and Fryer, 1973), Etizolam (Shiroki et al., 1975), Furacylin (Povelista and Gural, 1973), Ribavirin (Sidwell et al., 1975), Hexaconazole (Shepherd, 1986), Triadimefon (Lye, 1987), Mycobutanil (Efthimiadis, 1988), Rizatriptan (Hart, 1999), Propiconazole (Reet et al., 1976), and Fluotrimazole (Worthington, 1984). 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles by virtue of their ambient nucleophilic centre are good starting materials for the synthesis of several interesting N-bridged heterocycles. A detailed literature survey revealed that, Schiff bases possess diverse type of biological activities (El-Sayed, 2006). Some Schiff's bases bearing aryl groups or heterocyclic residues exhibited interesting biological activities, which has attracted many researchers' attention in recent years (Holla et al., 2000; Isloor et al., 2009; Marina et al., 2002). Keeping in view of the above facts and in continuation of our search on biologically potent molecules, we herein report the synthesis of some new 1,2,4-triazole derivatives and their antimicrobial activity.

2. Experimental

2.1. Measurements

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) spectrometer using TMS as internal standard. Chemical shift values are given in δ scales. The mass spectra were recorded on LC–MS-Agilent 1100 series and API 2000 LC/MS system. Elemental analyses were performed on a Flash EA 1112 series CHNS–O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (Silica Gel 60 F254). Commercial grade solvents and reagents were used without further purification. The reaction pathway has been summarized in Scheme 1.

2.2. Synthesis

2.2.1. Synthesis of *N*-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide ***3a–b***

An equimolar mixture of *N*-benzoylglycine/*N*-(*p*-tolyl)glycine **2a** (0.01674 mol) and thiocarbohydrazide **1** (0.01674 mol) were fused on oil bath for 1 h. Then the reaction mixture was cooled and treated with a cold solution of 5% $NaHCO_3$. The resulted solid was filtered, washed with water and recrystallised from ethanol.

2.2.1.1. N-[(4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]benzamide 3a (Hassan, 2009). Yield 63.63%, m.p. 279–280 °C. IR (cm^{-1}) 3447, 3316 (N–H str), 3096 (C–H str), 1652 (C═O str), 1601 (C═N). ^{1}H NMR (DMSO-d_6) δ = 13.58 (s, 1H, S–H), 8.95 (t, 1H, N–H), 7.46–7.88 (m, 5H, Ar-H), 5.61 (s, 2H, –NH_2), 4.49 (d, 2H, –CH_2). MS (EI). *m*/*z* 250 [M + 1].

2.2.1.2. N-[(4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-methylbenzamide 3b. Yield 46.90%, m.p. 220–221 °C. IR (cm^{-1}) 3321, 3258 (N–H str), 3079 (C–H str), 1682 (C═O str), 1617 (C═N). ^{1}H NMR (DMSO-d_6) δ = 13.47 (s, 1H, S–H), 8.51 (t, 1H, N–H), 7.18–7.78 (m, 4H, Ar-H), 5.48 (s, 2H, –NH_2), 4.51 (d, 2H, –CH_2). MS (EI). *m*/*z* 264 [M + 1].

2.2.2. Synthesis of *N*-[(4-{[(*E*)-substituted]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide ***4a–f***

Equimolar mixture of *N*-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide **3a–b** (0.0004 mol), substituted benzaldehyde (0.0004 mol) and 2–3 drops of concentrated sulfuric acid in ethanol–dioxane mixture was refluxed for 6 h. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallised from hot ethanol.

2.2.2.1. N-[(4-{[(E)-(4-Nitrophenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]benzamide 4a. Yield 88.88%, m.p. 276–279 °C. IR (cm^{-1}) 3322 (N–H str), 3046 (C–H stretch), 1642 (C═O str), 1596 (C═N), 1277 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.99 (s, 1H, S–H), 10.4 (s, 1H, N═CH), 9.01 (t, 1H, N–H), 7.42–8.33 (m, 9H, Ar-H), 4.67 (d, 2H, –CH_2). ^{13}C NMR (DMSO-d_6) δ = 166.3, 161.8, 158.9, 149.4, 148.8, 138.1, 133.6, 131.4, 129.6, 128.2, 127.2, 124.0, 34.2. MS [EI] *m/z* 383 [M + 1]. Elemental analysis ($C_{17}H_{14}N_6O_3S$); calcd. C, 53.40; H, 3.69; N, 21.98; found C, 53.36; H, 3.72; N, 21.95%.

2.2.2.2. N-[(4-{[(E)-(4-Methoxyphenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]benzamide 4b. Yield 66.34%, m.p. 232–235 °C. IR (cm^{-1}) 3282 (N–H str), 3057 (C–H stretch), 1641 (C═O str), 1603 (C═N), 1251 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.82 (s, 1H, S–H), 9.78 (s, 1H, N═CH), 8.97 (t, 1H, N–H), 7.04–7.84 (m, 9H, Ar-H), 4.60 (d, 2H, –CH_2), 3.84 (s, 3H, –OCH_3). ^{13}C NMR (DMSO-d_6) δ = 166.3, 163.2, 162.7, 161.6, 148.3, 133.7, 131.3, 130.6, 128.2, 127.2, 124.5, 114.5, 55.4, 34.3. MS [EI] *m/z* 368 [M + 1]. Elemental analysis ($C_{18}H_{17}N_5O_2S$); calcd. C, 58.84; H, 4.66; N, 19.06; found C, 58.80; H, 4.61; N, 19.09%.

2.2.2.3. 4-Methyl-N-[(4-{[(E)-(4-nitrophenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]benzamide 4c. Yield 64.88%, m.p. 269–272 °C. IR (cm^{-1}) 3318 (N–H str), 3035.8 (C–H str), 1640 (C═O str), 1603 (C═N), 1276 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.99 (s, 1H, S–H), 10.41 (s, 1H, N═CH), 8.93 (t, 1H, N–H), 7.24–8.34 (m, 8H, Ar-H), 4.67 (d, 2H, –CH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ = 166.2, 161.8, 158.8, 149.3, 148.8, 141.3, 138.1, 130.8, 129.6, 128.8, 127.2, 124.0, 34.2, 20.8. MS [EI] *m/z* 397 [M + 1]. Elemental analysis ($C_{18}H_{16}N_6O_3S$); calcd. C, 54.54; H, 4.07; N, 21.20; found C, 54.59; H, 4.01; N, 21.25%.

2.2.2.4. N-[(4-{[(E)-(4-Methoxyphenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-methylbenzamide 4d. Yield 75.11%, m.p. 246–250 °C. IR (cm^{-1}) 3283 (N–H str), 3053 (C–H stretch), 1634 (C═O str), 1604 (C═N), 1246 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.68 (s, 1H, S–H), 10.12 (s, 1H, N═CH), 8.69 (t, 1H, N–H), 6.96–7.83 (m, 8H, Ar-H), 4.62 (d, 2H, –CH_2), 3.86 (s, 3H, –OCH_3), 2.38 (s, 3H, –CH_3). MS [EI] *m/z* 382 [M + 1]. Elemental analysis ($C_{19}H_{19}N_5O_2S$); calcd. C, 59.82; H, 5.02; N, 18.36; found C, 59.78; H, 5.06; N, 18.31%.

2.2.2.5. N-[(4-{[(E)-(4-Chlorophenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-methylbenzamide 4e. Yield 62.10%, m.p. 257–258.5 °C. IR (cm^{-1}) 3295 (N–H str), 3045 (C–H str), 1637 (C═O str), 1585 (C═N), 1274 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.94 (s, 1H, S–H), 10.08 (s, 1H, N═CH), 8.94 (t, 1H, N–H), 7.24–7.93 (m, 8H, Ar-H), 4.61 (d, 2H, –CH_2), 2.33 (s, 3H, –CH_3). ^{13}C NMR (DMSO-d_6) δ = 166.2, 161.7, 161.3, 148.6, 141.4, 137.2, 131.0, 130.9, 130.2, 129.2, 128.8, 127.3, 34.2, 20.9. MS [EI] *m/z* 386. MS [EI] *m/z* 386 [M + 1]. Elemental analysis ($C_{18}H_{16}ClN_5OS$); calcd. C, 56.03; H, 4.18; N, 18.15; found C, 56.07; H, 4.12; N, 18.20%.

2.2.2.6. N-[(4-{[(E)-(3,4-Dimethoxyphenyl)methylidene]amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-methylbenzamide 4f. Yield 81.62%, m.p. 231–235 °C. IR (cm^{-1}) 3372 (N–H stretch), 3052 (C–H stretch), 1653 (C═O str), 1595 (C═N), 1259 (C═S). ^{1}H NMR (DMSO-d_6) δ = 13.88 (s, 1H, S–H), 9.82 (s, 1H, N═CH), 8.92 (t, 1H, N–H), 7.06–7.75 (m, 7H, Ar-H), 4.64 (d, 2H, –CH_2), 2.33 (s, 3H, –CH_3), 3.74 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3). ^{13}C NMR (DMSO-d_6) δ = 166.2, 163.0, 161.6, 152.7, 149.1, 148.4, 141.3, 131.0, 128.8, 127.3, 124.7, 124.6, 111.2, 108.8, 55.6, 55.3, 34.3, 20.9. MS [EI] *m/z* 412 [M + 1]. Elemental analysis ($C_{20}H_{21}N_5O_3S$); calcd. C, 58.38; H, 5.14; N, 17.02; found C, 58.41; H, 5.19; N, 17.07%.

2.3. Antimicrobial studies

All the newly synthesized compounds were evaluated for their antimicrobial activities against various microorganisms representing Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus (*Candida albicans*), using the Minimum Inhibition Concentration (MIC) method by the serial dilution technique (Mackie and Mc Cartney, 1989). Several colonies of *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* were picked off a fresh isolation plate and inoculated in corresponding tubes containing 5 mL of trypticase soya broth. The broth was incubated for 6 h at 37 °C until there was visible growth. Mc Farland No.5 standard was prepared by adding 0.05 mL of 1% w/v $BaCl_2{\cdot}2H_2O$ in Phosphate Buffered saline (PBS) to 9.95 mL of 1% v/v H_2SO_4 in PBS. The growth of all the four cultures was adjusted to Mc Farland No. 5 turbidity standard using sterile PBS. This gives a 10^8 cfu/mL suspension. The working inoculums of above mentioned four different microorganisms containing 10^5 cfu/mL suspension was prepared by diluting the 10^8 cfu/mL suspension, 10^3 times in trypticase soya broth.

Antimicrobial suspension was prepared by dissolving 0.5 µg of each compound in 10 mL of trypticase soya broth to get 50 µg/mL. This suspension was filter sterilized in syringe filters. To prepare the dilutions in all, for each of the eight anti-microbial compounds and standard antimicrobial i.e. Ceftriaxone, 24 tubes of 5 mL capacity were arranged in four rows with each row containing six tubes. Then 1.9 mL of trypticase soya broth was added in the first tube in each row and 1 mL in the remaining tubes. Now, 100 µl of filtered anti microbial suspension was added to the first tube in each row and after mixing the content, 1 mL was serially transferred from these tubes to the second tube in each of the rows. The contents in the second tube of each of the rows were mixed and transferred to the third tube in each of the rows. This serial dilution was repeated till the sixth tube in each of the rows. This provided anti microbial concentrations of 50, 25, 12.5, 6.25, 3.125, 1.6125 µg/mL in the first to sixth tube, respectively, in each row. Finally, 1 mL of 10^5 cfu/mL of *S. aureus, B. subtilis*, *E. coli, P. aeruginosa* and *C. albicans* suspension were added to the first, second, third and fourth rows of tubes, respectively. Along with the test samples and Ceftriaxone (standard), the inoculum control (without antimicrobial compound) and broth control (without antimicrobial compound and inoculum) were maintained. All the test sample and control tubes were then incubated for 16 h at 37 °C. The results of the antimicrobial studies are summarized in Table 1.

Table 1 Antimicrobial activity of newly synthesized compounds.

Compound No.	Antibacterial (MIC, μg/mL)				Antifungal (MIC, μg/mL)
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	3.125	3.125	3.125	3.125	1.6125
4b	3.125	3.125	3.125	3.125	3.125
4c	3.125	3.125	3.125	3.125	3.125
4d	3.125	3.125	3.125	3.125	3.125
4e	3.125	3.125	3.125	3.125	3.125
4f	3.125	3.125	25.00	25.00	1.6125
Ceftriaxone (standard)	3.125	1.6125	1.6125	1.6125	3.125
Inoculum control	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentration	Growth in all concentrations
Broth control	No growth	No growth	No growth	No growth	No growth

3. Results and discussion

3.1. Chemistry

Formation of *N*-[(4-{[(*E*)-substituted]amino}-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide (**4**) was confirmed by recording their IR, NMR, mass spectra and elemental analyses. IR spectrum of compound **4a** showed absorption bands at 3322, 3046, 1642, 1596 and 1277 cm^{-1} which is due to the N–H, C–H, C═O, C═N and C═S groups, respectively. The ^1H NMR spectrum of **4a** showed a singlet at δ 13.99 corresponds to S–H proton. A singlet at δ 10.4 was due to N═CH proton. A triplet was observed at δ 9.01 was due to N–H attached to $–CH_2$. The appearance of doublet at δ 4.67 and multiplet at δ 7.42–8.33 was due to $–CH_2$ and aromatic ring protons, respectively. Similarly the mass spectrum was recorded and reported as [M + 1] values. For the compound **4a**, molecular weight 383 is consistent with the molecular formula $C_{17}H_{14}N_6O_3S$. The values for the remaining compounds have been presented under the experimental part.

3.2. Antimicrobial activity

All the newly synthesized compounds were screened for their antibacterial and antifungal activities. For antibacterial studies, microorganisms employed were*S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. For antifungal studies, microorganism employed was *C. albicans*. Both antimicrobial studies were assessed by Minimum Inhibition Concentration (MIC) method by the serial dilution technique.

From the antimicrobial activity study, it was found that, compounds**4a**–**f** exhibited the same activity as that of the standard drug Ceftriaxone against *S. aureus* and moderate activity against other bacteria *B. subtilis, E. coli, P. aeruginosa* and also fungus *C. albicans*. The observed activity may be due to presence of chloro, methoxy and nitro groups in these compounds. The antifungal activity of compounds **4a** and **4f** against *C. albicans* was found to be higher than that for the standard drug. However, remaining compounds exhibited same activity as that of standard drug. The observed activity in **4a** and **4f** is may be due to the presence of nitro group alone for the **4a**, and in **4f**, it may be due to the number and orientation of methoxy group.

4. Conclusion

A novel series of Schiff bases bearing 1,2,4-triazole ring systems were synthesized. These were characterized by IR, NMR, mass spectrometry study and elemental analyses. All the compounds were screened for their antibacterial and antifungal activity by serial dilution method. Compounds **4a**–**f** exhibited the same activity as that of the standard drug Ceftriaxone against *S. aureus*. Compounds **4a** and **4f** have showed excellent antifungal activity.

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