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RESEARCH ARTICLE

Diclofenac 1,3,4-Oxadiazole Derivatives; Biology-Oriented Drug Synthesis (BIODS) in Search of Better Non-Steroidal, Non-Acid Antiinflammatory Agents

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Abstract: ***Background*:** Inflammation is defined as the response of immune system cells to damaged or injured tissues. The major symptoms of inflammation include increased blood flow, cellular influx, edema, elevated cellular metabolism, reactive oxygen species (ROS) nitric oxide (NO) and vasodilation. This normally protective mechanism against harmful agents when this normal mechanism becomes dysregulated that can cause serious illnesses including ulcerative colitis, Crohn's disease, rheumatoid arthritis, osteoarthritis, sepsis, and chronic pulmonary inflammation.

***Method*:** In this study synthetic transformations on diclofenac were carried out in search of better non-steroidal antiinflammatory drugs (**NSAIDs**), non-acidic, antiinflammatory agents. For this purpose diclofenac derivatives (**2-20**) were synthesized from diclofenac (**1**). All derivatives (**2-20**) and parent diclofenac (**1**) were evaluated for their antiinflammatory effect using different parameters including suppression of intracellular reactive oxygen species (ROS), produced by whole blood phagocytes, produced by neutrophils, and inhibition of nitric oxide (NO) production from J774.2 macrophages. The most active compound also evaluated for cytotoxicity activity.

***Results*:** Diclofenac (**1**) inhibited the ROS with an IC_{50} of 3.9 ± 2.8, 1.2 ± 0.0 µg/mL respectively and inhibited NO with an IC_{50} of 30.01 ± 0.01 µg/mL. Among its derivatives **4**, **5**, **11**, **16**, and **20**, showed better antiinflammatory potential. The compound **5** was found to be the most potent inhibitor of intracellular ROS as well as NO with IC_{50} values of 1.9 ± 0.9, 1.7 ± 0.4 µg/mL respectively and 7.13 ± 1.0 µg/mL, respectively, and showed good inhibitory activity than parent diclofenac. The most active compounds were tested for their toxic effect on NIH-3T3 cells where all compounds were found to be non-toxic compared to the standard cytotoxic drug cyclohexamide.

***Conclusion*:** Five derivatives were found to be active. Compound **5** was found to be the most potent inhibitor of ROS and NO compared to parent diclofenac **1** and standard drugs ibuprofen and L-NMMA, respectively. The most active compounds **1**, **4**, **5**, **11** and **20** were found to be non-toxic on NIH-3T3 cells. Compound **4**, **5**, and **20** also showed good antiinflammatory potential, compound **11** and **16** showed moderate and low level of inhibition, respectively.

ARTICLE HISTORY

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1. INTRODUCTION

Inflammation is defined as the response of immune system cells to damaged or injured tissues that involve migration of leucocytes such as neutrophils and macrophages from blood to damaged cells, irritants and pathogens *etc* for their repair and removal [1]. During inflammation, inflammatory mediators including reactive oxygen and nitrogen species, leukotrienes, prostaglandins, cytokines *etc* are released that increases the vascular permeability and leucocytes migration to the site of inflammation [2]. The major symptoms of inflammation include increased blood flow, cellular influx, edema, elevated cellular metabolism reactive oxygen species

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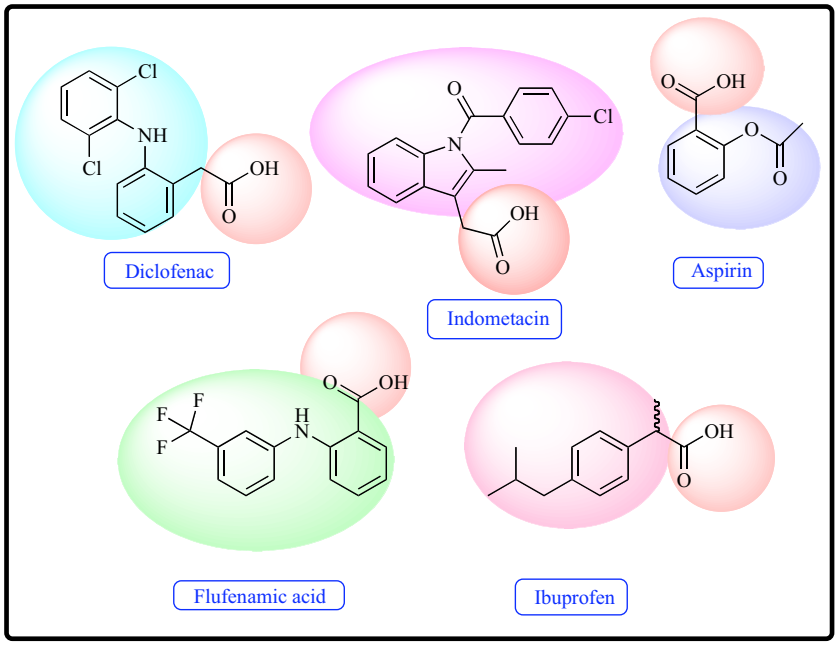


Fig. (1). Reported antiinflammatory drug having acidic moiety.

(ROS) nitric oxide (NO) and vasodilation [3]. This normally protective mechanism against harmful agents when this normal mechanism becomes dysregulated that can cause serious illnesses including ulcerative colitis, Crohn's disease, rheumatoid arthritis, osteoarthritis, sepsis, and chronic pulmonary inflammation [4].

The most favored class of drug in use for controlling various pathological conditions is the non-steroidal anti-inflammatory drugs (NSAIDs) [5]. The mode of action of NSAIDs involves the repression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) [6]. Among the most admired NSAIDs is diclofenac sodium. This drug is accepted in more than 120 countries across the world, and is ranked 30th among the top 200 drugs [7].

Chemical name of diclofenac is 2-(2, 6-dichloranilino) phenylacetic acid. Diclofenac has potential biological activities including antibacterial [8], antitumour [9], anticancer [10, 11], however, the major use of diclofenac is as an antiinflammatory drug [11, 12]. The suppressive effect of diclofenac on ROS produced from rat peritoneal neutrophils was also reported previously [13]. Unfortunately, all NSAIDs drugs such as diclofenac, indomethacin, aspirin, flufenamic acid, phenylbutazone, and ibuprofen have gastrointestinal tract side effects which is mainly due to the contact of open carboxylic moiety (pKa = 3.5-5.5) in these drugs to gastrointestinal tract [14, 15] (Fig. **1**). Therefore, our research group continuously engaged to combat this evil disease in an efficient manner by synthesizing different functionally oriented molecules and evaluate their antiinflammatory activities in past [16-21].

BIODS science consists of some modification of previously known drug to reduce its side effect, increase activity potential *etc*. In the current study, we used the BIODS approach [22], syntheses of 1,3,4-oxadiazole derivatives of marketed antiinflammatory drug (diclofenac) were carried out and evaluate their antiinflammatory potentials. As 1,3,4-oxadiazole also possess good antiinflammatory activity [23, 24]. To the best of our knowledge only compounds **1-4** were previously reported while remaining analogs are new.

2. MATERIALS AND METHOD

Methanol, hydrazine hydrate, carbon disulfide, phenacyl halide, aryl halides, and potassium hydroxide were purchased from Sigma-Aldrich, USA and used as received without purification. Diclofenac was donated by a local pharmaceutical company. TLC plates were pre-coated silica gel aluminium plates (Kieselgel 60, 254, E. Merck). Visualization of TLC chromatograms was carried out under UV light at wavelengths of 254 and 365 nm. Melting points of all the compounds were determined on Stuart® SMP10 melting point apparatus and are uncorrected. EI-MS has recorded on a Finnigan MAT-311A (Germany) mass spectrometer. ^{1}H-NMR experiments were carried out on Avance Bruker AM 300, 400, and 500 MHz machines. ^{13}C-NMR experiments were carried on Avance Bruker AM 300, 400 and 500 MHz instruments at 75, 100, and 125 MHz, respectively. Infrared (IR) spectra were recorded on JASCO IR-A-302 spectrophotometer.

3. EXPERIMENTAL

3.1. Antiinflammatory Assays

3.1.1. Oxidative Burst Inhibition by Chemiluminescence Technique

Oxidative burst studies using luminol enhanced chemiluminescence technique were performed as described by Mesaik *et al.* with some modifications [25]. The assay was performed on whole blood and neutrophils isolated from blood of healthy human volunteers using luminol as a probe

and zymosan as an activator. Briefly 25 μL of diluted whole blood in HBSS++ or 25 μL of isolated neutrophils (1 x 10^6 cells/mL) were incubated with 25 μL of different concentrations of compounds (1, 10, 25 and 100 μg/mL) each in triplicate in white half area 96 well plates (Costar, NY, USA). The plates were incubated at 37 °C for 15 min in the thermostat chamber of a luminometer (Labsystems, Helsinki, Finland). 25 μL of (7 x 10^{-5} M) luminol (Research Organics, Cleveland, OH, USA) and, 25 μL serum opsonized zymosan (SOZ) 2 mg/mL (Fluka, Buchs, Switzerland) was then added into the wells. The plates were then read in luminometer for 50 min and results were recorded as total integral readings as relative light units (RLU) [25].

3.1.2. Nitric Oxide Assay

The mouse macrophage cell line J774.2 (European Collection of Cell Cultures, UK) was cultured in 75 mL flasks IWAKI (Asahi Techno Glass, Japan) in DMEM Sigma-Aldrich (Steinheim, Germany) that contained 10% fetal bovine serum GIBCO (NY US) supplemented with streptomycin/penicillin 1%. Flasks were kept at 37 °C in atmosphere of humidified air containing 5% CO_2, cells were seeded in 96-well plate (1 x 10^6 cells/mL), and were stimulated by 30 μg/mL *E. coli* lipopolysaccharide (LPS) (DIFCO Laboratories Michigon, USA) and test compounds were added at three different concentrations (1, 10, 100 μg/mL). The plates were incubated at 37 °C in 5% CO_2 and humidified air for 48 hours. The cell culture supernatants were collected. nitrite accumulation in J774.2 cell culture supernatant was measured using the Griess method described previously [26].

3.1.3. MTT Cytotoxicity Assay

Cytotoxicity of compounds on NIH-3T3 fibroblast cells (ATCC, Manassas, USA) was evaluated by using the standard MTT colorimetric assay. Briefly, 100 µL of 6 × 10^4 cells/mL in DMEM supplemented with 10% FBS were plated into 96-wells flat bottom plate and incubated overnight at 37°C in 5% CO_2. Three different concentrations of test compound (1, 10 and 100 µg/mL) were added to the plate in triplicates and incubated for 48 h. 50 µL of 0.5 mg/mL MTT was added to each well and plates were then further incubated for 4 h. MTT was aspirated and 100 µL of DMSO was then added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using spectrophotometer (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxic activity was recorded as concentration causing 50% growth inhibition (IC_{50}) for 3T3 cells [27].

3.1.4. General Procedure

3.1.4.1. Diclofenac (1)

Diclofenac sodium (5 g) was dissolved in water with stirring followed by the addition of 2 mL of 2 N HCl drop wise to obtain the precipitate of diclofenac acid then addition of ice water for the termination of reaction. A solid was formed, filtered, washed with cold water and dry at room temperature. The structure was identified by using spectroscopic techniques including, ^{1}H-, ^{13}C-NMR, EI-MS, ESI-MS, HRMS, and IR.

3.1.4.2. Diclofenac Methyl Ester (2)

Diclofenac acid (5 g) was dissolved in methanol (80 mL) with stirring and sulfuric acid (3 mL) was added drop wise with continuous stirring and the mixture was heated under reflux for 15 min. After completion of the reaction (TLC analysis), it was cooled to room temperature and poured onto crushed ice (250 g) to obtain crystalline solid (**2**) which was filtered, washed with water followed by 5% sodium hydrogen carbonate solution, finally with water and dried in air.

3.1.4.3. Diclofenac Hydrazide (3)

Diclofenac ester (2 g) was dissolved in 20 mL methanol then hydrazine hydrate (10 mL) was added while keeping in ice bath and stirred for 48 h. Progress of the reaction was monitored through TLC. A solid was formed when poured on to ice, filtered and dried in air.

3.1.4.4. Diclofenac Oxadiazole-2-thione (4)

To a solution of diclofenac hydrazide (1.0 g in 50 mL of ethanol), potassium hydroxide (0.5 g) and carbon disulfide (12 mL) were added drop wise at room temperature and the reaction was allowed to reflux for 24 h. After completion of reaction (TLC analysis), a potassium salt of the product was obtained which was acidified with HCL (conc.) with stirring condition to obtain a solid product which was filtered, washed with distilled water, and dry at room temperature.

3.1.4.5. Derivatives of Diclofenac Oxadiazole Thione (5-20)

Diclofenac oxadiazole thione (0.352 g), ethanol (15 mL), triethyl amine (0.1 mL) and phenacyl halide and other halides (1 mmol) were added in a round-bottomed flask and refluxed at 100 °C for 5 h. After completion of the reaction, mixture was placed in refrigerator to obtain precipitates. The solid was filtered, washed with water and dried in vacuum.

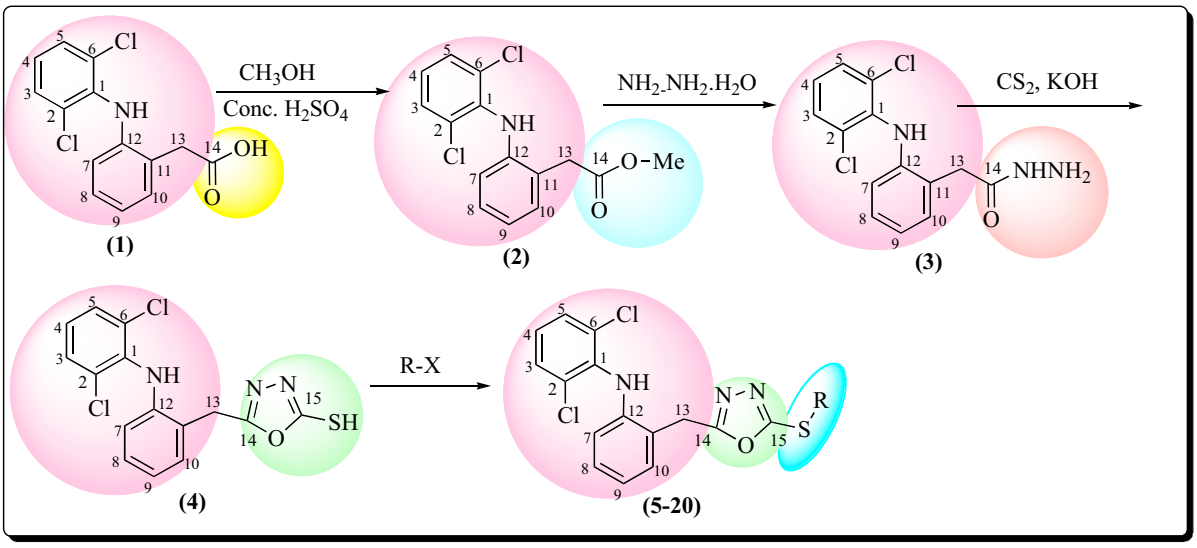
4. RESULT AND DISCUSSION

4.1. Chemistry

Diclofenac derivative was synthesized by different steps under refluxing conditions. The diclofenac (**1**) in the presence of methanol and sulphuric acid was converted into methyl ester **2** and then this ester converted hydrazide **3** with hydrazine hydrate in methanol. Resulting hydrazide **3** was transformed to oxadiazole derivative **4** in carbon disulfide under basic conditions. Final products **5**-**20** were obtained by reaction of oxadiazole derivative with different phenacyl halides and aryl halides in the presence of ethanol and triethyl amine (Scheme **1**). Chemical structures of all of these derivatives were established by ^{1}H-NMR, ^{13}C-NMR, EI-MS, and IR spectroscopy.

4.2. Antiinflammatory Activity of Diclofenac (1) and its Derivatives

Diclofenac **1** and all derivatives (Fig. **2**) were initially tested to check their inhibitory potential on intracellular reactive oxygen species (ROS) produced from human whole blood phagocytes at single concentration of 25 μg/mL. The compounds showing inhibitory potential were then tested on two different parameters of inflammation. The active com-



Scheme 1. Syntheses of oxadiazole derivatives of dicleofenac.

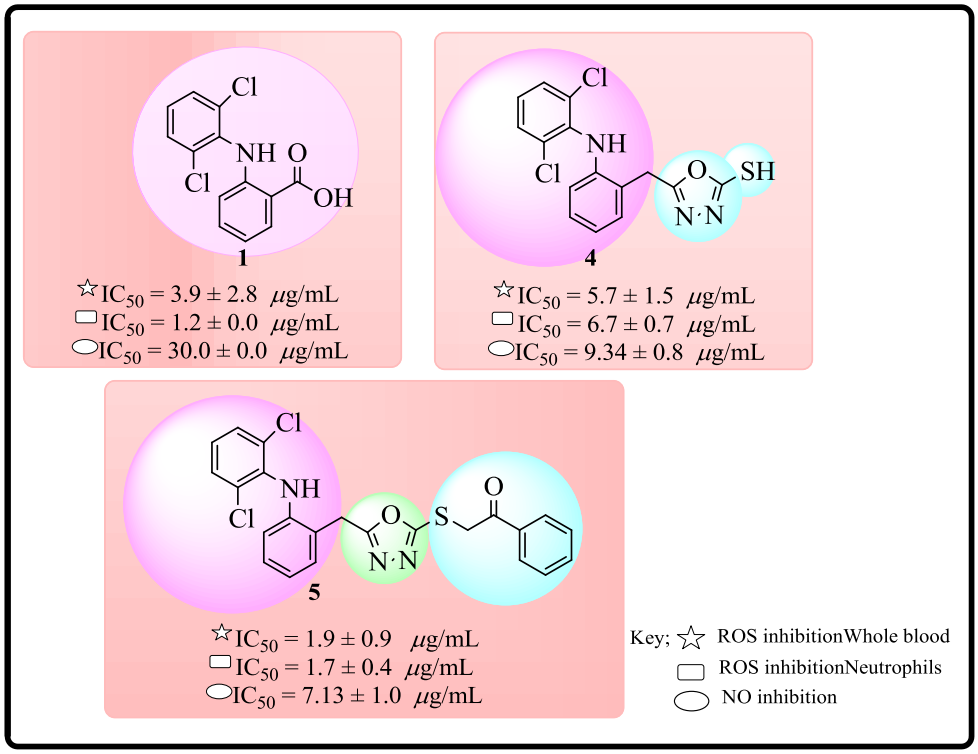


Fig. (2). Structure-activity relationship of diclofenac and oxadiazole **4** and compound **5**.

pounds were further evaluated for their inhibitory effect on the production of ROS from isolated neutrophils and on nitric oxide (NO) generated from lipopolysaccharide (LPS) stimulated J774.2 macrophages (Table **1**).

In this study diclofenac **1** inhibited the ROS with an IC_{50} value 3.9 ± 2.8 and 1.2 ± 0.0 μg/mL from human whole blood cells and isolated neutrophils, respectively. It also moderately inhibited NO with an IC_{50} value of 30.1 ± 0.0 μg/mL compared to the standard L-NMMA IC_{50} 24.2 ± 0.8 μg/mL. However, when the acid group of diclofenac was converted into ester **2** and hydrazide **3** the activity was found to be diminished. Conversion of hydrazide **3** into its oxadiazole derivative **4** it regains the inhibitory activity on ROS f **3.1.4.1.** rom whole blood and neutrophils with IC_{50} values of 5.7 ± 1.5 and 6.7 ± 0.7 μg/mL, respectively. However potent inhibition of NO IC_{50} 9.34 ± 0.8 μg/mL was observed as compared to parent **1** as well as standard L-NMMA. Conversion of **4** into **5** gave a most potent inhibitior of ROS as well as NO of this series having an IC_{50} of value 1.9 ± 0.9 and 1.7 ± 0.4 μg/mL on whole blood and neutrophils, respectively, and 7.13 ± 1.0 μg/mL on nitric oxide (NO) (Fig. **3**).

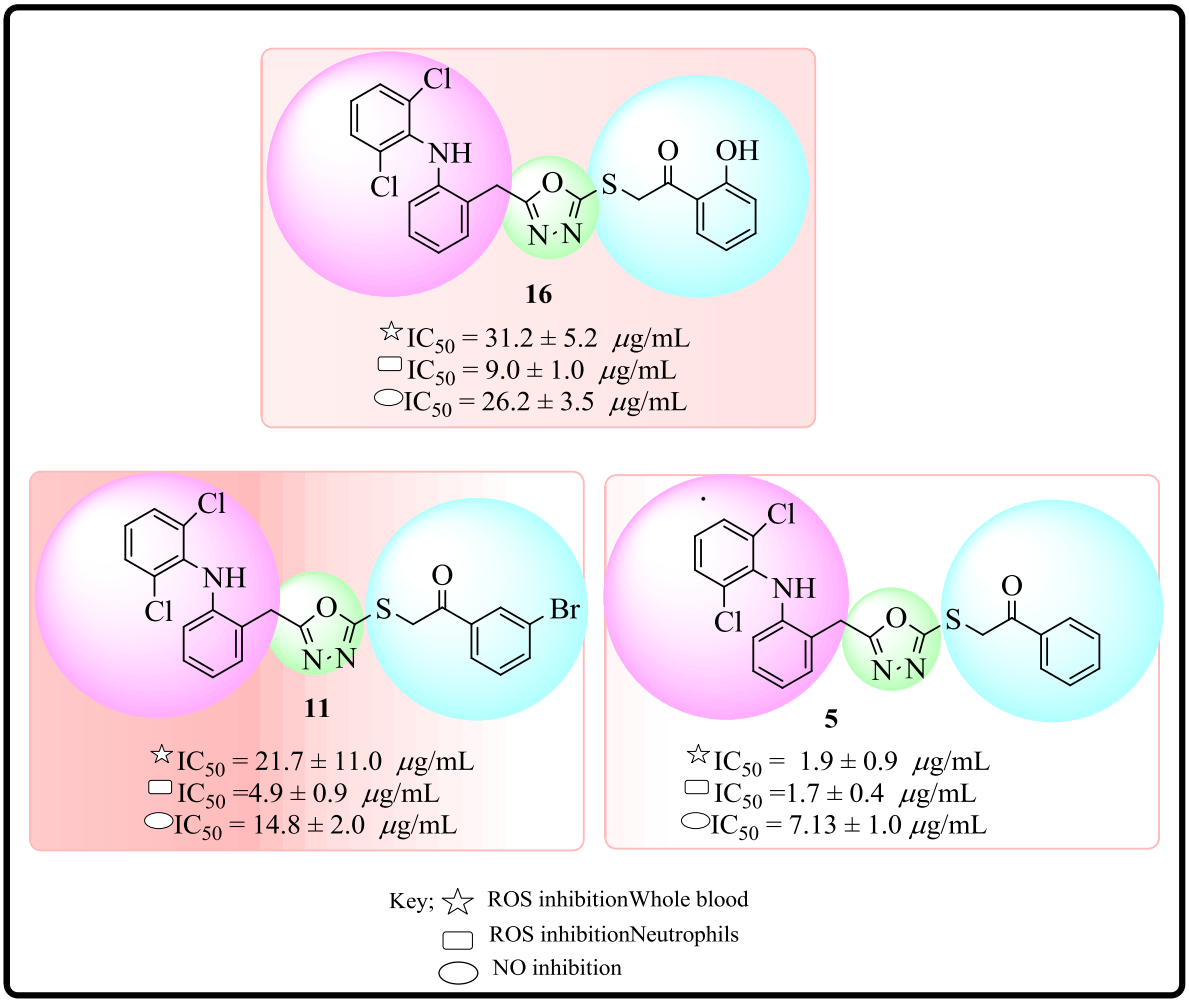


Fig. (3). Structure-activity relationship of phenacyl analogs of substituted oxadiazole dicleofenac **5**, **11**, and **16**.

The oxadiazole derivative of diclofenac **4** reacts with differents phenacyl halide to afford compounds **5**-**17**. Phenacyl derivative of 1,3,4-oxadiazole diclofenac **5** was found to be most active compound which showed superior antiinflammatory activity than parent diclofenac **1** as well as standard such as ibuprofen which is used for ROS inhibition having an IC_{50} values of 11.2 ± 1.9 and 2.5 ± 0.6 μg/mL for whole blood and isolated neutrophils, respectively, and from L-NMMA which was used as standard for nitric oxide (NO) inhibition IC_{50} = 24.2 ± 0.8 μg/mL. Compound **11**, a substituted phenacyl derivative of oxadiazole having a bromo residue at position 3 showed moderate level of inhibition having an IC_{50} value of 21.7 ± 11.0, 5.0 ± 0.9 and 14.8 ± 2.0 μg/mL for whole blood, neutrophils ROS, and nitric oxide inhibition, respectively. While compound **10** having a bromo group at postion 4 was found to be inactive. Hydroxy substituted phenacyl derivative **16** showed low level of inhibition on whole blood ROS and neutrophil ROS IC_{50} = 31.2 ± 5.2, 9.0 ± 1.0 μg/mL respectively. Where as moderate inhibition of nitric oxide IC_{50} = 26.2 ± 3.5 μg/mL was observed (Fig. **4**). The cytotoxicites of most active derivatives **1**, **4**, **5**, **11** and **20** were tested on NIH-3T3 cells where all compounds were found to be non-toxic as compared to the cyclohexamide which was used as a standard cytotoxic drug (Table **2**).

Chloro, nitro, methoxy and phenyl substituted phenacyl oxadiazoles **7**, **8**, **9**, **12**-**15**, and **17**, respectively were found to be inactive. 1,3,4-Oxadiazole diclofenac **4** was also reacted with varyingly substituted aryl halide and afforded dcompounds **18**, **19**, and **20**. Among them only compound **20** having a 2-bromo benzyl substituent showed good activity with IC_{50} values of 4.5 ± 1.7, 3.4 ± 0.5, and 21.9 ± 1.15 μg/mL for whole blood, neutrophil ROS and NO inhibition, respectively. While chloro substituted derivatives **18** and **19** were found to be inactive (Figs. **5, 6, 7**).

5. SPECTROSCOPIC DATA OF SYNTHETIC COMPOUNDS

5.1. 2-(2-(2,6-Dichlorophenylamino)phenyl)acetic Acid (1)

M.p. 152-154°C (lit. 153-154°C [28]); R_f: 0.31 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.7 (s, 1H, OH), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 7.8 Hz, H-3, H-5),

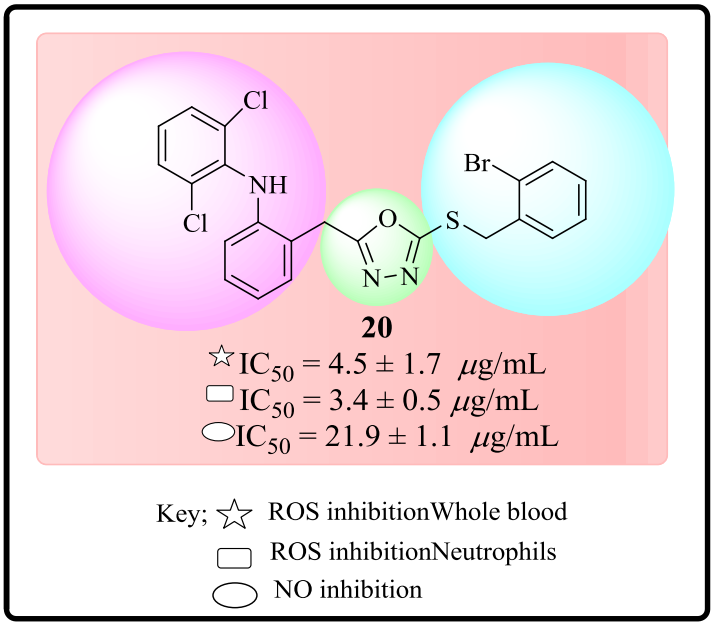


Fig. (4). Structure-activity relationship of benzyl analogs of oxadiazole dicleofenac **20**.

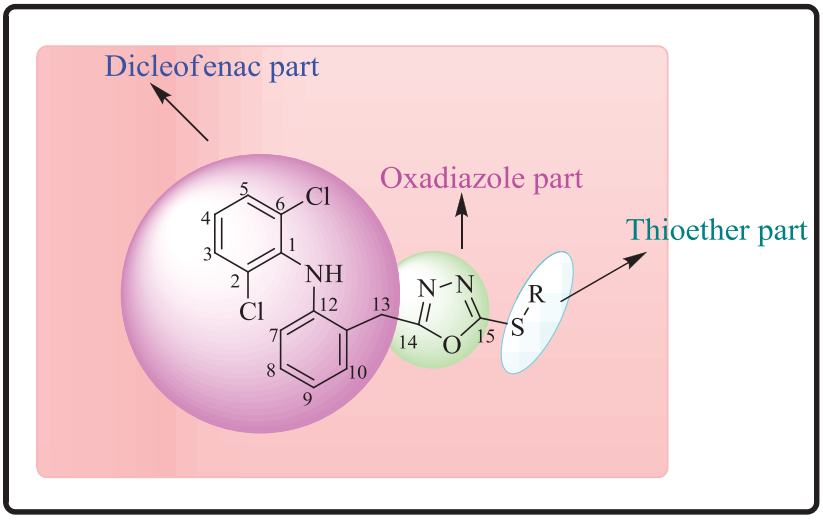


Fig. (5). 1,3,4-Oxadiazole derivative of dicleofenac.

Table 1. Anti-inflammatory activity of diclofenac (1) and its derivatives (2-20).

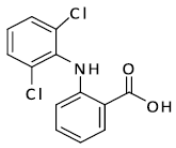
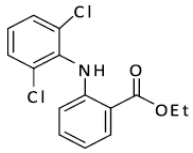
Compound	Structure	ROS Inhibition Whole Blood IC_{50} ± SD (μg/mL)	ROS Inhibition Neutrophils IC_{50} ± SD (μg/mL)	NO Inhibition IC_{50} ± SD (μg/mL)
1		3.9 ± 2.8	1.2 ± 0.0	30.0 ± 0.0
2		NA[b]	ND[c]	ND[c]

Table 1. contd…

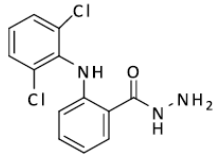
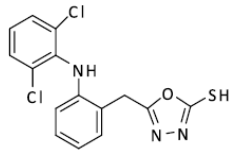
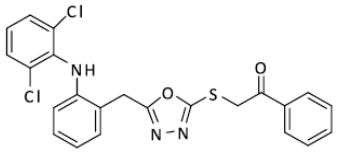
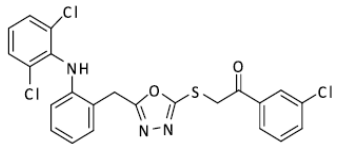
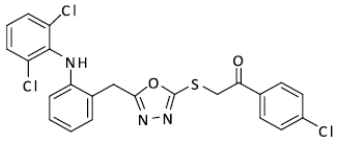
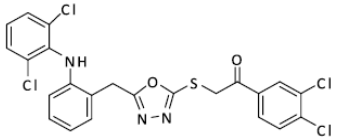
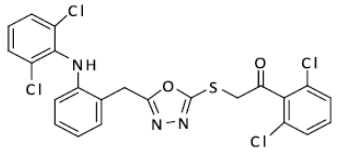
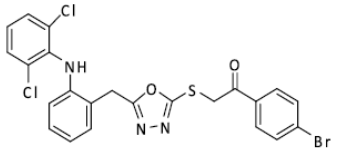
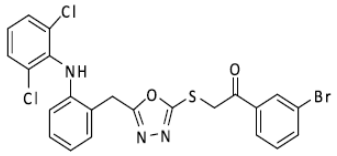
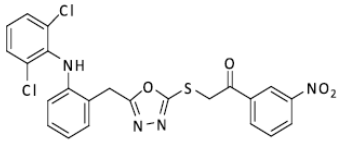
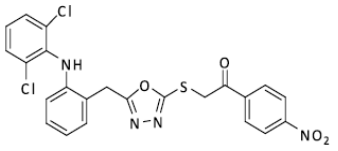
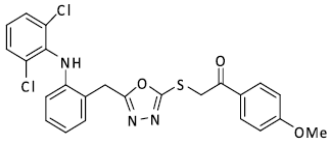
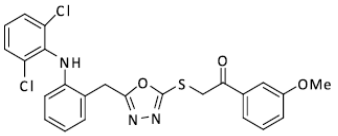
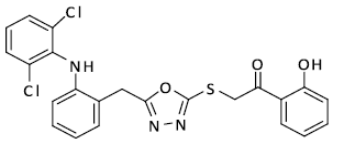
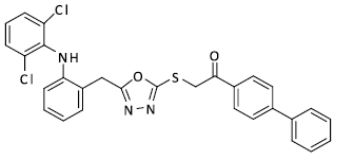
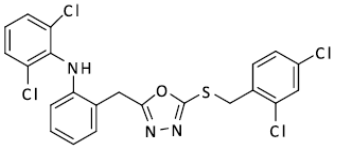
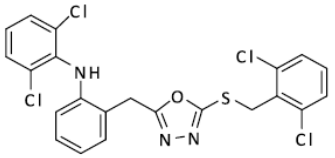
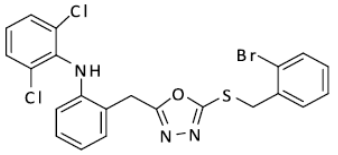
Compound	Structure	ROS Inhibition Whole Blood IC_{50} ± SD (μg/mL)	ROS Inhibition Neutrophils IC_{50} ± SD (μg/mL)	NO Inhibition IC_{50} ± SD (μg/mL)
3		NA[b]	ND[c]	ND[c]
4		5.7 ± 1.5	6.7 ± 0.7	9.34 ± 0.8
5		1.9 ± 0.9	1.7 ± 0.4	7.13 ± 1.0
6		NA[b]	ND[c]	ND[c]
7		NA[b]	ND[c]	ND[c]
8		NA[b]	ND[c]	ND[c]
9		NA[b]	ND[c]	ND[c]
10		NA[b]	ND[c]	ND[c]
11		21.7 ± 11.0	4.9 ± 0.9	14.8 ± 2.0
12		NA[b]	ND[c]	ND[c]

Table 1. contd…

Compound	Structure	ROS Inhibition Whole Blood IC_{50} ± SD (μg/mL)	ROS Inhibition Neutrophils IC_{50} ± SD (μg/mL)	NO Inhibition IC_{50} ± SD (μg/mL)
13		NA[b]	ND[c]	ND[c]
14		NA[b]	ND[c]	ND[c]
15		NA[b]	ND[c]	ND[c]
16		31.2 ± 5.2	9.0 ± 1.0	26.2 ± 3.5
17		NA[b]	ND[c]	ND[c]
18		NA[b]	ND[c]	ND[c]
19		NA[b]	ND[c]	ND[c]
20		4.5 ± 1.7	3.4 ± 0.5	21.9 ± 1.1

SD[a] means Standard deviation; NA[b] = Not Active; ND[c] = Not determined; Standard: Ibuprofen, (whole blood) IC_{50} = 11.2 ± 1.9 μg/mL; (neutrophil) IC_{50} = 2.5 ± 0.6 μg/mL; N[G] monomethyl L-arginine acetate (L-NMMA) nitric oxide IC_{50}= 24.2 ± 0.8.

Table 2. Cytotoxicity of active compounds on NIH-3T3 cells. The IC_{50} values were calculated using three doses (1, 10, 100 μg/mL) of each compound. Values are expressed as mean ± SD of experiments done in triplicate. Results were compared with cyclohexamide as a standard cytotoxic drug.

Compounds	IC_{50} ± SD[a] (μg/mL)
1	29.8 ± 0.4
4	32.0 ± 0.6

Table 2. contd…

Compounds	$IC_{50} \pm SD^a$ (μg/mL)
5	76.7 ± 6.0
11	>100
20	>100
Cyclohexamide	0.13 ± 0.02

SD[a] means Standard deviation.

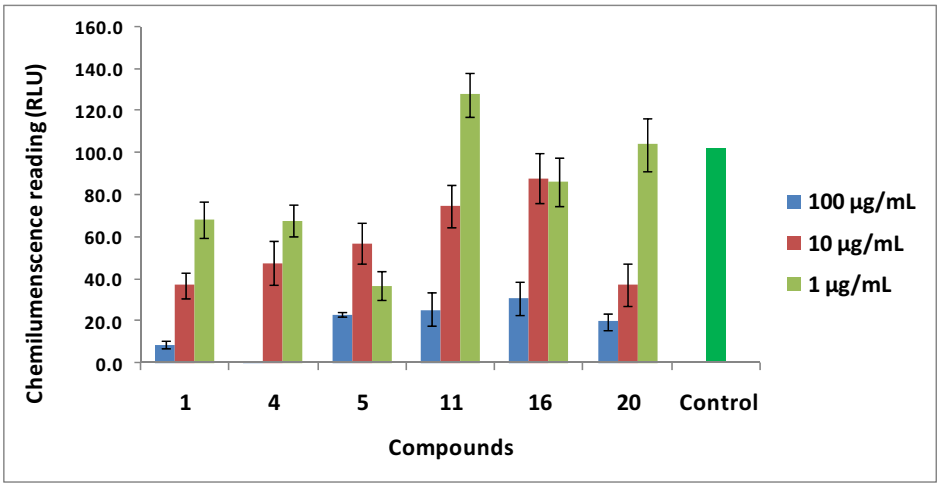


Fig. (6). The graph represents the inhibitory effect of compounds on oxidative burst. Compounds were tested at three different concentrations (1, 10 and 100 μg/mL). Results are presented in relative light units (RLU) and oxidative burst activity of whole blood phagocytes using luminol as a probe. Each vertical bar represents a mean of triplicate. Results were compared to the control. Where Control is zymosan activated whole blood phagocytes with no drug.

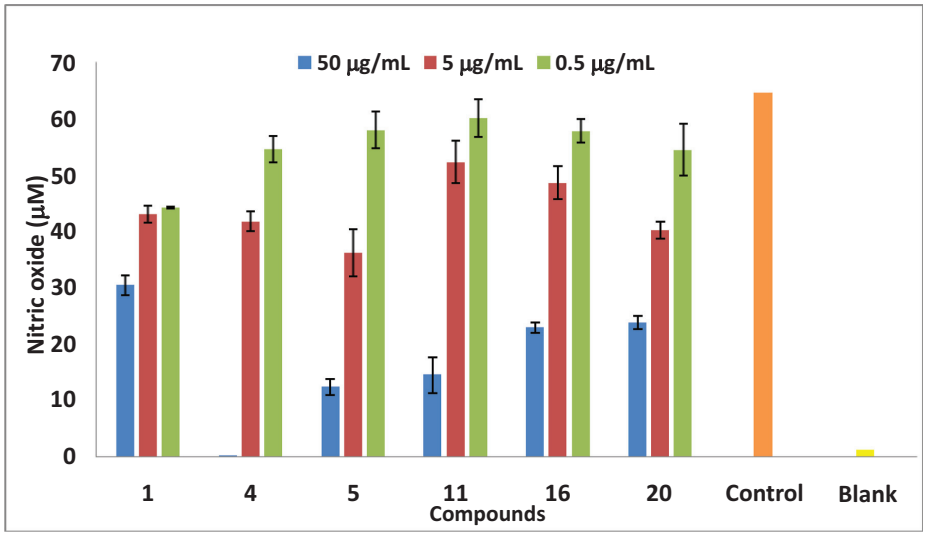


Fig. (7). The graph represents the inhibitory effect of compounds on Nitric oxide (NO) production from mouse macrophage J774.2 cell line. Compounds were tested at three different concentrations (0.5, 5 and 50 μg/mL). Each vertical bar represents a mean of triplicate. Results were compared to the control. Where Control = Cells activated by lipopolysaccharide (LPS) with no drug and Blank = Cells without LPS.

7.23 (s, 1H, NH), 7.20 (m, 2H, H-10, H-11), 7.07 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4), 6.86 (t, 1H, $J_{(9/10,11)}$ = 8.0 Hz, H-9), 6.28 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 3.68 (s, 2H, CH_2); EI-MS: *m/z* (rel. abund. %), 298 $[M+2]^+$ (29.4.7), 296 $[M]^+$ (18.5), 278 (4.7), 242 (40.0), 214 (100.0), 179 (26.3), 151 (12.5), 109 (6.9); HREI-MS: *m/z* Calcd for $C_{14}H_{11}Cl_2NO_2$ $[M]^+$ 296.1486, Found 296.1485; IR (KBr, cm^{-1}): 3347 (O-H), 3362 (N–H), 1670 (C=O), 1569 (C=C), 1259 (C-N), 1180 (C-O); Anal. Calcd for $C_{14}H_{11}Cl_2NO_2$: C, 56.78; H, 3.74; Cl, 23.94; N, 4.73; O, 10.80; Found: C, 56.77; H, 3.73; N, 4.72.

5.2. Methyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate (2)

Yield: 96 %; m.p. 98-100°C (lit. 97-99°C [28]); R_f: 0.36 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (400 MHz, DMSO-

d_6): δ 7.53 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.22 (m, 2H, H-9, H-10), 7.06 (d, 1H, $J_{(11,10)}$ = 6.0 Hz, H-11), 7.03 (t, 1H, $J_{(4/3,5)}$ = 7.6 Hz, H-4), 6.25 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 3.77 (s, 2H, CH_2), 3.64 (s, 3H, OCH_3); EI-MS: *m/z* (rel. abund. %), 311 $[M+2]^+$ (24.7), 309 $[M]^+$ (36.7), 277 (9.6), 242 (39.2), 214 (100.0), 179 (10.2), 151 (7.6); HREI-MS: *m/z* calcd for $C_{15}H_{13}Cl_2NO_2$ $[M]^+$ 309.0323, Found 309.0322; IR (KBr, cm^{-1}): 3382 (N-H), 1660 (C=O), 1579 (C=N), 1569 (C=C), 1259 (C-N), 1170 (C-O); Anal. Calcd for $C_{15}H_{13}Cl_2NO_2$: C, 58.08; H, 4.22; Cl, 22.86; N, 4.52; O, 10.32; Found: C, 58.07; H, 4.21; N, 4.51.

5.3. 2-(2-(2,6-Dichlorophenylamino)phenyl)acetohydrazide (3)

Yield: 97%; m.p. 132-134°C C (lit. 134-136°C [29]); R_f: 0.35 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H, NH), 9.51 (s, 1H, NH), 7.51 (d, 2H, $J_{(3,4),(5,4)}$ = 8.4 Hz, H-3, H-5), 7.16 (m, 2H, H-10, H-11), 7.04 (t, 1H, $J_{(4/3,5)}$ = 7.6 Hz, H-4), 6.85 (t, 1H, $J_{(9/10,11)}$ = 7.6 Hz, H-9), 6.29 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 4.30 (s, 2H, CH_2), 3.50 (s, 2H, NH_2); ^{13}C-NMR: (100 MHz, DMSO-d_6): δ_C, 170.7 (C=O), 142.9 (C-1), 137.1 (C-7), 130.2 (C-3, C-5),), 129.2 (C-3, C-5), 127.1 (C-8), 125.3 (C-9), 124.8 (C-10), 120.6 (C-10), 116.0 (C-4), 37.6 (CH_2); EI-MS: *m/z* (rel. abund. %), 311 $[M+2]^+$ (2.4), 309 $[M]^+$ (3.8), 278 (58.2), 242 (17.2), 214 (100.0), 179 (13.0), 151 (6.9); HREI-MS: *m/z* Calcd for $C_{14}H_{13}Cl_2N_3O$ $[M]^+$ 309.0436, Found 309.0435; IR (KBr, cm^{-1}): 3387 (N-H), 1670 (C=O), 1599 (C=N), 1560 (C=C), 1299 (C-N); Anal. Calcd for $C_{14}H_{13}Cl_2N_3O$: C, 54.21; H, 4.22; Cl, 22.86; N, 13.55; O, 5.16; Found: C, 54.20; H, 4.21; N, 13.54.

5.4. 5-(2-(2,6-Dichlorophenylamino)benzyl)-1,3,4-oxadiazole-2-thiol (4)

Yield: 97%; m.p. 160-162°C (lit. 162-164°C [30]); R_f: 0.38 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.28 (s, 1H, SH), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 8.4 Hz, H-3, H-5), 7.23 (m, 2H, H-10, H-11), 7.12 (s, 1H, NH), 7.09 (t, 1H, $J_{(4/3,5)}$ = 6.9 Hz, H-4), 6.86 (t, 1H, $J_{(9/10,11)}$ = 7.2 Hz, H-9), 6.19 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 4.24 (s, 2H, CH_2); ^{13}C-NMR: (100 MHz, DMSO-d_6): δ 177.8 (C-14), 162.5 (C-13), 143.1 (C-7), 136.9 (C-1), 131.8 (C-1), 131.0 (C-2, C-6), 129.1 (C-3, C-5), 128.3 (C-4), 126.4 (C-8), 121.3 (C-12), 120.3 (C-9), 115. (C-10), 27.6 (CH_2); EI-MS: *m/z* (rel. abund. %), 353 $[M+2]^+$ (24.3), 351 $[M]^+$ (32.6), 291 (63.6), 278 (54.6), 256 (19.6), 214 (100.0), 131 (35.4); HREI-MS: *m/z* Calcd for $C_{15}H_{11}Cl_2N_3OS$ $[M]^+$ 352.2383, Found 352.2382; IR (KBr, cm^{-1}): 3367 (N-H), 2979 (S-H), 1579 (C=N), 1500 (C=C), 1294 (C-N), 1286 (C=S); Anal. Calcd for $C_{15}H_{11}Cl_2N_3OS$: C, 51.15; H, 3.15; Cl, 20.13; N, 11.93; O, 4.54; S, 9.10; Found: C, 51.14; H, 3.14; N, 11.92.

5.5. 2-(5-(2-(2,6-Dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)-1-phenylethanone (5)

Yield: 95%; m.p 128-130°C; R_f: 0.55 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.01 (d, 2H, $J_{(3,4),(5,4)}$ = 7.5 Hz, H-3, H-5), 7.70 (t, 1H, $J_{(10/9,11)}$ = 7.5 Hz, H-10) 7.57 (t, 2H, $J_{(19/18,20),\ (21/20,22)}$ = 7.5 Hz, H-19, H-21), 7.52 (d, 2H, $J_{(18,19),(22,21)}$ = 7.5 Hz, H-18, H-22) 7.23 (t, 1H, $J_{(20/19,21)}$ = 8.0 Hz, H-20) 7.15 (m, 1H, H-11), 7.14 (s, 1H, NH), 7.06 (t, 1H, $J_{(4/3,5)}$ = 7.5 Hz, H-4) 6.83 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.19 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 5.05 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 470.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{17}Cl_2N_3O_2S$ $[M]^+$ 470.3710, found 470.3711; IR (KBr, cm^{-1}): 3214 (N-H), 1586 (C=N), 1509 (C=C), 1489 (C=S), 1170 (C-O); Anal. *m/z* Calcd $C_{23}H_{17}Cl_2N_3O_2S$: C, 58.73; H, 3.64; Cl, 15.07; N, 8.93; O, 6.80; S, 6.82 Found: C, 58.72; H, 3.63; N, 8.92.

5.6. 3-(3,4-Dichlorobenzyl)-5-(2-(2,6-dichlorophenylamino)benzyl)-1,3,4-oxadiazole-2(3*H*)-thione (6)

Yield: 91%; m.p 120-122°C; R_f: 0.51 (ethyl acetate/hexane, 2:8) ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.68 (d, 1H, $J_{(18,\ 21)}$ = 1.2 Hz, H-18), 7.52 (s, 1H, NH), 7.50 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.36 (dd, 1H, $J_{(22,18)}$ = 1.2 Hz $J_{(22,21)}$ = 8.4 Hz, H-22) 7.23 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4), 7.16 (d, 1H, $J_{(11,\ 10)}$ = 7.6 Hz, H-11), 7.09 (m, 2H, H-8, H-10), 6.85 (t, 1H, $J_{(9/8,10)}$ = 7.6 Hz, H-9), 6.19 (d, 1H, $J_{(21,22)}$ = 8.1 Hz, H-21), 4.44 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ^{13}C-NMR: (100 MHz, DMSO-d_6): δ 166.5 (C=S), 162.4 (C-14), 142.9 (C-2, C-6), 138.1 (C-17), 136.8 (C-19), 131.5 (C-20), 130.9 (C-18), 130.8 (C-21), 130.6 (C-22), 130.5 (C-3, C-5), 130.2 (C-7), 129.2 (C-1), 129.1 (C-11), 128.2 (C-9), 126.4 (C-10), 122.2 (C-8), 120.4 (C-12), 115.5 (C-4), 34.2 (13-CH_2), 27.3 (16-CH_2); ESI-MS *m/z*: 511.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{15}Cl_4N_3OS$ $[M]^+$ 515.2510, Found 511.2511; IR (KBr, cm^{-1}): 3211 (N-H), 1545 (C=N), 1543 (C=C), 1430 (C=S), 1187 (C-O); Anal. *m/z* Calcd $C_{22}H_{15}Cl_4N_3OS$: C, 51.68; H, 2.96; Cl, 27.74; N, 8.22; O, 3.13; S, 6.27, Found: C, 51.67; H, 2.95; N, 8.21.

5.7. 1-(4-Chlorophenyl)-2-(5-(2-(2,6-dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (7)

Yield: 91%; m.p 118-120°C; R_f: 0.50 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.03 (d, 2H, $J_{(20,19),(22,23)}$ = 8.4 Hz, H-20, H-22), 7.64 (d, 2H, $J_{(19,20),(23,22)}$ = 8.4 Hz, H-19, H-23), 7.53 (d, 2H, $J_{(3,4),(5,4)}$ = 8.1 Hz, H-3, H-5), 7.24 (t, 1H, $J_{(10/9,11)}$ = 7.5 Hz, H-10), 7.16 (d, 1H, $J_{(11,10)}$ = 7.5 Hz, H-11), 7.12 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 6.9 Hz, H-4), 6.84 (t, 1H, $J_{(9/8,10)}$ = 6.0 Hz, H-9), 6.21 (d, 1H, $J_{(8,9)}$ = 8.1 Hz, H-8), 5.03 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 504.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{16}Cl_3N_3O_2S$ $[M]^+$ 504.8160, Found 504.8161; IR (KBr, cm^{-1}): 3221 (N–H), 1678 (C=O), 1562 (C=N), 1528 (C=C),1423 (C=S), 1026 (C–O); Anal. *m/z* Calcd for for for $C_{23}H_{16}Cl_3N_3O_2S$: C, 54.72; H, 3.19; Cl, 21.07; N, 8.32; O, 6.34; S, 6.35 Found: C, 54.71; H, 3.18; N, 8.33.

5.8. 1-(3,4-Dichlorophenyl)-2-(5-(2-(2,6-dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (8)

Yield: 94%; m.p 178-180°C; R_f: 0.52 (ethyl acetate/hexanes, 2:8)^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.22 (d, 1H, $J_{(19,22)}$ = 1.6 Hz, H-19), 7.96 (dd, 1H, $J_{(22,19)}$ = 2.0 Hz $J_{(22,23)}$ = 8.4 Hz, H-22), 7.84 (d, 1H, $J_{(23,22)}$ = 8.4 Hz, H-23), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.23 (t, 1H, $J_{(10/9,11)}$ = 8.0 Hz, H-10), 7.16 (d, 1H, $J_{(11,10)}$ = 7.6 Hz, H-11), 7.11 (s, 1H, NH), 7.07 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4), 6.83 (t, 1H, $J_{(9/8,10)}$ = 7.6 Hz, H-9), 6.19 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 5.03

(s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 539.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{15}Cl_4N_3O_2S$ $[M]^+$ 539.2611, Found 539.2610; IR (KBr, cm^{-1}): 3265 (N-H), 1629 (C=O), 1532 (C=N), 1576 (C=C), 1425 (C=S), 1070 (C-O); Anal. *m/z* Calcd for for for $C_{23}H_{15}Cl_4N_3O_2S$: C, 51.23; H, 2.80; Cl, 26.30; N, 7.79; O, 5.93; S, 5.95 Found: C, 51.23; H, 2.82; N, 7.78.

5.9. 3-(4-Chlorobenzyl)-5-(2-(2,6-dichlorophenylamino) benzyl)-1,3,4-oxadiazole-2(3*H*)-thione (9)

Yield: 90%; m.p 110-112°C; R_f: 0.51 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.23 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.19 (d, 2H, $J_{(18,19),(21,22)}$ = 8.0 Hz, H-18, H-21) 7.37 (t, 1H, $J_{(20/21,22)}$ = 7.5 Hz, H-20), 7.23 (m, 2H, H-10, H-11) 7.12 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4) 6.86 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.21 (d, 1H, $J_{(8,9)}$ = 7.5 Hz, H-8), 4.42 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ^{13}C-NMR: (75 MHz, DMSO-d_6): δ_C, 166.4 (C=S), 142.9 (C-14), 136.8 (C-2, C-6), 135.8 (C-20), 132.2 (C-17), 131.5 (C-7), 130.7 (C-12), 130.5 (C-19, C-21), 129.1 (C-18, C-22), 128.4 (C-3, C-5), 128.1 (C-9), 126.4 (C-8), 122.2 (C-9), 120.4 (C-10), 34.8 (13-CH_2), 27.2 (16-CH_2); ESI-MS *m/z*: 476.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{16}Cl_3N_3OS$ $[M]^+$ 476.8059, Found 476.8058; IR (KBr, cm^{-1}): 3210 (N-H), 1546 (C=N), 1548 (C=C), 1436 (C=S), 1187 (C–O); Anal. *m/z* Calcd $C_{22}H_{16}BrCl_2N_3OS$: C, 55.42; H, 3.38; Cl, 22.31; N, 8.81; O, 3.36; S, 6.72 Found: C, 55.41; H, 3.36; N, 8.80.

5.10. 1-(4-Bromophenyl)-2-(5-(2-(2,6-dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)ethanone (10)

Yield: 92%; m.p 148-150°C; R_f: 0.53 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.95 (d, 2H, $J_{(23,22),(19,20)}$ = 8.4 Hz, H-23, H-19), 7.78 (d, 2H, $J_{(20,19),(22,23)}$ = 8.4 Hz, H-20, H-22), 7.53 (d, 2H, $J_{(3,4),(5,4)}$ = 8.1 Hz, H-3, H-5), 7.24 (t, 1H, $J_{(10/9,11)}$ = 8.2 Hz, H-10), 7.16 (m, 2H, H-11, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 7.5 Hz, H-4), 6.84 (t, 1H, $J_{(9/8,10)}$ = 7.2 Hz, H-9), 6.20 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 5.02 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 549.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{16}BrCl_2N_3O_2S$ $[M]^+$ 549.2670, Found 549.2671; IR (KBr, cm^{-1}): 3285 (N-H), 1689 (C=O), 1552 (C=N), 1568 (C=C), 1445 (C=S), 1090 (C-O); Anal. *m/z* Calcd for $C_{23}H_{16}BrCl_2N_3O_2S$: C, 50.29; H, 2.94; Br, 14.55; Cl, 12.91; N, 7.65; O, 5.83; S, 5.84 Found: C, 50.28; H, 2.92; N, 7.62.

5.11. 1-(3-Bromophenyl)-3-(5-(2-(2,6-dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)propan-1-one (11)

Yield: 94%; m.p 134-136°C; R_f: 0.52 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.15 (s, 1H, H-23), 8.01 (d, 1H, $J_{(21,20)}$ = 9.8 Hz, H-21), 7.90 (d, 1H, $J_{(19,20)}$ = 9.8 Hz, H-19), 7.55 (m, 3H, H-3, H-5, H-10), 7.24 (t, 1H, $J_{(20/19,21)}$ = 8.1 Hz, H-20), 7.16 (m, 1H, H-10), 7.12 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 7.5 Hz, H-4) 6.84(t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.21 (d, 1H, $J_{(8,9)}$ = 7.8 Hz, H-8), 5.04 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 476.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{16}Cl_3N_3OS$ $[M]^+$ 476.8059, Found 476.8058; IR (KBr, cm^{-1}): 3212 (N-H), 1650 (C=O), 1545 (C=N), 1540 (C=C), 1463 (C=S), 1165 (C-O); Anal. *m/z* Calcd $C_{24}H_{18}BrCl_2N_3O_2S$: C, 51.17; H, 3.22; Br, 14.19; Cl, 12.59; N, 7.46; O, 5.68; S, 5.69 Found: C, 51.16; H, 3.21; N, 7.45.

5.12. 2-(5-(2-(2,6-Dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)-1-(3-nitrophenyl)ethanone (12)

Yield: 92%; m.p 123-125°C; R_f: 0.50 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.70(s, 1H, H-23), 8.52 (d, 1H, $J_{(21,20)}$ = 8.4 Hz, H-21), 8.44 (d, 1H, $J_{(19,20)}$ = 8.0 Hz, H-19), 7.88 (t, 1H, $J_{(20/19,21)}$ = 8.0 Hz, H-20), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 7.5 Hz, H-3, H-5), 7.23 t, 1H, $J_{(10/9,11)}$ = 8.0 Hz, H-10), 7.16 (d, 1H, $J_{(11,10)}$ = 7.6 Hz, H-11), 7.12 (s, 1H, NH), 7.07 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4), 6.83 (t, 1H, $J_{(9/8,10)}$ = 7.6 Hz, H-9), 6.19 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8) 5.14 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 515.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{16}Cl_2N_4O_4S$ $[M]^+$ 515.3685, Found 515.3684; IR (KBr, cm^{-1}): 3215 (N–H), 1692 (C=O), 1572 (C=N), 1579 (C=C), 1423 (C=S), 1041 (C-O); Anal. *m/z* Calcd for for $C_{23}H_{16}Cl_2N_4O_4S$: C, 53.60; H, 3.13; Cl, 13.76; N, 10.87; O, 12.42; S, 6.22, Found: C, 53.62; H, 3.12; Cl, N, 10.88.

5.13. 5-(2-(2,6-Dichlorophenylamino)benzyl)-3-(2-(4-nitrophenyl)-2-thioxoethyl)-1,3,4-oxadiazol-2(3*H*)-one (13)

Yield: 90%; m.p 135-137°C; R_f: 0.49 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.37 (d, 2H, $J_{(20,19),(22,23)}$ = 9.0 Hz, H-20, H-22), 8.24 (d, 2H, $J_{(19,20),(23,22)}$ = 8.7 Hz, H-19, H-23) 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5) 7.24 (m, 2H, H-10, H-11), 7.11 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 6.9 Hz, H-4), 6.84 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.20 (d, 1H, $J_{(8,9)}$ = 8.1 Hz, H-8), 5.10 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 515.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{16}Cl_2N_4O_4S$ $[M]^+$ 515.3685, Found 515.3684; IR (KBr, cm^{-1}): 3215 (N-H), 1689 (C=O), 1557 (C=N), 1545 (C=C),1434 (C=S), 1180 (C–O); Anal. *m/z* Calcd for $C_{23}H_{16}Cl_2N_4O_4S$: C, 53.60; H, 3.13; Cl, 13.76; N, 10.87; O, 12.42; S, 6.22, Found: C, 53.62; H, 3.11; N, 10.86.

5.14. 2-(5-(2-(2,6-Dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)-1-(4-methoxyphenyl)ethanone (14)

Yield: 91%; m.p 130-132°C; R_f: 0.51 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.00 (d, 2H, $J_{(19,20),(23,22)}$ = 8.8 Hz, H-19, H-23), 7.53 (d, 2H, $J_{(20,21),(22,23)}$ = 8.0 Hz, H-20, H-23), 7.23 (t, 1H, $J_{(9/8,10)}$ = 8.0 Hz, $J_{(10/9,11)}$ = 8.0 Hz, H-9, H-10), 7.16 (d, 2H, $J_{(3,4),(5,4)}$ = 8.4 Hz, H-3, H-5), 7.07 (d, 1H, $J_{(11,10)}$ = 7.5 Hz, H-11), 7.04 (s, 1H, NH), 6.84 (t, 1H, $J_{(4/3,5)}$ = 6.9 Hz, H-4), 6.20 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 4.99 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ^{13}C-NMR: (75 MHz, DMSO-d_6): δ_C, 190.6 (C=O), 166.2 (C=S), 163.6 (C-14), 162.9 (C-2, C-6), 142.8 (C-1), 136.9 (C-19, C-23), 131.5 (C-20, C-22), 130.7 (C-3, C-5), 130.4 (C-7), 129.1 (C-18), 128.1 (C-21), 127.8 (C-12), 126.3 (C-4), 122.2 (C-8), 120.4 (C-9), 115.5 (C-10), 114.0 (C-4), 55.6 (OCH_3), 40.9 (13-CH_2), 27.1 (C-16); ESI-MS *m/z*: 499.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{24}H_{19}Cl_2N_3O_3S$ $[M]^+$

499.0524, Found 499.0522; IR (KBr, cm^{-1}): 3221 (N-H), 1678 (C=O), 1562 (C=N), 1528 (C=C),1423 (C=S), 1026 (C-O); Anal. *m/z* Calcd for for for $C_{24}H_{19}Cl_2N_3O_3S$; C, 57.61; H, 3.83; Cl, 14.17; N, 8.40; O, 9.59; S, 6.41 Found: C, 57.60; H, 3.82; N, 8.41.

5.15. 2-(5-(2-(2,5-Dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)-1-(3-methoxyphenyl)ethan-1-one (15)

Yield: 91 %; m.p 132-134°C; R_f: 0.48 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.61 (d, 1H, $J_{(10,11)}$ = 7.5 Hz, H-10), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.48 (s, 1H, NH), 7.47 (d, 1H, $J_{(19,20)}$ = 8.0 Hz, H-19), 7.27 (m, 2H, H-20, H-23), 7.16 (d, 2H, $J_{(11,10)} = J_{(21,20)}$ = 10.5 Hz, H-11, H-21) 7.06 (t, 1H, $J_{(4/3,5)}$ = 7.5 Hz, H-4), 6.83 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.20 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8) 5.03 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2), 3.81 (s, 3H, OCH_3); ESI-MS *m/z*: 500.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{24}H_{19}Cl_2N_3O_3S$ $[M]^+$ 500.3970, Found 500.3971; IR (KBr, cm^{-1}): 3315 (N-H), 1691 (C=O), 1579 (C=N), 1576 (C=C),1443 (C=S), 1049 (C-O); Anal. *m/z* Calcd for $C_{24}H_{19}Cl_2N_3O_3S$: C, 57.61; H, 3.83; Cl, 14.17; N, 8.40; O, 9.59; S, 6.41, Found: C, 57.62; H, 3.82; Cl, 14.15; N, 8.41.

5.16. 2-(5-(2-(2,6-Dichlorophenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2*H*)-yl)-1-(2-hydroxyphenyl)ethanone (16)

Yield: 94%; m.p 164-166°C; R_f: 0.47 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.13 (s, 1H, OH), 7.81 (dd, 1H, $J_{(23,22)}$ = 2.4 Hz $J_{(23,21)}$ = 6.3 Hz, H-23), 7.53 (d, 3H, $J_{(3,4),(5,4),\ (21,20)}$ = 8.1 Hz, H-3, H-5, H-21), 7.18 (m, 2H, H-10, H-11), 7.05 (d, 1H, $J_{(22,23)}$ = 7.2 Hz, H-22), 7.01 (s, 1H, NH), 6.97 (t, 1H, $J_{(4/3,5)}$ = 7.8 Hz, H-4), 6.85 (t, 1H, $J_{(9/8,10)}$ = 8.1 Hz, H-9), 6.19 (d, 1H, $J_{(8,9)}$ = 7.5 Hz, H-8), 4.97 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ESI-MS *m/z*: 486.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{23}H_{17}Cl_2N_3O_3S$ $[M]^+$ 486.3704, Found 486.3703; IR (KBr, cm^{-1}): 3215 (N-H), 1692 (C=O), 1572 (C=N), 1579 (C=C),1423 (C=S), 1041 (C–O); Anal. *m/z* Calcd for for $C_{23}H_{17}Cl_2N_3O_3S$: C, 56.80; H, 3.52; Cl, 14.58; N, 8.64; O, 9.87; S, 6.59 Found: C, 56.82; H, 3.51; N, 8.63.

5.17. 1-(Biphenyl-4-yl)-2-(5-(2-(phenylamino)benzyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethanone (17)

Yield: 90%; m.p 160-162°C; R_f: 0.56 (ethyl acetate/hexanes, 2:8): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.08 (d, 2H, $J_{(19,20),(23,22)}$ = 8.4 Hz, H-19, H-23), 7.87 (d, 2H, $J_{(20,19),(22,23)}$ = 8.0 Hz, H-20, H-22), 7.77 (d, 2H, $J_{(25,26),(29,28)}$ = 7.6 Hz, H-25, H-29), 7.35(d, 4H, $J_{(20,19),(22,23)}$ = 8.0 Hz, H-20, H-22), 7.53 (m, 4H, H-3, H-5, H-23, H-28), 7.45 (t, 1H, $J_{(10/9,11)}$ = 8.0 Hz, H-10), 7.23 (t, 1H, $J_{(27/26,28)}$ = 8.0 Hz, H-27), 7.17 (m, 2H, H-11, NH), 7.07 (t, 1H, $J_{(4/3,5)}$= 8.0 Hz, H-4), 6.84 (t, 1H, $J_{(9/8,10)}$ = 8.0 Hz, H-9), 6.20 (d, 1H, $J_{(8,9)}$ = 8.0 Hz, H-8), 5.08 (s, 2H, 16-CH_2), 4.36 (s, 2H, 13-CH_2); ESI-MS *m/z*: 546.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{29}H_{21}Cl_2N_3O_2S$ $[M]^+$ 546.4669, Found 546.4668; IR (KBr, cm^{-1}): 3281 (N-H), 1688 (C=O), 1562 (C=N), 1598 (C=C),1423 (C=S), 1086 (C-O); Anal. *m/z* Calcd for for for $C_{29}H_{21}Cl_2N_3O_2S$: C, 63.74; H, 3.87; Cl, 12.98; N, 7.69; O, 5.86; S, 5.87 Found: C, 63.73; H, 3.86; N, 7.68.

5.18. 3-(2,4-Dichlorobenzyl)-5-(2-(2,6-dichlorophenylamino)benzyl)-1,3,4-oxadiazole-2(3*H*)-thione (18)

Yield: 92%; m.p 128-130°C; R_f: 0.50 (ethyl acetate/hexanes, 2:8); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.23 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5), 7.19 (d, 2H, $J_{(18,19),(21,22)}$ = 8.0 Hz, H-18, H-21) 7.37 (t, 1H, $J_{(20/21,22)}$ = 7.5 Hz, H-20), 7.23 (m, 2H, H-10, H-11) 7.12 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4) 6.86 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.21 (d, 1H, $J_{(8,9)}$ = 7.5 Hz, H-8), 4.42 (s, 2H, 16-CH_2), 4.35 (s, 2H, 13-CH_2); ^{13}C-NMR: (75 MHz, DMSO-d_6): δ 166.4 (C=S), 142.9 (C-14), 136.8 (C-2, C-6), 135.8 (C-20), 132.2 (C-17), 131.5 (C-7), 130.7 (C-12), 130.5 (C-19, C-21), 129.1 (C-18, C-22), 128.4 (C-3, C-5), 128.1 (C-9), 126.4 (C-8), 122.2(C-9), 120.4 (C-10), 34.8 (13-CH_2), 27.2 (16-CH_2); ESI-MS *m/z*: 511.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{15}Cl_4N_3OS$ $[M]^+$ 511.251, Found 511.250; IR (KBr, cm^{-1}): 3215 (N-H), 1546 (C=N), 1549 (C=C), 1431 (C=S), 1185 (C-O); Anal. *m/z* Calcd $C_{22}H_{15}Cl_4N_3OS$: C, 51.68; H, 2.96; Cl, 27.74; N, 8.22; O, 3.13; S, 6.27 2 Found: C, 51.66; H, 2.94; N, 8.20.

5.19. 2,6-Dichloro-*N*-(2-((5-(2,6-dichlorobenzylthio)-1,3,4-oxadiazol-2-yl)methyl)phenyl)aniline (19)

Yield: 96%; m.p 132-134°C; R_f: 0.50 (ethyl acetate/hexanes, 2:8) ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.36 (d, 2H, $J_{(3,4),(5,4)}$ = 7.5 Hz, H-3, H-5), 7.34 (d, 2H, $J_{(19,20),(21,20)}$ = 8.0 Hz, H-19, H-21) 7.37 (t, 1H, $J_{(20/21,22)}$ = 7.5 Hz, H-20), 7.23 (m, 2H, H-10, H-11), 7.12 (s, 1H, NH), 7.08 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4) 6.86 (t, 1H, $J_{(9/8,10)}$ = 7.5 Hz, H-9), 6.21 (d, 1H, $J_{(22,\ 21)}$ = 8.0 Hz, H-22), 4.62 (s, 2H, 16-CH_2), 4.39 (s, 2H, 13-CH_2); ESI-MS *m/z*: 511.9 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{15}Cl_4N_3OS$ $[M]^+$ 511.2510, Found 511.2511; IR (KBr, cm^{-1}): 3217 (N-H), 1540 (C=N), 1544 (C=C),1430 (C=S), 1183 (C-O); Anal. *m/z* Calcd $C_{22}H_{16}BrCl_2N_3OS$: C, 51.68; H, 2.96; Cl, 27.74; N, 8.22; O, 3.13; S, 6.27, Found: C, 51.67; H, 2.97; N, 8.21.

5.20. *N*-(2-((5-(2-Bromobenzylthio)-1,3,4-oxadiazol-2-yl)methyl)phenyl)-2,6-dichloroaniline (20)

Yield: 93%; m.p 124-126°C; R_f: 0.52 (ethyl acetate/hexanes, 2:8) ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.63 (d, 1H, $J_{(19,\ 20)}$ = 8.4 Hz, H-19), 7.52 (d, 2H, $J_{(3,4),(5,4)}$ = 8.0 Hz, H-3, H-5) 7.47 (dd, 1H, $J_{(8,11)}$ = 1.3 Hz, $J_{(8,9)}$ = 6.0 Hz, H-8), 7.27 (m, 4H, H-10, H-11, H-20, H-21) 7.13 (s, 1H, NH), 7.50), 7.09 (t, 1H, $J_{(4/3,5)}$ = 8.0 Hz, H-4) 6.86 (t, 1H, $J_{(9/8,10)}$ = 8.0 Hz, H-9), 6.21 (d, 1H, $J_{(22,\ 21)}$ = 8.1 Hz, H-22), 4.51 (s, 2H, 16-CH_2), 4.37 (s, 2H, 13-CH_2); ^{13}C-NMR: (100.0 MHz, DMSO-d_6): δ 166.6 (C=S), 162.2 (C-14), 142.9 (C-2, C-6), 136.8 (C-17), 135.3 (C-18), 132.8 (C-7), 131.5 (C-12), 131.4 (C-21), 130.6 (C-22), 130.0 (C-3, C-5), 129.1 (C-19), 128.2 (C-20), 127.9 (C-11), 126.4 (C-8), 123.9 (C-9), 122.1 (C-10), 120.4 (C-1), 115.5 (C-4), 36.6 (13-CH_2), 27.2 (16-CH_2); ESI-MS *m/z*: 519.0 (M^+); HR-ESI-MS *m/z* Calcd for $C_{22}H_{16}BrCl_2N_3OS$ $[M]^+$ 521.2569, Found 521.2568; IR (KBr, cm^{-1}): 3219 (N-H), 1542 (C=N), 1540 (C=C),1438 (C=S), 1181 (C-O); Anal. *m/z* Calcd $C_{22}H_{16}BrCl_2N_3OS$: C,

50.69; H, 3.09; Br, 15.33; Cl, 13.60; N, 8.06; O, 3.07; S, 6.15, Found: C, 50.67; H, 3.08; N, 8.07.

CONCLUSION

In this study, nineteen analogs of diclofenac were synthesized using BIODS approach and antiinflammatory activities of diclofenac **1** and its derivatives were performed. Five derivatives were found to be active. Compound **5** was found to be the most potent inhibitor of ROS and NO compared to parent diclofenac **1** and standard drugs ibuprofen and L-NMMA, respectively. The most active compounds **1**, **4**, **5**, **11** and **20** were found to be non-toxic on NIH-3T3 cells. Compound **4**, **5**, and **20** also showed good antiinflammatory potential, compound **11** and **16** showed moderate and low level of inhibition, respectively. These compounds might have the potential to develop a novel non-steroidal (NSAIDs), non-acidic antiinflammatory agent. Therefore, optimization of these molecules, *in vivo* and detailed toxic studies are our next plans.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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