EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 7
575-587
Year: 2018

DESIGN AND SYNTHESIS OF NOVEL 1,3,4-OXADIAZOLE AND 1,2,4-TRIAZOLO[3,4-B]1,3,4-THIADIAZOLE DERIVATIVES AND THEIR ANTIMICROBIAL STUDIES

Settypalli Triloknadh[1], Chunduri Venkata Rao[1]*, Begari Nagaraju, Hari Balaji[2] and Meriga Balaji[2]

[1]Department of Chemistry,
[2]Department of Biochemistry,
Sri Venkateswara University-Tirupati-517502.

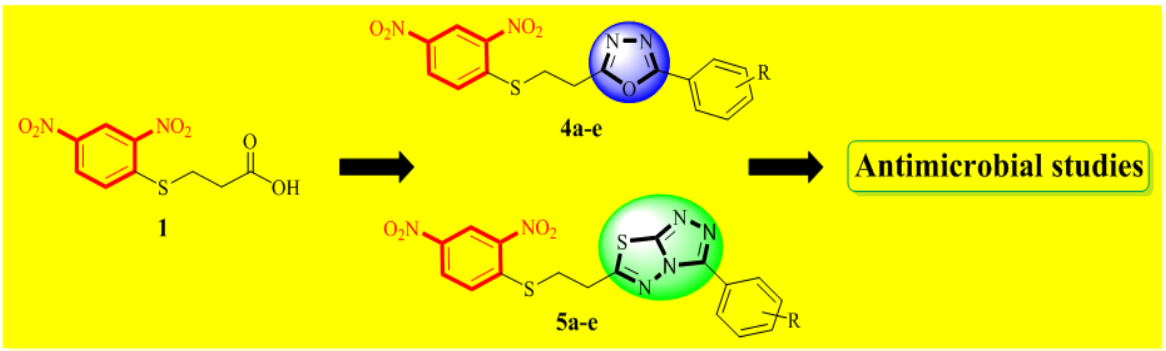
***Corresponding Author: Chunduri Venkata Rao**
Department of Chemistry, Sri Venkateswara University-Tirupati-517502.

Article Received on 08/05/2018 **Article Revised on 28/05/2018** **Article Accepted on 18/06/2018**

ABSTRACT
Design and synthesis of a series of 2,4-dinitro phenyl ring containing 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives. All of the compounds were characterized well by spectroscopy analysis (^{1}H-NMR, ^{13}C-NMR, FT-IR and LC-MS) and they were evaluated for their antimicrobial activity against four bacterial and three fungal strains using paper disc diffusion technique.

KEYWORDS: 1,3,4-oxadiazole, 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole, Antimicrobial activity.

Graphical Abstract



INTRODUCTION
Nitro (–NO_2) group is a unique functional group, it plays an important role in the action of certain drugs. It is one of the strong electron withdrawing groups, which creates localized or electron deficient sites within the molecules. The formed electrophilic sites may then react with a variety of intra or extracellular biological nucleophiles i.e. proteins, enzymes, amino acids, nucleic acids etc to produce biological changes which can be useful or harmful, depending upon the point of view used to judge them.[1] There are several –NO_2 group containing drugs available in the literature, which exert their pharmacological action due to the presence of aromatic nitro groups. Some of the market available important –NO_2 group containing drugs are Chloramphenicol (antibiotic), Nitrazepan (Tranquilizer), Metronidazole (Amebicide), Niridazole (Schistosomicide), Parathion (Insecticide) **I**, 2,4-dinitrophenol (Weed killer) **II** etc. Moreover, recently reported mycobacterial DprE1 inhibitors dinitrobenzamide **III**,[2] trinitroxanthone **IV**[3] and some antimycobacterial compounds (Benzazoles) were also containing dinitro phenyl aromatic rings in their structures.[4,5] Subsequently, some dinitro phenyl ring containing heterocyclic compounds were also synthesized and evaluated for their antimicrobial activities **VI**.[6-8]

1,3,4-Oxadiazole is an important pharmacophoric group, which shows a broad spectrum of biological activities like, antibacterial,[9] antifungal,[10] antiviral,[11] antiproliferative,[12] anticonvulsant,[13] anti-inflammatory and analgesic,[14] hypolipidemic,[15] antitubercular,[16] anticancer,[17] ulcerogenic[18] etc. It is a bioisostere of amide and ester. The good pharmacokinetic property of 1,3,4-oxadiazole is due to –N=C–O– group, which increases the lipophilicity that influence the capability of drug to reach the target by transmembrane diffusion. Additionally, reported 2,5-disubstituted 1,3,4-

oxadiazoles **VII & VIII,**[19,20] 3-ethyl pyridine linked 1,3,4-oxadiazole analogues **IX**,[21] pyrazole linked 1,3,4-oxadiazole derivatives **X**[22] were also found to exhibit good antimicrobial activities as compared to the standard drugs.

Nitrogen heterocycles play an important role in medicinal chemistry because of their utility in various applications. Among them 1,2,4 triazoles are important, some of the available drugs in the market incorporated the 1,2,4-triazole ring (eg. Vorozole, Letrozole, Anastrozole, Fluconazole, Ravuconazole, Voriconazole, Itraconazole, Posaconazole etc).[23] Furthermore, the combined heterocycle of 1,2,4-triazole and 1,3,4-thiadiazole i.e. 1,2.4-triazolo[3,4-b]1,3,4-thiadiazole also exhibited wide spread applications as antimicrobial,[24] antidepressant,[25] anticancer,[26] anti-inflammatory,[27] urease inhibition and antioxidant,[28] analgesic,[29] antitubercular[30] etc. And also S.N. Swamy *et al*, M.R. Maqsood *et al* & N. Aggarwal *et al* reported some potent antimicrobial compounds containing 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole moiety.[31-33]

Inspired by the above literature survey, herein, we designed, synthesized and screened some novel 1,3,4-oxadiazole, 1,2,4-triazolo[3,4-b][1,3,4]-thiadiazole derivatives bearing 2,4-dinitro phenyl ring for their *in vitro* antimicrobial activity.

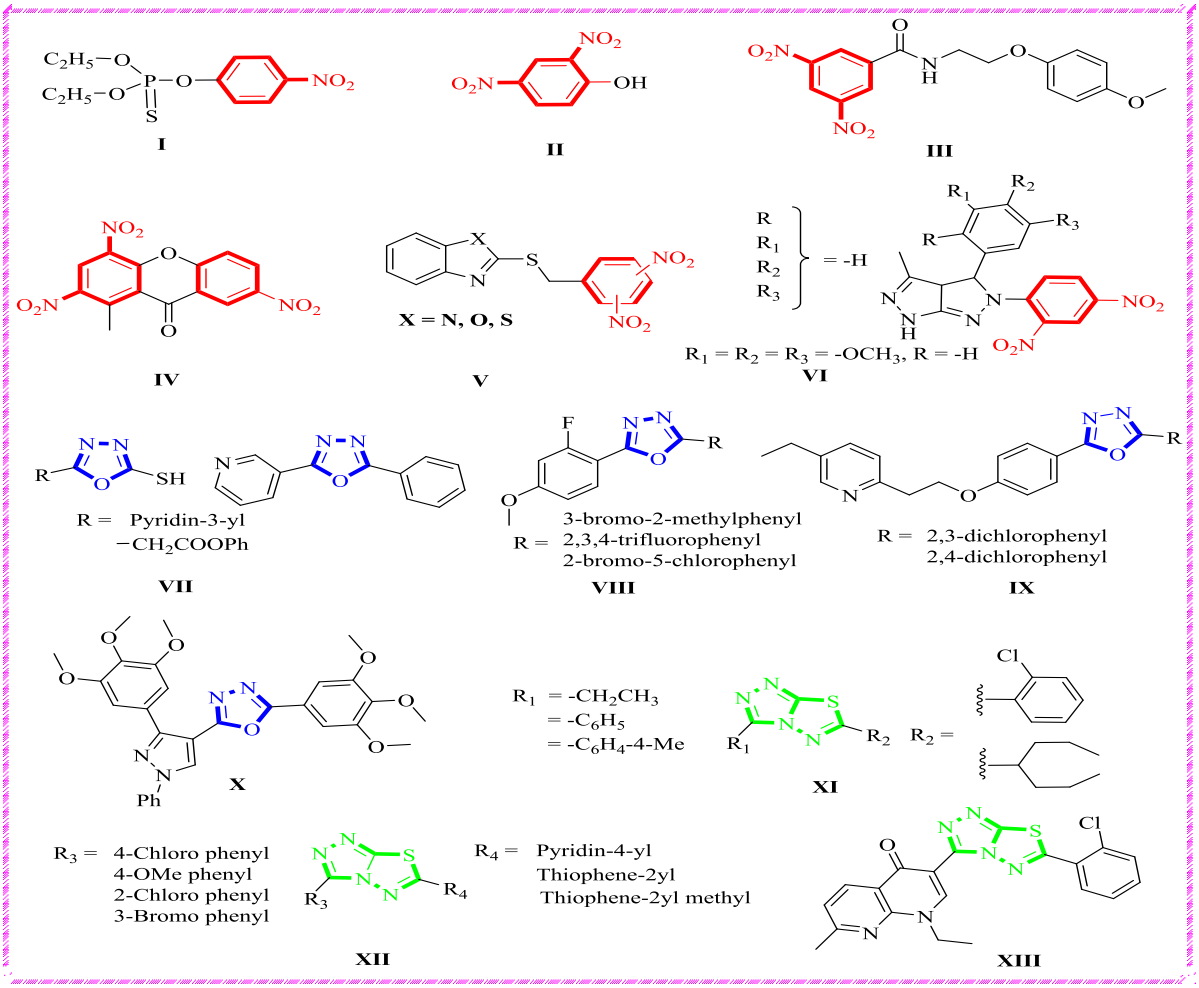


Figure 1: Reported drugs and some antimycobacterial & antimicrobial compounds.

MATERIALS AND METHODS

Chemistry

All Chemicals, reagents and solvents were obtained from commercial suppliers (Sigma Aldrich, Avra synthesis, SD-Fine) and used without purification. Progress of the reactions were monitored by thin layer chromatography using Merck silica gel GF_{254} aluminium plates and the spots were visualized with UV lamp (254 nm) or Iodine vapors. Column chromatography was performed using silica gel (60-120 mesh) purchased from Avra synthesis chemical Ltd and the combination of Pet.ether/Ethyl acetate (30-50%) used as eluents. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded in DMSO-d_6 using TMS as internal standard and the multiplicities were given as s (singlet), t (triplet), d (doublet), dd (double doublet), m (multiplet) etc in the data. LC-MS-2010A SHIMADZU spectrometer was used for mass spectra. FT-IR spectra were obtained using a BRUKER-FT-IR spectrometer and the values were represented as wave numbers cm^{-1}. Melting points were determined in open capillaries and these are uncorrected.

Synthesis of 3-((2,4-dinitrophenyl)thio)propanoic acid (1).

Methyl 3-((2,4-dinitrophenyl)thio)propanoate (1.5 g, 5.24 mmol) was dissolved in 10 ml of MeOH at 0°C and then aq. NaOH (0.83g, 20.96 mmol) 10 ml was added to the solution drop by drop for half an hour and stirred the resultant solution at rt for overnight. Finally 50 ml ice cold water was added to the above solution and neutralized with 50% HCl to pH 7 to obtained yellow color residue, filtered and dried to obtain the compound **1** in good yield. Yield: 92%; (Yellow color solid); m.p. = 208-210ºC; LC-MS (ESI): m/z = 273 $(M+H)^+$ for $C_9H_8N_2O_6S$.

General procedure for synthesis of compounds (4a) and (4b-e)

To the mixture of **1** (100 mg, 0.36 mmol) and simple acid hydrazide **2a** (49.9 mg, 0.36 mmol) phosphorous oxy chloride (4 ml) was added and refluxed for 5-6 h at 90ºC. After the completion of reaction it was poured into the chilled water 50 ml drop by drop and then basified with aq. ammonia. Compound **4a** yellow color solid obtained in good yield was purified with column chromatography.

Compounds **5a-e** were obtained from **3a-e** as per the above procedure.

2-(2-((2,4-dinitrophenyl)thio)ethyl)-5-phenyl-1,3,4-oxadiazole (4a): Yield: 72%; (Yellow color solid); m.p. = 120-122ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.43 (t, J = 7.2 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.78 (t, J = 7.2 Hz, 2H, −$CH_2\underline{CH_2}$−), 7.68-7.70 (m, 3H, Ar-H), 7.92 (d, J = 7.8 Hz, 2H, Ar-H), 8.00 (d, J = 7.6 Hz, 1H, Ar-H), 8.46 (dd, J = 7.8 Hz, J = 2.8 Hz, 1H, Ar-H), 8.84 (d, J = 3.2 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 23.82, 28.09, (−CH_2CH_2− carbons) 120.96, 121.99, 127.89, 128.48, 128.98, 129.36, 129.87 (Aromatic carbons), 143.71, 144.18, 144.98, (2 × C−NO_2, C−S carbons) 164.11, 165.01 (two oxadiazole carbons); LC-MS (ESI) : m/z = 373 $(M+H)^+$ for $C_{16}H_{12}N_4O_5S$.

2-(2-((2,4-dinitrophenyl)thio)ethyl)-5-(p-tolyl)-1,3,4-oxadiazole (4b): Yield: 78%; (Greenish yellow color solid); m.p. = 112-114ºC; IR (KBr) ν (cm^{-1}): 3099, 2923, 2857, 1588, 1511, 1454, 1330, 1255, 1084, 913, 805; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.40 (s, 3H, phenyl −CH_3), 3.41 (t, J = 7.2 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.74 (t, J = 7.2 Hz, 2H, −$CH_2\underline{CH_2}$−), 7.39 (d, J = 7.6 Hz, 2H, Ar-H), 7.93 (d, J = 7.6 Hz, 2H, Ar-H), 7.99 (d, J = 7.6 Hz, 1H, Ar-H), 8.42 (dd, J = 7.8 Hz, J = 2.8 Hz, 1H, Ar-H), 8.82 (d, J = 3.2 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 21.02, 23.85, 28.06, (−CH_3, −CH_2CH_2− carbons) 120.49, 121.22, 126.29, 127.43, 128.23, 129.84 (Aromatic carbons), 142.01, 143.73, 144.16, 144.90, (2 × C−NO_2, C−S, C−CH_3 carbons) 164.15, 164.36 (two oxadiazole carbons); LC-MS (ESI) : m/z = 387 $(M+H)^+$ for $C_{17}H_{14}N_4O_5S$.

2-(2-((2,4-dinitrophenyl)thio)ethyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (4c): Yield: 76%; (Dark yellow color solid); m.p. = 132-134ºC; IR (KBr) ν (cm^{-1}): 3075, 2927, 2856, 1508, 1330, 1233, 1155, 1004, 823, 720; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.40 (t, J = 7.2 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.76 (t, J = 7.2 Hz, 2H, −$CH_2\underline{CH_2}$−), 3.83 (s, 3H, phenyl −OCH_3), 7.16 (d, J = 7.6 Hz, 2H, Ar-H), 7.92 (d, J = 7.6 Hz, 2H, Ar-H), 8.00 (d, J = 7.2 Hz, 1H, Ar-H), 8.41 (dd, J = 7.4 Hz, J = 2.2 Hz, 1H, Ar-H), 8.82 (d, J = 3.2 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 23.84, 28.10, 55.44 (−CH_2CH_2−, −OCH_3, carbons) 114.77, 115.58, 121.23, 127.44, 128.17, 128.25 (Aromatic carbons), 143.74, 144.16, 144.92 (2 × C−NO_2, C−S carbons), 161.92 (C−O carbon), 163.99, 164.05 (two oxadiazole carbons); LC-MS (ESI) : m/z = 403 $(M+H)^+$ for $C_{17}H_{14}N_4O_6S$.

2-(4-bromophenyl)-5-(2-((2,4-dinitrophenyl)thio)ethyl)-1,3,4-oxadiazole (4d): Yield: 69%; (Light brown color solid); m.p. = 196-198 ºC; IR (KBr) ν (cm^{-1}): 3088, 3003, 2920, 2848, 1585, 1518, 1340, 1222, 1082, 818, 725; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.38 (t, J = 7.2 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.79 (t, J = 7.2 Hz, 2H, −$CH_2\underline{CH_2}$−), 7.58 (d, J = 7.6 Hz, 2H, Ar-H), 7.91 (d, J = 7.4 Hz, 2H, Ar-H), 8.03 (d, J = 7.2 Hz, 1H, Ar-H), 8.43 (dd, J = 7.4 Hz, J = 2.2 Hz, 1H, Ar-H), 8.81 (d, J = 2.8 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 23.82, 28.09, (−CH_2CH_2− carbons) 120.82, 122.96, 125.23, 127.99, 128.29, 129.32, 130.22 (Aromatic carbons), 143.71, 144.17, 144.89, (2 × C−NO_2, C−S carbons) 164.12, 164.26 (two oxadiazole carbons); LC-MS (ESI): m/z = 451 $(M+H)^+$ for $C_{16}H_{11}BrN_4O_5S$.

2-(4-chlorophenyl)-5-(2-((2,4-dinitrophenyl)thio)ethyl)-1,3,4-oxadiazole (4e): Yield: 79%; (Light yellow color flakes); m.p. = 129-131ºC; IR (KBr) ν (cm^{-1}): 3093, 2927, 2854, 1589, 1516, 1338, 1260, 1086, 917, 819, 728; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.40 (t, J = 7.2 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.75 (t, J = 7.2 Hz, 2H, −$CH_2\underline{CH_2}$−), 7.64 (d, J = 7.6 Hz, 2H, Ar-H), 8.00 (m, 3H, Ar-H), 8.41 (dd, J = 7.4 Hz, J = 2.2 Hz, 1H, Ar-H), 8.83 (d, J = 3.2 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 23.87, 28.00, (−CH_2CH_2− carbons) 121.23, 122.09, 127.45, 128.14, 128.23, 129.52 (Aromatic carbons), 136.64 (C−Cl carbon), 143.75, 144.12, 144.91 (2 × C−NO_2, C−S carbons), 163.34, 164.87 (two oxadiazole carbons); LC-MS (ESI) : m/z = 407 $(M+H)^+$ for $C_{16}H_{11}ClN_4O_5S$.

6-(2-((2,4-dinitrophenyl)thio)ethyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5a): Yield: 66%; (Yellow color solid); m.p. = 168-170 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.54 (t, J = 7.8 Hz, 2H, −$\underline{CH_2}CH_2$−), 3.82 (t, J = 7.8 Hz, 2H, −$CH_2\underline{CH_2}$−), 7.55-7.62 (m, 3H, Ar-H), 8.02 (d, J = 7.6 Hz, 1H, Ar-H), 8.26 (d, J = 7.8 Hz, 2H, Ar-H), 8.43 (dd, J = 7.8 Hz, J = 2.6 Hz, 1H, Ar-H), 8.82 (d, J = 2.8 Hz, 1H, Ar-H); LC-MS (ESI) : m/z = 429 $(M+H)^+$ for $C_{17}H_{12}N_6O_4S_2$.

6-(2-((2,4-dinitrophenyl)thio)ethyl)-3-(p-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5b)
Yield: 72%; (Yellow color solid); m.p. = 172-174ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.43 (s, 3H, phenyl $-CH_3$), 3.59 (t, J = 7.2 Hz, 2H, $-\underline{CH_2}CH_2-$), 3.78 (t, J = 7.2 Hz, 2H, $-CH_2\underline{CH_2}-$), 7.43 (d, J = 8.2 Hz, 2H, Ar-H), 7.99 (d, J = 7.6 Hz, 1H, Ar-H), 8.21 (d, J = 7.8 Hz, 2H, Ar-H), 8.40 (dd, J = 7.8 Hz, J = 2.6 Hz, 1H, Ar-H), 8.80 (d, J = 2.8 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 21.09, 29.68, 30.42, ($-CH_3$, $-CH_2CH_2-$ carbons) 120.68, 122.41, 126.58, 127.38, 128.43, 129.34 (Aromatic carbons), 141.01, 143.79, 143.98, 145.18, (2 × C−NO_2, C−S, C−CH_3 carbons) 149.19, 153.86, 168.74 (triazolothiadiazole carbons); LC-MS (ESI) : m/z = 443 $(M+H)^+$ for $C_{18}H_{14}N_6O_4S_2$.

6-(2-((2,4-dinitrophenyl)thio)ethyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5c): Yield: 82%; (Yellow color solid); m.p. = 176-178ºC; IR (KBr) ν (cm^{-1}): 3014, 2837, 1520, 1347, 1225, 1090, 987, 836, 727; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.58 (t, J = 7.2 Hz, 2H, $-\underline{CH_2}CH_2-$), 3.79 (t, J = 7.2 Hz, 2H, $-CH_2\underline{CH_2}-$), 3.81 (s, 3H, phenyl $-OCH_3$), 7.12 (d, J = 7.2 Hz, 2H, Ar-H), 8.00 (d, J = 7.6 Hz, 1H, Ar-H), 8.22 (d, J = 7.4 Hz, 2H, Ar-H), 8.42 (dd, J = 7.4 Hz, J = 2.6 Hz, 1H, Ar-H), 8.79 (d, J = 2.8 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 29.68, 30.54, 55.28 ($-CH_2CH_2-$, $-OCH_3$, carbons) 114.86, 117.32, 121.19, 127.89, 128.32, 129.15 (Aromatic carbons), 143.79, 143.86, 145.22 (2 × C−NO_2, C−S carbons), 148.99, 154.12 (triazolothiadiazole carbons) 161.78 (C−O carbon), 168.69 (triazolothiadiazole carbon); LC-MS (ESI): m/z = 459 $(M+H)^+$ for $C_{18}H_{14}N_6O_5S_2$.

3-(4-bromophenyl)-6-(2-((2,4-dinitrophenyl)thio)ethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5d): Yield: 70%; (Light brown color solid); m.p. = 199-201 ºC; IR (KBr) ν (cm^{-1}): 3092, 2949, 2838, 1518, 1341, 1219, 1050, 909, 829, 729; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.64 (t, J = 7.2 Hz, 2H, $-\underline{CH_2}CH_2-$), 3.79 (t, J = 7.2 Hz, 2H, $-CH_2\underline{CH_2}-$), 7.64 (d, J = 7.8 Hz, 2H, Ar-H), 7.98 (d, J = 7.6 Hz, 1H, Ar-H), 8.22 (d, J = 7.8 Hz, 2H, Ar-H), 8.40 (dd, J = 7.6 Hz, J = 2.8 Hz, 1H, Ar-H), 8.80 (d, J = 2.8 Hz, 1H, Ar-H); LC-MS (ESI): m/z = 507 $(M+H)^+$ for $C_{17}H_{11}BrN_6O_4S_2$.

3-(4-chlorophenyl)-6-(2-((2,4-dinitrophenyl)thio)ethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (5e): Yield: 84%; (Greenish yellow color solid); m.p. = 202-204 ºC; IR (KBr) ν (cm^{-1}): 3100, 2980, 2836, 1520, 1344, 1218, 1093, 972, 829, 724; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.59 (t, J = 7.2 Hz, 2H, $-\underline{CH_2}CH_2-$), 3.79 (t, J = 7.2 Hz, 2H, $-CH_2\underline{CH_2}-$), 7.67 (d, J = 7.2 Hz, 2H, Ar-H), 8.00 (d, J = 7.2 Hz, 1H, Ar-H), 8.20 (d, J = 7.2 Hz, 2H, Ar-H), 8.41 (dd, J = 7.4 Hz, J = 2.2 Hz, 1H, Ar-H), 8.80 (d, J = 3.2 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 29.64, 30.36, ($-CH_2CH_2-$ carbons) 121.16, 124.18, 127.34, 128.55, 129.24, 129.26 (Aromatic carbons), 134.89 (C−Cl carbon), 143.80, 143.92, 145.17 (2 × C−NO_2, C−S carbons), 149.16, 153.21, 168.86 (triazolothiadiazole carbons); LC-MS (ESI): m/z = 463 $(M+H)^+$ for $C_{17}H_{11}ClN_6O_4S_2$.

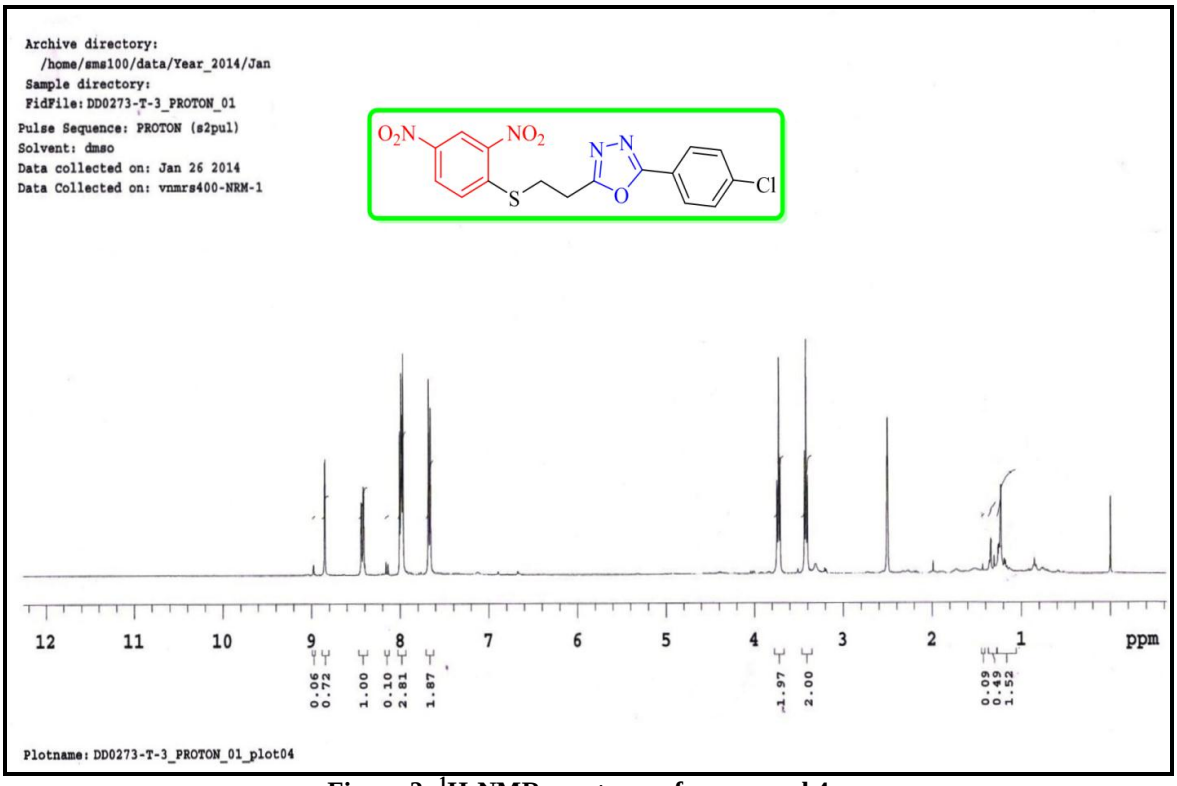


Figure 2: ^{1}H-NMR spectrum of compound 4e.

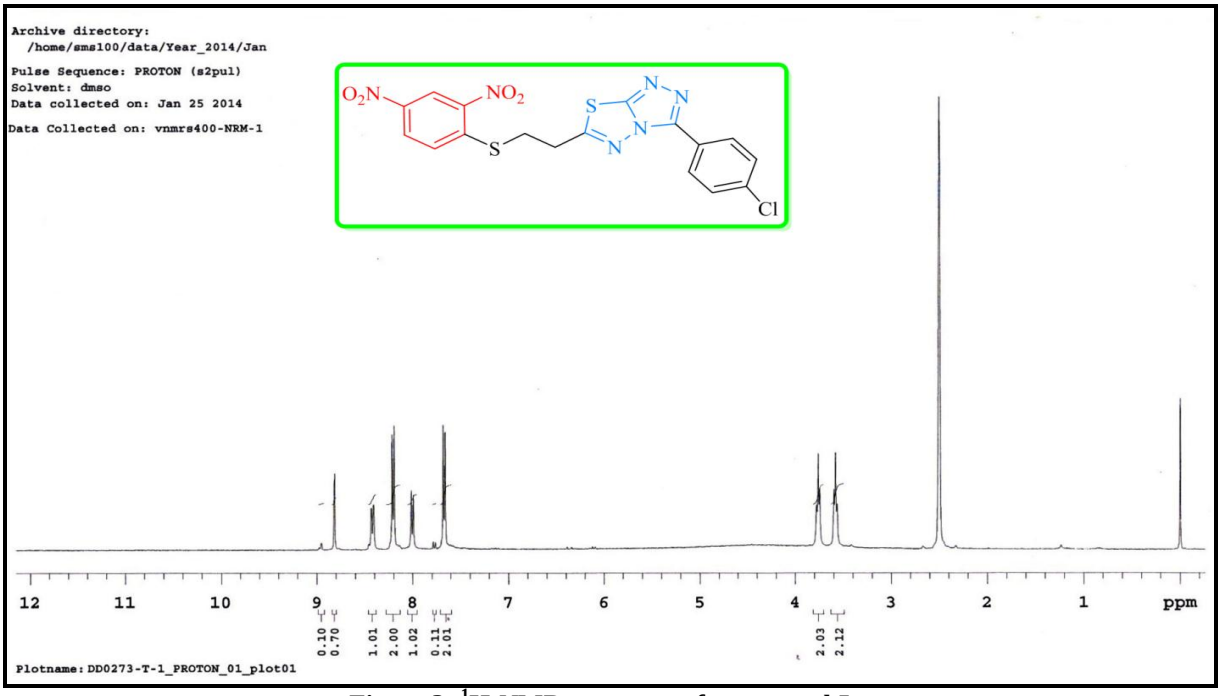


Figure 3: ^{1}H-NMR spectrum of compound 5e.

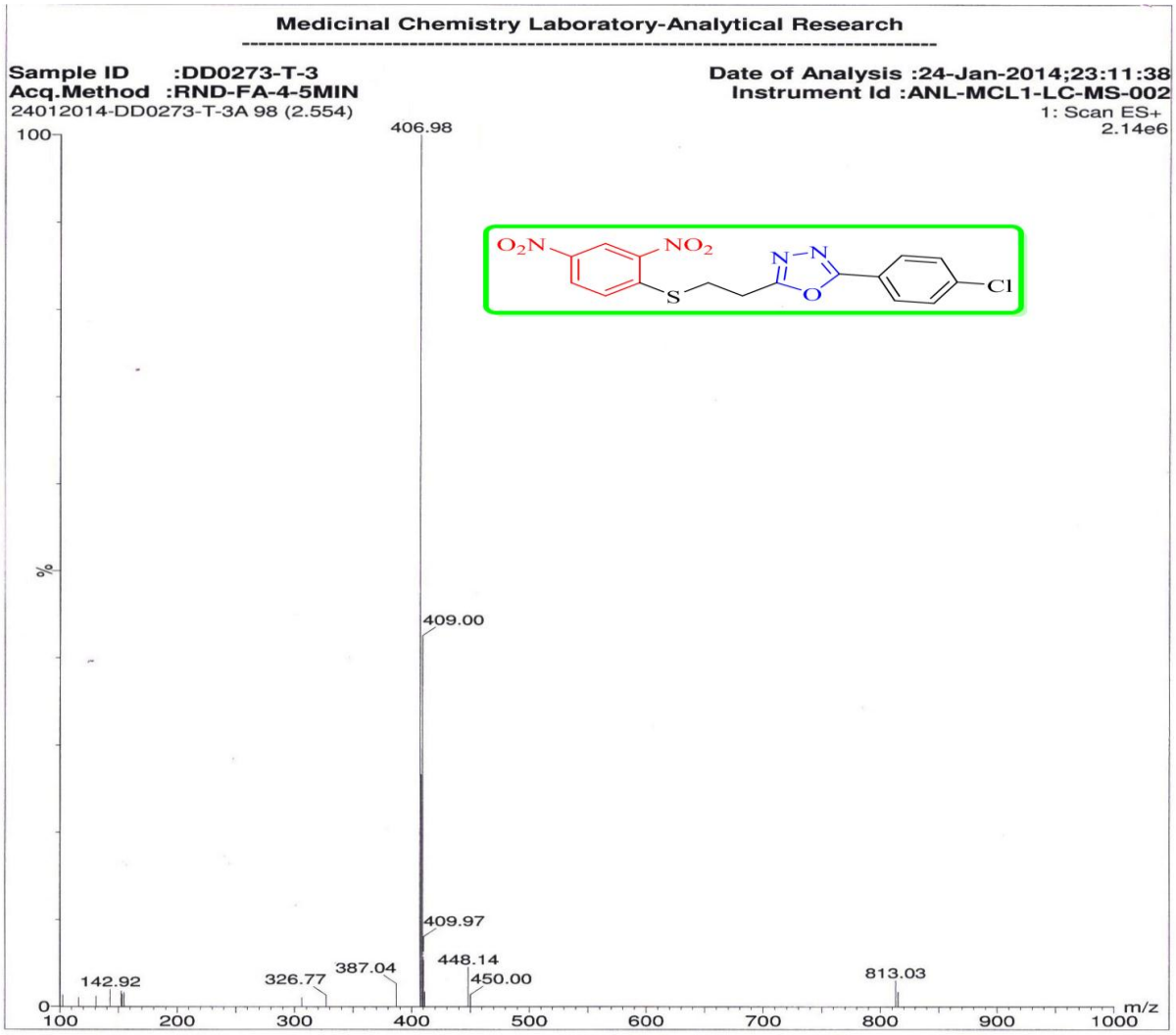


Figure 4: LC-MS spectrum of compound 4e.

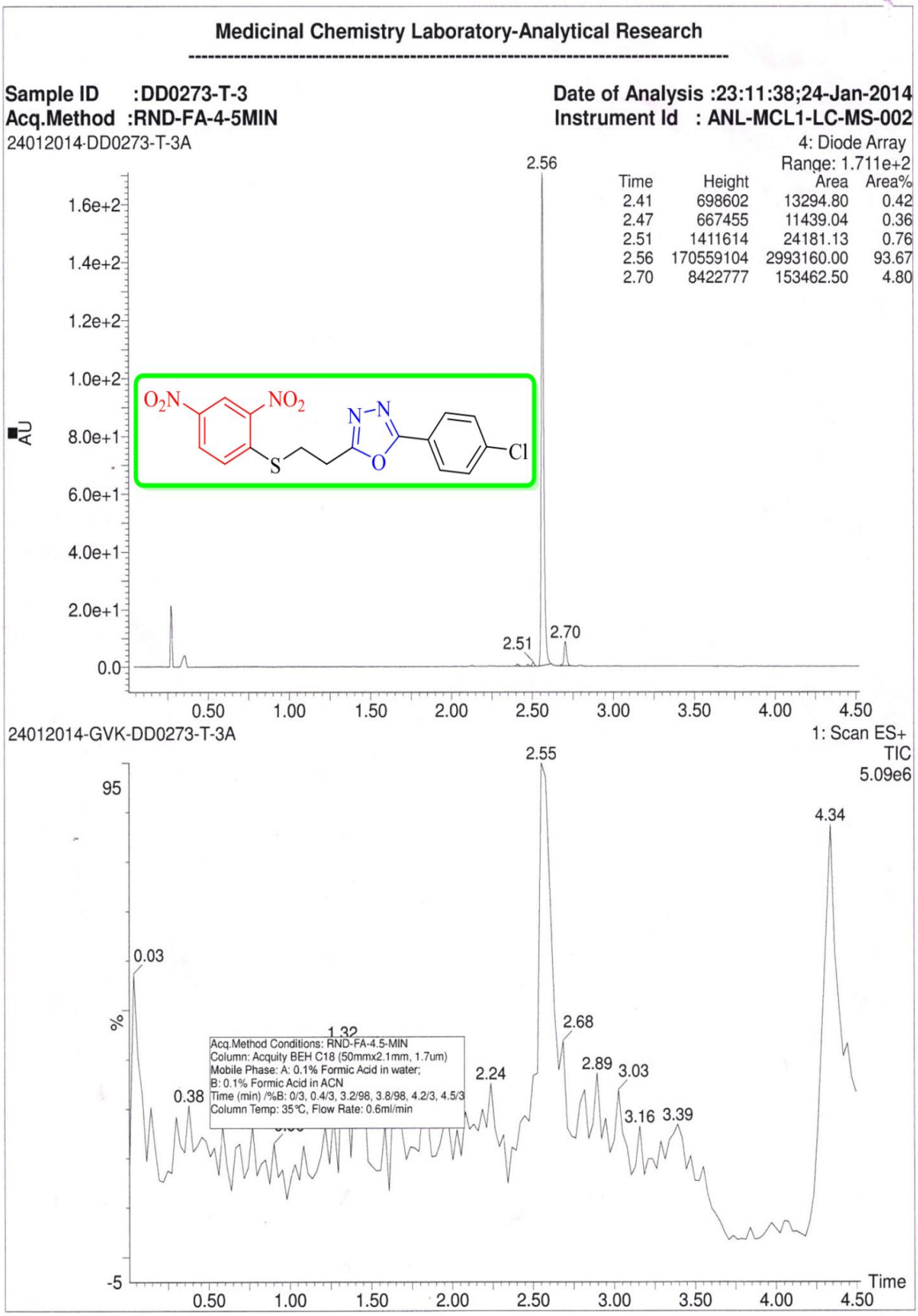


Figure 5: LC-MS spectrum of compound 4e.

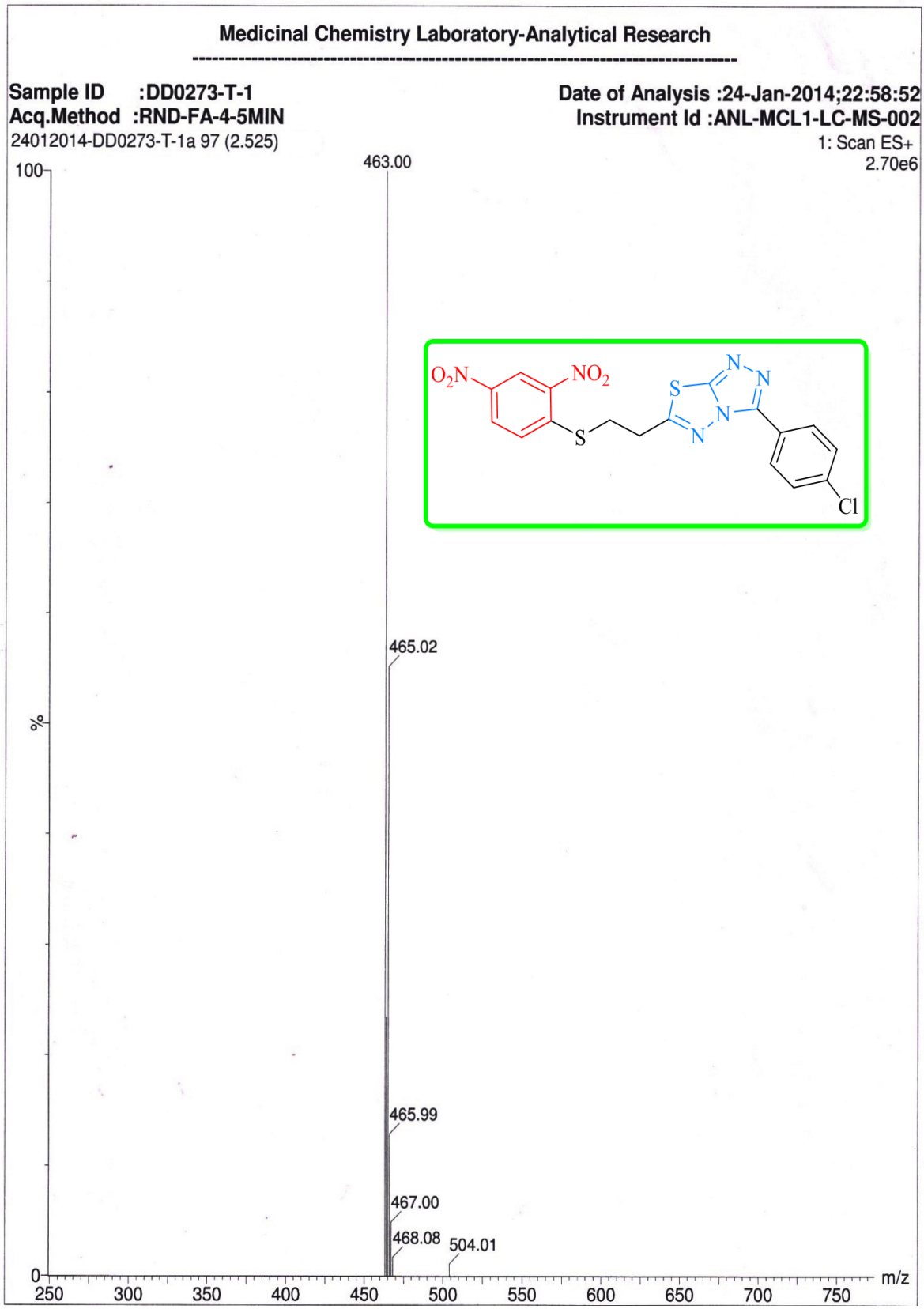


Figure 6: LC-MS spectrum of compound 5e.

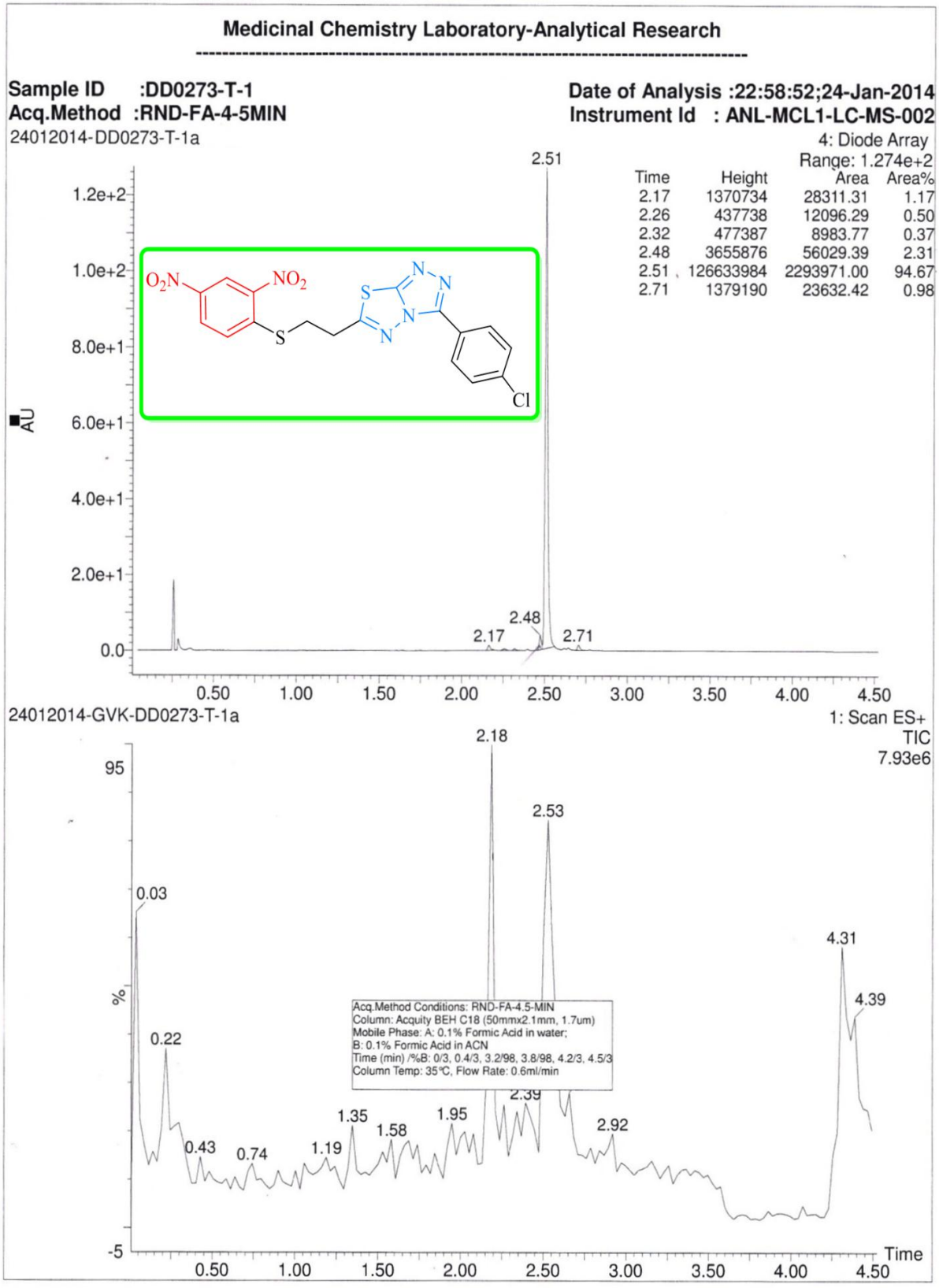


Figure 7: LC-MS spectrum of compound 5e.

Biological Assay

Anti microbial activity

Preparation of the Media

Weighed 14 gm of Nutrient Agar (Hi media) was dissolved in the 500 ml of distilled water. The medium was sterilized under 15 lbs pressure for 15 minutes in an auto clave. 30ml of this sterilized semi -solid nutrient agar medium was poured in pre-sterilized 90mm glass Petri plates under aseptic conditions in Laminar Flow.

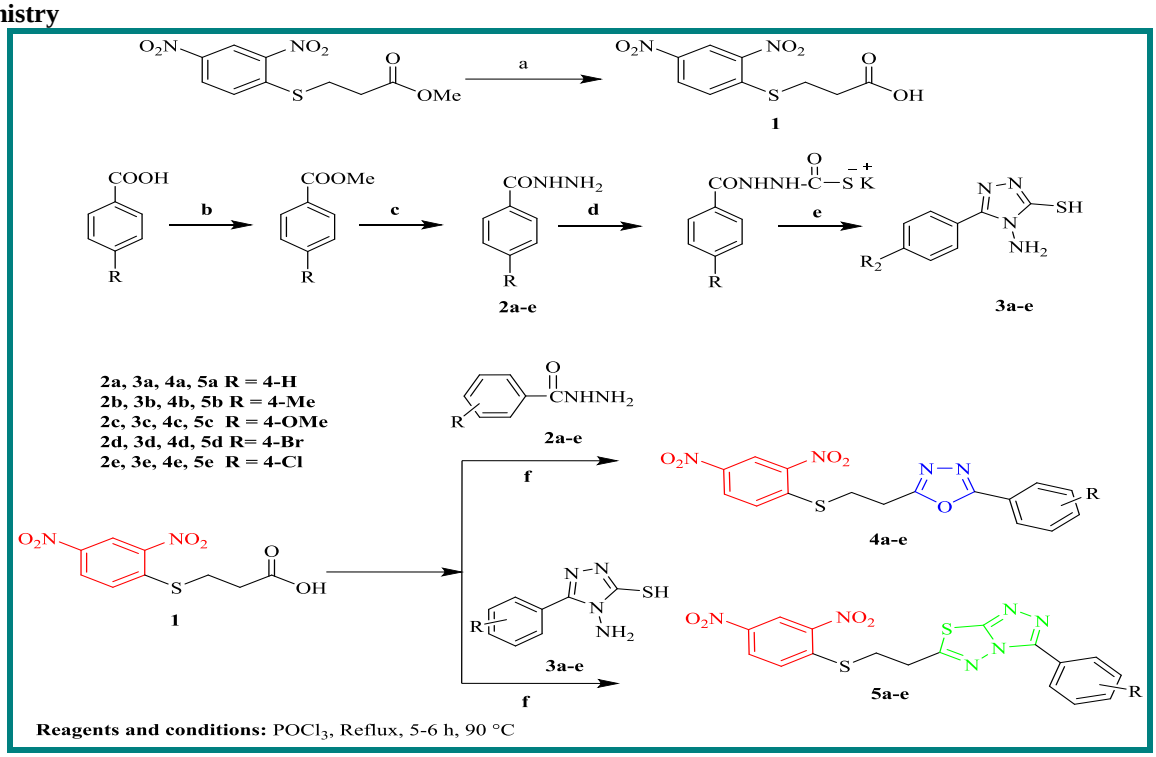
The plate were allowed to cool at room temperature to solidify the medium.

Paper disc diffusion technique

These assays are used to establish a dose response curve for a given strain of bacterium against a given test compound. The following protocol is employed. 20 ml of agar medium was poured in to the 90 mm glass Petri plate and seeded with the bacterium, in which case the inoculums density should be known 100 µl. The inoculum was evenly distributed throughout the agar medium using pipettes or a sterile glass rod. Then test discs are loaded with a fixed volume of a known concentration of the test compound and are then dried. The disks are placed on the agar plates; each plate should include a positive control in addition to the test compounds treated discs. Finally the petri plates were incubated at 37ºC in incubator for 24hour and the zone of inhibition was measured (Diameter in mm) to the nearest 0.1 mm, using a pair of vernier calipers. This should be performed across two perpendicular diameters for each disc.

RESULTS AND DISCUSSION

Chemistry



Scheme 1: Synthesis of 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives.

The synthetic route of 2,4-dinitro phenyl ring linked 1,3,4-oxadiazole (**4a-e**) and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives (**5a-e**) was depicted in the **Scheme 1**. Methyl 3-((2,4-dinitrophenyl)thio) propanoate was hydrolyzed with NaOH/MeOH at rt. The substituted aromatic acid hydrazides (**2a-e**), amino mercapto 1,2,4-triazoles (**3a-e**) and Methyl 3-((2,4-dinitrophenyl)thio) propanoate was prepared as per the previously reported well known procedures.[34-36] Reaction of acid **1** with **2a-e** or **3a-e** in $POCl_3$ under reflux at 90ºC afforded the final compounds **4a-e** or **5a-e**. All of the synthesized compounds were well characterized by spectral analysis (^{1}H-NMR, ^{13}C-NMR, FT-IR and LC-MS).

Formation of compound **4b** was confirmed by appearance of two extra A_2B_2 pattern doublets at δ 7.39, 7.93 ppm and −Me group singlet resonates at δ 2.40 ppm in the ^{1}H-NMR spectrum and C_2, C_5 carbons at δ 164.15, 164.36 ppm and −Me group signal at δ 21.02 ppm in the ^{13}C-NMR spectrum. In addition 2 × C−NO_2, C−S, C−CH_3 carbons of **4b** appeared at δ 142.01, 143.73, 144.16, 144.90 ppm. Ethylene chain of **4b** was confirmed by appearance of two triplets (δ 3.41, 3.74 ppm) and two singlets (δ 23.85, 28.06 ppm) in the ^{1}H-NMR & ^{13}C-NMR spectra respectively. C_3−H doublet of (2,4-dinitrophenyl)thio moiety appeared at far downfield (δ 8.82 ppm) in the ^{1}H-NMR spectrum due to deshielding effect of two ortho −NO_2 groups and it is involved J^4coupling with C_5−H of same aromatic ring. C_5−H proton was involved coupling with C_3−H & C_6−H protons, that is why it showed double doublet at δ 8.42 ppm (J = 7.8 Hz, 2.8 Hz). C−OCH_3, −OCH_3 carbons of **4c** resonates at δ 161.92, 55.44 ppm and chemical shift value of one of the A_2B_2 pattern doublets (δ 7.16 ppm) shifted to slight upfield due to +M effect of –OCH_3 group. Moreover molecular ion peaks appeared at m/z $(M+H)^+$ = 373, 387, 403 showed good agreement with molecular weights of **4a-c** respectively. –Br, −Cl atoms in **4d**, **4e** (**Fig. 4**) were confirmed by appearance of $(M+H)^+$ $(M+2+H)^+$ ion peaks in 1:1, 3:1 ratio in the Mass spectrum respectively. Triazolothiadiazole

formation in **5e** was corroborated by appearance of extra three triazolothiadiazole carbons signals at δ 149.16, 153.21, 168.86 ppm and A_2B_2 doublets at δ 7.67, 8.20 ppm (**Fig. 3**) in the ^{13}C-NMR & ^{1}H-NMR spectrum respectively. Seven aromatic benzene ring carbon signals and two aliphatic ethylene chain signals can be visualized at δ 121.16, 124.18, 127.34, 128.55, 129.24, 129.26 (Aromatic carbons), 134.89 (C−Cl carbon) and 29.64, 30.36 ppm respectively. Compound **5e** (**Fig. 6**) mass spectrum showed $(M+H)^+$ m/z = 463.00, $(M+2+H)^+$ m/z = 465.02 molecular ion peaks in 3:1 ratio, this is a simple indication of –Cl atom. Compounds **5b**, **5c** –Me & $–OCH_3$ group signals resonates at δ 2.43, 3.81 (^{1}H-NMR), 21.09, 55.28 ppm (^{13}C-NMR) respectively.

Antimicrobial activity

Synthesized compounds were evaluated *in vitro* for their antimicrobial activity against various pathogenic bacteria and fungal strains by paper disc method. The anti bacterial activities are carried out against two Gram-positive bacteria strains, *Bacillus subtilis* and Staphylococcus *aureus* and two Gram-negative bacteria strains, *Escheriachia coli* and *Salmonella*. Antifunal activity was carried out with three fungal strains, *Aspergillus niger* and *Aspergillus flavus and Rhizopus.* Streptomycin and Fluconazole were used as antibacterial and antifungal reference drugs respectively. Synthesized compounds were found to be moderately active, slightly active or inactive compared to the standard drugs (See **Table 1**). **4a** (ZOI[*S.a*] = 1.1 mm); **5a** (ZOI[*B.s*] = 1.0 mm**,** ZOI**[***E.c***] =** 1.0 mm); **5d** (ZOI[*S.a*] = 1.0 mm) were showed moderate activity. But compounds **4b**, **4d**, **5b** showed low antibacterial activity and **4c**, **4e**, **5c**, **5e** are in active. Based on the above bacterial activity results the following observations can be made. Unsubstituted phenyl ring or 4-Br substitution suitable for bacterial activity. But 4-OMe, 4-Cl groups on the phenyl ring not tolerated for good bacterial activity. That is why **4c**, **4e**, **5c**, **5e** showed inactivity against the most of the bacterial strains.

Concerning to antifungal activity, compounds **4e**, **5d** showed moderate activity against three fungal strains. However compound **5b** showed moderate activity against *Aspergillus flavus.* Remaining compounds were exhibited low antifungal activity at the ZOI range of 0.9−0.6 mm. 4-Cl, 4-Br groups are favorable for antifungal activity. Some of the Petri plate images were shown in **Fig 8** & **Fig 9** in which we could see the inhibition zone formed around the each active compound loaded disc.

Table 1: Antimicrobial activity of compounds [Zone of inhibition: (Paper Disc Method)].

Compounds	Zone of Inhibition (mm)						
	Bacteria				Fungi		
	Gram- positive		Gram-negative				
	B. s	***S. a***	***E. c***	***S***	***A. n***	***A. f***	***R***
4a	0.5	**1.1**	0.5	0	0.8	–	0.8
4b	0.7	0.8	0.7	0.8	0.7	0.6	0.7
4c	–	–	0.5	–	0.8	0.7	–
4d	0.5	0.6	0.5	0.5	0.6	–	0.9
4e	–	–	0.6	–	**1.2**	**1.1**	**1.2**
5a	**1.0**	0.5	**1.0**	0.5	0.7	0.6	0.7
5b	0.8	0.8	0	0.6	–	**1.7**	0.8
5c	0.7	–	–	–	0.6	0.6	0.6
5d	0.7	**1.0**	–	0.6	**1.1**	**1.5**	**0.8**
5e	–	–	–	0.5	–	–	0.6
Streptomycin[a]	2.4	3.1	3.5	3.9			
Fluconazole[b]					f_1	f_2	f_3

B.s: *Bacillus subtilis*; ***S. a*****:** *Staphylococcus aureus*; ***E. c***: *Escheriachia coli*; ***S:*** *Salmonella*; ***A. n***: *Aspergillus niger;* ***A. f***: *Aspergillus flavus* ***R***: *Rhizopus*;

"– No Activity;

a Reference Drug(Antibacterial activity);

b Reference Drug (Antifungal activity).

f_1, f_2, f_3: Standard value 2.9 mm for compounds **4a-e**; 3.2 mm for **5a-e.**

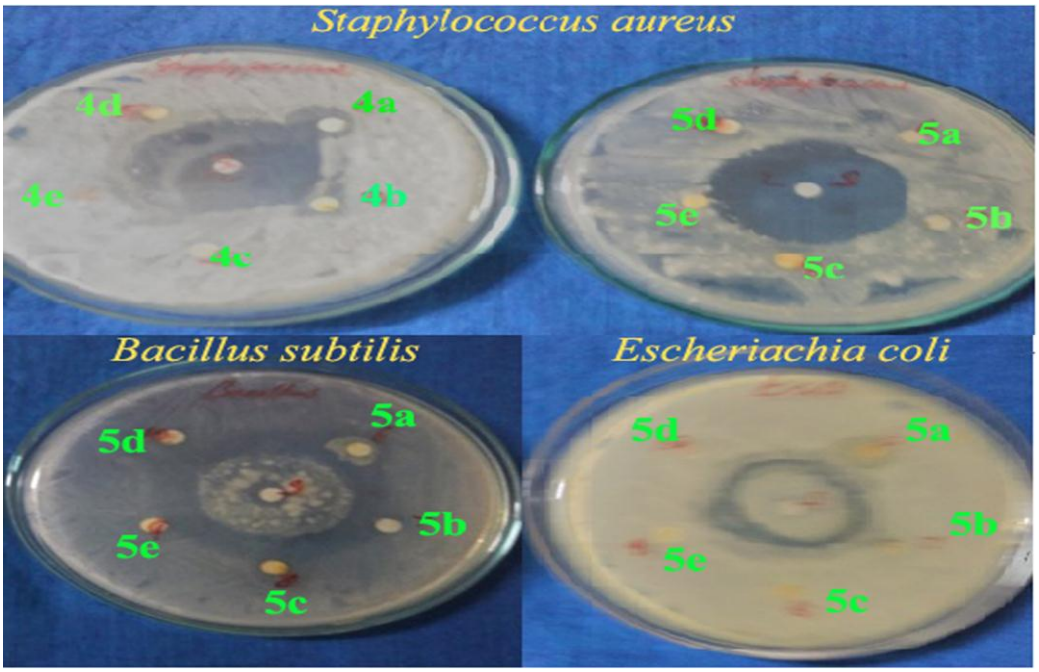


Figure 8: Petri plate images of three bacterial strains: Compounds 4a (ZOI[*S.a*] = 1.1 mm); 5a (ZOI[*B.s*] = 1.0 mm, ZOI[*E.c*] = 1.0 mm); 5d (ZOI[*S.a*] = 1.0 mm) showed moderate bacterial inhibition.

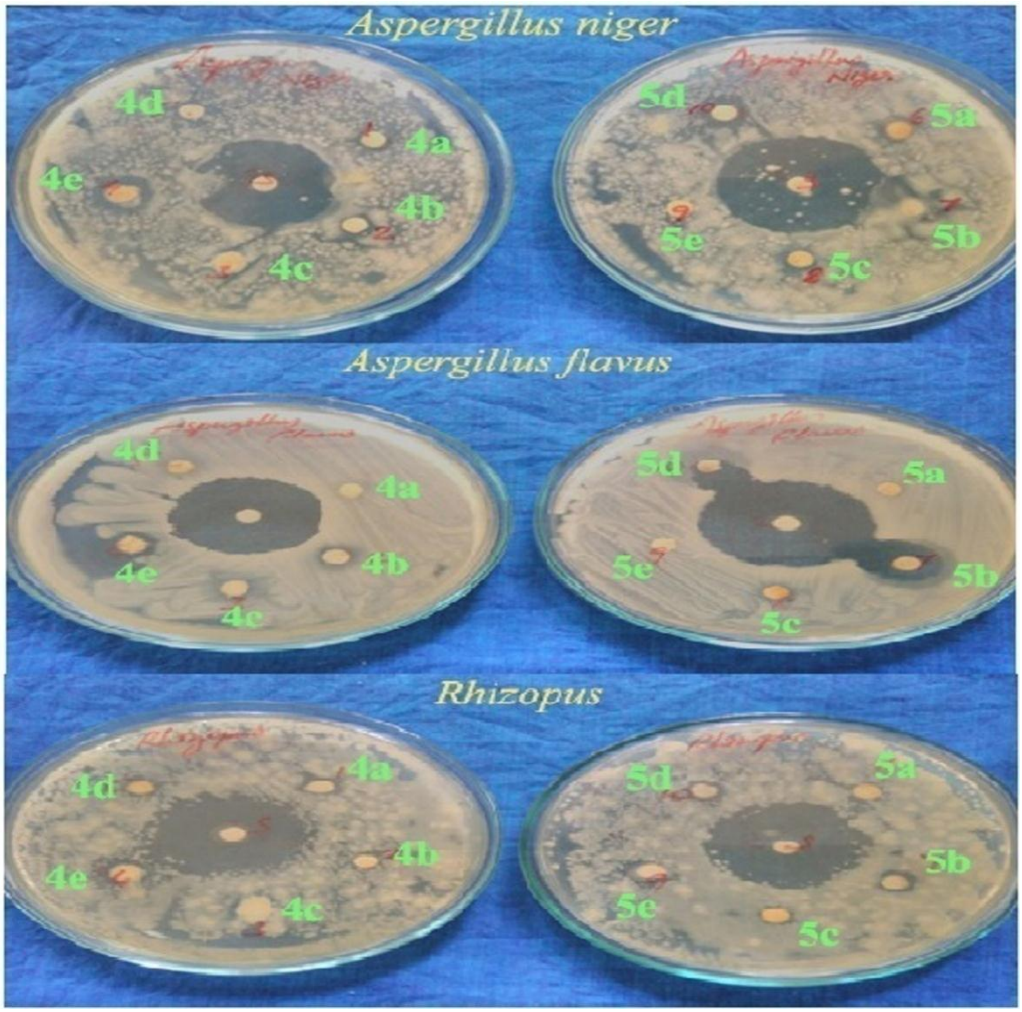


Figure 9: Petri plate images of three fungal strains: 4e (ZOI[*A.n*] = 1.2 mm); (ZOI[*A.f*] = 1.1 mm); (ZOI[*R*] = 1.2 mm); 5d (ZOI[*A.n*] = 1.1 mm); (ZOI[*A.f*] = 1.5 mm); (ZOI[*R*] = 0.8 mm); 5b (ZOI[*A.f*] = 1.7 mm) showed moderate inhibition against fungal strains.

CONCLUSION

In this study, a series of 2,4-dinitro phenyl ring containing 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives **4a-e** & **5a-e** were synthesized and were well characterized by spectroscopically. The antimicrobial activity was performed on the target compounds by using disk diffusion technique. The results showed that some of the targets are moderate antimicrobials. Hence, this study may be helpful to develop potent antibiotics from the source of dinitro phenyl ring linked 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives.

ACKNOWLEDGEMENTS

One of the authors Mr. S. Triloknadh thankful to University Grants Commission, New Delhi for providing the financial assistance for JRF & SRF through the award Lt. No. F.17-142/98(SA-I).

REFERENCES

1. Strauss MJ. The nitro aromatic group in drug design, pharmacology and toxicology (for nonpharmacologists). Ind Eng Chem Prod Res Dev, 1979; 18(3): 158-165.
2. Christophe T, Jackson M, Jeon HK, Fenistein D, Dominguez MC, Kim J, Genovesio A, Carralot JP, Ewann F, Kim EH, Lee SY, Kang S, Seo MJ, Park EJ, Skovierova H, Pham H, Riccardi G, Nam JY, Marsollier L, Kempf M, Joly-Guillou ML, Oh T, Shin WK, No Z, Nehrbass U, Brosch R, Cole ST, Brodin P. High content screening identifies decaprenyl-phosphoribose 2' epimerase as a target for intracellular antimycobacterial inhibitors. Plos Pathog, 2009; 5(10): e1000645.
3. Schaper KJ, Pickert M, Frahm A.W. Substituted xanthones as antimycobacterial agents Part 3: QSAR investigations. Arch Pharm, 1999; 332: 91-102.
4. Klimesova V, Koci J, Pour M, Stachel J, Waisser K, Kaustova J. Synthesis and preliminary evaluation of benzimidazole derivatives as antimicrobial agents. Eur J Med Chem, 2002; 37: 409-418.
5. Koci J, Klimesova V, Waisser K, Kaustova J, Dahse HM, Mollmann U. Heterocyclic benzazole derivatives with antimycobacterial in vitro activity. Bioorg Med Chem Lett, 2002; 12: 3275-3278.
6. Bhaskar VH, More VS, and Kumar M. Biological activities of 4-(substituted phenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole. Asian J Chem, 2008; 20(7): 5474-5482.
7. Kanagarajan V, Thanusu J, Gopalakrishnan M. Synthesis, spectral characterization, In-vitro antibacterial and antifungal activities of novel (2*e*)-Ethyl-2-(2-(2,4-dinitrophenyl) hydrazono)-4-(naphthalen-2-yl)-6-Arylcyclohex-3-enecarboxylates. Iran J Pharm Res, 2011; 10(4): 711-725.
8. Hamid A, Sadiq Z, Yaqub G, Khan N, Iqbal S, Iqbal K, Ijaz Z, Bajwa A. Synthesis and bioassay of novel phenylhydrazine and 2,4-dinitrophenylhydrazide substituted carbazoles. Asian J Chem, 2013; 25(10): 5412-5414.
9. Xu WM, Han FF, He M, Hu DY, He J, Yang S, Song BA. Inhibition of tobacco bacterial wilt with sulfone derivatives containing an 1,3,4-oxadiazole moiety. J Agric Food Chem, 2012; 60: 1036-1041.
10. Liu F, Luo XQ, Song BA, Bhadury PS, Yang S, Jin LH, Xue W, Hu DY. Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety. Bioorg Med Chem, 2008; 16: 3632-3640.
11. Li Z, Zhan P, Liu X. 1,3,4-oxadiazole: a privileged structure in antiviral agents. Mini Rev Med Chem, 2011; 11(13): 1130-1142.
12. Kumar BNP, Mohana KN, Mallesha L, Veeresh B. Synthesis and in vitro antiproliferative activity of 2,5-disubstituted-1,3,4-oxadiazoles containing trifluoromethyl bezenesulfonamide moiety. Med Chem Res, 2014; 23: 3363-3373.
13. Tabatabai SA, Lashkari SB, Zarrindast MR, Gholibeikian M, Shafiee A. Design, synthesis and anticonvulsant activity of 2-(2-phenoxy) phenyl-1,3,4-oxadiazole derivatives. Iran J Pharm Res, 2013; 12: 105-111.
14. Akhter M, Husain A, Azad B, Ajmal M. Aroylpropionic acid based 2,5-disubstituted-1,3,4-oxadiazoles: synthesis and their anti-inflammatory and analgesic activities. Eur J Med Chem, 2009; 44: 2372-2378.
15. Idrees GA, Aly OM, Abuo-Rahma GAA, Radwan MF. Design, synthesis and hypolipidemic activity of novel 2-(naphthalene-2-yloxy)propionic acid derivatives as desmethyl fibrate analogs. Eur J Med Chem, 2009; 44: 3973-3980.
16. Ahsan MJ, Samy JG, Khalilullah H, Nomani MS, Saraswat P, Gaur R, Singh A. Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. Bioorg Med Chem Lett, 2011; 21: 7246-7250.
17. Mochona B, Qi X, Euynni S, Sikazwi D, Mateeva N, Soliman KF. Design and evaluation of novel oxadiazole derivatives as potential prostate cancer agents. Bioorg Med Chem Lett, 2016; 26: 2847-2851.
18. Bhandari SV, Bothara KG, Raut MK, Patil AA, Sarkate AP, Mokale VJ. Design, synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1,3,4-oxadiazole-2-thiol and Schiff bases of diclofenac acid as nonulcerogenic derivatives. Bioorg Med Chem lett, 2008; 16: 1822-1831.
19. Jha KK, Samad A, Kumar Y, Shaharyar M, Khosa RL, Jain J, Kumar V, Singh P. Design, synthesis and biological evaluation of 1,3,4-oxadiazole derivatives. Eur J Med Chem., 2010; 45: 4963-4967.
20. Chandrakantha B, Shetty P, Nambiyar V, Isloor N, Isloor AM. Synthesis, characterization and biological activity of some new 1,3,4-oxadiazole

bearing 2-flouro-4-methoxy phenyl moiety. Eur J Med Chem, 2010; 45: 1206-1210.
21. Gaonkar SL, Rai KML, Prabhuswamy B. Synthesis and antimicrobial studies of a new series of 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles. Eur J Med Chem, 2006; 41: 841-846.
22. Ningaiah S, Bhadraiah UK, Doddaramappa SD, Keshavamurthy S, Javarasetty C. Novel pyrazole integrated 1,3,4-oxadiazoles: Synthesis, characterization and antimicrobial evaluation. Bioorg Med Chem Lett, 2014; 24: 245.
23. Badr SMI, Barwa RM. Synthesis of some new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles starting from 5-nitro-2-furoic acid and evaluation of their antimicrobial activity. Bioorg Med Chem., 2011; 19: 4506-4512.
24. Demirbas N, Demirbas A, Karaoglu SA, Celik E. Synthesis and antimicrobial activities of some new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-b] [1,3,4]thiadiazines. Arkivoc, 2005; i: 75-91.
25. Jubie S, Ramesh PN, Dhanabal P, Kalirajan R, Muruganantham N, Antony AS. Synthesis, antidepressant and antimicrobial activities of some novel stearic acid analogues. Eur J Med Chem, 2012; 54: 931-935.
26. Husain A, Rashid M, Shaharyar M, Siddiqui AA, Mishra R. Benzimidazole clubbed with triazolo-thiadiazole and triazolo-thiadiazines: New anticancer agents. Eur J Med Chem, 2013; 62: 785-798.
27. Malladi S, Isloor AM, Shetty P, Fun HK, Telkar S, Mahmood R, Isloor N. Synthesis and anti-inflammatory evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety. Med Chem Res, 2012; 21: 3272-3280.
28. Hanif AM, Saleem M, Hussain MT, Rama NH, Zaib S, Aslam MAM, Jones PG, Iqba J. Synthesis, urease inhibition, antioxidant and antibacterial studies of some 4-amino-5-aryl-3H-1,2,4-triazole-3-thiones and their 3,6-disubstituted 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives. J Braz Chem Soc, 2012; 23: 854-860.
29. Chawla G, Kumar U, Bawa S, Kumar J. Synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenic activities of 1,3,4-oxadiazole and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives. J Enzyme Inhib Med Chem, 2012; 27: 658-665.
30. Karthikeyan MS, Holla BS, Kalluraya B, Kumari NS. Biological studies of some 2,4-dichloro-5-fluorophenyl containing triazolothiadiazoles. Monatsh chem, 2007; 138: 1309-1316.
31. Swamy SN, Basappa, Priya BS, Prabhuswamy B, Doreswamy BH, Prasad JS, Rangappa KS. Synthesis of pharmaceutically important condensed heterocyclic 4,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives as antimicrobials. Eur J Med Chem, 2006; 41: 531-538.
32. Maqsood MR, Hanif M, Rafiq M, Saleem M, Zaib S, Ahmedkhan A, Iqbal M, Iqbal J, Rama NH, Seo SY, Hwan Lee K. Some pyridyl and thiophenyl-substituted 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives as potent antibacterial. Bull Korean Chem Soc, 2012; 33(12): 4180-4184.
33. Aggarwal N, Kumar R, Dureja P, Khurana JM. Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives. Eur J Med Chem, 2011; 46: 4089-4099.
34. Mathew V, Giles D, Keshavayya J, Vaidya VP. Studies on synthesis and pharmacological activities of 1,2,4-triazolo[3,4-b]1,3,4-thiadiazoles and their dihydro analogues. Arch Pharm Chem Life Sci, 2009; 342(4): 210-222.
35. Chidananda N, Poojary B, Sumangala V, Kumari NS, Shetty P, Arulmoli T. Facile synthesis, characterization and pharmacological activities of 3,6-disubstituted 1,2,4-triazolo[3,4-b][1,3,4] thiadiazoles and 5,6-dihydro-3,6-disubstituted-1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles. Eur J Med Chem, 2012; 51: 124-136.
36. Dudova K, Castek F, Machacek V, Simunek P. Preparation of substituted methyl *O*-nitrophenyl sulfides. Molecules, 2002; 7: 7-17.