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ORIGINAL ARTICLE

Synthesis, antimicrobial evaluation and hemolytic activity of 2-[[5-alkyl/aralkyl substituted-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl) phenyl]acetamide derivatives

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Abstract 2,5-Disubstituted 1,3,4-oxadiazole compounds are one of the most attractive classes for researchers due to their pharmacological activities. In the current research, a new series of 2-[[5-alkyl/aralkyl-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl)phenyl]acetamides (**6a**–**m**) were prepared by converting different aryl/aralkyl organic acids (**1a**–**m**) successively into corresponding esters (**2a**–**m**), hydrazides (**3a**–**m**) and 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols (**4a**–**m**). Finally, the target compounds **6a**–**m** were synthesized by stirring 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols (**4a**–**m**) with 2-bromo-*N*-[4-(4-morpholinyl)phenyl]acetamide (**5**) in the presence of *N*,*N*-dimethyl-formamide (DMF) and sodium hydride (NaH). The structures of the synthesized compounds were elucidated through IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The compounds were also screened for antimicrobial and hemolytic activity and most of them were found to be active against the selected microbial species at variable extent relative to reference standards. The compounds, **6d** and **6f** were active against the selected panel of microbes and the former was the most potent one.

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This series showed less toxicity and may be considered for further biological screening and application trial except **6h** and **6l**, possessing higher cytotoxicity.

1. Introduction

Therapeutically active and new drug constituents are needed for the treatment of different diseases and disorders due to increased resistance against the existing drugs, so organic chemists and pharmacists are endeavoring to contribute in this field for new compounds with great therapeutic potential [8,17].

Heterocyclic class of organic compounds consisting of a five membered ring containing one oxygen and two nitrogen atoms is called Oxadiazole. Therapeutically most important and active isomer among all the oxadiazoles is the 1,3,4-oxadiazole ring [6]. During the last decade a variety of oxadiazole derivatives were prepared and analyzed for antimicrobial and enzyme inhibition potential [12]. Oxadiazole class of compounds is known for its antitubercular, antiviral, antibacterial, insecticidal, anticancer, anticonvulsant, anti-inflammatory, and analgesic potential [15,10,18,7,20].

Six membered heterocyclic compound, morpholine and its derivatives are important in pharmaceutics due to their great enzyme inhibition activity and antimicrobial potential [13]. C-substituted morpholine moiety, chiral and achiral derivatives of morpholine are known for their biological activity [9]. Synthesis of new morpholine containing compounds is the area of interest for the pharmacists because therapeutically potent compounds are the need of hour to cure different malfunctions and diseases [19].

2. Experimental protocol

2.1. Materials and methods

All the chemical reagents and solvents of analytical grade purchased from local supplier were of Sigma Aldrich and Alfa Aesar. By the open capillary method with the help of Griffin & George melting point apparatus, melting points were measured for all the compounds and were uncorrected. To confirm the purity of synthesized compounds, F_{256} 20×20 cm, silica coated TLC plates were utilized with *n*-Hexane and EtOAc as solvent system. ^{1}H-NMR spectra were recorded by utilizing 400 MHz Bruker spectrometers while ^{13}C-NMR spectra were taken at 75 and 100 MHz Bruker spectrometers, indicating chemical shift value on ppm scale. TMS was taken as reference standard. Jasco-320-A spectrophotometer was used to record IR spectra by utilizing the KBr pellet method. JMS-HX-110 spectrometer was utilized for EIMS spectra.

2.2. General procedure for synthesis of different ethyl aryl/aralkyl carboxylates (*2a*–*m*)

Aryl/aralkyl carboxylic acids (**1a**–**m**, 0.02 mol), the absolute ethanol (50 mL) and conc. H_2SO_4 (5 mL) were taken in a 250 mL round bottom (RB) flask fitted with reflux condenser. The reaction mixture was refluxed for 1.5 h. The reaction completion was established by thin layer chromatography (TLC). After the completion, reaction mass was poured into a separating funnel containing distilled water (50 mL). Diethyl ether (15 mL) was added to the separating funnel and mixture was neutralized by conc. aq. sodium carbonate solution. The solution was shaken and kept still for some time. Lower aqueous layer was discarded and upper ether layer containing required ester was taken in a distillation flask. Diethyl ether was distilled off and the transparent esters (**2a**–**m**) were collected from the flask.

2.3. General procedure for synthesis of different aryl/aralkyl carbohydrazides (*3a*–*m*)

Ethyl aryl/aralkyl carboxylates (**2a**–**m**, 0.018 mol) were dissolved in 30 mL methanol in a 100 mL RB flask. 80% Hydrazine hydrate (0.018 mol) was added and the mixture was stirred or refluxed for 4–5 h till completion, monitored by TLC. Excess of methanol was distilled off and cold distilled water was added along with shaking till the appearance of precipitates. The aryl/aralkyl carbohydrazides, **3a**–**m**, were filtered, washed with distilled water and dried.

2.4. General procedure for synthesis of different 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols (*4a*–*m*)

Aryl/aralkyl carbohydrazides (**3a**–**m**, 0.015 mol) were dissolved in absolute ethanol (30 mL) in a 100 mL RB flask. Carbon disulfide (0.015 mol) was added to the flask followed by the addition of potassium hydroxide (0.03 mol). The mixture was refluxed for 6–7 h along with proper stirring. The reaction completion was monitored by TLC. After complete reaction, the mixture was diluted with distilled water (30 mL) and acidified with dilute HCl to a pH of 2. The precipitated products, 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols **4a**–**m**, were then filtered, washed with distilled water and re-crystallized from ethanol.

2.5. Procedure for synthesis of 2-bromo-N-[4-(4-morpholinyl) phenyl]acetamide (*5*)

4-(4-Morpholinyl)benzenamine (0.1 mmol) was taken in an iodine flask (100 mL). The sodium carbonate solution was added to maintain a pH of 9–10. Equimolar 2-bromoacetyl bromide was introduced drop wise along with vigorous shaking. The liberated gases were released by removing stopper. After complete addition, flask was set to stirring for half an hour and monitored by TLC. The precipitates of title compound were filtered, washed with distilled water and dried for further reactions.

2.6. General procedure for synthesis of 2-[(5-aryl/aralkyl-1,3,4-oxadiazol-2-yl)thio]-N-[4-(4-morpholinyl)phenyl]acetamide (*6a*–*m*)

5-Aryl/aralkyl-1,3,4-oxadiazol-2-thiols (**4a**–m, 0.001 mol) were dissolved in 10 mL DMF in a 100 mL RB flask at room

temperature. After the addition of sodium hydride (0.001 mol) to the reaction mixture, stirring for 0.5 h at room temperature was done. The electrophile, 2-bromo-*N*-[4-(4-morpholinyl)phenyl]acetamide (**5**), was added to reaction mixture in an equimolar ratio and further stirred for 3–5 h. Reaction course was monitored by TLC. After reaction completion, the ice cold distilled water was poured into reaction contents with shaking till the formation of precipitates. The precipitates were filtered, washed with distilled water and dried to afford the title compounds.

2.7. Bioactivity assays

2.7.1. Microbial strains

All the synthesized compounds were tested against microorganisms, including Gram-positive bacteria: *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria: *Pasteurella multocida* (*P. multocida*) and *Escherichia coli* (*E. coli*); and four pathogenic fungi, *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*), *Alternaria alternate* (*A. alternate*) and *Ganoderma lucidum* (*G. lucidum*). All bacterial and fungal strains were obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity of the microorganisms were verified by the Department of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured at 37 °C in Nutrient agar (NA) overnight and fungal strains were cultured at 28 °C using potato dextrose agar (PDA) overnight [5].

2.7.2. Disk diffusion method

Disk diffusion method was used to find out the antimicrobial activity of the synthesized compounds. 100 µL suspension of tested microorganisms was spread on PDA medium for 10^6 spores/mL of fungi and on NA medium for 10^7 colony-forming units/mL of bacteria cells. The filter disks of 6 mm diameter were saturated with compound solution and placed on the agar plates inoculated with the tested microorganisms. Filter disks without samples were employed as negative control. Fluconazole (30 µg/disk) and streptomycin (30 µg/disk) were applied as positive reference for bacterial strains and fungal strains, respectively. Plates were placed 4 °C for 2 h and then incubated at 37 °C for 18 h for bacterial strains and at 28 °C for 24 h for fungal strains. Antimicrobial activity was justified after comparison of diameter of growth inhibition zone measured in mm for organisms and the controls [5].

2.7.3. Hemolytic activity

Hemolytic activity was studied by the reported method [16,14]. 3.0 mL fresh heparin added human blood was obtained from voluntaries after guidance from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Plasma was disposed off after centrifuging blood at 1000*g* for 5 min, and blood cells were washed three times by using cold aseptic isosmotic phosphate-buffered saline (PBS) having pH 7.4. The RBCs for each assay were kept 10^8 cells per mL. 100 µL of each compound was poured in each assay. Then the incubation of the assays was carried out at 37 °C for 35 min followed by agitation after 10 min. All the samples were kept on cold ice for 5 min and then again centrifuged for 5 min at 1000*g*. 100 µL was skimmed off from every tube and followed by 10 time dilution with cold PBS. Two controls were employed i.e. PBS as negative control and Triton X-100 (0.1% v/v) as positive control. The %RBCs lysis was computed for every sample by noting the absorbance at 576 nm using UV spectrophotometer.

2.7.4. Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± sem.

2.8. Spectral characterization of the synthesized compounds

2.8.1. 2-[[5-(4-Methylphenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6a**)

White amorphous solid; Yield: 87%; M.P. 224 °C; Mol. formula: $C_{21}H_{22}N_4O_3S$; Mol. weight: 410 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3038 (Ar-H stretching), 1657 (C═O str.), 1619 (C═N stretching), 1476 (Ar C═C stretching), 1237, 1063 (C—O—C bond str.), 658 (C—S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.86 (d, J = 8.0 Hz, 2H, H-2‴ & H-6‴), 7.69 (d, J = 7.6 Hz, 2H, H-2′ & H-6′), 7.65 (d, J = 8.0 Hz, 2H, H-3‴ & H-5‴), 7.29 (d, J = 7.6 Hz, 2H, H-3′ & H-5′), 4.01 (s, 2H, H-2), 3.96 (br. s, 2H, H_e-2″ & H_e-6″), 3.85 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.42 (br. s, 2H, H_e-3″ & H_e-5″), 3.00 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″), 2.41 (s, 3H, CH_3-4‴); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.4 (C-1), 166.3 (C-2‴), 164.9 (C-5‴), 142.7 (C-4′), 140.7 (C-4‴), 129.7 (C-2‴ & C-6‴), 126.5 (C-3‴ & C-5‴), 121.0 (C-2′ & C-6′), 119.9 (C-1′), 116.5 (C-1‴), 111.4 (C-3′ & C-5′), 66.3 (C-2″ & C-6″), 48.7 (C-3″ & C-5″), 36.3 (C-2), 21.4 (CH_3-4‴); EIMS (m/z): 410 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 205 $[C_{11}H_{13}N_2O_2]^+$, 192 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 119 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 117 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 91 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

2.8.2. 2-[[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl] acetamide (**6b**)

White amorphous solid; Yield: 81%; M.P. 180 °C; Mol. formula: $C_{20}H_{19}ClN_4SO_3$; Mol. weight: 430.5 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3036 (Ar-H stretching), 1655 (C═O str.), 1617 (C═N stretching), 1474 (Ar C═C stretching), 1236, 1061 (C—O—C bond str.), 656 (C—S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.90 (d, J = 7.6 Hz, 1H, H-6‴), 7.78 (d, J = 8.0 Hz, 1H, H-3‴), 7.76 (d, J = 8.0 Hz, 2H, H-2′ & H-6′), 7.54 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 7.48 (t, J = 7.6 Hz, 1H, H-5‴), 7.40 (t, J = 7.6 Hz, 1H, H-4‴), 4.06 (s, 2H, H-2), 4.04–3.96 (m, 4H, H-2″ & H-6″), 3.48 (br. s, 2H, H_e-3″ & H_e-5″), 3.27 (br. s, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.5 (C-1), 167.3 (C-5‴), 166.2 (C-2‴), 147.3 (C-4′), 133.6 (C-1′),131.2 (C-4‴), 131.0 (C-2‴), 129.1 (C-3‴), 127.8 (C-6‴), 127.0 (C-5‴), 126.7 (C-1‴), 119.7 (C-2′ & C-6′), 111.6 (C-3′ & C-5′), 66.4 (C-2″ & C-6″), 47.2 (C-3″ & C-5″), 33.2 (C-2); EIMS (m/z): 430 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 212 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 139 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 137 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 111 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.3. 2-[[5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl] acetamide (**6c**)*

Off white amorphous solid; Yield: 76%; M.P. 226 °C; Mol. formula: $C_{20}H_{19}ClN_4SO_3$; Mol. weight: 430.5 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3030 (Ar-H stretching), 1649 (C═O str.), 1611 (C═N stretching), 1468 (Ar C═C stretching), 1229, 1055 (C─O─C bond str.), 650 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.87 (d, J = 7.6 Hz, 1H, H-6‴), 7.80 (s, 1H, H-2‴), 7.51 (d, J = 7.2 Hz, 1H, H-4‴), 7.45 (t, J = 7.6 Hz, 1H, H-5‴), 7.40 (d, J = 8.4 Hz, 2H, H-2′ & H-6′), 6.71 (d, J = 8.4 Hz, 2H, H-3′ & H-5′), 4.05 (s, 2H, H-2), 4.02–3.94 (m, 4H, H-2″ & H-6″), 3.48 (br. s, 2H, H_e-3″ & H_e-5″), 3.26 (br. s, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.3 (C-1), 166.2 (C-2‴), 165.8 (C-5‴), 147.6 (C-4′), 134.6 (C-3‴), 133.3 (C-1′), 130.7 (C-1‴), 130.5 (C-2‴), 128.5 (C-4‴), 127.4 (C-6‴), 127.4 (C-5‴), 119.5 (C-2′ & C-6′), 111.6 (C-3′ & C-5′), 66.8 (C-2″ & C-6″), 47.3 (C-3″ & C-5″), 33.2 (C-2); EIMS (m/z): 430 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 212 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 139 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 137 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 111 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.4. 2-[[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl] acetamide (**6d**)*

Off white amorphous solid; Yield: 72%; M.P. 238 °C; Mol. formula: $C_{20}H_{19}ClN_4SO_3$; Mol. weight: 430.5 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3027 (Ar-H stretching), 1647 (C═O str.), 1609 (C═N stretching), 1466 (Ar C═C stretching), 1227, 1053 (C─O─C bond str.), 647 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.91 (d, J = 8.4 Hz, 2H, H-2‴ & H-6‴), 7.47 (d, J = 8.4 Hz, 2H, H-3‴ & H-5‴), 7.36 (d, J = 9.2 Hz, 2H, H-2′ & H-6′), 6.95 (d, J = 9.2 Hz, 2H, H-3′ & H-5′), 3.99 (s, 2H, H-2), 3.98 (br. s, 2H, H_e-2″ & H_e-6″), 3.83 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.14 (br. s, 2H, H_e-3″ & H_e-5″), 2.98 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.6 (C-1), 166.1 (C-2‴), 164.7 (C-5‴), 147.5 (C-4′), 137.7 (C-4‴), 133.6 (C-1′), 129.8 (C-3‴ & C-5‴), 129.6 (C-1‴), 129.0 (C-2‴ & C-6‴), 119.7 (C-2′ & C-6′), 111.8 (C-3′ & C-5′), 66.9 (C-2″ & C-6″), 47.2 (C-3″ & C-5″), 33.5 (C-2); EIS (*m/z*): 430 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 212 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 139 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 137 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 111 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.5. 2-[[5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6e**)*

Off white amorphous solid; Yield: 78%; M.P. 210 °C; Mol. formula: $C_{20}H_{19}N_5O_5S$; Mol. weight: 441 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3025 (Ar-H stretching), 1645 (C═O str.), 1607 (C═N stretching), 1464 (Ar C═C stretching), 1225, 1051 (C─O─C bond str.), 645 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 9.47 (s, 1H, H-2‴), 8.39 (d, J = 8.4 Hz, 1H, H-6‴), 8.33 (d, J = 7.6 Hz, 1H, H-4‴), 7.73 (t, J = 8.0 Hz, 1H, H-5‴), 7.39 (d, J = 8.4 Hz, 2H, H-2′ & H-6′), 6.71 (d, J = 8.4 Hz, 2H, H-3′ & H-5′), 4.11 (s, 2H, H-2), 4.07–4.01 (m, 4H, H-2″ & H-6″), 3.49–3.37 (m, 4H, H-3″ & H-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 168.5 (C-1), 167.4 (C-5‴), 166.2 (C-2‴), 148.6 (C-3‴), 147.3 (C-4′), 135.6 (C-6‴), 133.6 (C-1′), 132.6 (C-1‴), 131.6 (C-5‴), 125.2 (C-4‴), 121.4 (C-2‴), 119.7 (C-2′ & C-6′), 110.6 (C-3′ & C-5′), 66.4 (C-2″ & C-6″), 47.4 (C-3″ & C-5″), 33.2 (C-2); EIMS (*m/z*): 441 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 223 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 150 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 148 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 122 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.6. 2-[[5-(3-Aminophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6f**)*

White amorphous solid; Yield: 71%; M.P. 223 °C; Mol. formula: $C_{20}H_{21}N_5O_3S$; Mol. weight: 411 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3029 (Ar-H stretching), 1643 (C═O str.), 1615 (C═N stretching), 1467 (Ar C═C stretching), 1224, 1051 (C─O─C bond str.), 651 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.97 (d, J = 8.0 Hz, 2H, H-2′ & H-6′), 7.76 (s, 1H, H-2‴), 7.72 (d, J = 8.8 Hz, 1H, H-6‴), 7.53 (t, J = 7.2 Hz, 1H, H-5‴), 7.50 (d, J = 7.6 Hz, 2H, H-3′ & H-5′), 7.37 (d, J = 7.2 Hz, 1H, H-4‴), 4.03 (s, 2H, H-2), 3.98 (br. s, 2H, H_e-2″ & H_e-6″), 3.91 (br. s, 2H, H_a-2″ & H_a-6″), 3.46 (br. s, 2H, H_e-3″ & H_e-5″), 3.10 (br. s, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.6 (C-1), 167.5 (C-5‴), 166.3 (C-2‴), 147.4 (C-4′), 146.7 (C-3‴), 133.5 (C-1′), 132.1 (C-5‴), 129.5 (C-1‴), 123.7 (C-6‴), 119.8 (C-2′ & C-6′), 117.5 (C-4‴), 116.3 (C-2‴), 111.5 (C-3′ & C-5′), 66.5 (C-2″ & C-6″), 47.3 (C-3″ & C-5″), 33.3 (C-2); EIMS (*m/z*): 411 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 205 $[C_{11}H_{13}N_2O_2]^+$, 193 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 120 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 118 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 92 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.7. 2-[[5-(4-Aminophenyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6g**)*

White amorphous solid; Yield: 71%; M.P. 223 °C; Mol. formula: $C_{20}H_{21}N_5O_3S$; Mol. weight: 411 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3032 (Ar-H stretching), 1646 (C═O str.), 1618 (C═N stretching), 1470 (Ar C═C stretching), 1227, 1054 (C─O─C bond str.), 654 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.94 (d, J = 8.0 Hz, 2H, H-2‴ & H-6‴), 7.88 (d, J = 8.8 Hz, 2H, H-2′ & H-6′), 7.79 (d, J = 8.0 Hz, 2H, H-3‴ & H-5‴), 7.71 (d, J = 8.8 Hz, 2H, H-3′ & H-5′), 4.11 (s, 2H, H-2), 4.02–3.91 (m, 4H, H-2″ & H-6″), 3.55–3.41 (m, 4H, H-3″ & H-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 169.6 (C-1), 166.1 (C-2‴), 164.7 (C-5‴), 153.1 (C-4‴), 147.7 (C-4′), 133.4 (C-1′), 125.6 (C-2‴ & C-6‴), 125.3 (C-1‴), 119.5 (C-2′ & C-6′), 114.1 (C-3‴ & C-5‴), 111.2 (C-3′ & C-5′), 66.1 (C-2″ & C-6″), 46.9 (C-3″ & C-5″), 32.9 (C-2); EIMS (*m/z*): 411 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 205 $[C_{11}H_{13}N_2O_2]^+$, 193 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 120 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 118 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 92 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.8. 2-[[5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6h**)*

White amorphous solid; Yield: 68%; M.P. 212 °C; Mol. formula: $C_{21}H_{21}N_4O_4S$; Mol. weight: 425 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3037 (Ar-H stretching), 1651 (C═O str.), 1623 (C═N stretching), 1475 (Ar C═C stretching), 1232, 1059 (C─O─C bond str.), 659 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.61 (d, J = 8.8 Hz, 2H, H-2′ & H-6′),

7.26 (d, J = 8.4 Hz, 2H, H-3‴ & H-5‴), 6.97 (d, J = 8.4 Hz, 2H, H-2‴ & H-6‴), 6.90 (d, J = 8.8 Hz, 2H, H-3′ & H-5′), 5.19 (s, 2H, H-7‴), 3.99 (s, 2H, H-2), 3.94 (br. s, 2H, H_e-2″ & H_e-6″), 3.83 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.32 (br. s, 2H, H_e-3″ & H_e-5″), 2.98 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 166.0 (C-1), 164.7 (C-2‴), 164.5 (C-5‴), 155.7 (C-1‴), 147.1 (C-4′), 143.2 (C-4‴), 133.2 (C-1′), 129.4 (C-3‴ & C-5‴), 121.0 (C-2′ & C-6′), 116.0 (C-2‴ & C-6‴), 110.8 (C-3′ & C-5′), 65.6 (C-7‴), 59.7 (C-2″ & C-6″), 49.2 (C-3″ & C-5″), 36.4 (C-2); EIMS (m/z): 460 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 242 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 169 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 167 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$,162 $[C_{10}H_{12}NO]^{\cdot+}$, 141 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 111 $[C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.9. 2-[[5-(1,3-Benzodioxol-5-yl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl] acetamide (**6i**)*

White amorphous solid; Yield: 68%; M.P. 212 °C; Mol. formula: $C_{21}H_{20}N_4O_5S$; Mol. weight: 440 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3035 (Ar-H stretching), 1649 (C═O str.), 1613 (C═N stretching), 1477 (Ar C═C stretching), 1224, 1059 (C─O─C bond str.), 651 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.65 (d, J = 2.4 Hz, 1H, H-2‴), 7.51 (d, J = 8.4 Hz, 2H, H-2′ & H-6′), 7.36 (dd, J = 8.4, 2.4 Hz, 1H, H-6‴), 6.95 (d, J = 8.4 Hz, 1H, H-5‴), 6.89 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 6.05 (s, 2H, H-7‴), 3.98 (s, 2H, H-2), 3.95 (br. s, 2H, H_e-2″ & H_e-6″), 3.84 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.27 (br. s, 2H, H_e-3″ & H_e-5″), 2.98 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 165.8 (C-1), 164.9 (C-2‴), 163.8 (C-5‴), 150.8 (C-3‴), 148.2 (C-4‴), 147.3 (C-4′), 133.6 (C-1′), 122.8 (C-1‴), 121.8 (C-6‴), 121.0 (C-2′ & C-6′), 116.4 (C-3′ & C-5′), 108.7 (C-5‴), 106.4 (C-2‴), 101.8 (C-7‴), 66.5 (C-2″ & C-6″), 49.3 (C-3″ & C-5″), 36.3 (C-2); EIMS (m/z): 440 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 222 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$,205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 149 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 147 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 121 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.10. 2-[[5-(Phenylmethyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6j**)*

White amorphous solid; Yield: 65%; M.P. 168 °C; Mol. formula: $C_{21}H_{22}N_3O_3S$; Mol. weight: 396 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3038 (Ar-H stretching), 1652 (C═O str.), 1624 (C═N stretching), 1476 (Ar C═C stretching), 1233, 1060 (C─O─C bond str.), 660 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.59 (d, J = 8.0 Hz, 2H, H-2′ & H-6′), 7.33–7.27 (m, 5H, H-2‴ to H-6‴), 6.95 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 4.16 (s, 2H, H-7‴), 3.94 (s, 2H, H-2), 3.88 (br. s, 2H, H_e-2″ & H_e-6″), 3.84 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.34 (br. s, 2H, H_e-3″ & H_e-5″), 2.99 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 166.9 (C-1), 165.0 (C-2‴), 164.8 (C-5‴), 132.9 (C-4′), 129.0 (C-1‴), 128.8 (C-1′), 128.6 (C-3‴ & C-5‴), 128.5 (C-2‴ & C-6‴), 127.6 (C-4‴), 121.0 (C-2′ & C-6′), 116.6 (C-3′ & C-5′), 66.4 (C-2″ & C-6″), 49.3 (C-3″ & C-5″), 36.2 (C-2), 31.5 (C-7‴); EIMS (m/z): 410 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 205 $[C_{11}H_{13}N_2O_2]^+$, 192 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 119 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 117 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 91 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.11. 2-[[5-[1-(Phenylsulfonyl)-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6k**)*

White amorphous solid; Yield: 71%; M.P. 220 °C; Mol. formula: $C_{25}H_{24}N_4O_5S_2$; Mol. weight: 492 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3035 (Ar-H stretching), 1647 (C═O str.), 1617 (C═N stretching), 1474 (Ar C═C stretching), 1221, 1057 (C─O─C bond str.), 657 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.75 (d, J = 7.2 Hz, 2H, H-2′ & H-6′), 7.61–7.54 (m, 5H, H-2‴′ to H-6‴′), 6.97 (d, J = 7.2 Hz, 2H, H-3′ & H-5′), 4.23 (s, 2H, H-2), 3.94 (br. s, 2H, H_e-2″ & H_e-6″), 3.78–3.76 (m, 4H, H-2‴ & H-6‴), 3.63 (br. s, 2H, H_a-2″ & H_a-6″), 2.86 (br. s, 2H, H_e-3″ & H_e-5″), 2.54 (br. s, 2H, H_a-3″ & H_a-5″), 2.13–2.08 (m, 1H, H-4‴), 1.98–1.95 (m, 4H, H-3‴ & H-5‴); ^{13}C-NMR ($CDCl_3$ & CD_3OD, 100 MHz): δ/ppm 170.0 (C-5‴), 169.8 (C-1), 168.4 (C-2‴), 147.2 (C-4′), 145.7 (C-1‴′), 133.9 (C-1′), 133.2 (C-4‴′), 129.3 (C-3‴′ & 5‴′), 127.0 (C-2‴′ & 6‴′), 118.9 (C-2′ & C-6′), 110.8 (C-3′ & C-5′), 65.9 (C-2″ & C-6″), 48.9 (C-3″ & C-5″), 42.9 (C-2‴ & C-6‴), 33.3 (C-2), 32.9 (C-4‴), 29.0 (C-3‴ & C-5‴); EIMS (m/z): 543 $[M]^{\cdot+}$, 325 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 252 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 250 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 224 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 141 $[M\text{-}C_{19}H_{24}N_5O_3S]^{\cdot+}$, 77 $[M\text{-}C_{19}H_{24}N_5O_5S_2]^{\cdot+}$, 86 $[C_4H_8NO]^+$.

*2.8.12. 2-[[5-[2-(1,3-Benzodioxol-5-yl)ethenyl]-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide (**6l**)*

Off white amorphous solid; Yield: 73%; M.P. 222 °C; Mol. formula: $C_{23}H_{22}N_4O_5S$; Mol. weight: 466 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3034 (Ar-H stretching), 1648 (C═O str.), 1620 (C═N stretching), 1472 (Ar C═C stretching), 1229, 1056 (C─O─C bond str.), 656 (C─S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 7.36 (d, J = 8.0 Hz, 2H, H-2′ & H-6′), 7.03 (d, J = 1.2 Hz, 1H, H-2‴), 6.98 (dd, J = 8.0, 1.6 Hz, 1H, H-6‴), 6.91 (d, J = 8.0 Hz, 1H, H-5‴), 6.81 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 6.01 (s, 2H, H-7‴), 3.99 (s, 2H, H-2), 3.95 (br. s, 2H, H_e-2″ & H_e-6″), 3.86 (t, J = 4.4 Hz, 2H, H_a-2″ & H_a-6″), 3.43 (br. s, 2H, H_e-3″ & H_e-5″), 3.01 (t, J = 4.4 Hz, 2H, H_a-3″ & H_a-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 168.9 (C-1), 165.1 (C-2‴), 163.5 (C-5‴), 149.4 (C-4‴), 148.9 (C-3‴), 147.8 (C-4′), 141.7 (C-8‴), 132.9 (C-1′), 131.5 (C-1‴), 121.0 (C-9‴), 120.5 (C-6‴), 120.1 (C-2′ & C-6′), 110.6 (C-3′ & C-5′), 107.8 (C-5‴), 106.8 (C-2‴), 101.0 (C-7‴), 65.9 (C-2″ & C-6″), 46.9 (C-3″ & C-5″), 32.6 (C-2); EIMS (m/z): 466 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 248 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 175 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 173 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$, 162 $[C_{10}H_{12}NO]^{\cdot+}$, 147 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 121 $[M\text{-}C_{16}H_{17}N_4O_3S]^+$, 91 $[M\text{-}C_{17}H_{19}N_4O_4S]^+$, 86 $[C_4H_8NO]^+$.

*2.8.13. 2-[[5-(2-Naphthylmethyl)-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl] acetamide (**6m**)*

Off white amorphous solid; Yield: 77%; M.P. 209 °C; Mol. formula: $C_{25}H_{24}N_4O_3S$; Mol. weight: 460 $gmol^{-1}$; IR (KBr, ν_{max}/cm^{-1}): 3035 (Ar-H stretching), 1649 (C═O str.), 1621 (C═N stretching), 1473 (Ar C═C stretching), 1230, 1057

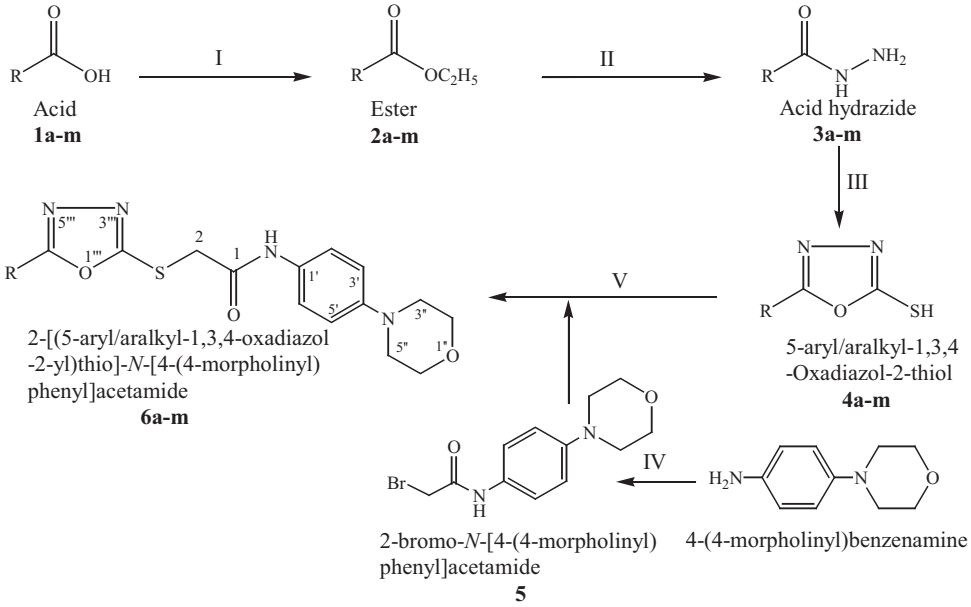
(C—O—C bond str.), 657 (C—S bond str.); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ/ppm 9.18 (s, 1H, H-1‴), 8.05 (d, J = 8.0 Hz, 1H, H-4‴), 7.86 (d, J = 7.6 Hz, 1H, H-8‴), 7.82 (t, J = 4.4 Hz, 1H, H-5‴), 7.55 (d, J = 8.0 Hz, 1H, H-3‴), 7.53–7.49 (m, 2H, H-6‴ & H-7‴), 7.44 (d, J = 8.0 Hz, 2H, H-2′ & H-6′), 6.97 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 4.61 (s, 2H, H-11‴), 3.93 (s, 2H, H-2), 3.87–3.83 (m, 4H, H-2″ & H-6″), 3.23–3.16 (m, 4H, H-3″ & H-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ/ppm 167.9 (C-1), 167.2 (C-2‴), 166.1 (C-5‴), 146.9 (C-4′), 133.8 (C-1′), 133.1 (C-10‴), 130.5 (C-2‴), 130.0 (C-8‴), 129.6 (C-9‴), 129.4 (C-5‴), 128.6 (C-4‴), 127.3 (C-7‴), 125.8 (C-6‴), 125.4 (C-3‴), 125.1 (C-1‴), 118.8 (C-2′ & C-6′), 112.1 (C-3′ & C-5′), 67.2 (C-2″ & C-6″), 47.8 (C-3″ & C-5″), 33.1 (C-2), 32.8 (C-11‴); EIMS (m/z): 460 $[M]^{\cdot+}$, 277 $[C_{13}H_{15}N_3O_2S]^{\cdot+}$, 251 $[C_{12}H_{15}N_2O_2S]^+$, 242 $[M\text{-}C_{12}H_{14}N_2O_2]^{\cdot+}$, 205 $[C_{11}H_{13}N_2O_2]^+$, 177 $[M\text{-}C_{10}H_{13}N_2O]^+$, 169 $[M\text{-}C_{13}H_{15}N_4O_2S]^+$, 167 $[M\text{-}C_{13}H_{15}N_3O_3S]^{\cdot+}$,162 $[C_{10}H_{12}NO]^{\cdot+}$, 141 $[M\text{-}C_{14}H_{15}N_4O_3S]^+$, 86 $[C_4H_8NO]^+$.

3. Results and discussion

The 2-[[5-aryl/aralkyl-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl)phenyl]acetamides, **6a**–**m**, were competently synthesized according to the protocol sketch in Scheme 1 and different substituted aryl/aralkyl groups are mentioned in Table 1. The general reaction procedures along with necessary conditions and the spectral characterization are described in the experimental section. In continuation of our previous work [1–3,4,11], the synthesis and biological screening of new 2-[[5-alkyl/aralkyl-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl)phenyl]acetamide compounds were performed with an objective to detect the anti-microbial and hemolytic activity of the synthesized compounds. All the synthesized derivatives were screened for anti-microbial and cytotoxic potential and found that most of the compounds exhibited significant activity against the selected microbial species relative to reference standard drugs.

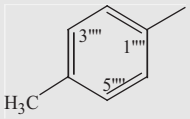
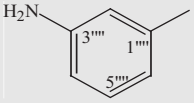
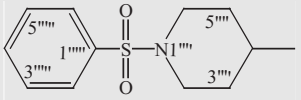
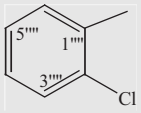
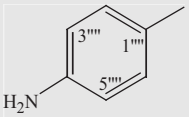
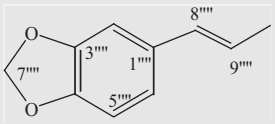
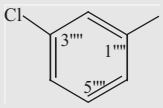
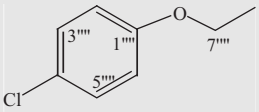
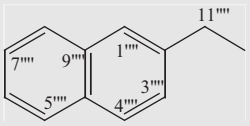
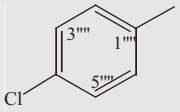
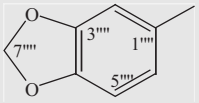
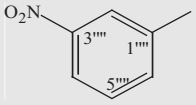
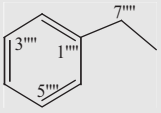
3.1. Chemistry

In the present research, the compounds, **6a**–**m**, were synthesized in a series of steps. The different aryl/aralkyl organic acids were subjected to reflux with ethanol in the presence of concentrated H_2SO_4 to yield corresponding ethyl esters. Carbohydrazides were synthesized from corresponding esters by reacting with hydrated hydrazine in methanol. 5-Aryl/aralkyl-1,3,4-oxadiazol-2-thiols, **4a**–**m**, were prepared by intermolecular cyclization of different aryl/aralkyl carbohydrazides with carbon disulfide in the presence of potassium hydroxide in ethanol, as solvent. The target compounds were synthesized by electrophilic substitution reaction of 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols, **4a**–**m**, with 2-bromo-*N*-[4-(4-morpholinyl)phenyl] acetamide (**5**). The structural elucidation of all the synthesized compounds was brought about by IR, EIMS, ^{1}H-NMR and ^{13}C-NMR and screened them for biological activities. For compound, **6a**, the IR spectra showed the presence of aromatic C-H stretching at 3038 cm^{-1}, C=N stretching at 1619 cm^{-1}, C=C stretching at 1476 cm^{-1} while C-O-C stretching appeared at 1237 and 1063 cm^{-1}. In ^{1}H-NMR, for the morpholine ring, the most deshielded equatorial proton of position-2 and position-6, due to adjacent electronegative oxygen, appeared around δ 3.96 as broad singlet integrated for two protons while axial protons of position-2 and postion-6 appeared around δ 3.85 as triplet with coupling constant 4.4 Hz with integration of two protons. Similarly equatorial protons of 3rd and 5th position, adjacent to nitrogen atom, revealed around δ 3.42 as broad singlet having integration of two protons while axial protons resonated around δ 3.00 as



Scheme 1 Outline for the synthesis of 2-[(5-aryl/aralkyl-1,3,4-oxadiazol-2-yl)thio]-*N*-[4-(4-morpholinyl)phenyl]acetamide. Reagents and conditions: (I) Ethanol, Conc. H_2SO_4, reflux for 6–8 h. (II) Hydrazine hydrate, methanol, reflux for 4–5 h. (III) CS_2, KOH, Ethanol, Reflux for 6–7 h. (IV) 2-Bromoacetyl bromide, Aq. Na_2CO_3 soln., stir for 0.5 h. (V) Compound **5**, DMF, NaH, stir for 4–5 h.

Table 1 Different 5-substituted aryl/aralkyl groups of 1,3,4-oxadiazole moiety.

C. No.	R	C. No.	R	C. No.	R
6a		**6f**		**6k**	
6b		**6g**		**6l**	
6c		**6h**		**6m**	
6d		**6i**			
6e		**6j**			

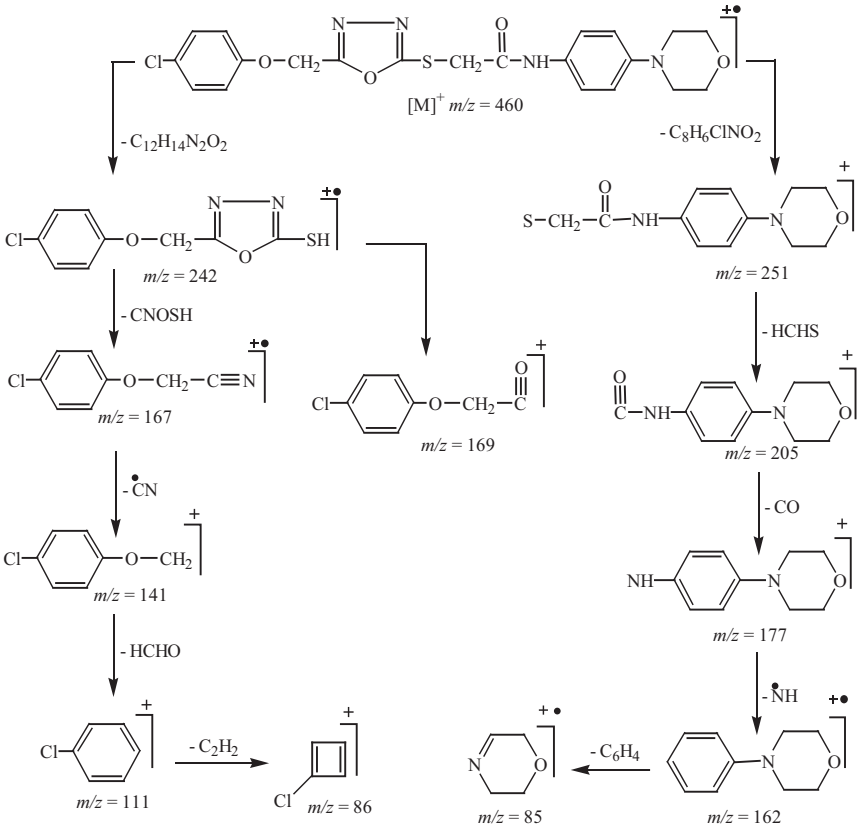


Figure 1 The mass fragmentation pattern of 2-[[5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl)phenyl]acetamide (**6h**).

triplet having *J*-coupling 4.4 Hz with integration of two protons. Signals of the *N*-substituted *p*-amino phenyl ring attached to the morpholine ring appeared as two doublets around δ 7.69 and 7.29 with coupling constant 7.6 Hz and each integrated for two protons. Methylene of acetamide linkage appeared as singlet around δ 4.01 ppm integrated for two protons, due to deshielding effect of neighboring sulfur atom and electron withdrawing effect of the carbonyl group. The four protons of the *p*-substituted phenyl ring attached to 1,3,4-oxadiazole moiety showed characteristic two doublets (*J*-coupling of 8.0 Hz) with double integration at δ 7.86 for two protons adjacent to the 1,3,4-Oxadiazole ring and at δ 7.65 for two protons in vicinity of the methyl group. The singlet of three protons of the methyl group resonated at δ 2.41. In ^{13}C-NMR, quaternary carbons of the Oxadiazole ring appeared at δ 166.3 (C-2‴) and 164.9 (C-5‴); that of acetamide linkage appeared at δ 169.4 (C-1); that of the phenyl ring adjacent to the morpholine ring resonated at δ 142.7 (C-4′) and 119.9 (C-1′); and that of the phenyl ring linked to Oxadiazole moiety resonated at δ 140.7 (C-4⁗) and 116.5 (C-1⁗). The methine carbon signals with double intensity appeared for both phenyl rings at δ 129.7 (C-2⁗ & C-6⁗), 126.5 (C-3⁗ & C-5⁗), 121.0 (C-2′ & C-6′) and 111.4 (C-3′ & C-5′). The methylene carbons of the morpholine ring resonated at δ 63.3 (due to vicinal oxygen) and 48.7 (due to vicinal nitrogen); and that of the acetamoyl group at δ 36.5 (due to neighboring carbonyl group and sulfur atom). The only methyl carbon revealed signal at δ 21.4. In EIMS molecular ion peak appeared at *m*/*z* 410. Other characteristic peaks appeared at *m*/*z* 277, 251, 205, 177, 162 and 86. The characteristic peak at *m*/*z* 177 corresponds to *p*-aminophenyl substituted morpholine moiety while *m*/*z* 162 corresponds to phenyl substituted morpholine while *m*/*z* 86 is related to morpholine cation. The mass fragmentation pattern of the 2-[[5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazol-2-yl]thio]-*N*-[4-(4-morpholinyl)phenyl]acetamide (**6h**) is sketched in Fig. 1. All this spectroscopic analysis helped to elucidate the structure of **6a** and all the other synthesized compounds.

3.2. Biological activities

All the synthesized compounds were analyzed for antibacterial activity against two gram positive bacteria (*S. aureus, B. subtilis*) and two gram negative bacteria (*P. multocida* and *E. coli*); antifungal activity (*A. niger, A. flavus, G. lucidum* and *A. alternata*) and hemolytic activity. In antibacterial and antifungal activities, zone of inhibition for all the compounds was calculated in millimeter (mm) (Table 2 and 3) and for hemolytic activity%age lysis was calculated.

3.2.1. Antibacterial activity

Compound **6d** showed better inhibition against all the bacterial strains relative to reference standard, streptomycin, while compound **6f** exhibited better results against three bacterial strains, *S. aureus, B. subtilis* and *E. coli* with zones of inhibition as 24, 25, and 23 mm respectively relative to reference. Compound **6e** was found to be active against only one gram negative bacterial strain with zone of inhibition as 23 mm. Compounds, **6c** and **6j** were also active against all the bacterial strains but not with appreciable values. Compounds, **6a** and **6b** were active against only two gram positive bacterial strains *S. aureus* and *B. subtilis*. The compounds, **6g**, **6h**, **6i**, **6k**, **6l** and **6m** were found inactive against all bacterial strains.

3.2.2. Antifungal activity

All the compounds were also screened for antifungal potential. Results revealed that compound **6d** possessed good antifungal activity against all the four fungal strains, specifically excellent against *A. niger* strain with inhibition value of 25 mm which was equal to the reference standard fluconazole. Compound, **6f** was found to exhibit good results against three fungal strains, *A. flavus, G. lucidum* and *A. alternata* but excellent against *A. flavus* with inhibition of 26 mm, equal to that of reference. Compounds, **6c**, **6e**, **6j** and **6k** were found to be active against all the fungal strains but results were not appreciable. The compounds, **6a**, **6b**, **6g**, **6h**, **6i**, **6l** and **6m** were found inactive against all fungal strains.

3.2.3. Hemolytic activity

The result of hemolytic activity (Table 4) rendered the compounds **6e** and **6k**, the least cytotoxic because of the lowest hemolytic activity as 5.35% and 4.61%, respectively relative to standard triton-X with that of 100%, as positive control. Due to lowest hemolytic activity these compounds can be

Table 2 Antibacterial activity of synthesized compounds.

C. No.	Zone of inhibition (mm)			
	S. aureus	*B. subtilis*	*P. multocida*	*E. coli*
6a	19	18	–	–
6b	17	20	–	–
6c	20	20	21	22
6d	25	25	23	24
6e	20	21	21	23
6f	24	25	21	23
6g	–	–		–
6h	–	–	–	–
6i	–	–	–	–
6j	20	21	15	16
6k	–	–	–	–
6l	–	–	–	–
6m	–	–	–	–
Streptomycin	27	28	24	25

Table 3 Antifungal activity of synthesized compounds.

C. No.	Zone of inhibition (mm)			
	A. niger	*A. flavus*	*G. lucidum*	*A. alternata*
6a	–	–	–	–
6b	–	–	–	–
6c	22	20	19	21
6d	25	24	22	22
6e	18	22	20	22
6f	23	26	22	22
6g	–	–		–
6h	–	–	–	–
6i	–	–	–	–
6j	18	20	18	15
6k	24	23	13	16
6l	–	–	–	–
6m	–	–	–	–
Fluconazole	25	26	25	26

Table 4 Hemolytic activity of synthesized compounds.

Compound	Hemolytic activity (Mean)% ± S.D
6a	31.46
6b	6.23
6c	21.815
6d	13.04
6e	5.35
6f	8.49
6g	7.04
6h	35.00
6i	24.07
6j	8.04
6k	4.61
6l	33.35
6m	20.05
PBS	0
Triton-X (toxicity)	100

PBS = Phosphate buffered saline.

utilized in pharmacy for drug development programs. Compounds, **6h** and **6l** were found to possess the highest cytotoxic effect among all the synthesized compounds.

4. Conclusion

All the derivatives of 2-bromo-*N*-[4-(4-morpholinyl)phenyl] acetamide were synthesized in laboratory in appreciable yields and structure elucidation was brought about by IR, ^{1}H-NMR, ^{13}C-NMR and EIMS spectral data. All the synthesized derivatives were screened for antibacterial activity against gram-negative and gram-positive bacterial strains; for antifungal potential and cytotoxic activity. The compound, **6d**, possessed the significant results of antibacterial and antifungal activities and that might be because of the 4-chlorophenyl group present in the molecule. The results indicated that most of the synthesized compounds have moderately good inhibitory potential for both bacterial strains and fungal strains. Some of the derivatives possess good cytotoxic potential. So these derivatives can be considered by the pharmacists in drug development process.

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