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Synthesis of new *S*-substituted derivatives of 5-[3-(1*H*-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-ylhydrosulfide as suitable antibacterial and anticancer agents with moderate cytotoxicity

Majid Nazir[1], Muhammad Athar Abbasi[1]*, Aziz-ur-Rehman[1], Sabahat Zahra Siddiqui[1], Syed Adnan Ali Shah[2,3], M Shahid[4], H Fatima[4], Sunniya Iftikhar[5] and Rahman Shah Zaib Saleem[5]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Faculty of Pharmacy & [3]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Punca kAlam Campus, Bandar Punca Kalam, Selangor Darul Ehsan, Malaysia
[4]Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan
[5]Department of Chemistry & Chemical Engineering, SBA School of Sciences & Engineering, Lahore University of Management Sciences, Lahore, Pakistan

Abstract: In the study presented here, the nucleophilic substitution reaction of 5-[3-(1*H*-indol-3-yl)propyl]-1,3,4-oxadiazol-2-ylhydrosulfide was carried out with different alkyl/aralkyl halides (5a-r) to form its different *S*-substituted derivatives (6a-r), as depicted in scheme 1. The structural confirmation of all the synthesized compounds was done by IR, ^{1}H-NMR, ^{13}C-NMR and CHN analysis data. Ba**c**terial biofilm inhibitory activity of all the synthesized compounds was carried out against *Bacillus subtilis* and *Escherichia coli*. The anticancer activity of these molecules was ascertained using anti-proliferation (SRB) assay on HCT 116 Colon Cancer Cell lines while the cytotoxicity of these molecules was profiled for their haemolytic potential. From this investigation it was rational that most of the compounds exhibited suitable antibacterial and anticancer potential along with a temperate cytotoxicity.

Keywords: 5-[3-(1*H*-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl hydro sulfide; alkyl/aralkyl halides; bacterial biofilm inhibition; antibacterial; anticancer; cytotoxicity.

INTRODUCTION

The word Indole is a composite of Oleum and Indigo, as it was synthesized by the reaction of an oleum and indigo dye. Indole is composed of a benzo-pyrrole in which the benzene and pyrrole rings are fused together through the 2- and 3-positions of the pyrrole ring. Indole is a solid with flowery smell and finds use as a constituent in many perfumes. The indole ring is found in many natural products such as fungal metabolites, the indole alkaloids, and marine natural products. Indole can undergo aromatic electrophillic substitution reactions. The C-3 position of pyrrole ring is the most nucleophillic, followed by the *N* and C-2 positions. Indole can be deprotonated at nitrogen of pyrrole ring. The salts thus formed can act as good nucleophiles. Highly ionic salts (e.g. Li^+, K^+) favor the *N*-substitutions. Softer counter ions favor C-3 substitution (Hardick *et al.,* 2012).

There are many examples of indole containing drugs and clinical candidates in the recent literature. These include antimicrobial like Apaziquone (Puri *et al.,* 2006), antihypertensive like Vincamine (Cook and James, 1981), antidepressant like Binedaline (Faltus *et al.,* 1984), antipsychotic like Oxypertine (Somohano *et al.,* 1976), antischizophrenia like Roxindole (Grunder *et al.,* 1993). The development of microbial resistance against many antibiotics has ringed the alarm bell for researchers to prepare the new drug molecules. Several species of bacteria are pathogenic and cause infectious diseases (Bahiru *et al.,* 2013; Lowy, 1998). The term cancer scientifically known as malignant neoplasm is used for a set of complex diseases in which cell division takes place without control and these cells are able to invade nearby tissues of the body. Cancer cells also spread to other parts of body through blood and lymph systems (Brancale *et al.,* 2007; Islam and Iskander, 2004).

Indole derivatives played a significant role in a diversified array of markets such as plastics, dyes, vitamin supplements, agriculture, over-the-counter drugs, flavor enhancers and perfumery. In addition, 1,3,4-oxadiazoles derivatives have a vital rule in the heterocyclic chemistry and also used widely in organic synthesis (Patil and Dandagvhal, 2016; Yar *et al.,* 2014; Chande, 2003). Considerable attention has been focused on 2,5-di-substituted-1,3,4-oxadiazole containing compounds due to their remarkable activity and also importance in pharmaceutical field. These compounds are effectively being utilized as antibacterial. Some of these compounds have also been recognized as anticancer, anti-Parkinson, anti-HIV and anti-proliferative agents as well (Holla *et al.,* 2004; Khan *et al.,* 2004; Amir; 1998; Rai, 2009).

The bioactivity of indole and oxadiazole moieties prompted us to synthesize some new molecules bearing

**Corresponding author:* e-mail: atrabbasi@yahoo.com

these moieties together. So in continuation of our previous efforts on the Chemistry and bioactivity of oxadiazoles (Rubab *et. al.*, 2016a; Rubab *et al*., 2016b; Abbasi *et al*. 2014), in the present investigation, the designed heterocyclic molecules were screened to explore their antibacterial and anticancer potential. Moreover, their cytotoxicity was also assessed to find their utility as possible therapeutic agents in drug development programs.

MATERIALS AND METHODS

General

Chemicals were purchased from Sigma Aldrich & Alfa Aesar (Germany) and solvents of analytical grades were supplied by local suppliers. By using open capillary tube method, melting points were taken on Griffin and George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate and *n*-hexane (30:70) as mobile phase), initial purity of compounds was detected at 254 nm. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. ^{1}H-NMR signals were recorded at 600 MHz and ^{13}C-NMR at 150 MHz in DMSO-d_6 using Bruker spectrometers.

Synthesis

Synthesis of ethyl 4-(1H-indol-3-yl)butanoate (2)

4-(1*H*-Indol-3-yl)butanoic acid (0.2 mol.; 1) dissolved in absolute ethanol (70 mL) and catalytic amount of concentrated sulfuric acid (20 mL) was taken in a 500 mL round bottomed (RB) flask and refluxed for 8 hrs until the maximum completion of reaction, supervised through TLC. At the end, reaction mixture was neutralized with 10% aqueous sodium carbonate (40 mL). The product was isolated by solvent extraction by chloroform (50 mL × 3). The solvent was distilled off and the required ester, 2, was obtained as reddish brown liquid.

Synthesis of 4-(1H-indol-3-yl)butanohydrazide (3)

4-(1*H*-Indol-3-yl)butanoate (0.15 mol.; 2) in 60 mL methanol and hydrazine monohydrate (80 %; 25 mL) was taken in a 500 mL round bottomed flask. The reaction mixture was stirred for 14 hrs at room temperature (RT). After absolute conversion, the acid hydrazide was obtained by distilling methanol off from the reaction mixture. The precipitates were filtered, washed with cold *n*-hexane and air-dried to get pure 4-(1*H*-indol-3-yl)butanohydrazide (3).

Synthesis of 5-[3-(1H-indol-3-yl)propyl]-1,3,4-oxadiazol-2-ylhydrosulfide (4)

4-(1H-indol-3-yl)butanohydrazide (0.13 mol.; 3) in absolute ethanol (30 mL) and KOH (0.13 mol) were taken in a RB flask. Carbon disulfide (0.26 mol.) was added subsequently. Mixture was refluxed for 16 hrs. On completion of the reaction excess chilled distilled water and dil. HCl was added to adjust pH 5-6. The precipitates were filtered, washed and dried to get desired cyclized product, 4.

Synthesis of 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-ylhydrosulfidederivatives (6a-r)

5-[3-(1*H*-Indol-3-yl)propyl]-1,3,4-oxadiazol-2-ylhydrosulfide (0.2 g, 4) was added in DMF (5 mL) contained in a 250 mL round bottom flask at room temperature, added one pinch of LiH and stirred for 30 min. Then different alkyl/aralkyl halides (5a-r) were added in equimolar amounts separately in each respective reaction and stirred for 6-8 hours. Single spot TLC showed completion of the reaction, the reaction mixture was quenched with ice cold water (100 mL). The respective derivatives, 6a-r, were collected through filtration or solvent extraction according to nature of the product.

Biological studies

Assessment of bacterial biofilm inhibition

The inhibition of bacterial (*Bacillus subtilis/Escherichia coli)* biofilm formation was assessed by the microliter-plate method as described by (Stepanovic *et al.,* 2000). The wells of a sterile 24-well flat bottomed plastic tissue culture plate were filled with 100 µL of nutrient broth (Oxoid, UK). Two concentrations, that is, 2.5 and 5.0 µg of testing samples (dissolved in 1 mL of DMSO), were added in different wells. Finally, 20 µL of bacterial suspension containing 1×10^{9} CFU/mL was inoculated. Positive control well contained Ciprofloxacin and nutrient broth (Oxoid, UK) while negative control well contained nutrient broth and microbial strain. Afterwards, plates were covered and then incubated aerobically for 24 hours at 37 ^{o}C. There after, the contents of each well were beheld thrice with 220 µL of sterile phosphate buffer (pH: 7.2). To remove all non-adherent bacteria, plates were vigorously shaken. Then, attached leftover bacteria were fixed with 220 mL of 99% methanol per well. Next, after 15 min, plates were emptied and left to dry. Then, plates were stained for 5 min with 220 mL of 50% crystal violet per well. Surplus stain was rinsed of using distilled water. Then plates were air-dried and the bound dye was re-solubilized with 220 µL of 33% (v/v) glacial acetic acid per well. The optical density (OD) of each well was measured at 630 nm using micro-plate reader (Biotek, USA). All the tests were carried thrice against selected bacterial strains and the results were averaged. The bacterial growth inhibition (Inhibition %) was calculated using the following formula.

$$\text{Inhibition \%} = 100 - \frac{(OD_{630\ sample} \times 100)}{OD_{630\ control}}$$

***Anti-proliferative activity assay* (*anticancer activity*)**

The anti-proliferative activity of the compounds against HCT 116 human colon cancer cell line was evaluated by Sulforhodamine B (SRB) assay (Al-Samaraie *et al.,* 2005; Vichai *et al.,* 2006). The HCT 116 cells were trypsinized,

counted and diluted to 15000 cells per mL. 100 µL of diluted cells were added to each well of 96 well culture plates (SPL life science®) which yielded final concentration of 1500 cell per well. These plates were incubated at 37 °C with 5% CO_2 for 24 hours. For the screening, the seeded cells were treated with 50 µM and 25 µM of the compound using DMSO as control. The cells were incubated for 72 hours at 37 °C before the cytotoxicity assay. Then trichloroacetic acid was added in each well to fix the cells. After incubation for 2 hours, the plates were washed four times with water and dried. Then 100 µL of 0.06% SRB dye was added in each well followed by a 30 minutes incubation at room temperature. Excess SRB dye was washed with 1% v/v solution of acetic acid in water. The plates were dried for 15 minutes at 37 °C and the dye in the cells was solubilized in 10 mM *Tris* base solution (pH 10.5) with five minutes shaking. Absorbance of solubilized dye was measured at 490 nm in micro-plate reader (Bio Tek®). The calculation was made by using following formula.

$$\text{Anti} - \text{proliferation } (\%) = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Hemolytic activity

Bovine blood samples was collected in EDTA that was diluted with saline (0.9% NaCl), and centrifuge at 1000xg for 10 min. The erythrocytes separated diluted in phosphate buffer saline of pH 7.4 and a suspension was made. Add 20 µL of synthetic compounds solution (10 mg/mL) in 180 µL of RBCs suspension and incubate for 30 min at room temperature. PBS was used as negative control and Triton 100-X was taken as positive control (Sharma *et al.* 2001; Powell *et al.*, 2000). The %age of hemolysis was taken as by using formula:

$$(\%)\ of\ Hemolysis = \frac{Absorbance\ of\ Sample - Absorbance\ of\ Negative\ Control}{Absorbance\ of\ Positive\ Control} \times 100$$

STATISTICAL ANALYSIS

All the measurements were carried out in triplicate and statistical analysis was performed by Microsoft Excel 2010. The results are presented as mean ± SEM with 96 % CL.

Spectral characterization of synthesized compounds

Ethyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6a)

Light yellow solid; Mol. formula $C_{15}H_{17}N_3OS$; Mol. mass: 287 $gmol^{-1}$; % yield: 61%; melting point 122°C; IR (KBr, cm^{-1}) *υ*: 3210 (N-H str.), 2926 (C-H str. of aromatic ring), 1587 (C=C aromatic str.), 680 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.79 (s, 1H, NH-1), 7.50 (br.d, *J* = 7.86 Hz, 1H, H-7), 7.33 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (s, 1H, H-2), 7.06 (dist.t, *J* = 7.4 Hz, 1H, H-5), 6.96 (dist.t, *J* = 7.5 Hz, 1H, H-6), 3.19 (q, *J* = 7.3 Hz, 2H, CH_2-1'"), 2.86 (t, *J* = 7.5 Hz, 2H, CH_2-3'), 2.76 (t, *J* = 7.3 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.2 Hz, 2H, CH_2-2'), 1.3 (t, *J* = 7.3 Hz, 3H, CH_3-2'"); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.75 (C-5"), 162.90 (C-2"), 136.27 (C-8), 127.00 (C-9), 122.48 (C-2), 120.85 (C-6), 118.18 (C-4), 118.15 (C-5), 113.26 (C-3), 111.34 (C-7), 26.46 (C-1'"), 26.46 (C-1'), 24.25 (C-3'), 23.80 (C-2'), 14.81 (C-2'"); Anal. Calc. for $C_{15}H_{17}N_3OS$ (287.11): C, 62.69; H, 5.96; N, 14.62. Found: C, 62.63; H, 5.91; N, 14.58.

2-Chloroethyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6b)

Light yellow solid; Mol. formula $C_{15}H_{16}ClN_3OS$; Mol. mass: 321 $gmol^{-1}$; % yield 62%; melting point 188°C; IR (KBr, cm^{-1}): *υ* 3224 (N-H str.), 2928 (C-H str. of aromatic ring), 2870 (C-H str. of aliphatic), 1586 (C=C aromatic str.), 1156 (C-N-C bond str.), 697 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.79 (s, 1H, NH-1), 7.50 (dist.d, *J* = 7.2 Hz, 1H, H-7), 7.34 (dist.d, *J* = 8.7 Hz, 1H, H-4), 7.14 (dist.d, *J* = 2.2, 1H, H-2), 7.07 (m, 1H, H-5), 6.97 (m, 1H, H-6), 3.94 (t, *J* = 6.9 Hz, 2H, CH_2-2'"), 3.60 (t, *J* = 6.9 Hz, 2H, CH_2-2'"), 2.88 (t, *J* = 7.2 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.2 Hz, 2H, CH_2-1'), 2.06 (q, *J* = 7.2 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.04 (C-5"), 162.18 (C-2"), 136.28 (C-8), 127.01 (C-9), 122.48 (C-2), 120.85 (C-6), 118.18 (C-4), 118.15 (C-5), 113.27 (C-3), 111.34 (C-7), 34.01 (C-2'"), 26.48 (C-1'), 26.46 (C-2'"), 24.28 (C-3'), 23.80 (C-2'); Anal. Calc. for $C_{15}H_{16}ClN_3SO$ (321.07): C, 55.98; H, 5.01; N, 13.06. Found: C, 55.90; H, 4.95; N, 13.11.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 1-propyl sulfide (6c)

Yellow solid; Mol. formula $C_{16}H_{19}N_3SO$; Mol. mass: 301 $gmol^{-1}$; % yield 81%; melting point 96°C; IR (KBr, cm^{-1}): *υ* 3222 (N-H str.), 2926 (C-H str. of aromatic ring), 2876 (C-H str. of aliphatic), 1585 (C=C aromatic str.), 1154 (C-N-C bond str.), 680 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.79 (s, 1H, NH-1), 7.51 (br.d, *J* = 7.7 Hz, 1H, H-7), 7.33 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (d, *J* = 2.2 Hz, 1H, H-2), 7.06 (br.dt, *J* = 1.2, 7.4 Hz, 1H, H-5), 6.96 (br.dt, *J* = 1.0, 7.5 Hz, 1H, H-6), 3.17 (t, *J* = 7.1 Hz, 2H, CH_2-1'"), 2.87 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.77 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.70 (sext., *J* = 7.2 Hz, 2H, CH_2-2'"), 0.96 (t, *J* = 7.3 Hz, 3H, CH_3-3'"). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.74 (C-5"), 163.02 (C-2"), 136.28 (C-8), 127.01 (C-9), 122.47 (C-2), 120.85 (C-6), 118.18 (C-4), 118.15 (C-5), 113.27 (C-3), 111.34 (C-7), 33.79 (C-1'"), 26.55 (C-1'), 24.26 (C-3'), 23.80 (C-2'), 22.37 (C-2'"), 12.73 (C-3'"); Anal. Calc. for $C_{16}H_{19}N_3SO$ (301.12): C, 63.76; H, 6.35; N, 13.93. Found: C, 63.70; H, 6.26; N, 13.88.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 3-iodopropyl sulfide (6d)

Brown solid; Mol. formula $C_{16}H_{18}IN_3OS$; Mol. mass:427 $gmol^{-1}$; % yield 86%; melting point 107°C; IR (KBr, cm^{-}

^{1}): *υ* 3223 (N-H str.), 2924 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1589 (C=C aromatic str.), 1152 (C-N-C bond str.), 605 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.78 (s, 1H, NH-1), 7.50 (br.dd, *J* = 1.6, 7.8 Hz, 1H, H-7), 7.35 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.06 (br.t, *J* = 7.4 Hz, 1H, H-5), 6.97 (m, 1H, H-6), 3.29 (t, *J* = 6.6 Hz, 2H, CH_2-3'"), 2.88 (t, *J* = 7.3 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.3 Hz, 2H, CH_2-1'), 2.74 (t, *J* = 8.0 Hz, 2H, CH_2-1'"), 2.05 (q, *J* = 7.3 Hz, 2H, CH_2-2'), 1.90 (m, 2H, CH_2-2'"). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.85 (C-5"), 162.68 (C-2"), 136.29 (C-8), 127.02 (C-9), 122.47 (C-2), 120.85 (C-6), 118.18 (C-4), 118.15 (C-5), 113.28 (C-3), 111.35 (C-7), 32.73 (C-2'"), 28.81 (C-1'"), 26.49 (C-1'), 24.28 (C-3'), 23.82 (C-2'), 17.81 (C-3'"); Anal. Calc. for $C_{16}H_{18}IN_3SO$ (427.02): C, 44.97; H, 4.25; N, 9.83. Found: C, 44.91; H, 4.22; N, 9.77.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 2-propyl sulfide (6e)

Yellow solid; Mol. formula $C_{16}H_{19}N_3SO$; Mol. mass: 301 $gmol^{-1}$; % yield 60%; melting point 109°C; IR (KBr, cm^{-1}): *υ* 3213 (N-H str.), 2926 (C-H str. of aromatic ring), 2874 (C-H str. of aliphatic), 1586 (C=C aromatic str.), 1148 (C-N-C bond str.), 636 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.80 (s, 1H, NH-1), 7.51 (br.d, *J* = 8.0 Hz, 1H, H-7), 7.35 (br.d, *J* = 8.0 Hz, 1H, H-4), 7.14 (s, 1H, H-2), 7.07 (dist.t, *J* = 7.5 Hz, 1H, H-5), 6.97 (dist.t, *J* = 7.5 Hz, 1H, H-6), 3.78 (m, 1H, H-2'"), 2.88 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.3 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.40 (d, *J* = 6.8 Hz, 6H, CH_3-1'"& CH_3-3'"); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 167.91 (C-5"), 162.19 (C-2"), 136.30 (C-8), 127.02 (C-9), 122.46 (C-2), 120.85 (C-6), 118.18 (C-4), 118.15 (C-5), 113.27 (C-3), 111.35 (C-7), 38.73 (C-1'"), 26.54 (C-1'), 24.28 (C-3'), 23.81 (C-2'), 23.00 (2CH_3-C-2'"); Anal. Calc. for $C_{16}H_{19}N_3SO$ (301.12): C, 63.76; H, 6.35; N, 13.94. Found: C, 63.81; H, 6.44; N, 13.88.

Allyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6f)

Brown solid; Mol. formula $C_{16}H_{17}N_3SO$; Mol. mass: 299 $gmol^{-1}$; % yield 56%; melting point 101°C; IR (KBr, cm^{-1}): *υ* 3210 (N-H str.), 2926 (C-H str. of aromatic ring), 2877 (C-H str. of aliphatic), 1588 (C=C aromatic str.), 1153 (C-N-C bond str.), 679 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.81 (s, 1H, NH-1), 7.51 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.34 (br.d, *J* = 8.0 Hz, 1H, H-4), 7.14 (dist.d, *J* = 2.1 1H, H-2), 7.07 (br.t, *J* = 7.4 Hz, 1H, H-5), 6.97 (br.t, *J* = 7.4 Hz, 1H, H-6), 5.96-5.92 (m, 1H, CH-2'"), 5.31 (br.d, *J* = 16.9 Hz, 1H, H_b-3'"), 5.13 (br.d, *J* = 10.0 Hz, 1H, H_a-3'"), 3.86 (d, *J* = 8.0 Hz, 2H, CH_2-1'"), 2.86 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.76 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.4 Hz, 2H, CH_2-2'). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.02 (C-5"), 162.30 (C-2"), 136.28 (C-8), 132.60 (C-2'"), 127.00 (C-9), 122.47 (C-2), 120.86 (C-6), 119.10 (C-3'"), 118.18 (C-4), 118.16 (C-5), 113.25 (C-3), 111.35 (C-7), 34.57 (C-1'"), 26.51 (C-1'), 24.28 (C-3'), 23.79 (C-2'); Anal. Calc. for $C_{16}H_{17}N_3SO$ (299.11): C, 64.19; H, 5.72; N, 14.04. Found: C, 64.13; H, 5.67; N, 14.0.

2-Butyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6g)

Yellow solid; Mol. formula $C_{17}H_{21}N_3SO$; Mol. mass: 315 $gmol^{-1}$; % yield 88%; melting point 106°C; IR (KBr, cm^{-1}): *υ* 3228 (N-H str.), 3056 (C-H str. of aromatic ring), 2872 (C-H str. of aliphatic), 1585 (C=C aromatic str.), 1145 (C-N-C bond str.), 675 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.81 (s, 1H, NH-1), 7.50 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.33 (br.d, *J* = 8.0 Hz, 1H, H-4), 7.13 (dist.d, *J* = 2.4 Hz, 1H, H-2), 7.06 (br.t, *J* = 7.5 Hz, 1H, H-5), 6.96 (br.t, *J* = 7.5 Hz, 1H, H-6), 3.60 (m, 1H, H-2'"), 2.88 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.68 (m, 2H, CH_2-3'"), 1.37 (d, *J* = 6.8 Hz, 3H, CH_3-1'"), 0.96 (t, *J* = 7.3 Hz, 3H, CH_3-4'"); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.91 (C-5"), 162.90 (C-2"), 136.30 (C-8), 127.01 (C-9), 122.47 (C-2), 120.85 (C-6), 118.14 (C-4 & C-5), 113.11 (C-3), 111.35 (C-7), 45.22 (C-2'"), 29.03 (C-3'"), 26.55 (C-1'), 24.28 (C-3'), 23.81 (C-2'), 20.71 (C-1'") 11.03 (C-4'"); Anal. Calc. for $C_{17}H_{21}N_3SO$ (315.14): C, 64.73; H, 6.71; N, 13.32. Found: C, 64.78; H, 6.77; N, 13.42.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 1-pentyl sulfide (6h)

Light yellow solid; Mol. formula $C_{18}H_{23}N_3SO$; Mol. mass: 329 $gmol^{-1}$; % yield 86%; melting point 109°C; IR (KBr, cm^{-1}): *υ* 3225 (N-H str.), 2951 (C-H str. of aromatic ring), 2867 (C-H str. of aliphatic), 1586 (C=C aromatic str.), 1107 (C-N-C bond str.), 650 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.79 (s, 1H, NH-1), 7.51 (br.d, *J* = 8.0 Hz, 1H, H-7), 7.34 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (d, *J* = 2.3 Hz, 1H, H-2), 7.06 (br.dt, *J* = 1.1, 8.1 Hz, 1H, H-5), 6.96 (br.dt, *J* = 1.0, 8.0 Hz, 1H, H-6), 3.18 (t, *J* = 7.3 Hz, 2H, CH_2-1'"), 2.87 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.77 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.06 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.70 (quin, *J* = 7.4 Hz, 2H, CH_2-2'"), 1.37-1.25 (m, 4H, CH_2-3'" & CH_2-4'"), 0.85 (t, *J* = 7.2 Hz, 3H, CH_3-5'"). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.73 (C-5"), 163.04 (C-2"), 136.29 (C-8), 127.01 (C-9), 122.45 (C-2), 120.85 (C-6), 118.14 (C-4 & C-5), 113.27 (C-3), 111.34 (C-7), 31.87 (C-1'"), 29.93 (C-2'"), 28.62 (C-3'"), 26.54 (C-1'), 24.26 (C-3'), 23.80 (C-2'), 21.47 (C-4'"), 13.70 (C-5'"); Anal. Calc. for $C_{18}H_{23}N_3SO$ (329.16): C, 65.62; H, 7.04; N, 12.75. Found: C, 65.74; H, 7.17; N, 12.83.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 2-pentyl sulfide (6i)

Yellow solid; Mol. formula $C_{18}H_{23}N_3SO$; Mol. mass: 329 $gmol^{-1}$; % yield 59%; melting point 113°C; IR (KBr, cm^{-1}): *υ* 3214 (N-H str.), 2925 (C-H str. of aromatic ring), 2869 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1148 (C-N-C bond str.), 682 (C-S str.) cm^{-1}; ^{1}H-NMR

(600 MHz, DMSO-d_6, δ/ppm): δ 10.79 (s, 1H, NH-1), 7.50 (br.d, *J* = 7.9 Hz, 1H, H-7), 7.33 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (d, *J* = 2.2 Hz, 1H, H-2), 7.06 (br.dt, *J* = 1.0, 7.4 Hz, 1H, H-5), 6.96 (dt, *J* = 1.0, 7.5 Hz, 1H, H-6), 3.65 (m, 1H, CH-2'''), 2.87 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.06 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.67-1.6 (m, 4H, CH_2-3''' & CH_2-4'''), 1.38 (d, *J* = 7.3 Hz, 3H, CH_3-1'''), 0.87 (t, *J* = 7.3 Hz, 3H, CH_3-5'''). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.91 (C-5''), 162.25 (C-2''), 136.29 (C-8), 127.01 (C-9), 122.45 (C-2), 120.85 (C-6), 118.14 (C-4 & C-5), 113.27 (C-3), 111.35 (C-7), 43.46 (C-3'''), 38.11 (C-2'''), 26.55 (C-1'), 24.28 (C-3'), 23.80 (C-2'), 21.28 (C-1'''), 13.49 (C-5'''); Anal. Calc. for $C_{18}H_{23}N_3SO$ (329.16): C, 65.62; H, 7.04; N, 12.75. Found: C, 65.77; H, 7.12; N, 12.82.

1-Heptyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6j)

Light yellow solid; Mol. formula $C_{20}H_{27}N_3SO$; Mol. mass: 357 gmol^{-1}; % yield 96%; melting point 97^{o}C; IR (KBr, cm^{-1}): *υ* 3224 (N-H str.), 2922 (C-H str. of aromatic ring), 2861 (C-H str. of aliphatic), 1585 (C=C aromatic str.), 1138 (C-N-C bond str.), 681 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.81 (s, 1H, NH-1), 7.51 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.34 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.14 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.07 (br dt, *J* = 1.2 & 8.1 Hz, 1H, H-5), 6.97 (br.dt, *J* = 1.0, 8.1 Hz, 1H, H-6), 3.18 (t, *J* = 7.2 Hz, 2H, CH_2-1'''), 2.88 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 1.71 (quint, *J* = 7.4 Hz, 2H, CH_2-2'''), 1.36 (quint, *J* = 7.4 Hz, 2H, CH_2-3'''), 1.34-1.21 (m, 6H, CH_2-4''', CH_2-5''' & CH_2-6'''), 0.84 (t, *J* = 7.2 Hz, 3H, CH_3-7'''). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.70 (C-5''), 163.03 (C-2''), 136.29 (C-8), 127.01 (C-9), 122.45 (C-2), 120.83 (C-6), 118.13 (C-4), 118.12 (C-5), 113.25 (C-3), 111.34 (C-7), 31.89 (C-5'''), 30.97 (C-3'''), 28.94 (C-2'''), 28.09 (C-4'''), 27.72 (C-1'''), 26.55 (C-1'), 24.26 (C-3'), 23.81 (C-2'), 21.94 (C-6'''), 13.84 (C-7'''); Anal. Calc. for $C_{20}H_{27}N_3SO$ (357.19): C, 67.19; H, 7.61; N, 11.75. Found: C, 67.28; H, 7.78; N, 11.89.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 1-octyl sulfide (6k)

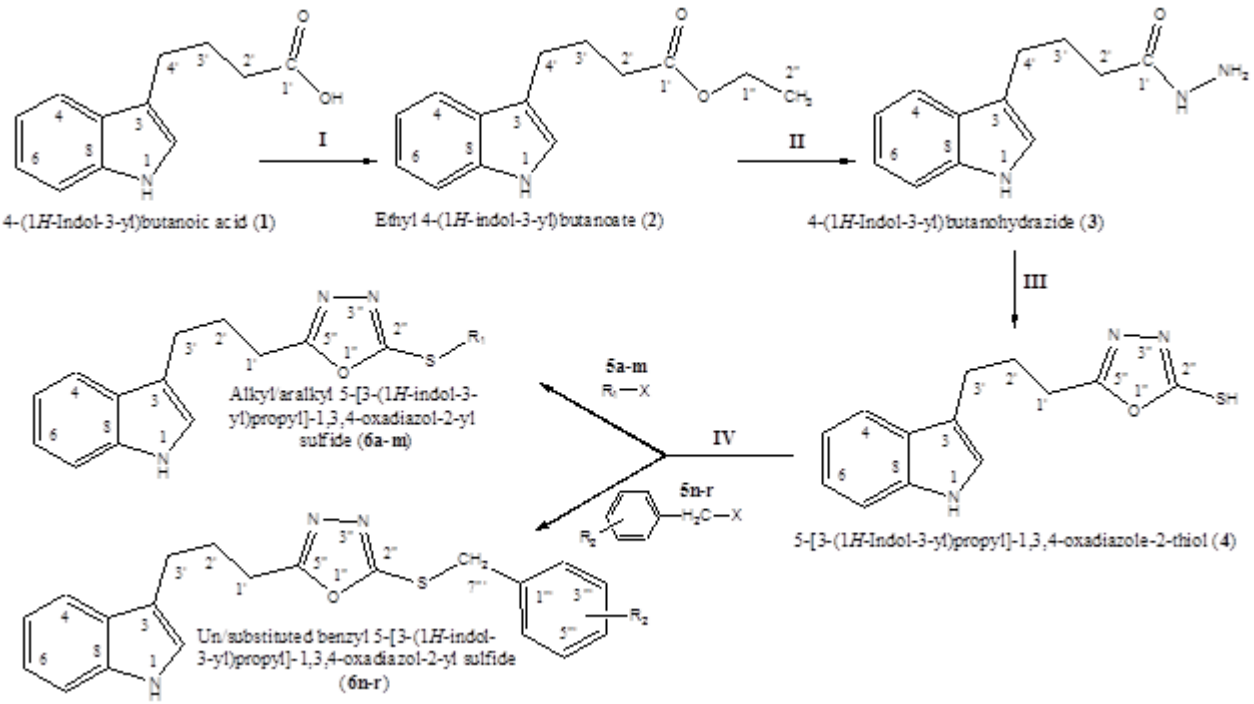
Reddish orange solid; Mol. formula $C_{21}H_{29}N_3SO$; Mol. mass: 371 gmol^{-1}; % yield 64%; melting point 103 ^{o}C; IR (KBr, cm^{-1}): *υ* 3219 (N-H str.), 2917 (C-H str. of aromatic ring), 2850 (C-H str. of aliphatic), 1586 (C=C aromatic str.), 1151 (C-N-C bond str.), 683 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.78 (s, 1H, NH-1), 7.50 (br.d, *J* = 7.9 Hz, 1H, H-7), 7.33 (br. d, *J* = 8.1 Hz, 1H, H-4), 7.13 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.06 (br.dt, *J* = 1.3, 8.1 Hz, 1H, H-5), 6.96 (br.dt, *J* = 1.02, 7.5 Hz, 1H, H-6), 3.18 (t, *J* = 7.3 Hz, 2H, CH_2-1'''), 2.86 (t, *J* = 7.5 Hz, 2H, CH_2-3'), 2.77 (t, *J* = 7.5 Hz, 2H, CH_2-1'), 2.03 (q, *J* = 7.5 Hz, 2H, CH_2-2'), 1.74-1.66 (m, 4H, CH_2-2''' & CH_2-3'''), 1.38-1.33 (m, 4H, CH_2-4''' & CH_2-5'''), 1.27-1.24 (m, 4H, CH_2-6''' & CH_2-7'''), 0.84 (t, *J* = 7.1 Hz, 3H, CH_3-8'''). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.71 (C-5''), 163.03 (C-2''), 136.30 (C-8), 127.01 (C-9), 122.46 (C-2), 120.83 (C-6), 118.16 (C-4), 118.12 (C-5), 113.26 (C-3), 111.34 (C-7), 31.91 (C-5'''), 31.11 (C-6'''), 28.93 (C-4'''), 28.45 (C-3'''), 28.30 (C-2'''), 27.75 (C-1'''), 26.55 (C-1'), 24.26 (C-3'), 23.81 (C-2'), 22.00 (C-7'''), 13.86 (C-8'''); Anal. Calc. for $C_{21}H_{29}N_3SO$ (371.20): C, 67.89; H, 7.87; N, 11.31. Found: C, 67.96; H, 7.91; N, 11.36.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl phenethyl sulfide (6l)

Light brown solid, Mol. formula $C_{23}H_{24}N_4SO$; Mol. mass: 363 gmol^{-1}; yield: 88%; melting point: 109^{o}C; IR (KBr, cm^{-1}): *υ* 3224 (N-H str.), 3053 (C-H str. of aromatic ring), 2843 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1150 (C-N-C bond str.), 638 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.81 (dist.d, *J* = 2.0 Hz, 1H, NH-1), 7.52 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.34 (br d, *J* = 8.1 Hz, 1H, H-4), 7.28 (br.t, *J* = 7.6 Hz, 2H, H-3'''& H-5'''), 7.24 (br.d, *J* = 8.5 Hz, 2H, H-2''' &H-6'''), 7.21 (br.t, *J* = 7.2 Hz, 1H, H-4'''), 7.14 (dist.d, *J* = 2.0 Hz, 1H, H-2), 7.07 (br.t, *J* = 7.06 Hz, 1H, H-5), 6.97 (br.t, *J* = 7.4 Hz, 1H, H-6), 3.45 (t, *J* = 7.5 Hz, 2H, CH_2-8'''), 3.02 (t, *J* = 7.5 Hz, 2H, CH_2-7'''), 2.88 (t, *J* = 7.5 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.05 (q, *J* = 7.5 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.75 (C-5''), 162.83 (C-2''), 139.14 (C-1'''), 136.29 (C-8), 128.57 (C-3''' & 5'''), 128.36 (C-2''' & 6'''), 126.48 (C-4'''), 127.02 (C-9), 122.48 (C-2), 120.86 (C-6), 118.18 (C-4), 118.16 (C-5), 113.28 (C-3), 111.35 (C-7), 34.94 (C-7'''), 33.10 (C-8'''), 26.51 (C-1'), 24.26 (C-3'), 23.80 (C-2'); Anal. Calc. for $C_{21}H_{21}N_3SO$ (363.14): C, 69.39; H, 5.82; N, 11.56. Found: C, 69.48; H, 5.93; N, 11.64.

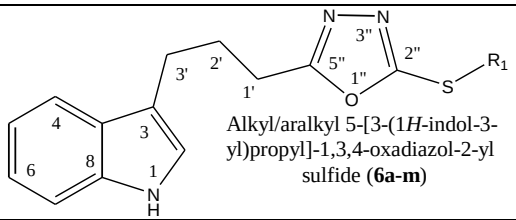
5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 3-phenylpropyl sulfide (6m)

Brown solid; Mol. formula $C_{22}H_{23}N_3SO$, Mol. mass: 337 gmol^{-1}; yield: 81%; melting point: 110^{o}C; IR (KBr, cm^{-1}): *υ* 3223 (N-H str.), 3084 (C-H str. of aromatic ring), 2879 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1151 (C-N-C bond str.), 621 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.82 (d, *J* = 2.0 Hz, 1H, NH-1), 7.52 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.35 (br.d, *J* = 8.0 Hz, 1H, H-4), 7.26 (br. t, *J*=7.56 Hz, 2H, H-3'''& H-5'''), 7.20-7.15 (m, 3H, H-2''', H-4''' & 6'''), 7.14 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.07 (br.t, *J* = 7.4 Hz, 1H, H-5), 6.97 (br.t, *J* = 7.3 Hz, 1H, H-6), 3.17 (t, *J* = 7.26 Hz, 2H, CH_2-9'''), 2.85 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.77 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.69 (t, *J* = 7.6, 2H, CH_2-7'''), 2.05 (m, 4H, CH_2-2' & CH_2-8'''); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 167.77 (C-5''), 162.88 (C-2''), 140.71 (C-1'''), 136.30 (C-8), 128.31 (C-2''' &C-6'''), 128.17 (C-3''' &C-5'''), 125.91 (C-4'''), 127.02 (C-9), 122.47 (C-2), 120.88 (C-6), 118.16 (C-4 & C-5), 113.27 (C-3), 111.36 (C-7), 33.70 (C-7'''), 31.42 (C-8'''), 30.60 (C-9'''), 26.52 (C-1'), 24.27 (C-3'), 23.82 (C-2'); Anal. Calc. for $C_{22}H_{23}N_3SO$ (377.16): C, 70.00; H, 6.14; N, 11.13. Found: C, 70.16; H, 6.29; N, 11.27.



Scheme 1: Outline for the synthesis of alkyl/aralkyl substituted (**6a-m**) and un/substituted benzyl derivatives (**6n-r**) of 5-[3-(1*H*-Indol-3-yl)propyl]-1,3,4-oxadiazole-2-thiol (**4**). Reagents & Conditions: (I) EtOH/H_2SO_4/refluxing for **8** hrs. (II) MeOH/N_2H_4•H_2O/refluxing for **14** hrs. (III) EtOH/CS_2/KOH/refluxing for **16** hrs. (IV) DMF/LiH/stirring for **6-8** hrs.

Table 1: Different substituents (**-R_1**) in **5a-m** & **6a-m** of scheme **1**.



Compd.	-R_1	Compd.	-R_1
5a, 6a	—CH_2—CH_3	5g, 6g	—CH(—CH_3)—CH_2—CH_3
5b, 6b	—CH_2—CH_2—Cl	5h, 6h	—CH_2—CH_2—CH_2—CH_2—CH_3
5c, 6c	—CH_2—CH_2—CH_3	5i, 6i	—CH(—CH_3)—CH_2—CH_2—CH_3
5d, 6d	—CH_2—CH_2—CH_2—I	5j, 6j	—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_3
5e, 6e	—CH(—CH_3)(—CH_3)	5k, 6k	—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_3
5f, 6f	—CH_2—CH=CH_2	5l, 6l	—H_2C—H_2C—(benzyl)
5m, 6m	—H_2C—H_2C—H_2C—(benzyl)		

Table 2: Different substituents (**-R_2**) in **5n-r** & **6n-r** of scheme **1**.

Compd.	5n, 6n	5o, 6o	5p, 6p	5q, 6q	5r, 6r
-R_2	-H	2-CH_3	2-Cl	3-Cl	4-Br

Table 3: *Bacillus subtilis* and *Escherichia coli* bacterial biofilm inhibition studies

Compound	*Bacillus subtilis* Bio film Inhibition		*Escherichia coli* Bio film Inhibition	
	Absorbance	% Inhibition	Absorbance	% Inhibition
6a	0.839	17.82	0.698	28.77
6b	0.218	78.64	0.204	79.18
6c	0.278	69.24	0.886	9.59
6d	0.285	72.08	0.217	77.85
6e	0.787	22.91	0.823	16.02
6f	0.124	87.95	0.44	44.1
6g	0.867	15.08	0.62	36.73
6h	0.383	62.48	0.882	10.0
6k	1.009	1.175	0.855	12.75
6l	0.764	25.17	0.263	73.16
6m	0.305	70.12	0.332	66.12
6n	0.222	78.25	0.698	28.75
6o	0.254	75.12	0.479	51.12
6p	0.932	8.71	0.868	11.42
6q	0.569	44.27	0.849	13.36
6r	0.354	65.32	0.102	89.59
Ciprofloxacin	0.195	80.9	0.131	86.63

Note: Ciprofloxacin was used as a positive control. Negative control (% Inhibition) = 1.021.

Table 4: The percentage inhibition of the HCT 116 cell lines at 25 µM and 50 µM concentrations

Compound	%age inhibition		Compound	%age inhibition	
	25 µm	50 µm		25 µm	50 µm
6a	16.15	20.51	6k	10.25	11.02
6b	2.63	5.12	6l	8.97	-6.66
6c	8.53	4.80	6m	7.44	9.47
6d	35.12	26.92	6n	12.30	6.41
6e	31.02	3.58	6o	10.25	17.69
6f	19.37	26.82	6p	0	10.76
6g	6.35	42.63	6q	16.41	34.10
6h	15.96	27.75	6r	10.25	24.61
6i	7.90	18.14	DMSO	0	0
6j	10.38	23.10			

Table 5: Cytotoxic potential through hemolytic activity

Compound	% Hemolysis	Compound	% Hemolysis	Compound	% Hemolysis
6a	17.15	6g	6.25	6m	37.85
6b	18.85	6h	0.65	6n	43.35
6c	15.65	6i	-	6o	44.85
6d	2.75	6j	-	6p	30.15
6e	30.75	6k	25.55	6q	11.15
6f	9.95	6l	28.65	6r	22.25
Note: PBS (% Hemolysis) = 1.03.				Triton X	87.67

Benzyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6n)

Yellow solid; Mol. formula$C_{20}H_{19}N_3SO$; Mol. mass 349 $gmol^{-1}$; yield: 64%; melting point 147°C; IR (KBr, cm^{-1}): υ 3217 (N-H str.), 3055 (C-H str. of aromatic ring), 2878 (C-H str. of aliphatic), 1583 (C=C aromatic str.), 1156 (C-N-C bond str.), 680 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.81 (s, 1H, NH-1), 7.52 (br.d, *J* = 7.9 Hz, 1H, H-7), 7.42 (br.d, *J* = 7.0 Hz, 2H, H-2''' & H-6'''), 7.33 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.31 (br.t, *J* = 6.9

Hz, 2H, H-3''' & H-5'''), 7.28 (br.t, *J* = 7.3 Hz, 1H, H-4'''), 7.14 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.07 (br.dt, *J* = 1.2, 8.1 Hz, 1H, H-5), 6.98 (br.dt, *J* = 1.0, 8.0 Hz, 1H, H-6), 4.46 (s, 2H, CH_2-7'''), 2.87 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.76 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.04 (q, *J* = 7.4 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.00 (C-5''), 162.44 (C-2''), 136.50 (C-1'''), 136.30 (C-8), 128.91 (C-3''' & 5'''), 128.52 (C-2''' &C-6'''), 127.66 (C-4'''), 127.02 (C-9), 122.47 (C-2), 120.86 (C-6), 118.18 (C-4), 118.15 (C-5), 113.26 (C-3), 111.35 (C-7), 35.85 (C-7'''), 26.50 (C-1'), 24.26 (C-3'), 23.80 (C-2'); Anal; Calc. for $C_{20}H_{19}N_3SO$ (349.12): C, 68.74; H, 5.48; N, 12.02. Found: C, 68.86; H, 5.55; N, 12.19.

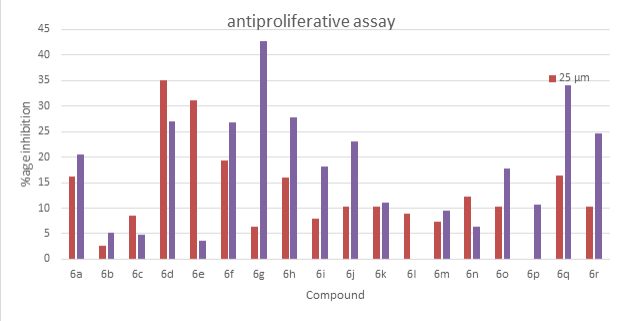


Fig. 1: Graphic representation of anti-proliferation activity of the compounds at 25 µM and 50 µM concentrations against HCT 116 cell lines.

5-[3-(1H-Indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl 2-methylbenzyl sulfide (6o)

Reddish orange solid; Mol. formula $C_{21}H_{21}N_3SO$; Mol. Mass: 363 gmol^{-1}; yield: 97%; melting point: 157^{o}C; IR (KBr, cm^{-1}): *υ* 3185 (N-H str.), 3080 (C-H str. of aromatic ring), 2928 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1153 (C-N-C bond str.), 680 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.82 (s, 1H, NH-1), 7.51 (br.d, *J* = 8.1 Hz, 1H, H-7), 7.35 (br.dd, *J* = 6.6, 8.0 Hz, 1H, H-6'''), 7.34 (br.dd, *J* = 6.6, 7.5 Hz, 1H, H-4), 7.2-7.17 (m, 2H, H-3'''& H-4'''), 7.14 (dist.d, *J* = 2.28 Hz, 1H, H-2), 7.13-7.1 (m, 1H, H-5'''), 7.07 (br.dt, *J* = 1.2, 7.9 Hz, 1H, H-6), 6.98 (br.dt, *J* = 1.2, 8.0 Hz, 1H, H-5), 4.48 (s, 2H, CH_2-7'''), 2.88 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.77 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.04 (q, *J* = 7.4 Hz, 2H, CH_2-2'), 2.37 (s, 3H, CH_3-2'''); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.06 (C-5''), 162.29 (C-2''), 136.69 (C-1'''), 136.30 (C-8), 133.75 (C-2'''), 130.41 (C-3'''), 129.88 (C-4'''), 128.11 (C-6'''), 127.02 (C-9), 126.02 (C-5'''), 122.48 (C-2), 120.86 (C-6), 118.18 (C-7), 118.15 (C-5), 113.26 (C-3), 111.35 (C-4), 34.43 (C-7'''), 26.51 (C-2'), 24.28 (C-1'), 23.81 (C-3'), 18.65 (CH_3-C-2'''); Anal. Calc. for $C_{21}H_{21}N_3SO$ (363.14): C, 69.39; H, 5.82; N, 11.56. Found: C, 69.46; H, 5.75; N, 11.50.

2-Chlorobenzyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6p)

Light yellow solid; Mol. Formula $C_{20}H_{18}ClN_3SO$; Mol. mass: 383 gmol^{-1}; yield: 72%; melting point 142^{o}C; IR (KBr, cm^{-1}): *υ* 3224 (N-H str.), 3088 (C-H str. of aromatic ring), 2928 (C-H str. of aliphatic), 1585 (C=C aromatic str.), 1153 (C-N-C bond str.), 667 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.80 (s, 1H, NH-1), 7.54 (br.dd, *J* = 1.8, 7.4 Hz, 2H, H-6'''), 7.5 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.46 (br.dd, *J* = 1.3, 7.8 Hz, 2H, H-3'''), 7.34 (br.d, *J* = 7.9 Hz,, 1H, H-4), 7.33-7.26 (m, 2H, H-4''' & H-5'''), 7.13 (dist.d, *J* = 2.22 Hz, 1H, H-2), 7.07 (br.dt, *J* = 1.2, 8.0 Hz, 1H, H-5), 6.97 (br.dt, *J* = 1.0, 8.0 Hz, 1H, H-6), 4.5 (s, 2H, CH_2-7'''), 2.86 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.76 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.04 (q, *J* = 7.4 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.29 (C-5''), 161.98 (C-2''), 136.29 (C-8), 133.84 (5'''), 133.22 (3'''), 131.43 (C-1'''), 129.85 (C-6'''), 129.53 (C-2'''), 127.37 (C-4'''), 127.01 (C-9), 122.47 (C-2), 120.87 (C-6), 118.19 (C-4), 118.16 (C-5), 113.25 (C-3), 111.35 (C-7), 34.17 (C-7'''), 26.49 (C-2'), 24.28 (C-1'), 23.40 (C-3'); Anal. Calc. for $C_{20}H_{18}ClN_3SO$ (383.09): C, 62.57; H, 4.73; N, 10.95. Found: C, 62.65; H, 4.86; N, 10.99.

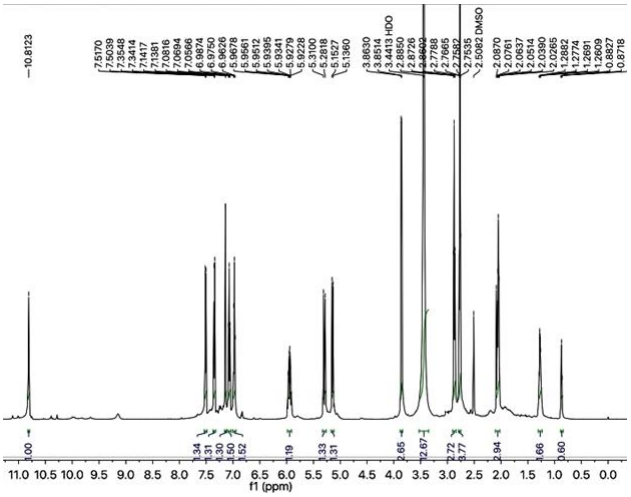


Fig. 2: ^{1}H-NMR spectrum of **6f**

3-Chlorobenzyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6q)

Yellow solid; Mol. formula $C_{20}H_{18}ClN_3SO$; Mol. mass: 383 gmol^{-1}; yield: 65%; melting point: 150^{o}C; IR (KBr, cm^{-1}): *υ* 3216 (N-H str.), 3082 (C-H str. of aromatic ring), 2843 (C-H str. of aliphatic), 1585 (C=C aromatic str.), 1158 (C-N-C bond str.), 678 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.80 (d, *J* = 1.9 Hz, 1H, NH-1), 7.56-7.51 (m, 2H, H-7 & H-2'''), 7.4-7.37 (m, 2H, H-4'''& H-5'''), 7.34 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.32 (dist.d, *J* = 6.6 Hz, 1H, H-6'''), 7.13 (dist.d, *J* = 2.2 Hz, 1H, H-2), 7.07 (br.t, *J* = 7.5 Hz, 1H, H-5), 6.97 (br.t, *J* = 7.5 Hz, 1H, H-6), 4.45 (s, 2H, CH_2-7'''), 2.86 (t, *J* = 7.4 Hz, 2H, CH_2-3'), 2.76 (t, *J* = 7.4 Hz, 2H, CH_2-1'), 2.04 (q, *J* = 7.4 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.11 (C-5''), 162.27 (C-2''), 139.32 (C-1'''), 136.30 (C-8), 132.95 (C-3'''), 130.28 (C-4'''), 128.76 (C-5'''), 127.61 (C-2'''), 127.58 (C-6'''), 127.02 (C-9), 122.47 (C-2), 120.86 (C-6), 118.18 (C-4), 118.16 (C-5), 113.24 (C-3), 111.35 (C-7), 35.04 (C-7'''), 26.49 (C-1'), 24.26 (C-

3'), 23.80 (C-2'); Anal. Calc. for $C_{20}H_{18}ClN_3SO$ (383.09): C, 62.57; H, 4.73; N, 10.95. Found: C, 62.69; H, 4.83; N, 11.16.

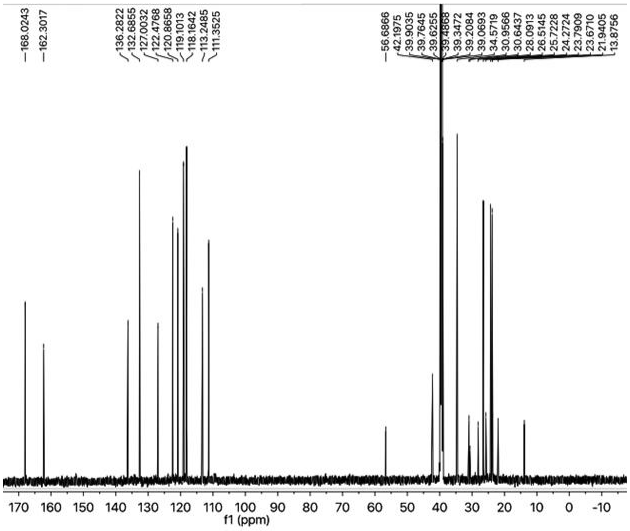


Fig. 3: ^{13}C-NMR spectrum of **6f** molecule.

4-Bromobenzyl 5-[3-(1H-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-yl sulfide (6r)

Light yellow solid; Mol. formula: $C_{20}H_{18}N_3BrSO$; Mol. mass: 428 $gmol^{-1}$; yield: 58%; melting point: 165^oC; IR (KBr, cm^{-1}): υ 3214 (N-H str.), 2925 (C-H str. of aromatic ring), 2869 (C-H str. of aliphatic), 1587 (C=C aromatic str.), 1151 (C-N-C bond str.), 680 (C-S str.) cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): δ 10.80 (s, 1H, NH-1), 7.50 (br.d, *J* = 7.8 Hz, 1H, H-7), 7.45 (br.d, *J* = 8.0 Hz, 2H, H-2''' & H-6'''), 7.38 (dist.d, *J* = 8.3 Hz, 2H, H-3''' & H-5'''), 7.34 (br.d, *J* = 8.1 Hz, 1H, H-4), 7.13 (s, 1H, H-2), 7.07 (br.t, *J* = 7.4 Hz, 1H, H-5), 6.97 (br.t, *J* = 7.4 Hz, 1H, H-6), 4.4 (s, 2H, CH_2-7'''), 2.86 (t, *J* = 7.5 Hz, 2H, CH_2-3'), 2.78 (t, *J* = 7.3 Hz, 2H, CH_2-1'), 2.03 (q, *J* = 7.5 Hz, 2H, CH_2-2'); ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): δ 168.08 (C-5''), 162.27 (C-2''), 136.29 (C-8), 135.84 (C-1'''), 132.29 (C-4'''), 131.36 (C-3''' & 5'''), 131.13 (C-2''' & 6'''), 127.01 (C-9), 122.47 (C-2), 120.86 (C-6), 120.82 (C-4), 118.16 (C-5), 113.24 (C-3), 111.35 (C-7), 35.05 (C-7'''), 26.47 (C-1'), 24.26 (C-3'), 23.79 (C-2'); Anal. Calc. for $C_{20}H_{18}N_3BrSO$ (428.35): C, 56.08; H, 4.24, N, 9.81. Found: C, 56.15; H, 4.33, N, 9.92.

RESULTS

In the presented research work, different derivatives of 5-[3-(1*H*-indol-3-yl)propyl]-1,3,4-oxadiazol-2-ylhydrosulfide were synthesized according to the outline illustrated in Scheme 1; table 1& table 2. The synthesis was accomplished by reacting 5-[3-(1*H*-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-ylhydrosulfide (4) with different alkyl/aralkyl halides (5a-r) in DMF and in the presence of LiH to obtain various *S*-substituted derivatives (6a–r). The structures of the targeted molecules were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectral techniques and the spectral data is given in the experimental section. The CHN analysis data also supported their structural assignments. Their antibacterial potential was then ascertained by the biofilm inhibition study against two bacterial strains i.e. *Bacillus subtilis* and *Escherichia coli* and these results are tabulated in table 3. The anti-proliferative activity results of the different derivatives are shown in fig. 1 and table 4. Their cytotoxicity profile was also studied through hemolytic study and the results are shown in table 5.

DISCUSSION

Chemistry

The synthesis of targeted *S*-substituted derivatives (6a–r) was carried out successfully in very good yields. First the, 4-(1*H*-Indol-3-yl)butanoic acid(1) was subjected to esterification by ethanol in the presence of concentrated sulphuric acid taken in catalytic amount. The ethanol is used as reactant and also solvent in order to push the equilibrium towards product side, as it is a reversible reaction. The product was collected by solvent extraction after addition of a weak base and excess of water. The addition of base neutralized the unreacted carboxylic acid and the catalytic sulphuric acid. The salts of these acids were transferred to the aqueous layer while the resulting ester was partitioned to the organic phase during solvent extraction. Thus, ethyl 4-(1*H*-indol-3-yl) butanoate (2) was obtained as brownish liquid (solid at refrigeration). The second step was performed to convert 2 into respective carbohydrazide 3 by the nucleophillic hydrazine in the presence of an organic solvent like methanol or ethanol and stirring for 14 hrs at room temperature. This nucleophillic substitution reaction is generally carried out at room temperature but sometimes a little bit higher temperature in the form of reflux might be required. The completion of this reaction yielded 4-(1*H*-indol-3-yl)butanohydrazide (3) as light brown solid. The third step in this synthesis was to form a heterocyclic ring through reaction with CS_2 in a basic alcoholic medium. The resulting cyclized product was 5-[3-(1*H*-indol-3-yl) propyl]-1, 3, 4-oxadiazol-2-ylhydrosulfide (4) having a mercapto group at its second carbon. Then, in last step, the acidic proton of this mercapto group was replaced with different alkyl/aralkyl groups by reaction with different alkyl/aralkyl halides, **5a-r,** in the presence of LiH using aprotic polar medium to get required derivatives, 6a-r, as sketched in Scheme-1, table 1 and table 2. The structures of all these derivatives were confirmed by spectral techniques, IR, ^{1}H-NMR, and ^{13}C-NMR. The CHN analysis data also augmented these structural assignments. The structural analysis of one of the compounds is discussed here in detail for the benefit of the reader. The molecule 6f was obtained as brown solid. Its molecular formula was established by counting the number of protons in its ^{1}H-NMR spectrum and

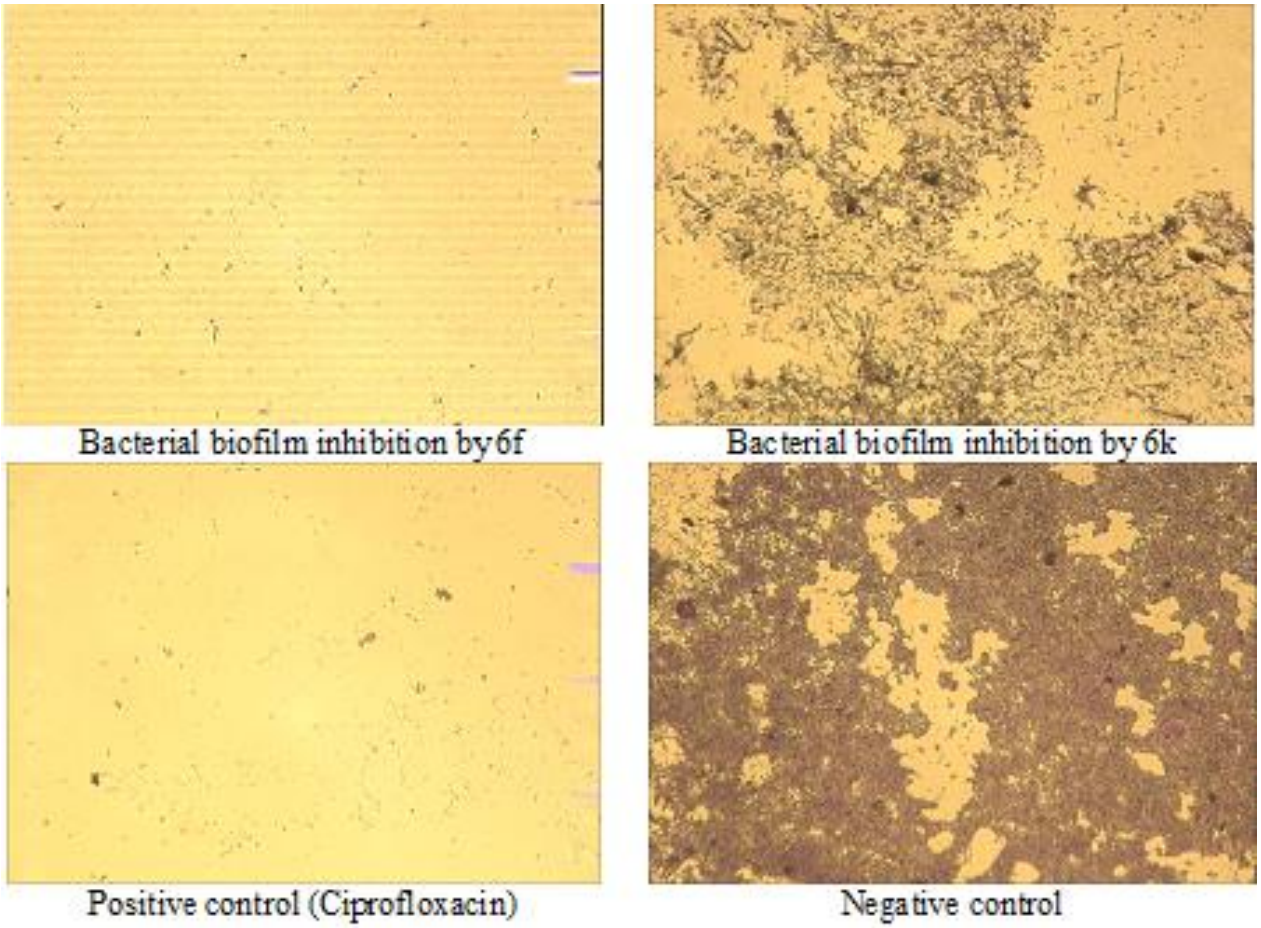


Fig. 4: Phase contrast microscopic view of inhibition of *Bacillus subtilis* Bacterial biofilm.

number of carbon resonances in its ^{13}C-NMR spectrum. The CHN analysis data was also in agreement with its molecular formal, $C_{16}H_{17}N_3SO$. Different functionalities in this molecule were depicted by absorption bands in its IR spectrum at n 3210 (N-H streching), 2926 (C-H Ar streching), 1588 (C=C Ar streching), 1153 (C-N-C streching), 736 (C-N streching) and 679 (C-S streching) cm^{-1}. With the help of ^{1}H-NMR spectrum of this molecule, the indole heterocyclic core was identified clearly by the characteristic signals at δ 10.81 (s, 1H, NH-1), 7.51 (br.d, J = 7.8 Hz, 1H, H-7), 7.34 (br.d, J = 8.0 Hz, 1H, H-4), 7.14 (dist.d, J = 2.1 1H, H-2), 7.07 (br.t, J = 7.4 Hz, 1H, H-5), 6.97 (br.t, J = 7.4 Hz, 1H, H-6) (Rubab *et. al.*, 2016a). Similarly, the resonances at δ 5.96-592 (m, 1H, CH-2'''), 5.31 (br.d, J = 16.9 Hz, 1H, H_b-3'''), 5.13 (br.d, J = 10.0 Hz, 1H, H_a-3'''), and 3.86 (d, J = 8.0 Hz, 2H, CH_2-1''') were an attribute of the substitution of an allyl group in the molecule (Abbasi *et. al.*, 2013). In the up-field region of spectrum, the signals of three intervening methylene groups at δ 2.86 (t, J = 7.4 Hz, 2H, CH_2-3'), 2.76 (t, J=7.4 Hz, 2H, CH_2-1'), and 2.05 (q, J = 7.4 Hz, 2H, CH_2-2'), were helpful to ascertain the connectivity of indole moiety from its 3-position to the 5-position of oxadiazole scaffold. The ^{1}H-NMR spectrum of this compound has been shown in fig. 2. The carbon skeleton of this molecule was also fully supported by its ^{13}C-NMR spectrum, shown in the fig. 3. The ^{13}C-NMR spectrum depicted the signals of all the sixteen carbons, where the most downfield quaternary carbons signals at δ 168.02 (C-5'') and 162.30 (C-2'') belonged to the cyclized 1,3,4-oxadiazole ring, thus confirming the formation of this ring. The other three quaternary carbons appearing at δ 136.28 (C-8), 127.00 (C-9), and 113.25 (C-3) ppm, were attribute of the indole core. The methine carbon resonances appearing at δ 122.47 (C-2), 120.86 (C-6), 118.18 (C-4), 118.16 (C-5), 111.35 (C-7) were also coherent with the indole moiety. The allyl group substituted at the sulfur atom was demonstrated by three signals at δ 132.60 (C-2'''), 119.10 (C-3'''), 34.57 (C-1''') while three methylene signals appearing in up-field region at δ 26.51 (C-1'), 24.28 (C-3'), and 23.79 (C-2') were assignable to those methylene groups, which were connecting the indole and oxadiazole moieties together. So, on the basis of aforesaid cumulative evidences, the structure of 6f was confirmed as allyl 5-[3-(1*H*-indol-3-yl)propyl]-1,3,4-oxadiazol-2-yl sulfide. A similar protocol was exercised for the structural characterization of other derivatives in the synthesized series.

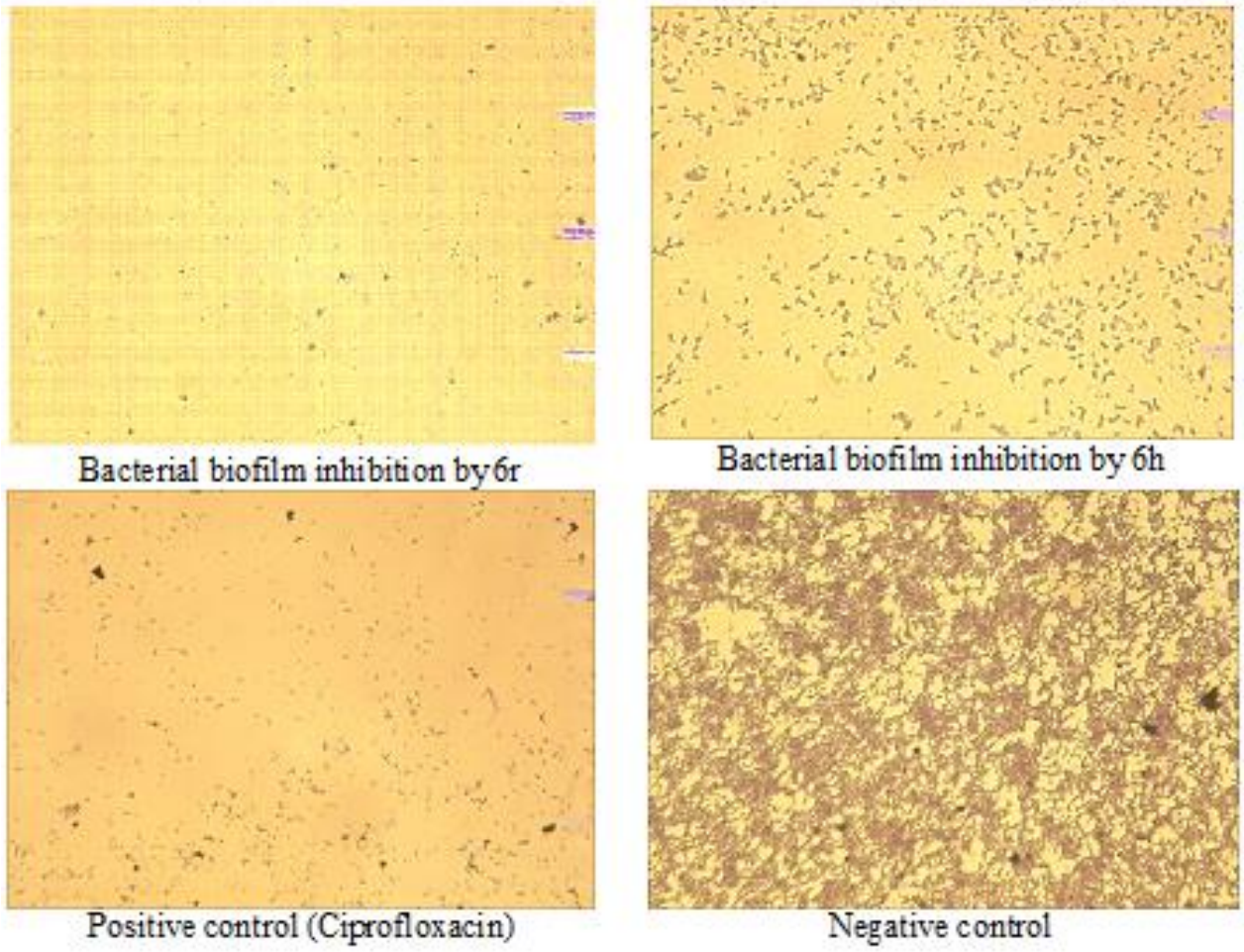


Fig 5: Phase contrast microscopic view of inhibition of *Escherichia coli* Bacterial biofilm.

Biofilm inhibition and structure-activity relationship

The antibacterial activity of the derivatives, 6a-r, was checked by bacterial biofilm inhibition method using two bacterial pathogenic strains, one of which was gram positive (*Bacillus subtilis*) and other was a gram negative (*Escherichia coli*) strain. Ciprofloxacin was used as a standard drug in both assays to compare the antibacterial potential of the synthesized molecules. From the results (table 3), an increase or decrease in the antibacterial potential was observed with the variations of substituents in molecules, 6a–r. Here, it was noted that among the series, maximum inhibition (89.85%) was given by 6f against *B. subtilis*. The percentage inhibition of this molecule was even higher than the standard Ciprofloxacin having value of 80.9%. The greater potential of 6f can be attributed to the substitution of allyl group at sulfur atom in this molecule. The other compounds 6m, 6r, 6o, 6n, 6d, 6b, 6c and 6h also showed good bacterial biofilm inhibition against this strain with percentage of 70.12%, 65.32%, 75.12%, 78.25%, 72.08%, 78.64%, 69.24% and 62.48%, respectively, owing to the respective substitutions. The phase contrast microscopic views of inhibition of *Bacillus subtilis* biofilm by 6f and 6k are shown in fig. 4. Against *Escherichia coli*, the molecule 6r exhibited an excellent antibacterial potential with percentage inhibition of 89.59%, relative to Ciprofloxacin (86.63%.The potent activity of this molecule might be an outcome of the substitution of 4-bromobenzyl group at the sulfur atom of oxadiazole scaffold. The other compounds 6m, 6l, 6d and 6b also showed good biofilm inhibition against this strain with percentage of 66.12%, 73.16%, 77.85 and 79.18%, respectively. The phase contrast microscopic views of inhibition of *Escherichia coli* biofilm by 6r and 6h are shown in fig. 5.

Anticancer activity and structure-activity relationship

All the synthesized derivatives, 6a-r, were tested for their anti-proliferation activity against HCT-116 cell lines at 25 µM and 50 µM concentrations (fig. 1; table 4). Among the series, the molecule 6d was found as most active compound with 35.12% inhibition at 25 µM concentration while 6g was identified as better inhibitor with inhibition of 42.63% at 50 µM concentration relative of other analogues. As these compounds differ from one another only in the substitution at the sulfur atom of oxadiazole unit, yet the varying results at different concentrations lead to the fact that these molecules exhibit a dose dependent response.

Hemolytic activity

All the synthesized compounds, 6a-r, were also subjected to hemolytic assay to find out their cytotoxicity profile. Results of percentage hemolysis are shown in table 5. Our results showed that all compounds of this series have moderate toxicity towards red blood cell membrane. Maximum membrane toxicity was shown by the compound 6o (44.85%) while minimum toxicity was recorded in 6h (0.65%). Precisely, a low toxicity was observed for molecule 6d (2.75%), 6g (6.25%), 6f (7.95%), 6q (11.15%), 6c (15.65%), 6a (17.15%) and 6b (18.85%) relative to Triton-X having % hemolysis of 87.67%.

CONCLUSION

On the basis of bacterial biofilm inhibition study, it was concluded that most of the synthesized molecules exhibited very good antibacterial potential against *Bacillus subtilis* and *Escherichia* coli. Some of the compounds revealed reasonable anticancer potentials, however, their response was dependent on the concentration. The compound 6f against *B. subtilis* can be used as new drug candidates. Furthermore, most of the molecules also have moderate cytotoxicity, so it can be concluded that these molecules are suitable therapeutic candidates for further structure optimizations and drug designing studies.

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