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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 2-(3′-aryl-sydnon-4′-ylidene)-5′-substituted-[1,3,4]-thiadiazolylamines and [1,3,4]-thiadiazol-2′-yl-3-oxo-[1,2,4]-triazoles as antimicrobial agents

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Abstract The title compounds (**3g–n**) and (**6g–n**) were synthesized using 3-arylsydnones as synthons, and the structures were confirmed by IR, ^{1}H NMR, FAB mass and CHN analysis. These compounds were evaluated for their antibacterial and the antifungal activities in terms of minimum inhibitory concentrations (MICs) against the bacterial strains *E. coli*, *B. cereus*, and the fungal strains *A. niger*, *C. albicans*. Some of the compounds have shown significant activities.

Keywords Sydnone · 1,3-dipolar cycloaddition · 1,3,4-thiadiazole · 1,2,4-triazole · MIC

Introduction

Essential components in drug designing programme and search for new leads is the synthesis of molecules, which are novel yet resemble biologically active molecules by virtue of presence of critical structural features. Heterocyclic molecules act as functionalised scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules (Silverman, 1992; Thompson and Ellman, 1996). Sydnones form a class of meso-ionic compounds which contain the azomethine system as a part of an aromatic ring. Due to their aromatic nature, they undergo electrophilic substitution at 4th position and also behave as potential 1,3-dipolar systems, undergoing addition reactions with different dipolarophiles (Angadiyavar and George, 1971). The 1,3-dipolar nature of sydnone in cycloaddition reactions to form five membered nitrogen and oxygen containing heterocycles has been the focus of the considerable attention. Our interest in the chemistry of sydnones including their use as synthons, and limited reports as source of 1,3-dipoles prompted us to investigate formation of bi heterocycles (Kamble and Sudha, 2006; Kamble *et al*., 2007). In the present study, we have utilized 1,3-dipolar cycloaddition reaction of the *N*-arylsydnone for the formation of 1,3,4-oxadiazole which intern was used for the formation of the title compounds. In the past few years, pivotal number of 1,2,4-triazoles (Bing *et al*., 2003; Chadha *et al*., 1998; Gokce *et al*., 2001 Holla *et al*., 2001; Hui *et al*., 2005; Marina *et al*., 2002; Sakata *et al*., 2000; Varvaresou *et al*., 2000) and schiff bases have been studied extensively for their broad spectrum activities such as fungicidal, herbicidal anticonvulsant, plant growth regulatory activities, anti-inflammatory, analgesic, anthelminthic, anti-tubercular, anti-cancer and antipyretic activities (Baluja *et al*., 2006; Pandeya *et al*., 1999; Patai 1970; Jungreis and Thabet, 1969).

On the other hand, 1,3,4-thiadiazoles (Padmavati *et al*., 2008; Pintilie *et al*., 2007) have also been proved to be the most important group of fungicides with systemic activity and are well known for their pronounced ability to control a large number of fungal diseases. Some of the 2-substituted-1,3,4-thiadiazoles have been found to produce the inhibition of growth of transplanted tumours and leukaemias in mice (Krakoff *et al*., 1959). In the interest of above, we have designed and synthesised a system that combines bio-labile molecules having Schiff bases and 1,2,4-triazoles derivatised with the 1,3,4-thiadiazoles. The free amino group of 1,3,4-thiadiazole can be easily exploited for condensation and ring insertion reactions. 1,3,4-Thiadiazole was chosen

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in this study because of the effect of its basic functions on non specific antifungal agents.

Results and discussion

Chemistry

3-Arylsydnone was reacted with a stirred mixture of *N*-methylformanilide and phosphorus oxychloride to get 3-aryl-4-formyl-sydnone (**1a–d**). The compound (**1a–d**) was then reacted with 2-amino-5-substituted [1,3,4]-thiadiazole (**2e–f**) to get the schiff base, 2-(3′-aryl-sydnon-4′-ylidene)-5-substituted-[1,3,4]-thiadiazol-2-yl-amine (**3g–n**). The efforts to get azetidinone derivatives (**4g–n**) by the reaction of (**3g–n**) with chloroacetylchloride were unsuccessful (Scheme 1).

In another set of reactions, the 3-arylsydnone was subjected to 1,3-dipolar cycloaddition with acetic anhydride initiated by bromination to form 3-aryl-5-methyl-2-oxo-Δ^4-1,3,4-oxadiazole (**5a–d**). The compound (**5a–d**) when refluxed with 1,3,4-thiadiazole derivative (**2e–f**) in ethanol at about 80°C gave the compound (**6g–n**) (Scheme 2).

The possible mechanism of the ring insertion of nitrogen of the 2-amino-5-substituted-1,3,4-thiadiazole into the 3-aryl-5-methyl-2-oxo-Δ^4-1,3,4-oxadiazole (**5a–d**) is proposed in the Scheme 3.

Spectral analyses

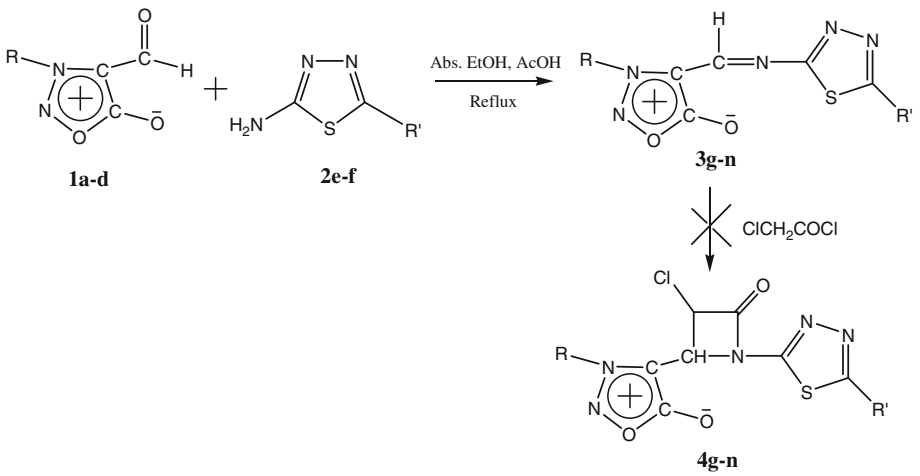
The structures of all the newly synthesised compounds were confirmed by spectral studies and CHN analyses. The IR of compounds (**3g–n**) showed two characteristic bands in the range 1740–1743 and 1660–1663 cm^{-1} due to sydnone carbonyl and C=N stretching, respectively. The absence of sharp band around 1675 cm^{-1} due to formyl carbonyl of compound (**1a–d**) confirmed the formation of the Schiff's base. The proton NMR spectral studies of the compound (**3g–j**) showed a singlet responsible for one proton in the range δ 9.46–9.8 ppm due to imine proton. The other protons viz., the aromatic protons and the substituents attached to the phenyl group of sydnone and substituent on the C_5-carbon atom of 1,3,4-thiadiazole appeared in their respective ranges.

The IR spectral analysis of another set of title compounds viz., (**6g–n**) has shown the following features.

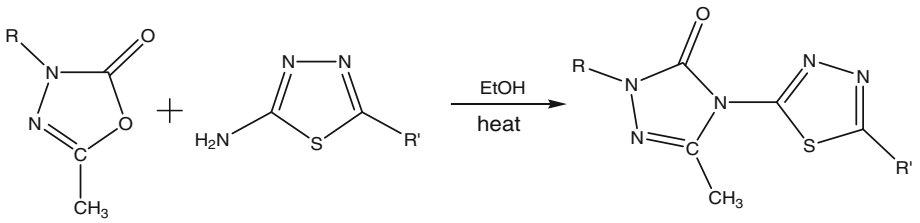
A sharp band around 1700–1715 cm^{-1} was observed due to carbonyl group present in 1,2,4-triazole ring. Its precursor (**5a–d**) has shown carbonyl stretching frequency in range 1775 cm^{-1} (Mallur and Badami, 2000). The decrease in carbonyl stretching frequency is a strong indication of formation of the compound (**6g–n**). The proton NMR studies of the compounds (**6g–n**) showed aromatic protons in their respective regions. Also, these compounds (**6g–n**) have shown a singlet for three protons in the range δ 2.29–2.38 ppm due to C_5-methyl protons. Whereas, the compounds (**6k–n**) have shown another singlet for the three protons due to methyl group attached to 1,3,4-thiadiazole ring. The compounds (**6g–n**) were also confirmed by the FAB mass spectral studies and have exhibited the corresponding molecular ion peaks.

In vitro antibacterial and antifungal activity

In this study, antimicrobial activities of the newly synthesized compounds were evaluated against four test microorganisms viz., bacteria *Escherichia coli*, *Bacillus cereus*, fungi *Candida albicans* and *Aspergillus niger*. The comparative activities of the newly synthesized compounds (**3g–n**) and (**6g–n**) and the control antibiotics chloramphenicol and ketoconazole on bacterial and fungal strains, respectively, are summarised in Table 1.



Scheme 1 Synthetic pathway of 2-(3′-aryl-5′-sydnon-4′-ylidene)-5-substituted-[1,3,4]-thiadiazol-2-yl-amines (**3g–n**). **a** R = phenyl, **b** R = *p*-tolyl, **c** R = *p*-anisyl, **d** R = *p*-chlorophenyl, **e** R′ = phenyl, **f** R′ = methyl, **g** R = phenyl, R′ = phenyl, **h** R = *p*-tolyl, R′ = phenyl, **i** R = *p*-anisyl, R′ = phenyl, **j** R = *p*-chlorophenyl, R′ = phenyl, **k** R = phenyl, R′ = methyl, **l** R = *p*-tolyl, R′ = methyl, **m** R = *p*-anisyl, R′ = methyl, **n** R = *p*-chlorophenyl, R′ = methyl



Scheme 2 Synthetic pathway of 2-aryl-5-methyl-3-oxo-(5′-substituted-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-[1,2,4]-triazoles (**6g–n**). **a** R = phenyl, **b** R = *p*-tolyl, **c** R = *p*-anisyl, **d** R = *p*-chlorophenyl, **e** R′ = phenyl, **f** R′ = methyl, **g** R = phenyl, R′ = phenyl, **h** R = *p*-tolyl, R′ = phenyl, **i** R = *p*-anisyl, R′ = phenyl, **j** R = *p*-chlorophenyl, R′ = phenyl, **k** R = phenyl, R′ = methyl, **l** R = *p*-tolyl, R′ = methyl, **m** R = *p*-anisyl, R′ = methyl, **n** R = *p*-chlorophenyl, R′ = methyl

Scheme 3 Proposed mechanism for the formation of 2-aryl-5-methyl-3-oxo-(5′-substituted-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-[1,2,4]-triazoles (**6g–n**)

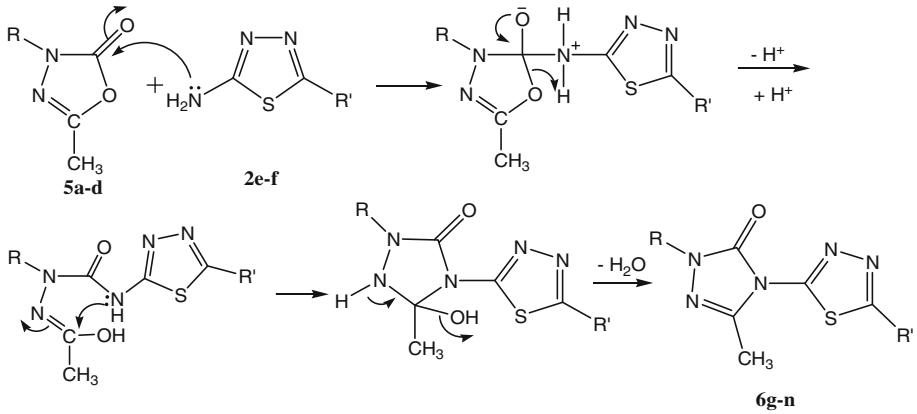


Table 1 The MICs of antibacterial activity of newly synthesized Schiff bases (**3g–n**) and triazole derivatives (**6g–n**)

Entry no.	*E. coli*	*B. cereus*	*C. albicans*	*A. niger*
3g	31.25	125	31.25	125
3h	62.5	125	3.9	125
3i	62.5	62.5	62.5	62.5
3j	31.25	62.5	7.8	31.25
3k	62.5	250	31.25	125
3l	62.5	125	31.25	125
3m	15.6	125	7.8	125
3n	62.5	62.5	7.8	125
6g	31.25	125	31.25	62.5
6h	15.6	62.5	125	62.5
6i	15.6	125	31.25	62.5
6j	31.25	15.6	7.8	15.6
6k	62.5	125	31.25	62.5
6l	62.5	62.5	62.5	62.5
6m	15.6	31.5	31.25	7.8
6n	15.6	15.6	7.8	7.8
Control 1	1.95	12.5	–	–
Control 2	–	–	0.97	0.97

Minimum inhibitory concentrations (MICs) of compounds and antibiotics expressed as (μg/ml); control 1, Chloramphenicol, control 2, Ketoconazole

The compounds **6h** with *p*-tolyl group and **6i**, **6m** with *p*-anisyl group and **6n** with *p*-chlorophenyl groups have shown remarkable activity against the *E. coli* whereas, the other compounds have shown weak to moderate activity against this bacterial species. Amongst the compounds tested for the activity against *B. cereus* only two compounds viz., **6j** and **6n** with *p*-chlorophenyl group have shown the potent activity (15.6 μg/ml) where, the standard drug has shown MIC value at 12.5 μg/ml. The compounds **3i**, **3j**, **3n**, **6h** and **6l** have exhibited a moderate inhibition activity against this bacterium.

The compound **3h** with *p*-tolyl group has shown promising activity against the fungal strain *C. albicans* with MIC value 3.9 μg/ml. Whereas, the compounds **3m–n**, **6j**, and **6n** have shown significant activity against this strain MIC (7.8 μg/ml). The remaining compounds have shown weak activity. In case of screening against *A. niger*, the compounds **6m–n** have exhibited the potent activity whereas, the compound **6j** has shown moderate activity. The activity of the other molecules against this fungus is less.

Conclusion

In summary, a series of sydnones derivatised with 1,3,4-thiadizoles (**3g–n**) and [1,3,4]thiadiazol-2'-yl-[1,2,4]-triazoles

(**6g–n**) were synthesized as potent antimicrobial agents. The structural design altered bioactivity profile of the title compounds and indicated a combination of SAR analysis towards the substituents attached to the sydnone and 1,2,4-triazoles.

Experimental

Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. The compounds were routinely checked for their homogeneity by thin layer chromatography (TLC) using hexane, chloroform and methanol (7:2:1) as eluents. The IR spectra were recorded on Nicolet Impact 410FT IR spectrophotometer using KBr pellets or Nujol. ^{1}H NMR spectra were recorded on Bruker 300-MHz FT NMR spectrometer in $CDCl_3$ with TMS as an internal standard. Mass spectral studies were carried out using Jeol SX-102 (FAB) and elemental analysis using Heraus CHN rapid analyser. 3-Aryl-4-formyl-sydnones (**1a–d**) (Thoman *et al*., 1964), 1,3,4-oxadiazolinones (**5a–d**) (Mallur and Badami, 2000) and 2-amino-5-substituted-[1,3,4]-thiadiazoles (**2e–f**) (Bernstein *et al*., 1957) were synthesized using published procedures.

General procedure for the preparation of 2-(3′-aryl-5′-sydnon-4′-ylidene)-5-substituted-[1,3,4]-thiadiazol-2-yl-amines (**3g–n**)

An equimolar mixture of 2-amino-5-substituted-1,3,4-thiadiazole (**2e–f**, 0.10 mol) and 3-aryl-4-formyl-sydnone (**1a–d**, 0.10 mol) in ethanol (50 ml) and acetic acid (2–3 drops) refluxed for 5–6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, excess solvent was distilled off, and the residue was washed with water. The solid separated was filtered and recrystallised from ethanol to get pale yellow crystals.

*2-(3′-Phenyl-sydnon-4′-ylidene)-5-phenyl-[1,3,4]-thiadiazol-2-yl-amine (**3g**)*

Recrystallisation from ethanol, yellow needles. Yield 80%, m.p. 157–159°C. IR 1743 cm^{-1} (C=O), 1663 cm^{-1} (H–C=N); ^{1}H NMR ($CDCl_3$) δ = 6.96–7.96 (m, 10H, Ar–H), 9.58 (s, 1H, H–C=N) ppm.

Anal. Calcd. for $C_{17}H_{11}O_2N_5S$: C, 58.45; H, 3.15; N, 20.05. Found: C, 58.40; H, 3.12; N, 20.00.

*2-(3′-p-Tolyl-sydnon-4′-ylidene)-5-phenyl-[1,3,4]-thiadiazol-2-yl-amine (**3h**)*

Recrystallisation from ethanol, yellow needles. Yield 66%, m.p. 171–173°C. IR 1742 cm^{-1} (C=O), 1664 cm^{-1} (H–C=N), 2950 cm^{-1} (C–H); ^{1}H NMR ($CDCl_3$) δ = 2.31 (s, 3H, CH_3), 6.96–7.96 (m, 9H, Ar–H), 9.52 (s, 1H, H–C=N) ppm.

Anal. Calcld. for $C_{18}H_{13}O_2N_5S$: C, 59.50; H, 3.58; N, 19.28. Found: C, 59.45; H, 3.54; N, 19.26.

*2-(3′-p-Anisyl-sydnon-4′-ylidene)-5-phenyl-[1,3,4]-thiadiazol-2-yl-amine (**3i**)*

Recrystallisation from methanol, yellow needles. Yield 76%, m.p. 200–202°C. IR 1741 cm^{-1} (C=O), 1662 cm^{-1} (H–C=N), 2950 cm^{-1} (–CH); ^{1}H NMR ($CDCl_3$) δ = 3.57 (s, 3H, OCH_3), 6.80–7.65 (m, 9H, Ar–H), 9.50 (s, 1H, H–C=N) ppm.

Anal. Calcd. for $C_{18}H_{13}O_3N_5S$: C, 56.99; H, 3.43; N, 18.46. Found: C, 56.94; H, 3.46; N, 18.45.

*2-(3′-p-Chlorophenyl-sydnon-4′-ylidene)-5-phenyl-[1,3,4]-thiadiazol-2-yl-amine (**3j**)*

Recrystallisation from methanol, yellow needles. Yield 76%, m.p. 103–105°C. IR 1743 cm^{-1} (C=O), 1660 cm^{-1} (H–C=N) 2950 cm^{-1} (–CH_3); ^{1}H NMR ($CDCl_3$) δ = 6.96–7.96 (m, 9H, Ar–H), 9.46 (s, 1H, H–C=N) ppm.

Anal. Calcld. for $C_{17}H_{10}O_2N_5SCl$: C, 53.26; H, 2.61; N, 18.28. Found: C, 53.24; H, 2.57; N, 18.25.

*2-(3′-Phenyl-sydnon-4′-ylidene)-5-methyl-[1,3,4]-thiadiazol-2-yl-amine (**3k**)*

Recrystallisation from ethanol, yellow needles. Yield 76%, m.p. 144–146°C. IR 1742 cm^{-1} (C=O), 1661 cm^{-1} (H–C=N); ^{1}H NMR ($CDCl_3$) δ = 2.25 (s, 3H, CH_3), 7.05–7.64 (m, 5H, Ar–H), 9.80 (s, 1H, H–C=N) ppm.

Anal. Calcd. for $C_{12}H_9O_2N_5S$: C, 50.17; H, 3.14; N, 24.39. Found: C, 50.14; H, 3.10; N, 24.40.

*2-(3′-p-Tolyl-sydnon-4′-ylidene)-5-methyl-[1,3,4]-thiadiazol-2-yl-amine (**3l**)*

Recrystallisation from methanol, yellow needles. Yield 66%, m.p. 171–173°C. IR 1743 cm^{-1} (C=O), 1660 cm^{-1} (H–C=N), 2940 cm^{-1} (–CH_3); ^{1}H NMR ($CDCl_3$) δ = 2.20 (s, 3H, CH_3, $ArCH_3$), 2.31 (s, 3H, CH_3), 6.84–7.63 (m, 4H, Ar–H), 9.54 (s, 1H, H–C=N) ppm.

Anal. Calcd. for $C_{13}H_{11}O_2N_5S$: C, 51.83; H, 3.65; N, 23.25. Found: C, 51.80; H, 3.63; N, 23.27.

*2-(3′-p-Anisyl-sydnon-4′-ylidene)-5-methyl-[1,3,4]-thiadiazol-2-yl-amine (**3m**)*

Recrystallisation from ethanol, yellow needles. Yield 76%, m.p. 200–202°C. IR 1741 cm^{-1} (C=O), 1661 cm^{-1} (H–C=N) 2950 cm^{-1} (–CH_3), 1301 cm^{-1} (C–O); ^{1}H NMR

($CDCl_3$-d_6) $\delta = 2.21$ (s, 3H, CH_3), 3.45 (s, 3H, OCH_3), 6.90–7.76 (m, 4H, Ar–H), 9.57 (s, 1H, H–C=N) ppm.

Anal. Calcld. for $C_{14}H_{13}O_3N_5S$: C, 49.21; H, 3.47; N, 22.08. Found: C, 49.24; H, 3.47; N, 22.10.

*2-(3-p-Chlorophenyl-sydnon-4-ylidene)-5-methyl-[1,3,4]-thiadiazol-2-yl-amine (**3n**)*

Recrystallisation from methanol, yellow needles. Yield 77%, m.p. 109–110°C. IR 1740 cm^{-1} (C=O), 1662 cm^{-1} (H–C=N), 2930 cm^{-1} (–CH_3); ^{1}H NMR ($CDCl_3$) $\delta = 2.20$ (s, 3H, CH_3), 6.80–7.24 (m, 4H, Ar–H), 9.45 (s, 1H, H–C=N) ppm.

Anal. Calcd. for $C_{12}H_8O_2N_5SCl$: C, 44.86; H, 2.49; N, 21.81. Found: C, 45.03; H, 2.45; N, 21.79.

General procedure for the preparation of 2-aryl-5-methyl-3-oxo-(5′-substituted-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-[1,2,4]-triazoles (**6g**–**n**)

A mixture of 3-aryl-5-methyl-2-oxo-Δ^4-1,3,4-oxadiazole (**5a**–**d**) (0.01 mol) and 2-amino-5-substituted-[1,3,4]-thiadiazole (**2e**–**f**) (0.01 mol) in absolute ethanol (25 ml) was refluxed for 6 h. The solvent was distilled off, and the residue was cooled. The solid separated was washed with water and filtered. Recrystallised from absolute ethanol to get yellow needles.

*5-Methyl-3-oxo-2-phenyl-4-(5′-phenyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-[1,2,4] triazole (**6g**)*

Recrystallisation from ethanol; pale yellow needles. Yield 77%. m. p. 185–187°C. IR 1710 cm^{-1} (C=O), 1643 cm^{-1} (C=N), 2923 cm^{-1} (C–H); ^{1} H NMR ($CDCl_3$) $\delta = 2.38$ (s, 3H, C_5–CH_3), 7.12–7.94 (m, 10H, Ar–H) ppm; MS (FAB) 335 (20), 291 (10), 283 (15), 249 (20), 232 (10), 190 (60), 181 (70), 148 (100), 124 (75), 106(70), 91 (60).

Anal. Calcd. for $C_{17}H_{13}ON_5S$: C, 58.11; H, 3.70; N, 19.94. Found: C, 58.07; H, 3.66; N, 19.78.

*5-Methyl-3-oxo-4-(5′-phenyl-[1,3,4]-thiadiazol-2′-yl)-2-p-tolyl-2,4-dihydro-[1,2,4]-triazole (**6h**)*

Recrystallisation from ethanol, pale yellow needles. Yield 72.40%. m. p. 211–213°C. IR 1705 cm^{-1} (C=O), 1640 cm^{-1} (C=N–H), 2945 cm^{-1} (C–H); ^{1}H NMR ($CDCl_3$) $\delta = 2.24$ (s, 3H, $ArCH_3$), 2.29 (s, 3H, C_5–CH_3), 7.28–7.94 (m, 9H, Ar-H); MS (FAB) 349 (15), 305 (10), 297 (15), 263 (15), 246 (10), 204 (35), 195 (70), 162 (100), 138 (80), 120 (85), 105 (70).

Anal. Calcd. for $C_{18}H_{15}ON_5S$: C, 59.18; H, 4.11; N, 19.18. Found: C, 59.19; H, 4.15; N, 19.15.

*2-p-Anisyl-5-methyl-3-oxo-[4-(5′-phenyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro[1,2,4]-triazole (**6i**)*

Recrystallisation from ethanol; pale yellow needles. Yield 74%. m.p. 144–145°C. IR 1700 cm^{-1} (C=O), 1633 cm^{-1} (C=N), 1300 cm^{-1} (C–O ether); ^{1}H NMR ($CDCl_3$) $\delta = 2.32$ (s, 3H, C_5–CH_3), 3.50 (s, 3H, OCH_3), 7.28–7.94 (m, 9H, Ar–H) ppm; MS (FAB) 365 (15), 321 (5), 313 (10), 279 (20), 262 (5), 220 (30), 211 (75), 178 (100), 154 (85), 136 (80), 121 (70).

Anal. Calcd. for $C_{18}H_{15}ON_5S$: C, 56.69; H, 3.94; N, 18.37. Found: C, 56.68; H, 3.87; N, 18.34.

*2-p-Chlorophenyl-5-methyl-3-oxo-4-(5′-phenyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro[1,2,4]-triazole (**6j**)*

Recrystallisation from ethanol; pale yellow needles. Yield 82%. m. p. 192–194°C. IR 1712 cm^{-1} (C=O), 1638 cm^{-1} (C=N), ^{1}H NMR ($CDCl_3$) $\delta = 2.37$ (s, 3H, C_5–CH_3) 7.28–7.94 (m, 9H, Ar–H) ppm; MS (FAB) 399 (20), 290 (5), 282 (10), 248 (20), 231 (5), 189 (30), 180 (70), 147 (100), 123 (80) 105 (80), 90 (72).

Anal. Calcd. for $C_{17}H_{12}ON_5SCl$: C, 52.98; H, 3.12; N, 18.19. Found: C, 52.94; H, 3.19; N, 18.24.

*5-Methyl-3-oxo-2-phenyl-4-(5′-methyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-[1,2,4]-triazole (**6k**)*

Recrystallisation from methanol; pale yellow needles. Yield 72%. m.p. 175–176°C. IR 1711 cm^{-1} (C=O), 1645 cm^{-1} (C=N), ^{1}H NMR ($CDCl_3$) $\delta = 2.20$ (s, 3H, C_5'–CH_3), 2.38 (s, 3H, C_5–CH_3), 7.28–7.94 (m, 5H, Ar–H); MS (FAB) 217 (20), 173 (6), 165 (15), 131 (30), 114 (100), 72 (75), 63 (60), 54 (12), 21 (60).

Anal. Calcd. for $C_{12}H_{11}ON_5S$: C, 52.74; H, 4.02; N, 25.64. Found: C, 52.76; H, 4.00; N, 25.63.

*5-Methyl-4-(5′-methyl-[1,3,4]-thiadiazol-2′-yl)-3-oxo-2-p-tolyl-2,4-dihydro-[1,2,4] triazole (**6l**)*

Recrystallisation from methanol; pale yellow needles. Yield 73%. m.p. 197–198°C. IR 1708 cm^{-1} (C=O), 1641 cm^{-1} (C=N), 2940 cm^{-1} (C–H); ^{1}H NMR ($CDCl_3$) $\delta = 2.25$ (s, 3H, C_5'–CH_3), 2.30 (s, 3H, $ArCH_3$), 2.41 (s, 3H, C_5–CH_3), 7.28–7.80 (m, 4H, Ar–H) ppm; MS (FAB) 231 (25), 187 (10), 179 (18), 145 (35), 128 (100), 86 (75), 77 (65), 44 (10), 20 (65).

Anal. Calcd. for $C_{13}H_{13}ON_5S$: C, 54.35; H, 4.52; N, 24.39. Found: C, 54.32; H, 4.50; N, 24.40.

*2-p-Anisyl-5-methyl-4-(5′-methyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-3-oxo-[1,2,4]-triazole (**6m**)*

Recrystallisation from ethanol; pale yellow needles. Yield 75%. m.p. 132–133°C. IR 1709 cm^{-1} (C=O), 1644 cm^{-1} (C=N), 1299 cm^{-1} (C–O), 2940 cm^{-1} (C–H); ^1H NMR ($CDCl_3$) $\delta = 2.20$ (s, 3H, C_5'–CH_3), 2.38 (s, 3H, C_5–CH_3) 3.28 (s, 3H, OCH_3), 7.20–7.80 (m, 4H, Ar–H) ppm; MS (FAB) 247 (12), 203 (15), 195 (20), 161 (30), 171 (100), 102 (70), 93 (60), 60 (15), 36 (62).

Anal. Calcd. for $C_{13}H_{13}O_2N_5S$: C, 51.48; H, 4.29; N, 23.10. Found: C, 51.45; H, 4.30; N, 23.11.

*2-p-Chlorophenyl-5-methyl-4-(5′-methyl-[1,3,4]-thiadiazol-2′-yl)-2,4-dihydro-3-oxo-[1,2,4]-triazole (**6n**)*

Recrystallisation from methanol; pale yellow needles. Yield 80%. m.p. 189–190°C. IR 1708 cm^{-1} (C=O), 1635 cm^{-1} (C=N), 2942 cm^{-1} (C–H); ^1H NMR ($CDCl_3$) $\delta = 2.21$ (s, 3H, C_5'–CH_3), 2.37 (s, 3H, C_5–CH_3), 7.28–7.94 (m, 4H, Ar–H) ppm; MS (FAB) 241, 197 (20), 189 (25), 155 (40), 138 (100), 96 (65), 87 (55), 54 (20), 30 (65) Anal. Calcd. for $C_{12}H_{10}ON_5SCl$: C, 44.58; H, 3.10; N, 21.67. Found: C, 44.54; H, 3.12; N, 21.61.

Antimicrobial assay

Antimicrobial activities of newly synthesized compounds were evaluated using microbroth dilution method (Koneman *et al*., 1997). Micro organisms used were *E. coli*, *B. cereus*, for antibacterial and *C. albicans*, *A. niger* for antifungal activity analyses. Microbroth dilution susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in DMF. Dilution series using sterile distilled water were prepared from 4 to 0.007 mg/ml in micro test tubes and were transferred to 96-well microtitre plates. Bacterial and fungal suspensions that were grown overnight in double strength Muller-Hinton broth were standardised to 10^8 CFU/ml using Mc Farland no. 0.5 standard solution. 100 µl of each organism suspension was then added into wells. The last well chain without any micro-organisms was used as negative control. Sterile distilled water and the medium served as positive growth control. After incubation at 37°C for 18–24 h, the first well without turbidity, was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 1.

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