Synthesis and antimicrobial activity of styryl/pyrrolyl/pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl amines and styryl/pyrrolyl/pyrazolyl sulfonylmethyl-1,3,4-thiadiazolyl amines

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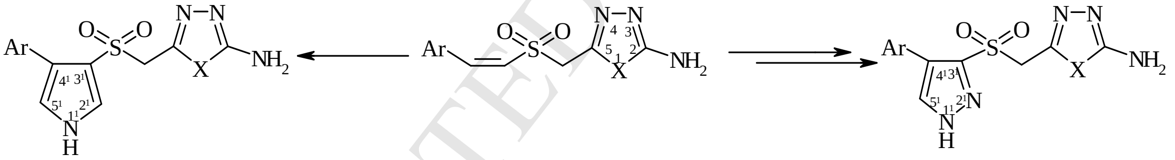
Graphical abstract

Synthesis and Antimicrobial Activity of Styryl / Pyrrolyl / Pyrazolyl Sulfonylmethyl-1,3,4-Oxadiazolyl Amines and Styryl / Pyrrolyl / Pyrazolyl Sulfonylmethyl-1,3,4-Thiadiazolyl Amines

Gundala Sravya, Gudi Yamini, Venkatapuram Padmavathi, Adivireddy Padmaja*

A new class of mono and bis heterocycles - styryl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines, pyrrolyl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines and pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines were prepared from the synthetic intermediate *Z*-styrylsulfonylacetic acid adopting simple and well versed synthetic methodologies and studied their antimicrobial activity. Amongst all the tested compounds styryl thiadiazole **5c** exhibited promising antimicrobial activity against *P. aeruginosa* and *P. chrysogenum*.



6 / 7

4 / 5

10 / 11

4 / 6 / 10 : X = O
5 / 7 / 11 : X = S

Synthesis and antimicrobial activity of styryl / pyrrolyl / pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl amines and styryl / pyrrolyl / pyrazolyl sulfonylmethyl-1,3,4-thiadiazolyl amines

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ABSTRACT

A new class of mono and bis heterocycles - styryl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines, pyrrolyl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines and pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl amines were prepared from the synthetic intermediate *Z*-styrylsulfonylacetic acid adopting simple and well versed synthetic methodologies and studied their antimicrobial activity. Amongst all the tested compounds styryl thiadiazole **5c** exhibited promising antimicrobial activity against *P. aeruginosa* and *P. chrysogenum*.

Keywords: Antimicrobial activity / Semicarbazide / Thiosemicarbazide / 1,3-Dipolar cycloaddition

1. Introduction

Nitrogen containing heteroarenes have great utility in synthetic medicinal and material chemistry. One such class of compounds are oxadiazoles, thiadiazoles, pyrroles and pyrazoles. 1,3,4-Oxadiazoles are associated with potent pharmacological activities due to the presence of toxophoric –N=C–O linkage and display antimicrobial [1], antimycobacterial [2], antiviral [3] and anticancer [4] activities. Further, they are very good bioisosteres of amides and esters, which can contribute substantially in increasing pharmacological activity by participating in hydrogen bonding interactions with the receptors. Thiadiazole derivatives are known to possess anticancer [5], antibacterial [6], antifungal [7], anti-inflammatory [8] and analgesic [9] activities. On the other hand, pyrroles have been widely used as building blocks in total synthesis of many natural products and exhibit biological activities such as powerful antibacterial, antiviral, anti-inflammatory, antitumor, and antioxidant activities [10]. Pyrrole containing drugs Netropsin and Distamycin are naturally anticancer antibiotics [11] (Fig. 1). Among the various synthetic methods, the notable classical ones are Knorr [12] and Hantzsch [13] including multicomponent and metal-catalyzed routes [14]. Literature survey indicates that pyrazole derivatives are well known for their antibacterial [15], antifungal [16], antimicrobial [17], anticancer [18], analgesic and anti-inflammatory [19, 20] activities. Pyrazole moiety makes the core structure of various drugs such as Difenamizole [21], Celecoxib [22], Tepoxalin [23] etc (Fig. 1). The 1,3-dipolar cycloaddition methodology is widely used for the syntheses of pyrazoles using diverse synthons such as nitrilimines and alkynes [24] hydrazones and nitroolefins [25] and azomethine imines and alkynes [26]. In recent years there is still a great demand for designing new compounds and broad spectrum antimicrobial agents remains a challenge for medicinal chemistry researchers. Motivated by the aforesaid findings and in continuation of our studies towards the development of a variety of biologically potent heterocycles [27], herein we plan to synthesize a new class of mono and bis heterocycles – styryl / pyrrolyl / pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl amines and styryl / pyrrolyl / pyrazolyl sulfonylmethyl-1,3,4-thiadiazolyl amines and to study their antimicrobial activity.

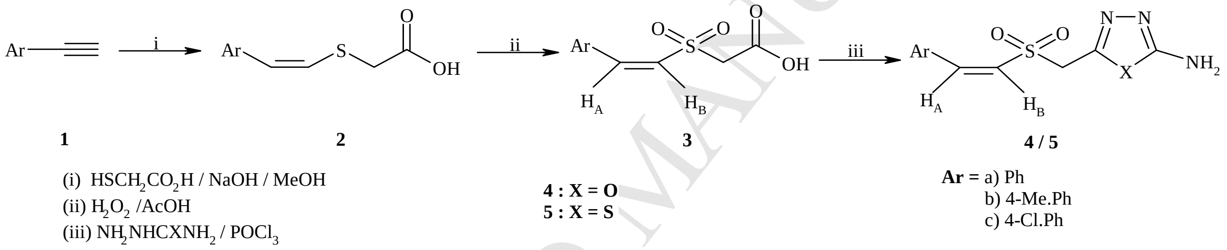
2. Chemistry

A new class of mono and bis heterocycles- 5-*Z*-styrylsulfonylmethyl-1,3,4-oxadiazolyl / thiadiazolyl amines, pyrrolyl sulfonylmethyl-1,3,4-oxadiazolyl / thiadiazolyl amines and pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl / thiadiazolyl amines from the synthetic intermediate

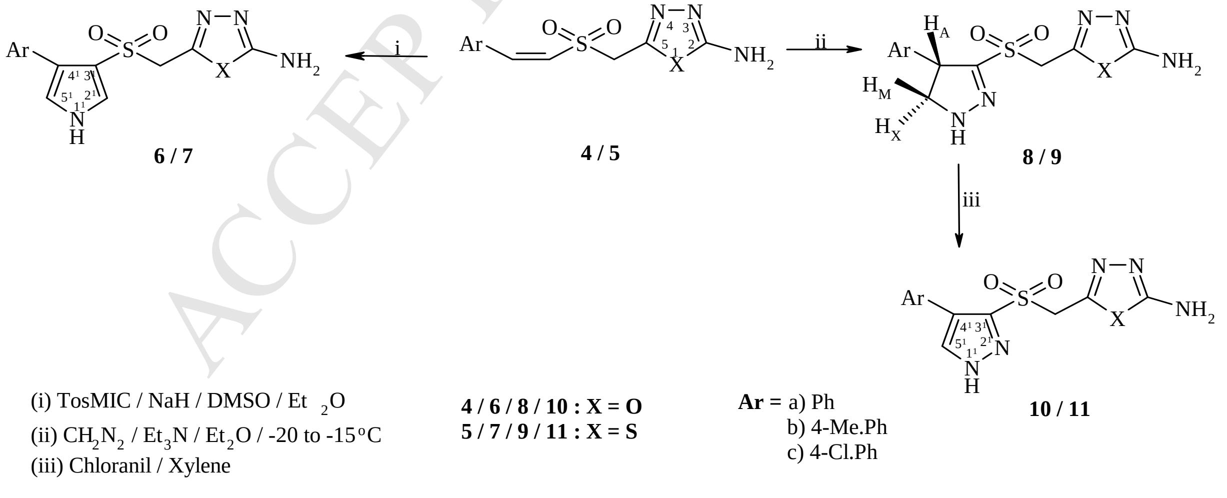
Z-styrylsulfonylacetic acid (**3**) (Schemes 1 and 2). The cyclocondensation of compound **3** with semicarbazide / thiosemicarbazide in the presence of $POCl_3$ produced 5-(2-arylethenesulfonylmethyl)-1,3,4-oxadiazol-2-amine (**4**) / 5-(2-arylethenesulfonylmethyl)-1,3,4-thiadiazol-2-amine (**5**). The ^1H NMR spectra of **4a** and **5a** displayed a singlet at δ 5.20 and 5.24 due to methylene protons attached to C-5. Apart from this, a doublet was observed in these compounds at 6.84 and 6.93 ppm due to olefin proton H_B and another doublet due to olefin proton, H_A adjacent to aryl group appeared at downfield region and merged with aromatic protons. The coupling constant value $J \approx 9.3$ Hz indicated that they possess *cis* geometry. Moreover, a broad singlet appeared at 4.25 and 4.30 ppm in **4a** and **5a** was assigned to NH_2 which disappeared on deuteration.

The olefin moiety present in compounds **4** and **5** was utilized to develop five membered heterocycles- pyrroles and pyrazoles. The 5-(4-aryl-1*H*-pyrrole-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**6**) and 5-(4-aryl-1*H*-pyrrole-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**7**) were prepared by the treatment of **4** and **5** with tosylmethyl isocyanide in the presence of sodium hydride in a solvent mixture of DMSO and ether. The ^1H NMR spectra of **6a** and **7a** exhibited a singlet at δ 5.02 and 5.08 ppm due to methylene protons attached to C-5 of oxadiazole and thiadiazole. In addition to these, a singlet was observed at 6.64 and 6.69 due to C_5'-H of pyrrole ring and another singlet due to C_2'-H appeared at downfield region and merged with aromatic protons. Moreover, in **6a** and **7a** two broad singlets appeared at 5.38, 8.70 and 5.43, 8.73 ppm were attributed to NH_2 and NH of pyrrole ring, respectively. The signals of highly acidic protons disappeared when D_2O was added. The cycloadditon of diazomethane to **4** and **5** in the presence of Et_3N in ether at -20 to -15ºC for 42-48 h gave 5-(4,5-dihydro-4-aryl-1*H*-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**8**) / 5-(4,5-dihydro-4-aryl-1*H*-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**9**). In the ^1H NMR spectra of **8a** and **9a** an AMX splitting pattern was observed due to pyrazoline ring protons. Thus three double doublets present at δ 3.54, 4.21, 4.56 in

8a and at 3.56, 4.29, 4.60 ppm in **9a** were assigned to H_X, H_M and H_A, respectively. The coupling constant values $J_{AM} \approx 12.2$, $J_{MX} \approx 10.4$ and $J_{AX} \approx 6.1$ Hz indicated that H_A, H_M are *cis*, H_A, H_X are *trans* while H_M, H_X are *geminal*. Further **8a** and **9a** displayed signals at δ 4.91, 4.95 (CH_2), 5.51, 5.55 (NH_2) and 10.08, 10.35 ppm (NH) respectively. The signals due to NH_2 and NH disappeared on deuteration. Aromatization of the compounds **8** and **9** with chloranil in xylene provided 5-(4-phenyl-1*H*-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**10**) and 5-(4-phenyl-1*H*-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**11**). The absence of AMX splitting pattern due to pyrazoline ring protons in **10** and **11** indicated that aromatization took place. The structures of all the compounds were further confirmed by IR, ^{13}C NMR, mass spectra and elemental analyses.



Scheme 1. Synthesis of styrylsulfonylmethyl-1,3,4-oxadiazolylamines and styrylsulfonylmethyl-1,3,4-thiadiazolylamines.



Scheme 2. Synthesis of pyrrolyl / pyrazolyl- sulfonylmethyl-1,3,4,-oxadiazolylamines and pyrrolyl / pyrazolyl- sulfonylmethyl-1,3,4,-thiadiazolylamines

3. Biology

3.1. Antimicrobial activity

The compounds **4–11** were screened for antibacterial activity at four different concentrations 12.5, 25, 50 and 100 μg/well.

4. Results and discussion

4.1 Antibacterial activity

The results of antibacterial activity shown in Table 1 indicated that Gram-negative bacteria were more susceptible towards the tested compounds than Gram-positive ones. It was observed that mono heterocyclic compounds (**4** and **5**) displayed slightly higher activity than the respective bis heterocyclic systems (**6-11**). This may be due to the presence of electron withdrawing styryl moiety in mono heterocyclic derivatives. The compounds having thiadiazole moiety (**5**, **7**, **9**, **11**) were more active than those having oxadiazole moiety (**4**, **6**, **8**, **10**). In fact, the compound **5c** showed excellent activity against *P. aeruginosa* when compared with the standard drug Chloramphenicol. This may be due to the presence of more electronegative chlorine on the aromatic ring. Among bis heterocyclic compounds aromatized derivatives (**6**, **7**, **10** and **11**) were more effective. On the other hand the non-aromatized compounds (**8** and **9**) were inactive. Moreover, it was observed that pyrazole containing bis heterocycles **10** and **11** displayed slightly higher activity than the respective pyrrole containing bis heterocycles **6** and **7**.

4.2. Antifungal activity

All the tested compounds inhibited the spore germination against tested fungi except compound **8**. In general, most of the compounds showed slightly higher antifungal activity towards *P. chrysogenum* than *A. niger*. Amongst all the compounds **5c** displayed greater inhibitory activity particularly against *P. chrysogenum* when compared with the standard drug Ketoconazole (Table 2). Moreover compounds **7c** and **11c** exhibited good activity. In fact compounds having pyrazole and pyrrole in combination with thiadiazole were displayed high inhibitory activity than others.

4.3. MIC, MBC and MFC of compounds 5c, 7c and 11c

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of the compounds tested are listed in Table 3. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. (But it is not sure that the microorganisms are completely killed.) The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungi. The MBC/MFC involves an additional set of steps performed once the minimum inhibitory concentration (MIC) is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC. The compound **5c** exhibited low MIC values when compared with **7c** and **11c**. In addition MBC value in **5c** was 2×MIC in case of *P. aeruginosa* and MFC value was 2×MIC in case of *P. chrysogenum*. However, the other compounds showed bactericidal and fungicidal effects greater than 2×MIC. The structure–antimicrobial activity relationship of the synthesized compounds revealed that mono heterocyclic compounds with extended conjugation displayed greater activity than the corresponding bis heterocycles. Amongst bis heterocyclic systems, the aromatized compounds having thiadiazole moiety (**7**, **11**) were more effective when compared with those having oxadiazole unit (**6**, **10**). The chloro substituted styryl-1,3,4-thiadiazole **5c** exhibited excellent antibacterial activity against *P. aeruginosa* with an inhibition zone of 34 mm at 100 µg/well and MIC and MBC of 6.25 and 12.5 µg/ml, respectively. The compound **5c** also displayed strong antifungal activity against *P. chrysogenum* with an inhibition zone of 41 mm at 100 µg/well and MIC and MFC of 12.5 and 25 µg/ml, respectively. Moreover, it was observed that the compounds having chloro substituent on aromatic ring enhanced the activity when compared with methyl and unsubstitued compounds.

5. Conclusion

In conclusion we have prepared a new class of mono and bis heterocycles-styrylsulfonymethyl-1,3,4-oxadiazolyl / thiadiazolyl amines, pyrrolyl sulfonylmethyl-1,3,4-oxadiazolyl / thiadiazolyl amines and pyrazolyl sulfonylmethyl-1,3,4-oxadiazolyl / thiadiazolyl amines from the simple substrate Z-styrylsulfonylacetic acid adopting simple and well versed synthetic methodologies. All the new compounds were tested for antimicrobial activity. In general compounds having thiadiazole unit displayed higher antimicrobial activity than those with oxadiazole moiety. The compound **5c** having chloro substituent on the aromatic ring showed promising antimicrobial activity against *P. aeruginosa* and *P. chrysogenum*.

6. Experimental

6.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$ / DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$ / DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electrospray ionization. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. The starting compound *Z*-styrylsulfonylacetic acid (**3**) was prepared as per the literature precedent [28].

*6.1.1. General procedure for the synthesis of 5-(2-arylethenesulfonylmethyl)-1,3,4-oxadiazol-2-amine / 5-(2-arylethenesulfonylmethyl)-1,3,4-thiadiazol-2-amine (**4a-c / 5a-c**)*

To an equimolar mixture (1 mmol) of semicarbazide / thiosemicarbazide and *Z*-styrylsulfonylacetic acid (**3a-c**), $POCl_3$ (7 ml) was added and heated under reflux for 4-6 h. The excess $POCl_3$ was removed under vacuum and the residue was poured onto crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water. It was dried and recrystallized from ethanol.

*6.1.1.1. 5-(2-Phenylethenesulfonylmethyl)-1,3,4-oxadiazol-2-amine (**4a**).* White solid (0.117g, 67%); m.p. 109-111^{o}C; IR (KBr): 1150, 1324 (SO_2), 1571 (C=N), 1615 (C=C), 3337, 3445 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 4.25 (bs, 2H, NH_2), 5.20 (s, 2H, CH_2), 6.84 (d, 1H, H_B, J = 9.2 Hz), 7.15-7.23 (m, 6H, H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d$_6$): δ 50.5 (CH_2), 132.0 (C-H_B), 142.2 (C-H_A), 154.2 (C-5), 159.6 (C-2), 127.1, 128.0, 129.9, 130.0 (aromatic carbons) ppm; HRMS (m/z): 288.2774 [M+Na]; Anal. Calcd. for $C_{11}H_{11}N_3O_3S$: C, 49.87; H, 4.20; N, 15.88; Found: C, 49.80; H, 4.18; N, 15.84%.

*6.1.1.2. 5-(4-Methylstyrylsulfonylmethyl)-1,3,4-oxadiazol-2-amine (**4b**).* White solid (0.181g, 65%); m.p. 118-120^{o}C; IR (KBr): 1148, 1320 (SO_2), 1564 (C=N), 1610 (C=C), 3319, 3438 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 2.26 (s, 3H, Ar-CH_3), 4.21 (bs, 2H, NH_2), 5.16 (s, 2H,CH_2), 6.87 (d, 1H, H_B, J = 8.5 Hz), 7.06-7.20 (m, 5H, H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d$_6$): δ 24.5 (Ar-CH_3), 49.8 (CH_2), 131.4 (C-H_B), 141.7 (C-H_A), 154.0 (C-5), 159.2 (C-2), 126.8, 127.6, 128.4, 129.7 (aromatic carbons) ppm; HRMS (m/z): 302.3032 [M+Na]; Anal. Calcd. for $C_{12}H_{13}N_3O_3S$: C, 51.69; H, 4.70; N, 15.21; Found: C, 51.60; H, 4.69; N, 15.04%.

*6.1.1.3. 5-(4-Chlorostyrylsulfonylmethyl)-1,3,4-oxadiazol-2-amine (**4c**).* White solid (0.206g, 69%); m.p. 127-129^{o}C; IR (KBr): 1153, 1330 (SO_2), 1579 (C=N), 1620 (C=C), 3342, 3452 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 4.27 (bs, 2H, NH_2), 5.22 (s, 2H, CH_2), 6.99 (d, 1H, H_B, J = 9.2 Hz), 7.21-7.29 (m, 5H, H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 50.9 (CH_2), 132.4 (C-H_B), 142.6 (C-H_A), 154.8 (C-5), 160.2 (C-2), 127.6, 128.4, 130.5, 132.4 (aromatic carbons) ppm; HRMS (m/z): 322.7241 [M+Na]; Anal. Calcd. for $C_{11}H_{10}ClN_3O_3S$: C, 44.02; H, 3.35; N, 14.13; Found: C, 44.08; H, 3.36; N, 14.02%.

*6.1.1.4. 5-(2-Phenylethenesulfonylmethyl)-1,3,4-thiadiazol-2-amine (**5a**).* White solid (0.211g, 75%); m.p. 122-124°C; IR (KBr): 1160, 1336 (SO_2), 1580 (C=N), 1625 (C=C), 3339, 3455 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.30 (bs, 2H, NH_2), 5.24 (s, 2H, CH_2), 6.93 (d, 1H, H_B, J = 9.4 Hz), 7.17-7.42 (m, 6H, H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 51.2 (CH_2), 133.2 (C-H_B), 142.8 (C-H_A), 154.4 (C-5), 160.0 (C-2), 128.4, 129.2, 129.8. 130.6 (aromatic carbons) ppm; HRMS (m/z): 304.3431 [M+Na]; Anal. Calcd. for $C_{11}H_{11}N_3O_2S_2$: C, 47.07; H, 3.97; N, 14.99; Found: C, 46.96; H, 3.94; N, 14.93%.

*6.1.1.5. 5-(4-Methylstyrylsulfonylmethyl)-1,3,4-thiadiazol-2-amine (**5b**).* White solid (0.206g, 70%); m.p. 130-132°C; IR (KBr): 1161, 1325 (SO_2), 1563 (C=N), 1622 (C=C), 3332, 3432 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, Ar-CH_3), 4.28 (bs, 2H, NH_2), 5.18 (s, 2H, CH_2), 6.89 (d, 1H, H_B, J = 8.8 Hz), 7.08-7.22 (m, 5H, H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 24.7 (Ar-CH_3), 50.6 (CH_2), 131.9 (C-H_B), 142.5 (C-H_A), 154.2 (C-5), 159.5 (C-2), 127.0, 127.9, 128.7, 130.2 (aromatic carbons) ppm; HRMS (m/z): 318.3711 [M+Na]; Anal. Calcd. for $C_{12}H_{13}N_3O_2S_2$: C, 48.87; H, 4.46; N, 14.36; Found: C, 48.79; H, 4.44; N, 14.23%.

*6.1.1.6. 5-(4-Chlorostyrylsulfonylmethyl)-1,3,4-thiadiazol-2-amine (**5c**).* White solid (0.246, 78%); m.p. 141-143°C; IR (KBr): 1150, 1324 (SO_2), 1571 (C=N), 1615 (C=C), 3337, 3445 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.25 (bs, 2H NH_2), 5.20 (s, 2H CH_2), 6.90 (d, 1H H_B, J = 8.9 Hz), 7.15-7.23 (m, 6H H_A & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 50.5 (CH_2), 132.0 (C-H_B), 142.2 (C-H_A), 154.2 (C-5), 159.6 (C-2), 127.1, 128.0, 129.9, 130.0 (aromatic carbons) ppm; HRMS (m/z):

338.7882 [M+Na]; Anal. Calcd. for $C_{11}H_{10}ClN_3O_2S_2$: C, 41.89; H, 3.18; N, 13.43; Found: C, 41.84; H, 3.19; N, 13.3%.

*6.1.2. General method for the preparation of 5-(4-aryl-1H-pyrrole-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine / 5-(4-aryl-1H-pyrrole-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**6a-c / 7a-c**)*

A mixture of **4a-c / 5a-c** (0.5 mmol) and TosMIC (1 mmol) in Et_2O-DMSO (2:1) was added dropwise under stirring to a suspension of NaH (50 mg) in Et_2O (10 ml) at room temperature and stirring was continued for 4-6 h. Then water was added and the reaction mass was extracted with Et_2O. The ethereal layer was dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent *in vacuo* gave a solid which was purified by column chromatography using silica gel (hexane-ethyl acetate; 4:1).

*6.1.2.1. 5-(4-Phenyl-1H-pyrrole-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**6a**).* White solid (0.095g, 63%); m.p. 136-138°C; IR (KBr): 1136, 1319 (SO_2), 1560 (C=N), 1624 (C=C), 3237 (NH), 3315, 3439 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.02 (s, 2H, CH_2), 5.38 (bs, 2H, NH_2), 6.64 (s, 1H, C_5'-H), 7.17-7.78 (m, 6H, C_2'-H & Ar-H), 8.70 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 56.1 (CH_2), 104.1 (C-4'), 110.3 (C-3'), 117.6 (C-5'), 121.8 (C-2'), 154.5 (C-5), 156.4 (C-2), 127.4, 129.1, 132.5, 136.4 (aromatic carbons) ppm; HRMS (m/z): 327.3137 [M+Na]; Anal. Calcd. For $C_{13}H_{12}N_4O_3S$: C, 51.41; H, 4.00; N, 18.59; Found: C, 51.31; H, 3.97; N, 18.41%.

*6.1.2.2. 5-(4-(4-Methylphenyl)-1H-pyrrole-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**6b**).* White solid (0.105g, 66%); m.p. 144-146°C; IR (KBr): 1132, 1314 (SO_2), 1557 (C=N), 1621 (C=C), 3233 (NH), 3310, 3428 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, Ar-CH_3), 4.98 (s, 2H, CH_2), 5.29 (bs, 2H, NH_2), 6.55 (s, 1H, C_5'-H), 7.09-7.71 (m, 5H, C_2'-H & Ar-H), 8.69 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 23.4 (Ar-CH_3), 55.9 (CH_2), 103.7 (C-4'), 109.4 (C-3'), 116.6 (C-5'), 121.1 (C-2'), 154.6 (C-5), 155.8 (C-2), 126.4, 128.5, 130.2, 135.4 (aromatic carbons) ppm; HRMS (m/z):

341.3412 [M+Na]; Anal. Calcd. for $C_{14}H_{14}N_4O_3S$: C, 52.89; H, 4.44; N, 17.75; Found: C, 52.82; H, 4.43; N, 17.60%.

*6.1.2.3. 5-(4-(4-Chlorophenyl)-1H-pyrrole-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**6c**).* White solid (0.120g, 71%); m.p. 160-162^{o}C; IR (KBr): 1143, 1322 (SO_2), 1566 (C=N), 1629 (C=C), 3242 (NH), 3326, 3466 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.06 (s, 2H, CH_2), 5.42 (bs, 2H, NH_2), 6.67 (s, 1H, C_5'-H), 7.21-7.82 (m, 5H, C_2'-H & Ar-H), 8.75 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 56.4 (CH_2), 104.4 (C-4'), 110.6 (C-3'), 118.3 (C-5'), 122.2 (C-2'), 155.9 (C-5), 156.9 (C-2), 128.2, 130.5, 134.2, 136.9 (aromatic carbons) ppm; HRMS (m/z): 361.7588 [M+Na]; Anal. Calcd. for $C_{13}H_{11}ClN_4O_3S$: C, 46.18; H, 3.30; N, 16.73; Found: C, 46.09; H, 3.27; N, 16.54%.

*6.1.2.4. 5-(4-Phenyl-1H-pyrrole-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**7a**).* White solid 0.107g, 67%); m.p. 148-150^{o}C; IR (KBr): 1142, 1324 (SO_2), 1562 (C=N), 1623 (C=C), 3253 (NH), 3320, 3446 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.08 (s, 2H, CH_2), 5.43 (bs, 2H, NH_2), 6.69 (s, 1H, C_5'-H), 7.28-7.84 (m, 6H, C_2'-H & Ar-H), 8.73 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 56.6 (CH_2), 104.6 (C-4'), 110.9 (C-3'), 117.9 (C-5'), 122.2 (C-2'), 158.1 (C-5), 159.6 (C-2), 128.2, 130.4, 133.5, 136.1 (aromatic carbons) ppm; HRMS (m/z): 343.3792 [M+Na]; Anal. Calcd. for $C_{13}H_{12}N_4O_2S_2$: C; 48.73, H; 3.78, N; 17.49; Found: C, 48.73; H, 3.82; N, 17.70%.

*6.1.2.5. 5-(4-(4-Methylphenyl)-1H-pyrrole-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**7b**).* White solid (0.121g, 73%); m.p. 156-158^{o}C; IR (KBr): 1139, 1310 (SO_2), 1559 (C=N), 1619 (C=C), 3250 (NH), 3,312, 3,432 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, Ar-CH_3), 5.01 (s, 2H, CH_2), 5.32 (bs, 2H, NH_2), 6.62 (s, 1H, C_5'-H), 7.21-7.80 (m, 5H, C_2'-H & Ar-H), 8.71 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 23.8 (Ar-CH_3), 56.6 (CH_2), 104.1 (C-4'), 110.2 (C-3'), 116.9 (C-5'), 121.8 (C-2'), 157.9 (C-5), 159.1 (C-2), 127.1, 129.1, 131.2, 135.8 (aromatic carbons) ppm; HRMS (m/z): 357.4075 [M+Na]; Anal. Calcd. For $C_{14}H_{14}N_4O_2S_2$: C, 50.36; H, 4.15; N, 16.92; Found: C, 50.28; H, 4.22; N, 16.75%.

*6.1.2.6. 5-(4-(4-Chlorophenyl)-1H-pyrrole-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**7c**).* White solid (0.135g, 76%); m.p. 165-167^{o}C; IR (KBr): 1151, 1326 (SO_2), 1573 (C=N), 1630 (C=C), 3262 (NH), 3,331, 3,451 (NH_2) (cm^{-1}); ^{1}H NMR (DMSO-d$_6$): δ 5.10 (s, 2H, CH_2), 5.48 (bs, 2H, NH_2), 6.71 (s, 1H, C$_5$'-H), 7.25-7.88 (m, 5H, C$_2$'-H & Ar-H), 8.77 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d$_6$): δ 56.9 (CH_2), 104.9 (C-4'), 110.9 (C-3'), 118.9 (C-5'), 122.9 (C-2'), 158.4 (C-5), 159.8 (C-2), 129.1, 131.2, 134.9, 136.8 (aromatic carbons) ppm; HRMS (m/z): 377.8248 [M+Na]; Anal. Calcd. for $C_{13}H_{11}ClN_4O_2S_2$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79%.

*6.1.3. General method for the preparation of 5-(4,5-dihydro-4-aryl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine / 5-(4,5-dihydro-4-aryl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**8a-c / 9a-c**)*

To a well cooled solution of **4a-c / 5a-c** (0.5 mmol) in dichloromethane (20 ml), an ice-cold ethereal solution of diazomethane (40 ml, 0.4 M) and triethylamine (0.12 g) were added. The reaction mixture was kept at -20ºC to -15ºC for 42-48 h. The solvent was removed under *vacuum* and the resultant solid was purified by passing through a column of silica gel using hexane-ethyl acetate (3:1) as an eluent.

*6.1.3.1. 5-(4,5-Dihydro-4-phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**8a**).* White solid (0.105g, 68%); m.p. 138-140^{o}C; IR (KBr): 1135, 1321 (SO_2), 1572 (C=N), 1624 (C=C), 3271 (NH), 3354, 3472 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 3.54 (dd, 1H, H_X, J_{AX} = 5.9 Hz, J_{MX} = 10.4 Hz), 4.21 (dd, 1H, H_M, J_{AM} = 11.8 Hz), 4.56 (dd, 1H, H_A), 4.91 (s, 2H, CH_2), 5.51 (bs, 2H, NH_2), 7.16-7.69 (m, 5H, Ar-H), 10.08 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d$_6$): δ 47.1 (CH_2), 52.8 (C-5'), 66.1 (C-4'), 152.3 (C-3'), 158.2 (C-5), 159.5 (C-2), 126.9, 127.6, 129.8, 131.9 (aromatic carbons) ppm; HRMS (m/z): 330.3172 [M+Na]; Anal. Calcd. for $C_{12}H_{13}N_5O_3S$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79%.

*6.1.3.2. 5-(4-(4-Methylphenyl)-4,5-dihydro-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine **(8b)**.* White solid (0.105g, 65%); m.p. 151-153°C; IR (KBr): 1128, 1319 (SO_2), 1584 (C=N), 1620 (C=C), 3267 (NH), 3345, 3463 (NH_2) (cm^{-1}); ^1H NMR (DMSO-d_6): δ 2.26 (s, 3H, Ar-CH_3), 3.47 (dd, 1H, H_X, J_{AX} = 5.6 Hz, J_{MX} = 10.1 Hz), 4.11 (dd, 1H, H_M, J_{AM} = 11.6 Hz), 4.49 (dd, 1H, H_A), 4.81 (s, 2H, CH_2), 5.49 (bs, 2H, NH_2), 7.13-7.66 (m, 4H, Ar-H), 9.93 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 21.2 (Ar-CH_3), 45.9 (CH_2), 52.3 (C-5'), 64.9 (C-4'), 151.8 (C-3'), 157.8 (C-5), 158.6 (C-2), 126.1, 127.2, 128.5, 130.5 (aromatic carbons) ppm; HRMS (m/z): 344.3433 [M+Na]; Anal. Calcd. for $C_{13}H_{15}N_5O_3S$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79 %.

*6.1.3.3. 5-(4-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine **(8c)**.* White solid (0.129g, 76%); m.p. 161-163°C; IR (KBr): 1140, 1327 (SO_2), 1587 (C=N), 1627 (C=C), 3280 (NH), 3,350, 3,480 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.59 (dd, 1H, H_X, J_{AX} = 6.1 Hz, J_{MX} = 10.7 Hz), 4.25 (dd, 1H, H_M, J_{AM} = 12.1 Hz), 4.59 (dd, 1H, H_A), 4.99 (s, 2H CH_2), 5.58 (bs, 2H, NH_2), 7.19-7.75 (m, 4H, Ar-H), 10.14 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 47.4 (CH_2), 53.2 (C-5'), 66.4 (C-4'), 152.9 (C-3'), 158.7 (C-5), 159.6 (C-2), 128.4, 129.1, 130.8, 132.6 (aromatic carbons) ppm; HRMS (m/z): 364.7615 [M+Na]; Anal. Calcd. for $C_{12}H_{12}ClN_5O_3S$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79%.

*6.1.3.4. 5-(4,5-Dihydro-4-phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine **(9a)**.* White solid (0.105g, 65%); m.p. 142-144°C; IR (KBr): 1132, 1331 (SO_2), 1584 (C=N), 1628 (C=C), 3256 (NH), 3366, 3481 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.56 (dd, 1H, H_X, J_{AX} = 6.4 Hz, J_{MX} = 10.5 Hz), 4.29 (dd, 1H, H_M, J_{AM} = 12.6 Hz), 4.60 (dd, 1H, H_A), 4.95 (s, 2H, CH_2), 5.55 (bs, 2H, NH_2), 7.18-7.75 (m, 5H, Ar-H), 10.35 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 47.6 (CH_2), 53.5 (C-5'), 66.5 (C-4'), 152.7 (C-3'), 158.1 (C-5), 159.6 (C-2), 127.7, 128.5, 130.2, 132.4 (aromatic carbons) ppm; HRMS (m/z): 346.3829 [M+Na]; Anal. Calcd. for $C_{12}H_{13}N_5O_2S_2$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79%.

6.1.3.5. 5-(4-(4-Methylphenyl)-4,5-dihydro-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (9b). White solid (0.118g, 70%); m.p. 155-157^{o}C; IR (KBr): 1129, 1324 (SO_2), 1581 (C=N), 1622 (C=C), 3250 (NH), 3355, 3475 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 2.28 (s, 3H, Ar-CH_3), 3.52 (dd, 1H, H_X, J_{AX} = 6.1 Hz, J_{MX} = 10.3 Hz), 4.14 (dd, 1H, H_M, J_{AM}=12.20 Hz), 4.52 (dd, 1H, H_A), 4.87 (s, 2H, CH_2), 5.46 (bs, 2H, NH_2), 7.12-7.70 (m, 4H, Ar-H), 9.98 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d$_6$): δ 21.6 (Ar-CH_3), 46.5 (CH_2), 52.7 (C-5'), 65.4 (C-4'), 151.8 (C-3'), 157.5 (C-5), 158.8 (C-2), 126.4, 127.9, 129.5, 131.9 (aromatic carbons) ppm; HRMS (m/z): 360.4116 [M+Na]; Anal. Calcd. for $C_{13}H_{15}N_5O_2S_2$: C, 44.10; H, 3.16; N, 16.01 %. Found: C, 44.00; H, 3.12; N, 15.79%.

6.1.3.6. 5-(4-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (9c). White solid (0.139g, 78%); m.p. 168-170^{o}C; IR (KBr): 1157, 1335 (SO_2), 1583 (C=N), 1630 (C=C), 3269 (NH), 3374, 3489 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 3.62 (dd, 1H, H_X, J_{AX} = 6.7 Hz, J_{MX} = 10.9 Hz), 4.28 (dd, 1H, H_M, J_{AM} = 12.8 Hz), 4.64 (dd, 1H, H_A), 5.03 (s, 2H, CH_2), 5.60 (bs, 2H, NH_2), 7.22-7.87 (m, 4H, Ar-H), 10.39 (bs, 1H, NH) ppm; ^{13}C NMR (DMSO-d$_6$): δ 47.9 (CH_2), 53.9 (C-5'), 66.9 (C-4'), 153.1 (C-3'), 158.4 (C-5), 159.6 (C-2), 128.6, 129.8, 131.1, 133.4 (aromatic carbons) ppm; HRMS (m/z): 380.8274 [M+Na]; Anal. Calcd. for $C_{12}H_{12}ClN_5O_2S_2$: C, 44.10; H, 3.16; N, 16.01; Found: C, 44.00; H, 3.12; N, 15.79%.

*6.1.4. General method for the preparation of 5-(4-phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine / 5-(4-phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (**10a-c** / **11a-c**)*

A solution of **8a-c** / **9a-c** (1 mmol) and chloranil (1.4 mmol) in xylene (10 ml) was refluxed for 16-18 h. Then the reaction mixture was treated with 5% NaOH solution. The organic layer was separated and repeatedly washed with water. It was dried over anhydrous Na_2SO_4 and the solvent was removed on a rotary evaporator. The resultant solid was recrystallized from 2-propanol.

*6.1.4.1 5-(4-Phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (**10a**).* White solid (0.195g, 64%); m.p. 133-135°C; IR (KBr): 1145, 1328 (SO_2), 1579 (C=N), 1623 (C=C), 3282 (NH), 3329, 3455 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.06 (s, 2H, CH_2), 5.56 (bs, 2H, NH_2), 6.34 (bs, 1H, NH), 6.83-7.77 (m, 6H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 54.1 (CH_2), 133.2 (C-4'), 137.9 (C-5'), 147.5 (C-3'), 157.8 (C-5), 159.3 (C-2), 126.9, 127.3, 128.5, 135.7 (aromatic carbons) ppm; HRMS (m/z): 328.3016 [M+Na]; Anal. Calcd. for $C_{12}H_{11}N_5O_3S$: C, 47.32; H, 3.66; N, 23.14; Found: C, 47.21; H, 3.63; N, 22.94%.

6.1.4.2. 5-(4-(4-Methylphenyl)-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (10b). White solid (0.220g, 69%); m.p. 140-142°C; IR (KBr): 1131, 1323 (SO_2), 1585 (C=N), 1626 (C=C), 3275 (NH), 3318, 3443 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H, Ar-CH_3), 5.02 (s, 2H, CH_2), 5.51 (bs, 2H, NH_2), 6.32 (bs, 1H, NH),6.81-7.72 (m, 5H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 21.5 (Ar-CH_3), 53.9 (CH_2), 131.80 (C-4'), 137.4 (C-5'), 146.7 (C-3'), 157.4 (C-5), 159.1 (C-2), 125.8, 126.6, 128.1, 134.2 (aromatic carbons) ppm; HRMS (m/z): 342.3276 [M+Na]; Anal. Calcd. for $C_{13}H_{13}N_5O_3S$: C, 48.84; H, 4.11; N, 22.01; Found: C, 48.89; H, 4.10; N, 21.93%.

6.1.4.3. 5-(4-(4-Chlorophenyl)-1H-pyrazol-3-sulfonylmethyl)-1,3,4-oxadiazol-2-amine (10c). White solid (0.244g, 72%); m.p. 158-160°C; IR (KBr): 1140, 1334 (SO_2), 1565 (C=N), 1612 (C=C), 3288 (NH), 3325, 3471 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.12 (s, 2H, CH_2), 5.62 (bs, 2H, NH_2), 6.38 (bs, 1H, NH), 6.90-7.81 (m, 5H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 54.8 (CH_2), 133.9 (C-4'), 138.2 (C-5'), 148.4 (C-3'), 158.2 (C-5), 159.6 (C-2), 127.1, 129.6, 130.5, 136.2 (aromatic carbons) ppm; HRMS (m/z): 362.7482 [M+Na]; Anal. Calcd. for $C_{12}H_{10}ClN_5O_3S$: C, 42.51; H, 2.99; N, 20.76; Found: C, 42.42; H, 2.97; N, 20.61%.

6.1.4.4. 5-(4-Phenyl-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (11a). White solid (0.215g, 67%); m.p. 146-148°C; IR (KBr): 1146, 1337 (SO_2), 1563 (C=N), 1615 (C=C), 3267 (NH), 3338, 3465 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.10 (s, 2H, CH_2), 5.57 (bs, 2H, NH_2), 6.42

(bs, 1H, NH), 6.95-7.86 (m, 6H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 54.6 (CH_2), 133.8 (C-4'), 138.4 (C-5'), 148.2 (C-3'), 158.1 (C-5), 159.7 (C-2), 127.3, 128.9, 129.7, 136.2 (aromatic carbons) ppm; HRMS (m/z): 344.3685 [M+Na]; Anal. Calcd. for $C_{12}H_{11}N_5O_2S_2$: C, 44.92; H, 3.43; N, 21.92; Found: C, 44.85; H, 3.45; N, 21.79%.

6.1.4.5. 5-(4(4-Methylphenyl)-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (11b). White solid (0.248g, 74%); m.p. 155-157^{o}C; IR (KBr): 1134, 1332 (SO_2), 1567 (C=N), 1600 (C=C), 3259 (NH), 3327, 3454 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d6): δ 2.34 (s, 3H, Ar-CH_3), 5.07 (s, 2H, CH_2), 5.49 (bs, 2H, NH_2), 6.40 (bs, 1H, NH), 6.91-7.75 (m, 5H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 21.9 (Ar-CH_3), 54.1 (CH_2), 133.1 (C-4'), 137.4 (C-5'), 147.9 (C-3'), 157.7 (C-5), 159.5 (C-2), 126.8, 127.9, 128.6, 135.2 (aromatic carbons) ppm; HRMS (m/z): 358.3931 [M+Na]; Anal. Calcd. for $C_{13}H_{13}N_5O_2S_2$: C, 46.66; H, 3.93; N, 21.07; Found: C, 46.55; H, 3.91; N, 20.88%.

6.1.4.6. 5-(4-(4-Chlorophenyl)-1H-pyrazol-3-sulfonylmethyl)-1,3,4-thiadiazol-2-amine (11c). White solid (0.284g, 80%); m.p. 165-167^{o}C; IR (KBr): 1150, 1340 (SO_2), 1580 (C=N), 1618 (C=C), 3274 (NH), 3333, 3470 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.16 (s, 2H, CH_2), 5.63 (bs, 2H, NH_2), 6.44 (bs, 1H, NH), 7.04-7.87 (m, 5H, C_5'-H & Ar-H) ppm; ^{13}C NMR (DMSO-d_6): δ 55.1 (CH_2), 134.2 (C-4'), 139.5 (C-5'), 148.7 (C-3'), 158.4 (C-5), 159.9 (C-2), 128.2, 129.8, 130.5, 137.2 ppm (aromatic carbons) ppm; HRMS (m/z): 378.8129 [M+Na]; Anal. Calcd. for $C_{12}H_{10}ClN_5O_2S_2$: C, 40.57; H, 2.84; N, 19.81; Found: C, 40.51; H, 2.83; N, 19.68%.

6.2. Biological assays

6.2.1. Compounds

The compounds **4-11** were dissolved in DMSO at different concentrations of 12.5, 25, 50 and 100 µg/well.

6.2.2. Cells

Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative) and *fungi Aspergillus niger*, *Penicillium chrysogenum* were obtained from Department of Microbiology, S.V University, Tirupati, India.

6.2.3. Antimicrobial activity

The *in vitro* antimicrobial study was carried out by agar well diffusion method against test organisms [29, 30]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 µl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/ml and from this 2.5, 5, 10 and 20 µl (12.5, 25, 50, 100 µg/well) were added into the wells by using sterile pipettes. Simultaneously the standard antibiotics, Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO which showed no zone of inhibition acts as negative control. The plates were incubated at 37^{o}C for 24 h for bacteria and at 28^{o}C for 48 h for fungi. After appropriate incubation the diameter of zone of inhibition of each well was measured. Duplicates were maintained and the average values were calculated for eventual antimicrobial activity.

Broth dilution test was used to determine minimum inhibitory concentration (MIC) of the above mentioned samples [31, 32]. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus, B. subtilis, P. aeruginosa* and *K. pneumoniae* and the test fungi *A. niger* & *P. chrysogenum* were diluted 100 folds in nutrient broth (100 µl bacterial cultures in 10 ml NB). The stock solution of the synthesized compounds was prepared in dimethyl sulfoxide (DMSO) by dissolving 5 mg of the compound in 1 ml of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, 40 µl of stock solution contains 6.25, 12.5, 25, 50, 100, 200 µg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37^{o}C for 24 h for bacteria and at 28^{o}C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

To determine the minimum bactericidal concentration (MBC) [33] and minimum fungicidal concentration (MFC) [34] for each set of test tubes in the MIC determination a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37^{o}C for 24 h and at 28^{o}C for 48 h respectively. After incubation the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

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Table 1. The *in vitro* antibacterial activity of compounds **4−11**.

Compound	Zone of inhibition (mm)															
	Gram-positive bacteria								Gram-negative bacteria							
	S. aureus				*B. subtilis*				*P. aeruginosa*				*K. pneumoniae*			
	12.5 µg/ well	25 µg/ well	50 µg/ well	100 µg/ well	12.5 µg/ well	25 µg/ well	50 µg/ well	100 µg/ well	12.5 µg/ well	25 µg/ well	50 µg/ well	100 µg/ well	12.5 µg/ well	25 µg/ well	50 µg/ well	100 µg/ well
4a	11±2	13±1	15±3	18±2	12±3	13±1	14±1	16±2	14±3	16±3	18±2	20±2	15±2	17±3	19±2	21±3
4b	-	-	12±1	14±3	8±2	9±3	11±2	14±1	12±2	14±1	16±3	18±3	11±3	13±2	15±3	17±2
4c	12±3	14±1	16±3	20±1	13±2	14±2	15±3	17±3	15±1	17±2	19±1	21±2	16±1	18±1	20±2	22±1
5a	21±1	22±3	23±2	25±2	27±1	28±1	29±1	31±2	23±3	25±3	27±2	29±1	27±2	29±3	31±1	33±3
5b	19±3	20±2	21±3	23±1	25±3	26±1	27±2	29±3	21±2	23±2	25±3	27±2	23±1	25±2	27±2	29±1
5c	22±2	23±1	24±2	26±3	28±2	29±2	30±1	32±3	24±3	26±3	28±2	34±3	32±2	34±3	36±2	38±2
6a	-	-	-	11±1	-	-	-	12±1	08±1	10±2	12±1	14±2	-	-	11±3	13±3
6b	-	-	-	10±3	-	-	-	10±2	-	-	10±2	11±1	-	-	-	12±1
6c	07±1	08±1	09±1	12±2	-	-	11±3	13±1	09±3	11±3	13±1	15±3	08±1	10±2	12±2	14±2
7a	17±3	19±3	22±2	24±1	20±1	21±3	22±1	24±3	19±1	21±2	23±2	25±1	26±2	27±1	29±2	31±1
7b	15±2	17±2	19±2	22±3	18±2	19±1	20±2	22±2	17±2	19±1	21±3	23±3	20±3	22±3	24±3	26±3
7c	17±1	19±1	21±3	23±1	21±3	22±3	23±3	25±3	20±3	22±3	24±1	26±1	28±2	30±2	32±2	34±2
8a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
8c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10a	-	-	10±1	14±3	07±1	09±2	11±3	15±2	11±3	13±3	15±1	17±2	10±3	12±3	14±3	16±2
10b	-	-	-	12±2	06±2	07±1	09±2	11±1	09±1	10±1	12±2	14±2	-	-	12±2	14±1
10c	08±1	10±2	12±1	15±1	08±3	09±3	12±1	13±3	12±2	14±3	16±3	18±3	11±1	13±3	15±1	17±3
11a	20±3	21±1	22±3	24±3	25±1	26±2	27±3	29±1	22±1	23±2	25±2	27±1	26±2	28±2	30±3	32±1
11b	16±1	17±3	18±1	20±2	21±2	22±1	23±1	25±2	20±3	22±3	24±1	26±2	22±2	24±1	26±3	28±3
11c	21±3	22±1	23±3	25±2	26±3	27±3	28±3	30±3	24±2	26±1	28±3	31±3	30±3	32±2	34±2	36±3
Chloramphenicol	30±1	32±2	35±2	37±1	32±3	34±2	36±1	40±1	25±2	27±2	29±2	32±1	38±2	40±2	42±1	44±2
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-) No activity, (±) Standard deviation.

Table 2. The *in vitro* antifungal activity of compounds **4−11**.

Compound	Zone of inhibition (mm)							
	A. niger				*P. chrysogenum*			
	12.5 *µ*g/ well	25 *µ*g/ well	50 *µ*g/ well	100 *µ*g/ well	12.5 *µ*g/ well	25 *µ*g/ well	50 *µ*g/ well	100 *µ*g/ well
4a	09±2	11±3	13±3	15±3	11±3	13±3	15±2	17±3
4b	08±3	10±1	11±2	13±2	10±2	12±2	14±3	16±2
4c	13±3	15±2	17±1	19±1	15±1	16±1	18±2	20±1
5a	25±2	27±1	29±3	31±3	29±3	34±2	36±3	35±2
5b	22±1	24±3	26±2	28±2	25±2	27±3	29±2	31±3
5c	27±3	29±2	31±1	32±3	34±1	35±2	37±1	41±2
6a	-	08±1	10±2	12±1	11±1	12±3	14±3	16±1
6b	-	-	07±2	09±2	08±2	10±1	12±1	14±2
6c	-	10±3	09±2	14±3	12±2	14±3	16±2	18±3
7a	12±2	14±1	16±3	18±1	25±1	27±2	29±2	31±2
7b	09±3	11±2	13±2	15±2	22±3	24±1	26±3	28±1
7c	15±2	17±3	19±1	21±3	29±2	32±3	34±2	37±3
8a	-	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-
8c	-	-	-	-	-	-	-	-
9a	-	-	07±1	09±2	-	-	-	12±2
9b	-	-	4±3	08±1	-	-	-	11±1
9c	-	-	10±2	11±2	-	-	10±2	13±2
10a	-	09±2	11±1	13±1	-	12±2	14±3	16±2
10b	-	08±1	10±2	10±3	-	-	11±1	14±3
10c	-	09±2	11±2	13±3	12±1	13±3	15±2	17±2
11a	23±3	25±2	27±1	29±2	30±2	32±2	34±1	36±1
11b	21±2	23±3	25±3	27±3	28±3	30±1	32±2	34±3
11c	24±1	26±1	28±1	30±2	31±2	33±3	35±3	38±2
Ketoconazole	29±3	31±2	34±1	37±1	34±1	36±1	37±2	39±2
Control (DMSO)	-	-	-	-	-	-	-	-

(-) No activity, (±) Standard deviation.

Table 3. MIC, MBC and MFC of compounds **5c**, **7c** and **11c**.

Compound	Minimum inhibitory concentration					
	MIC (MBC / MFC) μg/ml					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K.pneumoniae*	*A. niger*	*P. chrysogenum*
5c	25(>100)	25(100)	6.25(12.5)	12.5(50)	12.5(50)	12.5(25)
7c	50(>200)	50(>200)	25(100)	25(>100)	25(100)	50(>200)
11c	50(200)	25(100)	12.5(50)	25(>100)	25(100)	12.5(50)
Chloramphenicol	6.25	6.25	6.25	12.5	-	-
Ketoconazole	-	-	-	-	6.25	12.5

(-) No activity

Figure 1. Drugs containing pyrrole and pyrazole moieties.

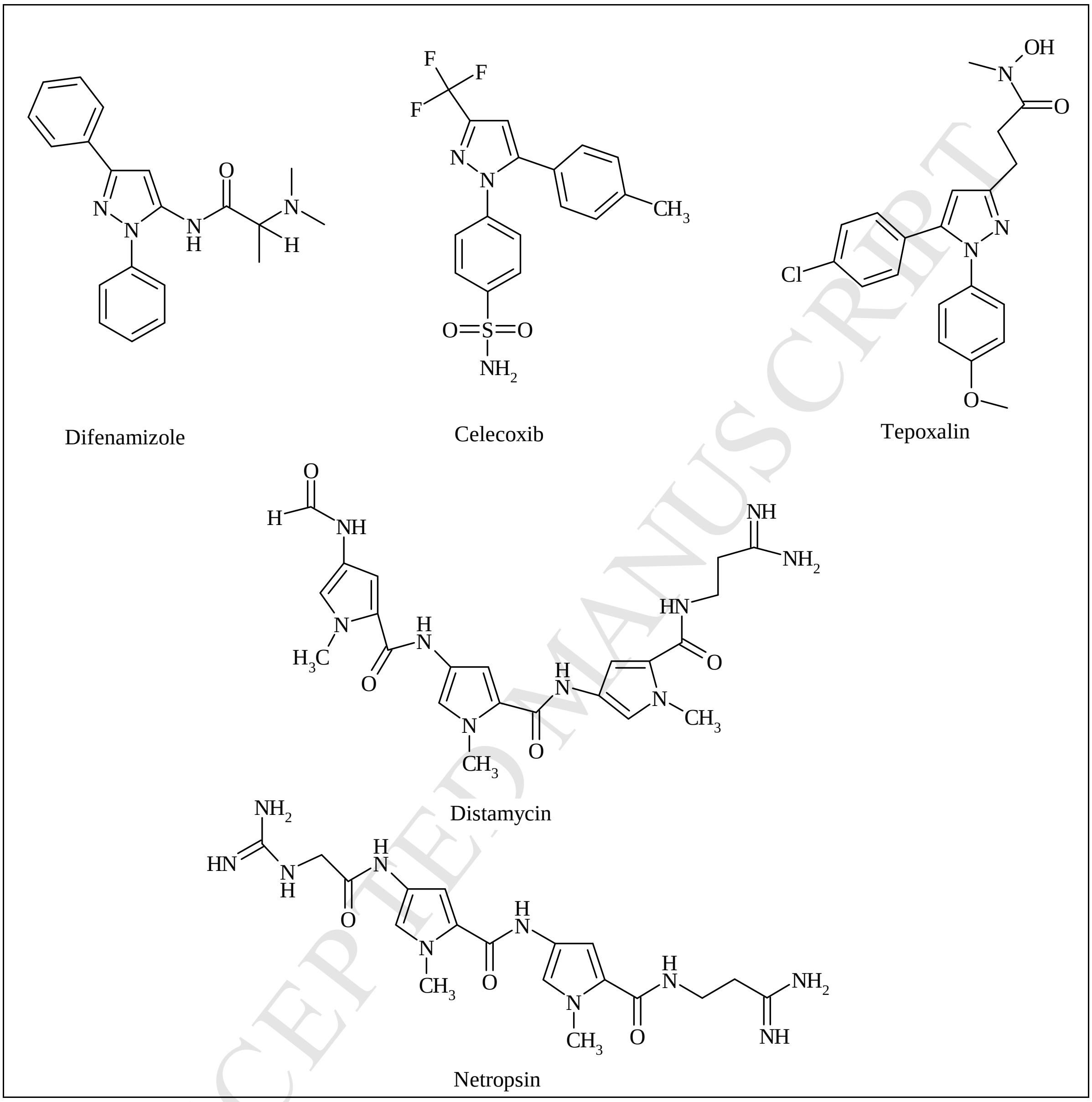


Figure 2. The *in vitro* antibacterial activity of compounds **4−11**.

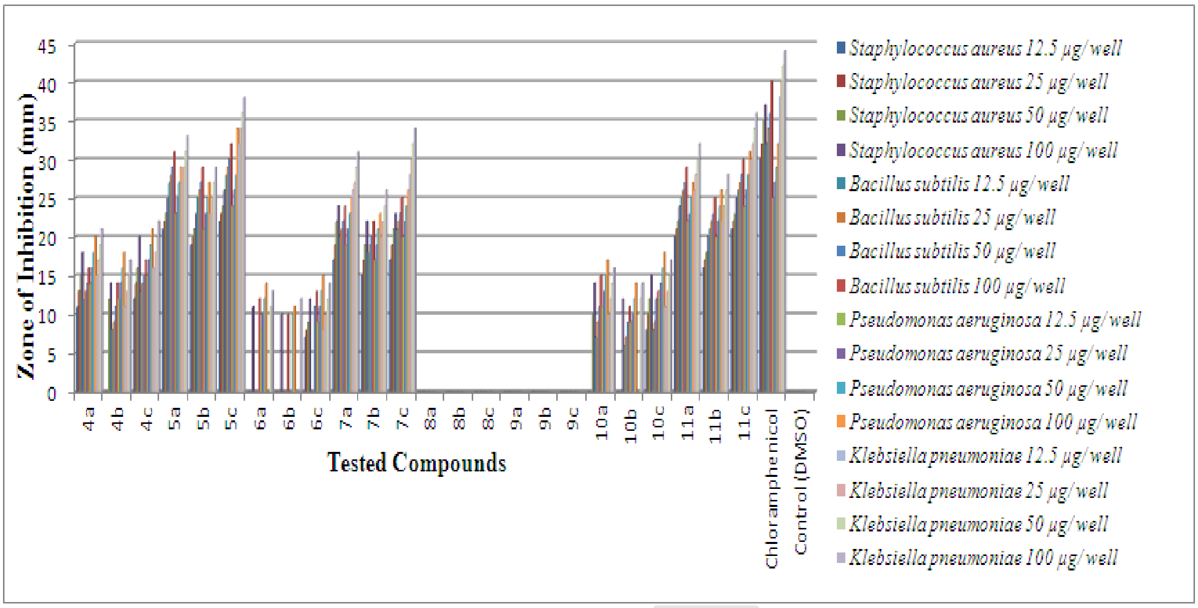
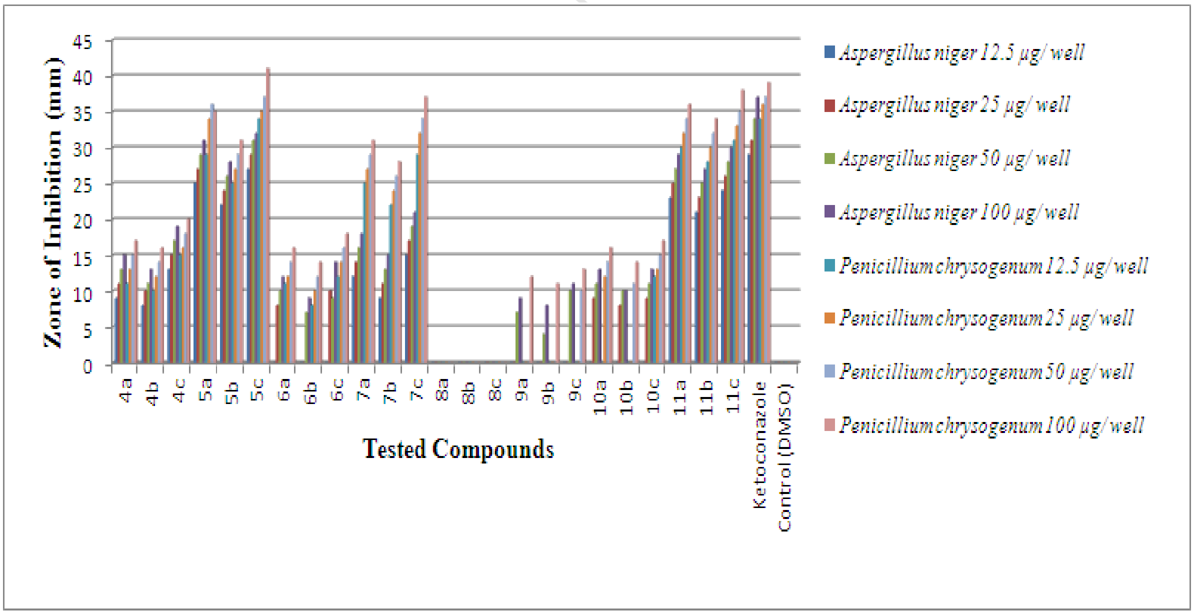


Figure 3. The *in vitro* antifungal activity of compounds **4−11**.



Research Highlights

- We have synthesized Pyrrolyl Sulfonylmethyl-1,3,4-Oxadiazolyl / 1,3,4-Thiadiazolyl Amines and Pyrazolyl Sulfonylmethyl-1,3,4-Oxadiazolyl / 1,3,4-Thiadiazolyl Amines
- Mono heterocyclic compounds (**4**, **5**) displayed slightly higher antimicrobial activity than the respective bis heterocyclic systems (**6-11**).
- In all the tested compounds 5-(4-Chlorostyrylsulfonylmethyl)-1,3,4-thiadiazol-2-amine showed greater antimicrobial activity.