

S-Methylene linkage comprising 1,3,4-oxadiazoles: synthesis, reaction optimization and *in vitro* anti-microbial potential

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CHRONICLE

Article history:

Received July 1, 2020

Received in revised form

October 26, 2020

Accepted October 31, 2020

Available online

November 2, 2020

Keywords:

1,3,4-Oxadiazoles

Anti-microbial study

Copper catalyst

S-linkage chemistry

Synthetic optimization

ABSTRACT

To generate a new class of scaffolds with amended anti-microbial potency, synthesis and *in vitro* biological evaluation of a series of 2-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-substitutedphenyl-1,3,4-oxadiazole (**5a-5o**) having S-Methylene linkage between the two 1,3,4-diisoxazole rings is reported. Anti-microbial properties of the entire compounds were investigated in a broad panel of some selected gram-positive bacterial strains, gram-negative bacterial strain, and fungal strains using the broth microdilution method. The results were dominant on –Cl and –NO₂ functionality than the used standard drug.

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1. Introduction

Despite a large number of antibiotics and chemotherapeutics available for medical use, the anti-microbial resistance created a substantial need for a new class of anti-microbial Agents in the last decades¹. A literature survey revealed that substances like 1,3,4-oxadiazole², 1,3,4-thiadiazole³, and 1,2,4-triazole⁴ moieties had occupied a unique position in the design and synthesis of biologically active agents with remarkable anti-bacterial, analgesic, and anti-inflammatory activities. Out of various isomeric form, the existence of the 1,3,4-oxadiazole ring affects the physicochemical and pharmacokinetic properties of the compound and show better metabolic stability, water solubility, and lower lipophilicity. The various application of the 1,3,4-oxadiazoles ring in the therapeutic area can be listed as anticoagulant⁵, antibacterial⁶, antifungal⁷, anticancer⁸, anti-inflammatory⁹, insecticidal¹⁰, antihypertensive¹¹, etc.¹².

Many routes of synthesis for the 1,3,4-oxadiazoles have been identified¹³⁻¹⁵ but, significantly fewer efforts have been made by the chemist to generate the ring using the metal-catalyzed process and environmentally fewer hazards recoverable catalysts. Moreover, one of the significant challenges in developing safe and effective antibody-drug conjugates (ADC) has been the generation of suitable chemical linkers between the drug and the antibody, but the linker chemistry is tricky. That's why less

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doi: 10.5267/j.ccl.2020.11.001

use is globally, and the S-methylene linker plays a vital role in the cytotoxic study. It was well studied that the oxadiazole ring connected via ester or amide linker exhibit promising biological efficacy against various cancer cell-lines and recently reported that the presence of a five-methylene linker turned out to be more effective for enhancing its efficiency¹⁶. Zhao *et al.* reported the evidence of small group linkers, e.g., methylene shows a significant role in strengthening the medicinal importance by which connect the anionic groups and aromatic groups to reduces the repulsive interaction¹⁷. Contour maps analyses indicated the importance of the linker between two aryl groups and the subtle differences of substituent groups¹⁸. Thus, the development of facile and efficient approaches to access these 1,3,4-oxadiazoles is highly valuable to early drug discovery¹⁹⁻²².

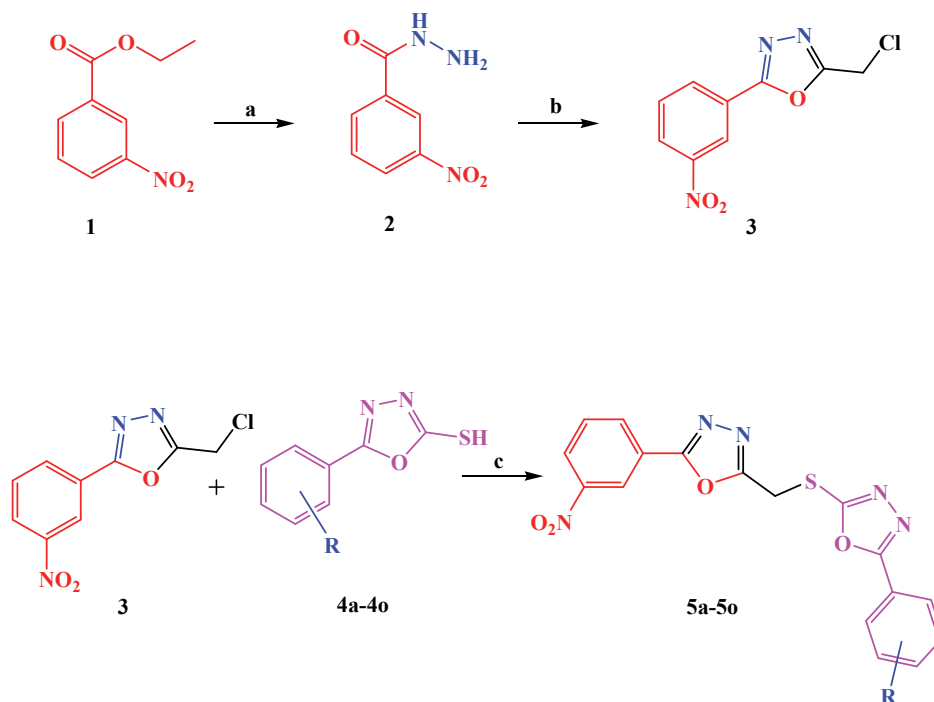
During designing efficient bio-active molecules, a new concept is used. Two active pharmacophores are coupled to generate one novel structure having unpredictable anti-microbial activity than any of the used pharmacophore. Therefore, these observations prompted us to connected two 1,3,4-oxadiazole rings having S-CH₂- linkage to enhance its potency. The synthesized diverse molecules were tested for their anti-microbial strength using some Gram (+ve) bacteria, Gram (-ve) bacteria, and some fungi strains.

2. Results and Discussion

2.1 Chemistry and Spectroscopic discussion

The multistep reaction sequences for the synthesis of the objective compounds **5a-5o** are framed in **Reaction scheme 1**.

Reaction Scheme 1. Synthetic scheme of 2-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-substitutedphenyl-1,3,4-oxadiazole (**5a-5o**)



Reaction Conditions: (a) Et-OH/NH₂NH₂, Reflux, 7h; (b) POCl₃/C₂O₂H₃Cl, Reflux, 6h; (c) CuI, Et₃N, THF, RT.

3-Nitrobenzohydrazide was synthesized by the reaction of 3-nitro acid ester and hydrazine hydrazide under reflux conditions followed by POCl₃ based cyclization to afford 2-(chloromethyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole (**3**) (92–97%). 5-Phenyl-1,3,4-oxadiazole-2-thiols (**4a-4o**) with variant substitution, commercially available, were synthesized as the previously reported process. The final adduct 2-(3-nitrophenyl)-5-(((5-phenyl-1,3,4-oxadiazol-2-yl)thio)methyl)-1,3,4-oxadiazole (**5a-5o**) were in turn prepared by soft metal (CuI) catalyzed reaction between intermediate three and 4a-4o

under primary conditions (TEA) gave “S-methylene linkage” containing novel product. It was found the analytically pure product (75-93%)²³.

As seen in **Table 1**, entry 1 is a traditional method, but it is suffering from low yield and also more consumption of time. But entry 2 shows the more product and less reaction time for the completion (1.5-2.0 Hours) due to a soft catalyst (CuI) with the best nucleophilic properties of iodine. While in the case of entry 3 and 4, the progress of the reaction was not observed in sodium iodide media and less yield in the case of CuBr/TEA reaction conditions, respectively^{23,24}.

Table 1. Reaction optimization in metal and non-metal catalyzed, and different alkaline media conditions

Entry	Compound Id	Catalyst	Base	Yield / Time
1		None	KOH/NaOH	60-70%/ 7-8 hours
2	5a	CuI	TEA	75-95%/ 1.5-2.0 hours
3		NaI	KOH	^a
4		CuBr	TEA	^b

^areaction not proceeded; ^bMinute quantity of yield (21%).

Spectral data, *i.e.*, ¹H NMR, ¹³C NMR, IR, and Mass of all the synthesized compounds, were recorded and found in full agreement with the proposed structures.

On a close look at the spectral data, IR spectral data obtained here lead to the approval of the establishment of targeted compounds. The IR spectral data obtained for the final derivatives **5a-5o** were suitable to a great extent in confirming their structure. Let us observe the spectral details of compound **5d** and correlate the frequencies with the functional group present in the structure. Absorption peaks at 1026 and 1350 cm⁻¹ helped confirm the presence of -C-O-C- in the oxadiazole nucleus. The absorption band at 1735 cm⁻¹ proved the presence of -C=C- in the aromatic ring. The -NO₂ functional group (nitro) was also confirmed by a two sharp peak in 1473 and 1527 cm⁻¹. An intense absorption band at 2931 cm⁻¹ helped assign the presence of -CH₂ (methylene group), and aromatic -C-H was noticed at 3078 cm⁻¹ in the compound **5d**. A sharp absorption peak at 717 cm⁻¹ helped to assign the presence of the C-Cl functional group.

The ¹H Nuclear Magnetic Resonance study was conducted for each of the derivatives, and the δ values were recorded. The δ values were recorded corresponding to the methylene group and the protons of the aromatic ring. For compound **5d**, δ value at 5.03 as a singlet proved the presence of two hydrogen atoms of the methylene group. A multiplet obtained at 7.53 to 8.64 ppm confirmed the presence of aromatic protons. ¹³C NMR spectra exhibited tight singlet at 24 δ ppm value, which showed the methylene bridge in the oxadiazole ring. The remaining aromatic carbons were in fair agreement with the theoretical values.

In mass spectra, the molecular ion peak observed at m/z 404.3 in **5d** confirmed the formation of the desired structure.

2.2 Biological Evaluation

An anti-microbial evaluation was carried out at the Department of Microbiology, RK University, Rajkot. The activity was expressed in the dose-response assay (MIC) against the affected organism using five various strains using ciprofloxacin as the reference anti-bacterial agent. The anti-fungal activity was carried out using two selected fungal strains using fluconazole as a reference drug. Results were expressed in minimum inhibition concentration (MIC). The detailed protocol, as stated in our last published paper, was repeated over here²⁵.

2.2.1 Anti-microbial evaluation

The newly synthesized entities (**5a-5o**) were screened for their anti-microbial assay against a broad panel of gram-positive bacteria *S. aureus* (ATCC No. 25923), *E. faecalis* (ATCC No. 29212), gram-negative bacteria *E. coli* (ATCC No. 25922), *P. aeruginosa* (ATCC No. 27853) and fungi *C. Albicans*

(ATCC No. 10231), *A. niger* (ATCC No. 1015). Broad panel of gram-positive bacteria (*S. aureus* and *E. faecalis*), gram-negative bacteria (*E. coli* and *P. aeruginosa*), and fungal strains (*C. Albicans* and *A. niger*) were utilized for testing the anti-microbial properties of the targeted compounds (**5a-5o**). The anti-bacterial and anti-fungal activities in MIC ($\mu\text{g/ml}$) have been reported in **Table 2**. It was observed that most of the compounds exhibited excellent activity compared to the standards used, and very few of them exhibited moderate or low activity. The MIC value of ciprofloxacin was recorded to be $62.5\mu\text{g/ml}$ against gram-positive bacterial strain *S. aureus*. The same standard responded at $125\mu\text{g/ml}$ against *E. faecalis*, *E. coli*, and *P. aeruginosa*. Fluconazole, when used as a standard drug against fungal strains *C. Albicans* and *A. niger*, MIC value was observed at $125\mu\text{g/ml}$ and $62.5\mu\text{g/ml}$, respectively. In general, it was observed that most of the compounds have resulted in being potent anti-bacterial, and very few of them were good anti-fungal.

Table 2. Anti-microbial screening of the synthesized bis-1,3,4-oxadiazoles (**5a-5o**)

Compounds	Minimum Inhibitory Concentration (MIC) in $\mu\text{g/ml}$					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231	<i>A. niger</i> ATCC 1015
5a	125	125	125	125	125	62.5
5b	62.5	125	62.5	62.5	250	62.5
5c	62.5	62.5	31.25	125	125	62.5
5d	31.25	62.5	31.25	31.25	250	31.25
5e	62.5	62.5	15.62	31.25	125	31.25
5f	250	125	125	250	250	125
5g	125	250	250	125	500	250
5h	250	250	125	125	250	125
5i	62.5	125	250	125	250	250
5j	125	125	250	125	500	500
5k	62.5	125	62.5	62.5	250	250
5l	62.5	62.5	31.25	125	125	250
5m	125	250	250	125	500	250
5n	250	250	125	125	250	125
5o	62.5	62.5	15.62	31.25	125	31.25
Fluconazole	-	-	-	-	125	62.5
Ciprofloxacin	62.5	125	125	125	-	-

MIC*: Minimum Inhibitory Concentration

2.2.2 Anti-bacterial activity

The anti-bacterial potential of the synthesized compounds (**5a-5o**) was tested against gram-positive bacteria *S. aureus* (ATCC no. 25923) and *E. faecalis* (ATCC no. 27853) and gram-negative bacteria *E. coli* (ATCC no. 25922) and *P. aeruginosa* (ATCC no. 27853). Compounds **5b** (3-NO₂), **5c** (4-NO₂), **5d** (2-Cl), **5e** (4-Cl), **5k** (-4-Br), **5l** (-3,4-diCl), and **5o** (-2,4-di-NO₂) were found to exhibit excellent or equivalent activity as compared to the standard ciprofloxacin. Other compounds **5a** (H), **5g** (2-I), **5i** (2-CH₃), **5j** (4-CH₃), **5m** (-3,4-di-OH), and **5n** (-4-CHF₂) were found to possess lower activity as compared to the standard drug and even poor than the formerly mentioned derivatives. When the same set of products **5a to 5o** were tested against another gram-positive bacterial strain *E. faecalis*, all the derivatives other than compound **5c** (4-NO₂), **5d** (2-Cl), and **5e** (4-Cl) resulted in equivalent or excellent activity. Compounds were exposed to gram-negative bacteria *E. coli*, and **5e** was found to show perfect activity with MIC value $15.62\mu\text{g/ml}$. Compounds **5c** (4-NO₂), **5d** (2-Cl), and **5o** (-2,4-di-NO₂) were exhibiting $31.25\mu\text{g/ml}$ MIC values, indicating better anti-bacterial potential than the standard ciprofloxacin. When the synthesized derivatives **5a to 5o** were tested against gram-negative bacteria *P. aeruginosa*, it was found that compound **5b** (2-Cl), **5e** (4-Cl), and **5o** (-2,4-di-NO₂) exhibited excellent activity ($31.25\mu\text{g/ml}$). The only derivative possessing insufficient activity than standard drug was compound **5f** (2,4-diCl) against the gram-negative bacterial strain *P. aeruginosa*.

2.2.3 Anti-fungal activity

The anti-fungal tests were performed against two different fungal strains, *A. niger*, and *C. Albicans*, where fluconazole was used as a standard drug. The anti-fungal property of the synthesized derivatives (**5a-5o**) was found to be more effective on *A. niger* as compared to *C. Albicans*. On testing the

compounds against *C. Albicans*, synthesized derivative 5d (2-Cl) was found to show excellent activity compared to the standard drug fluconazole. Few other derivatives 5a (H), 5c (4-NO₂), 5e (4-Cl), 5l (-3,4-diCl), and 5o (-2,4-di-NO₂) were found to possess activity equivalent to that of the standard drug, while others were found to be low inactivity. The same derivatives, when tested for their anti-fungal property against a different fungal strain, *A. niger*, resulted in comparatively much better results than the former (*C. Albicans*). Compounds 5d, 5e, and 5o possessing -Cl/ -dinitro as a substituent exhibited excellent activity (31.25 µg/ml), even better than the standard drug fluconazole. Also, the derivatives possessing -NO₂ were found to have equivalent activity (62.5µg/ml) to that of the standard fluconazole. Products were including -CH₃ as a functional group was found to show low activity (250 and 500 µg/ml) compared to standard drug.

3. Conclusions

In short, the idea of this report summarized that the compounds possessing electron-withdrawing substituents were found to exhibit excellent anti-bacterial and anti-fungal properties. Several derivatives were also showing even better results than the standard drug ciprofloxacin viz. compound 5e (4-Cl) showed MIC value even higher than standard drug against all bacterial and fungal strains (15.62-62.5 µg/ml) and the compound containing -NO₂ and -Cl functional group was found to exhibit excellent property against gram-negative and gram-positive bacteria.

Acknowledgment

The authors gratefully acknowledge Maharshi Dayanand Science College, Porbandar, and School of Science, RK University, Rajkot, India, for laboratory and financial support.

4. Experimental

4.1 Materials

Chemicals and solvents were purchased from the Sigma-Aldrich Chemical Co., Merck chemical, Finar, and Spectrochem Ltd. All the chemicals were used without further purification. Thin-layer chromatography was accomplished on 0.2 mm precoated plates of Silica gel G60 F₂₅₄ (Merck). Visualization was made under UV light (254 and 365nm). IR spectra were recorded on an “IR Affinity-1S spectrophotometer (Shimadzu)” [DRS method, SN ratio: 30,000:1, maximum resolution of 0.5 cm⁻¹, Optional extended wavenumber range: 25000 to 10 cm⁻¹; Standard wave number measurement range: 7800 to 350 cm⁻¹]. ¹H (400 MHz) and ¹³C (101.1 MHz) NMR spectra were recorded on a “Bruker AVANCE II spectrometer” in DMSO-d₆. Chemical shifts are expressed in δ ppm downfield from TMS. Mass spectra were determined by direct inlet probe on a “GC-MS-QP [Agilent 7820A-5977B; Ionization source: EI- 0.7 kV; Ion source temperature: 220 °C; Interface temperature: 240 °C] mass spectrometer”. Solvents were evaporated with a “Roteva rotary evaporator.” Melting points were measured in open capillaries and are uncorrected.

4.2 Method of Synthesis

4.2.1 Procedure for the synthesis of 3-nitrobenzohydrazide (2).

Dissolved 10 gm (0.082 mol) of ethyl 3-nitrobenzoate (**1**) in ethanol in a 100 ml clean, dry round bottom flask under heating condition. To this reaction mixture, 0.041 moles of hydrazine hydrate was added and was refluxed for 7 hrs at 70-80 °C temperature. Completion of the reaction was identified using Thin Layer Chromatography (TLC) using n-hexane: ethyl acetate (8:2 ml) as a mobile phase. After completing the reaction, it was poured into ice crushed water and stirred at a cooling temperature for 30 minutes. The solid separated was filtered off using wattmann filter paper and washed twice with the chilled water. The isolated powder material was crystalized using methanol to obtained an analytically pure product.

4.2.2 Procedure for the synthesis of 2-(chloromethyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole (3).

A vacuum dryer 100 ml RBF, added 21 ml POCl₃, 0.049 mole chloroacetic acid, and 0.017 moles of previously prepared intermediate **(2) (Acid hydrazide)**. The reaction mixture was refluxed for 6 hrs at 70-75 °C temperature. TLC was used to identify the completion of the reaction [Mobile phase: n-hexane: ethyl acetate (8:2 ml)]. After completing the reaction, it was poured into ice crushed water and stirred at a cooling temperature for 15 minutes. The solid separated was filtered off using wattmann filter paper and washed twice with the chilled water. The isolated powder material was crystallized using a binary system (Methanol: DMF) to obtain an analytically pure product.

4.2.3 General procedure for the synthesis of 2-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-substitutedphenyl-1,3,4-oxadiazole (5a-5o).

In a THF (3 volume) containing RBF, 2-(chloromethyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole **(3)** (0.01 mole) and various phenyl substituted 1,3,4-oxadiazole-2-thiol **(4a-4o)** (0.01 mole) were stirred at RT for 1.5-2.0 hrs using soft metal CuI (4 mol %) catalysed (0.02 mole) & Triethylamine (1 eq.) as a base catalyst. After completing the reaction [Monitored by TLC; Mobile phase: n-hexane: ethyl acetate (8:2 ml)], it was poured into crushed ice. The solid separated was isolated in dry powder by filtration and hot vacuum dryer. The product obtained was dried and recrystallized using ethanol.

The same method of synthesis was applied for all the derivatives. Various spectroscopic techniques were used to carry out the identification and characterization of derived products (5a-5o), *i.e.*, IR, NMR (¹H and ¹³C), elemental analysis, and MS.

4.3 Analytical and Physical Data

4.3.1 2-(3-Nitrophenyl)-5-(((5-phenyl-1,3,4-oxadiazol-2-yl)thio)methyl)-1,3,4-oxadiazole. (5a)

Yield: 87%. mp (°C): 126. IR (cm⁻¹): 3078 (C-H Aromatic stretching), 2931 (C-H Aliphatic stretching), 1638, 1578, 1456 (Aromatic ring skeleton), 1550 & 1399 (Aromatic -NO₂ stretching), 1255 (C-O stretching), 780 & 690 (Aromatic m-disubstitution), 701 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.69-8.67 (d, 1H, Aromatic proton), 8.57-8.56 (d, 1H, Aromatic proton), 8.50-8.45 (m, 2H, Aromatic proton), 8.01-7.99 (d, 1H, Aromatic proton), 7.89-7.85 (m, 1H, Aromatic proton), 7.65-7.61 (m, 2H, Aromatic proton), 7.34-7.29 (m, 1H, Aromatic proton), 5.48 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ(ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 133.71, 131.57, 130.33, 129.80, 129.06, 127.08, 126.66, 122.67, 121.29, 24.46.; Mass (m/z): 381 (M⁺).

4.3.2 2-(3-Nitrophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5b)

Yield: 81%. mp (°C): 225. IR (cm⁻¹): 3081 (C-H Aromatic stretching), 2945 (C-H Aliphatic stretching), 1621, 1588, 1442 (Aromatic ring skeleton), 1559 & 1385 (Aromatic -NO₂ stretching), 1259 (C-O stretching), 779 & 693 (Aromatic m-disubstitution), 698 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ (ppm): 8.64-8.60(d, 1H, Aromatic proton), 8.47-8.46 (d, 1H, Aromatic proton), 8.41-8.37 (d, 1H, Aromatic proton), 7.98-7.96 (d, 1H, Aromatic proton), 7.92-7.88 (m, 1H, Aromatic proton), 7.68-7.62 (m, 2H, Aromatic proton), 7.56-7.53 (m, 1H, Aromatic proton), 5.03 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 153.29, 149.11, 134.38, 133.71, 131.36, 130.33, 129.80, 127.60, 126.34, 122.67, 121.29, 24.46.. Mass (m/z): 426 (M⁺).

4.3.3 2-(4-Nitrophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5c)

Yield: 93%. mp (°C): 120. IR (cm⁻¹): 3075 (C-H Aromatic stretching), 2953 (C-H Aliphatic stretching), 1648, 1583, 1450 (Aromatic ring skeleton), 1560 & 1387 (Aromatic -NO₂ stretching), 1267 (C-O stretching), 868 (Aromatic p-disubstitution), 778 & 694 (Aromatic m-disubstitution), 689 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.69-8.62(m, 1H, Aromatic proton), 8.51-8.48 (d, 1H, Aromatic proton), 8.28-8.27 (d, 1H, Aromatic proton), 7.99-7.95 (m, 2H, Aromatic proton), 7.82-7.77 (m, 2H, Aromatic proton), 7.51-7.45 (m, 1H, Aromatic proton), 5.09 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 147.39,

133.71, 132.08, 130.33, 129.80, 127.45, 127.45, 124.68, 124.68, 122.67, 121.29, 24.46.; Mass (m/z): 426 (M⁺).

4.3.4 2-(2-Chlorophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5d)

Yield: 87 %. mp (⁰C): 112. IR (cm⁻¹): 3025 (C-H Aromatic stretching), 2947 (C-H Aliphatic stretching), 1656, 1587, 1459 (Aromatic ring skeleton), 1585 & 1380 (Aromatic –NO₂ stretching), 1260 (C-O stretching), 759 (Aromatic o-disubstitution), 786 & 688 (Aromatic m-disubstitution), 732 (C-Cl stretching), 696 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.65-8.59 (d, 2H, Aromatic proton), 8.47-8.38 (m, 3H, Aromatic proton), 7.92-7.88 (m, 3H, Aromatic proton), 5.06 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 153.29, 149.11, 149.04, 134.38, 133.71, 131.36, 130.33, 129.80, 127.60, 126.34, 122.67, 121.29, 24.46. Mass (m/z): 415 (M⁺).

4.3.5 2-(4-Chlorophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5e)

Yield: 92%. mp (⁰C): 124. IR (cm⁻¹): 3012 (C-H Aromatic stretching), 2958 (C-H Aliphatic stretching), 1663, 1578, 1444 (Aromatic ring skeleton), 1586 & 1369 (Aromatic –NO₂ stretching), 1252 (C-O stretching), 872 (Aromatic p-disubstitution), 791 & 696 (Aromatic m-disubstitution), 732 (C-Cl stretching), 690 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.78-8.76 (d, 1H, Aromatic proton), 8.59-8.51 (m, 2H, Aromatic proton), 7.90-7.83 (m, 2H, Aromatic proton), 7.61-7.55 (m, 3H, Aromatic proton), 5.05 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 137.31, 133.71, 130.33, 129.80, 129.46, 129.46, 128.39, 128.09, 128.09, 122.67, 121.29, 24.46.. Mass (m/z): 415 (M⁺).

4.3.6 2-(2,4-Dichlorophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5f)

Yield: 82%. mp (⁰C): 130. IR (cm⁻¹): 3070 (C-H Aromatic stretching), 2956 (C-H Aliphatic stretching), 1684, 1549, 1458 (Aromatic ring skeleton), 1557 & 1389 (Aromatic –NO₂ stretching), 1250 (C-O stretching), 861 (Aromatic p-disubstitution), 788 & 691 (Aromatic m-disubstitution), 735 (C-Cl stretching), 758 (Aromatic o-disubstitution), 698 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.32 (s, 1H, Aromatic proton), 8.03-8.02 (d, 1H, Aromatic proton), 7.63-7.62 (d, 1H, Aromatic proton), 7.50-7.44 (m, 2H, Aromatic proton), 7.82-7.77 (m, 1H, Aromatic proton), 7.36-7.30 (m, 1H, Aromatic proton), 5.11 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 153.29, 149.11, 149.04, 135.87, 134.93, 133.71, 131.73, 130.33, 129.80, 128.52, 128.48, 127.88, 122.67, 121.29, 24.46. Mass (m/z): 448 (M⁺).

4.3.7 2-(2-Iodophenyl)-5-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazole. (5g)

Yield: 80%. mp (⁰C): 106. IR (cm⁻¹): 3045 (C-H Aromatic stretching), 2938 (C-H Aliphatic stretching), 1698, 1540, 1468 (Aromatic ring skeleton), 1598 & 1382 (Aromatic –NO₂ stretching), 1258 (C-O stretching), 789 & 689 (Aromatic m-disubstitution), 768 (Aromatic o-disubstitution), 690 (C-S stretching), 561 (C-I stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.56 (s, 1H, Aromatic proton), 8.29-8.28 (d, 1H, Aromatic proton), 8.10-8.09 (d, 1H, Aromatic proton), 7.98-7.95 (m, 2H, Aromatic proton), 7.80-7.75 (m, 2H, Aromatic proton), 7.09-7.04 (m, 1H, Aromatic proton), 4.98 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 153.29, 149.17, 149.04, 140.05, 136.13, 133.71, 131.96, 130.33, 129.80, 129.54, 126.04, 122.67, 121.29, 24.46. Mass (m/z): 506 (M⁺).

4.3.8 2-(5-(((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)thio)-1,3,4-oxadiazol-2-yl) phenol. (5h)

Yield: 76%. mp (⁰C): 140. IR (cm⁻¹): 3459 (O-H stretching), 3058 (C-H Aromatic stretching), 2940 (C-H Aliphatic stretching), 1680, 1546, 1460 (Aromatic ring skeleton), 1591 & 1378 (Aromatic –NO₂ stretching), 1269 (C-O stretching), 781 & 685 (Aromatic m-disubstitution), 769 (Aromatic o-disubstitution), 697 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.51 (s, 1H, Aromatic proton), 8.25-8.24 (d, 1H, Aromatic proton), 7.94-7.93 (d, 1H, Aromatic proton), 7.67-7.61 (m, 1H, Aromatic proton), 7.40-7.33 (m, 1H, Aromatic proton), 7.19-7.12 (m, 1H, Aromatic proton), 6.98-6.91

(m, 2H, Aromatic proton), 6.72 (s, 1H, -OH proton), 4.88 (s, 2H, Methylene group). ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ(ppm): 165.16, 162.20, 158.44, 153.29, 150.08, 149.04, 133.71, 131.79, 130.33, 130.12, 129.80, 122.67, 121.29, 119.86, 118.80, 111.70, 24.46. Mass (m/z): 397 (M⁺).

4.3.9 2-(3-Nitrophenyl)-5-(((5-(*o*-tolyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1,3,4-oxadiazole. (5i)

Yield: 78%. mp (°C): 80. IR (cm⁻¹): 3012 (C-H Aromatic stretching), 2945 (C-H Aliphatic stretching), 1632, 1570, 1461 (Aromatic ring skeleton), 1548 & 1386 (Aromatic -NO₂ stretching), 1254 (C-O stretching), 778 & 699 (Aromatic m-disubstitution), 759 (Aromatic o-disubstitution), 712 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.52 (s, 1H, Aromatic proton), 8.24-8.22 (d, 1H, Aromatic proton), 7.94-7.92 (d, 1H, Aromatic proton), 7.67-7.62 (m, 2H, Aromatic proton), 7.31-7.28 (m, 3H, Aromatic proton), 4.89 (s, 2H, Methylene group), 2.59 (s, 3H, Methyl group). ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ(ppm): 165.16, 162.20, 153.29, 149.04, 148.76, 136.59, 133.71, 130.81, 130.33, 129.80, 129.37, 128.75, 128.51, 126.53, 122.67, 121.29, 24.46, 20.24. Mass (m/z): 395 (M⁺).

4.3.10 2-(3-Nitrophenyl)-5-(((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1,3,4-oxadiazole. (5j)

Yield: 85%. mp (°C): 115. IR (cm⁻¹): 3055 (C-H Aromatic stretching), 2926 (C-H Aliphatic stretching), 1630, 1549, 1455 (Aromatic ring skeleton), 1551 & 1398 (Aromatic -NO₂ stretching), 1245 (C-O stretching), 862 (Aromatic p-disubstitution), 788 & 688 (Aromatic m-disubstitution), 702 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.62 (s, 1H, Aromatic proton), 8.25-8.23 (d, 1H, Aromatic proton), 7.94-7.92 (d, 1H, Aromatic proton), 7.67-7.52-7.46 (m, 2H, Aromatic proton), 7.29-7.23 (m, 2H, Aromatic proton), 4.95 (s, 2H, Methylene group), 2.39 (s, 3H, Methyl group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ(ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 140.60, 133.71, 130.33, 129.80, 129.10, 129.10, 126.99, 126.99, 125.47, 122.67, 121.29, 24.46, 21.13. Mass (m/z): 395 (M⁺).

4.3.11 2-(((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-(4-bromophenyl)-1,3,4-oxadiazole. (5k)

Yield: 89%. mp (°C): 202. IR (cm⁻¹): 3056 (C-H Aromatic stretching), 2921 (C-H Aliphatic stretching), 1656, 1575, 1457 (Aromatic ring skeleton), 1551 & 1390 (Aromatic -NO₂ stretching), 1249 (C-O stretching), 870 (Aromatic p-disubstitution), 781 & 693 (Aromatic m-disubstitution), 715 (C-S stretching), 655 (C-Br stretching). ¹H NMR: (DMSO-*d*₆, 400 MHz) δ (ppm): 8.43 (s, 1H, Aromatic proton), 8.10-8.08 (d, 1H, Aromatic proton), 7.98-7.96 (d, 1H, Aromatic proton), 7.65-7.59 (m, 2H, Aromatic proton), 7.59-7.46 (m, 1H, Aromatic proton), 7.26-7.21 (m, 1H, Aromatic proton), 4.78 (s, 2H, Methylene group). ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ(ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 133.71, 132.80, 132.80, 130.33, 130.11, 130.11, 129.80, 127.74, 125.31, 122.67, 121.29, 24.46. Mass (m/z): 460 (M⁺).

4.3.12 2-(((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole. (5l)

Yield: 84%. mp (°C): 220. IR (cm⁻¹): 3011 (C-H Aromatic stretching), 2951 (C-H Aliphatic stretching), 1658, 1548, 1450 (Aromatic ring skeleton), 1554 & 1397 (Aromatic -NO₂ stretching), 1251 (C-O stretching), 869 (Aromatic p-disubstitution), 788 & 693 (Aromatic m-disubstitution), 742 (C-Cl stretching), 705 (C-S stretching).; ¹H NMR: (DMSO-*d*₆, 400 MHz) δ(ppm): 8.59 (s, 1H, Aromatic proton), 8.25-8.23 (d, 1H, Aromatic proton), 7.94-7.92 (d, 1H, Aromatic proton), 7.72-7.66 (m, 2H, Aromatic proton), 7.60 (s, 1H, Aromatic proton), 7.39-7.31 (m, 1H, Aromatic proton), 4.77 (s, 2H, Methylene group).; ¹³C NMR: (DMSO-*d*₆, 101.1 MHz) δ (ppm): 165.16, 162.20, 159.57, 153.29, 149.04, 133.71, 133.68, 132.85, 130.33, 129.80, 129.35, 129.02, 128.72, 126.80, 122.67, 121.29, 24.46.. Mass (m/z): 450 (M⁺).

4.3.13 4-(5-(((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-1,3,4-oxadiazol-2-yl) benzene-1,2-diol. (5m)

Yield: 85%. mp (°C): 185. IR (cm⁻¹): 3489 (O-H stretching), 3048 (C-H Aromatic stretching), 2935 (C-H Aliphatic stretching), 1632, 1579, 1461 (Aromatic ring skeleton), 1548 & 1389 (Aromatic -NO₂

stretching), 1250 (C-O stretching), 870 (Aromatic p-disubstitution), 779 & 688 (Aromatic m-disubstitution), 722 (C-S stretching).; ^1H NMR: (DMSO- d_6 , 400 MHz) δ (ppm): 8.58 (s, 1H, Aromatic proton), 8.22-8.20 (d, 1H, Aromatic proton), 7.92-7.90 (d, 1H, Aromatic proton), 7.67-7.60 (m, 1H, Aromatic proton), 7.07-7.01 (m, 1H, Aromatic proton), 6.94-6.85 (m, 2H, Aromatic proton), 4.93 (s, 2H, Methylene group), 3.34 (s, 1H, *p*-OH proton), 2.90 (s, 1H, *o*-OH proton). ^{13}C NMR: (DMSO- d_6 , 101.1 MHz) δ (ppm): 165.16, 162.20, 159.57, 153.29, 149.04, 148.26, 148.22, 133.71, 130.33, 129.80, 122.67, 121.45, 121.29, 119.07, 117.60, 112.31, 24.46. Mass (m/z): 413 (M^+).

4.3.14 2-((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-(4-(difluoromethyl)phenyl)-1,3,4-oxadiazole. (5n)

Yield: 91%. mp ($^{\circ}\text{C}$): 195. IR (cm^{-1}): 3052 (C-H Aromatic stretching), 2932 (C-H Aliphatic stretching), 1658, 1588, 1454 (Aromatic ring skeleton), 1555 & 1392 (Aromatic $-\text{NO}_2$ stretching), 1259 (C-O stretching), 1089 (C-F stretching), 863 (Aromatic p-disubstitution), 784 & 687 (Aromatic m-disubstitution), 698 (C-S stretching).; ^1H NMR: (DMSO- d_6 , 400 MHz) δ (ppm): 8.61 (s, 1H, Aromatic proton), 8.09-8.07 (d, 1H, Aromatic proton), 7.90-7.88 (d, 1H, Aromatic proton), 7.65-7.57 (m, 3H, Aromatic proton), 7.51-7.44 (m, 2H, Aromatic proton), 6.40-6.37 (t, 1H, Methine proton), 5.01 (s, 2H, Methylene group). ^{13}C NMR: (DMSO- d_6 , 101.1 MHz) δ (ppm): 165.16, 162.20, 159.39, 153.29, 149.04, 140.00, 133.71, 131.71, 130.33, 129.80, 126.36, 126.36, 125.44, 125.44, 122.67, 121.29, 116.13, 24.46. Mass (m/z): 431 (M^+).

4.3.15 2-((5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl)methylthio)-5-(2,4-dinitrophenyl)-1,3,4-oxadiazole. (5o)

Yield: 79%. mp ($^{\circ}\text{C}$): 188. IR (cm^{-1}): 3027 (C-H Aromatic stretching), 2989 (C-H Aliphatic stretching), 1634, 1579, 1454 (Aromatic ring skeleton), 1560 & 1398 (Aromatic $-\text{NO}_2$ stretching), 1258 (C-O stretching), 789 & 692 (Aromatic m-disubstitution), 765 (Aromatic o-disubstitution), 734 (C-S stretching).; ^1H NMR: (DMSO- d_6 , 400 MHz) δ (ppm): 9.12 (s, 1H, Aromatic proton of between two nitro group), 8.66-8.64 (d, 1H, Aromatic proton), 8.51 (s, 1H, Aromatic proton), 8.25-8.23 (d, 1H, Aromatic proton), 8.06-8.04 (d, 1H, Aromatic proton), 7.95-7.89 (m, 1H, Aromatic proton), 7.67-7.60 (m, 1H, Aromatic proton), 4.83 (s, 2H, Methylene group).; ^{13}C NMR: (DMSO- d_6 , 101.1 MHz) δ (ppm): 165.16, 162.20, 153.29, 150.56, 149.04, 147.61, 146.01, 133.71, 131.65, 130.33, 130.14, 129.80, 126.87, 122.67, 121.45, 121.29, 24.46. Mass (m/z): 471 (M^+).

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