Original Article

Synthesis and Evaluation of Insecticidal Activity of Bis-Semicarbazones and Bis-(5-Aryl Substituted-1, 3, 4-Oxadiazole)

Veerabhadraswamy Maruthyunjaya[1,*], Bhimashankar B Molkere[1], Prashantha Karunakar[2]

[1]Department of Science and Humanities, PES University, Bengaluru, Karnataka, INDIA.
[2]Department of Biotechnology, PES University, Bengaluru, Karnataka, INDIA.

ABSTRACT

Background: Various substituted semicarbazones and oxadiazols have been widely investigated for their insecticidal and pharmacological activities. Heterocyclic analogs of these types were extensively studied for their biological activities. In this study insecticidal activity of semicarbazones and oxadiaziole created our interest to prepare and screen the activity. **Objectives:** To prepare a series of substituted semicarbazones and oxadiazoles, with an observation to subjecting them for insecticidal and pharmacological screening. **Methodology:** An oxalic acid dicarbohydrazide compound was prepared by a mixture of diethyl oxalate (0.1 mole), hydrazine hydrate (99%) (0.2 mole) and 2-3 drops of Conc. HCl in absolute ethanol. Resulted compound was reflexed with different substituted aldehyde (0.05 moles) with addition of 1-2 drops of Conc. HCl in an absolute ethanol to acquired bis-semicarbazones. Derived bis-semicarbazones were subjected for oxidation using ferrichloride (20mg) and acetic acid (8mL) to obtain oxadiazole derivatives. **Results:** The compounds 2c-d and 3a showed high percentage of mortality in an insecticidal activity. Compounds 3a and 3b exhibited high anti-bacterial activity against *Kallipsi calla,* in case of *Escherichia coli* the compound 2d, 3a, 3c and 3d shows maximum activity. Compounds 3a and 3d showed high anti-fungal activity against *A. nigar* and 3c against *A. flavous.* The compounds 2a, 2b, 3c have shown good anti-inflammatory activity. Compound 3d showed a competitive inhibitory activity and act as lead molecule towards the drug designing. **Conclusion:** The present study exposed that the synthesized compounds exhibit significant insecticidal, anti-bacterial, anti-fungal, anti-inflammatory activity and act as lead molecule towards the drug designing.

Key words: Semicarbazone, Oxadiazole, Insecticidal activity, Biological activity, AutoDock.

INTRODUCTION

The semicarbazone and thio-semicarbazone derivatives have acknowledged significant attention from pharmaceutical industry due to their wide spectrum of their potential activities such as biological activities, anticonvulsant, anti-oxidant, anti-microbial activities, anti-viral, anti-HIV, anti-bacterial activities and anticancer.[1,2] In the recent years, some workers reviewed the bioactivity of semicarbazones exhibited anticonvulsant, anti-tubercular and aryl semicarbazones has been recognized as structurally novel class of compounds with remarkable anti-epileptic activity, anti-convulsant activity, with one of the important parameters for anti-convulsant activity.[3] The Present technology motivate and its derivatives embrace a vital session of biologically and pharmacologically active heterocyclic compounds such as anti-bacterial,[4] anti-fungal,[5,6] anti-inflammatory[7] and anti-convulsant activities.

The oxadiazole derivatives have its place in an important group of heterocyclic compounds for developing pharmaceutically significant molecules 1, 3, 4-oxadiazole derivatives have played a vital role in the medicinal chemistry. Abundant compounds with oxadiazole nucleus

Submission Date: 18-02-2019;
Revision Date: 13-06-2019;
Accepted Date: 28-06-2019

DOI: 10.5530/ijper.53.3s.115

Correspondence:
Dr. Veerabhadraswamy Maruthyunjaya,
Department of Science and Humanities, PES University, BSK III Stage, Bengaluru-560085, Karnataka, INDIA.
Phone: +91 9241503423
E-mail: vbs@pes.edu

www.ijper.org

used for anti-bacterial, anti-HIV, anti-fungal, anti-tubercular, veridical, anti-malarial, insecticidal, herbicidal, ant-imicrobial, anti-viral, anti-parkinsonian, anti-TB, anti-proliferative, anti-inflammatory and analgesic, muscle relaxant, anti-convulsant, sedative, hypnotic, anti-cancerand inhibition of lipid per-oxidation, hypertensive activities. Collected works on synthesis of oxa diazoles contain bromine oxidation of a semicarbazide derivative and the cyclo-desulfurization of acylthiosemicarbazide derivatives in solution using I_2/ NaOH or 1, 3- dicyclohexylcarbodimide (DCC) as well as mercury (II) acetate (Hg $(OAc)_2$) or yellow mercury (II) oxide HgO.[8-10] All these methods are usually carried out in different synthetic steps that require very dangerous reagents and produce undesirable mercury byproduct, at this time the insects and pests are getting more unaffected owed to use of insecticides.

In view of these observations, an attempt has been made to develop more dominant compounds with smaller amount of side effect and bio-products, we hereby report the synthesis, characterization and screened for biological activities of new substituted derivatives of a novel series of bis-semicarbazones and bis-(5-aryl substituted- 1, 3, 4-Oxadiazole) derivatives, as improved and potent insecticidal, anti-bacterial, anti-inflammatory, anti-fungal, Molecular docking studies.

MATERIALS AND METHODS

The homogeneity of all the newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G plates (silica gel 60F254) and spots were located by using iodine chamber. The IR spectra (in KBr disks) were recorded on Perkin Elmer and the characteristic bands obtained at the wave numbers are specific to the functional group of the molecular structure.[11] ^{1}H-NMR spectra were recorded by an instrument using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference standard. All chemical shift values were recorded at δ (ppm). Commercial grade solvents and reagents were used without further purification. The chemicals used in the synthesis of new compounds are diethyl oxalate, hydrazine hydrate, Ethanol, AR grade, Hydrochloric acid and so on.

The reaction scheme employed for the synthesis of compounds N'^{1}, N'^{2}–bis[phenylmethylidene]ethanedihydrazide (2a), N'^{1},N'^{2}-bis[(2-hydroxyphenyl)methylidene] ethanedihydrazide (2b), N'^{1}, N'^{2}- bis [(4-methoxyphenyl) methylidene] ethanedihydrazide (2c), N'^{1}, N'^{2}- bis [(4-hydroxy-3-methoxyphenyl) methylidene] ethanedihydrazide (2d), 5,5'-diphenyl-2, 2'-bi-1,3,4-oxadiazole (3a),2,2'-(2, 2'-bi-1,3,4-oxadiazole-5, 5'-diyl) diphenol (3b), 5,5'-bis(4-methoxyphenyl)-2,2'-bi-1,3,4-oxadiazole (3c), 4,4'-2,2'-bi-1,3,4-oxadiazole-5,5'-diyl)bis(2-methoxyphenol) (3d) are given in Figure 1-3 and the substituents and molecular formula of semicarbazones and oxadiazoles are summarized in Table 1. An intermediate compound oxalic acid dicarbohydrazidesis refluxed with substituted aldehyde with the addition of 1-2 drops of HCl in absolute ethanol media yielded bis-semicarbazones (2a-d) and the process of an oxidation of semicarbazones with ferrichloride gives bis-(5-aryl substituted - 1, 3, 4-Oxadiazole) (3a-d). The structures of the newly synthesized compounds are established by FT-IR and NMR spectra respectively.

Experimental Section

General procedure for the synthesis of oxalic acid dicarbohydrazide, bis-semicarbazonesandbis-(5-aryl substituted -1, 3, 4-Oxadiazole) alkanes

Oxalic acid dicarbohydrazide

An intermediate oxalic acid dicarbohydrazide compound (1) was prepared by refluxing the mixture of the diethyl oxalate (0.1 moles), hydrazine hydrate (99%) (0.2 mole) with 2-3 drop of concentrated hydrochloric acid (HCl) in a round bottom flask containing absolute ethanol (50 mL) for a period of 8-9 hr and cooled to room temperature. The resulted compound was filtered, dried and crystallized from Dimethylformamide DMF.

Bis-semicarbazones

The mixture of intermediate oxalic acid dicarbohydrazides (0.025 mole)(1), substituted aldehyde (0.05 mile) and 1-2 drops of conc. HCl in absolute ethanol (20-30mL) were refluxed for 7-8 hr, cooled to room temperature. Thus gained product was filtered, washed, dried and crystallized from D.M.F to yield bissemicarbazones compounds (2a-d).

Table 1: Substituent and Molecular formula of semicarbazones and Oxadiazoles.

Sl.No.	Compounds	Substituent	Molecular Formula
1	2a	$-C_6H_5$	$C_{16}H_{14}N_4O_2$
2	2b	$-2-OH-C_6H_4$	$C_{16}H_{14}N_4O_4$
3	2c	$-4-OCH_3-C_6H_4$	$C_{18}H_{18}N_4O_4$
4	2d	$-3-OCH_3-4-OH-C_6H_3$	$C_{18}H_{18}N_4O_6$
5	3a	$-C_6H_5$	$C_{16}H_{10}N_4O_2$
6	3b	$-2-OH-C_6H_4$	$C_{16}H_{10}N_4O_4$
7	3c	$-4-OCH_3-C_6H_4$	$C_{18}H_{14}N_4O_4$
8	3d	$-3-OCH_3-4-OH-C_6H_3$	$C_{18}H_{14}N_4O_6$

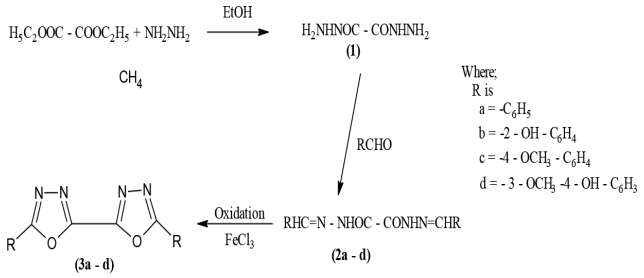


Figure 1: Synthetic route for the compounds 2a-d and 3a-d.

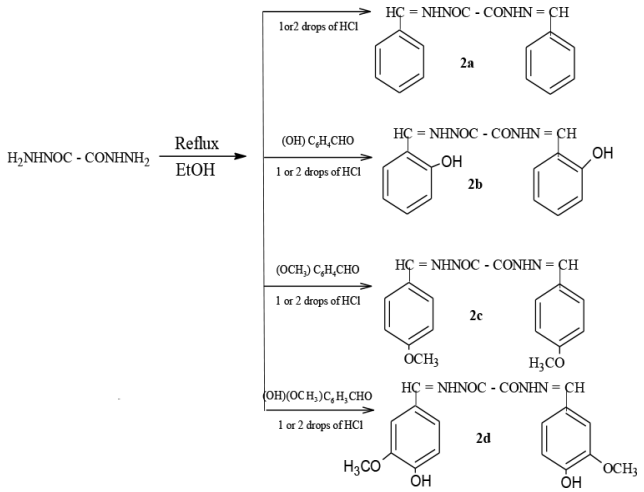


Figure 2: Synthetic route for the compounds 2a-d. Semicarbazones.

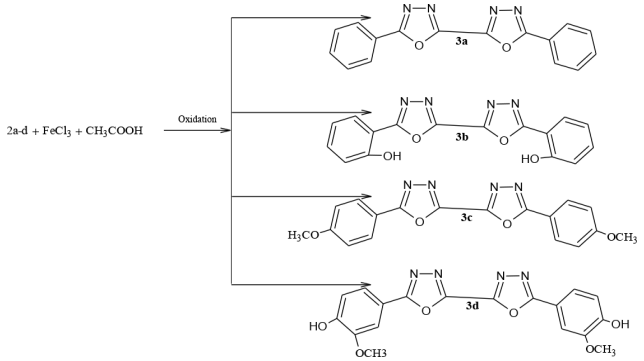


Figure 3: Synthetic route for the compounds 3a-d.Oxadiazoles.

Bis-(5-aryl substituted -1, 3, 4-Oxadiazole) alkanes

A mixture of bis-semicarbazones (2a-d) (0.002 mole), Ferrichloride (20 mg) and acetic acid (8 mL) were stirred for one hour, solution was poured over the water (15-20 mL) and left undisturbed for a period of 72 hr, the resulted solid product was filtered, washed with water, dried and crystallized from Dimethylforamide (D.M.F) to get compounds (3a-d).

Compound 2a

N'[1], N'[2]-bis [phenylmethylidene]ethanedihydrazide].

IR (ν cm^{-1}, KBr): 3247.9 (NH, m, stretch), 3055.0 (CH, s, stretch), 1959.5, 1658.7(C=O,s, stretch), 1234.4 (CO, s, stretch), 964.3, 879.5, 817.8, 756.0, 686.6 and 540.0(C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.3 - 7.6 (m, 10H, ArH), 8.0 (bs, 2H, NH_2), 8.1(s, 2H, CH), Molecular Formula; $C_{16}H_{14}N_4O_2$, Formula Weight; 294.30796, Anal. Cald for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04; O, 10.87. Density (g/cm^3); 1.19 ± 0.1 and Polarizability (cm^3); 33.73 ± 0.5 10^{-24}. Nominal Mass (Da); 294.

Compound 2b

N'[1],N'[2]-bis [(2-hydroxyphenyl) methylidene] ethanedihydrazide.

IR (ν cm^{-1}, KBr): 3155.3 (OH, m, stretch), 3062.7 (CH, s, stretch), 2368.4 (H-C=O, m, stretch), 1967.3, 1666.4 (C=O, s, stretch), 1257.5 (CO, s, stretch), 964.3, 871.8, 825.5, 756.0, 632.6 and 532.3 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.0 (s, 2H, OH), 6.8 –7.4 (m, 8H, ArH), 8.0 (bs, 2H, NH_2), 8.1 (s, 2H, CH), Molecular Formula; $C_{16}H_{14}N_4O_4$, Formula Weight; 326.30676, Anal. Cald for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17; O, 19.61. Density (g/cm^3); 1.35 ± 0.1, Polarizability (cm^3); 34.41 ± 0.5 10^{-24} and Nominal Mass (Da); 326.

Compound 2c

N'[1], N'[2]- bis [(4-methoxyphenyl) methylidene] ethanedihydrazide.

IR (ν cm^{-1}, KBr): 3247.9 (NH, m, stretch), 2908.5 (CH, s, stretch), 2839.0 (H-C=O, m, stretch), 1897.8, 1666.4 (C=O, s, stretch), 1303.8 (CO, s, stretch), 964.3, 817.8, 717.5, 586.3, 547.7 and 455.2 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.73 (s, 6H, CH_3), 6.8 – 7.5 (m, 8H, ArH), 8.0 (bs, 2H, NH_2), 8.1 (s, 2H, CH), Molecular Formula; $C_{18}H_{18}N_4O_4$, Formula Weight; 354.35992, Anal. Cald for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81; O, 18.06. Density (g/cm^3); 1.22 ± 0.1, Polarizability (cm^3); 38.34 ± 0.5 10^{-24} and Nominal Mass (Da); 354.

Compound 2d

N'[1], N'[2]- bis [(4-hydroxy-3-methoxyphenyl) methylidene] ethanedihydrazide.

IR (ν cm^{-1}, KBr): 3502.5 (OH, m, stretch), 3232.5 (NH, m, stretch), 2931.6 (CH, s, stretch), 2800.4, 2368.4 (H-C=O, m, stretch), 2044.4, 1658.7 (C=O, s, stretch), 1427.2, 1380.9, 1272.9 (CO, s, stretch), 964.3, 833.2, 740.6, 663.5, 624.9 and 532.3 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.73 (s, 6H, CH3), 5.0 (s, 2H, OH), 6.7 – 7.0 (m, 6H, ArH), 8.0 (bs, 2H, NH_2),

8.1 (s, 2H, CH), Molecular Formula; $C_{18}H_{18}N_4O_6$, Formula Weight; 386.35872, Anal. Cald for $C_{18}H_{18}N_4O_6$: C, 55.96; H, 4.70; N, 14.50; O, 24.85. Density (g/cm^3); 1.35 ± 0.1, Polarizability (cm^3); 39.02 ± 0.5 10^{-24} and Nominal Mass (Da); 386.

Compound 3a

5, 5'-diphenyl-2, 2'-bi-1, 3, 4-oxadiazole.

IR (ν cm^{-1}, KBr): 3247.9 (NH, m, stretch), 2360.7 (C-H, s, aromatics), 1658.7 (C=N), 1527.5 (C=C, aromatic), 1450.4, 1357.8 (C-N), 1234.4, 1195.8 (C-O-C), 1049.2, 964.3, 879.5, 817.8, 686.6 and 540.0 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.22 – 7.48 (m, 10H, ArH), Molecular Formula; $C_{16}H_{10}N_4O_2$, Formula Weight; 290.2762, Anal. Cald for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30; O, 11.02. Density (g/cm^3); 1.297 ± 0.06, Polarizability (cm^3); 30.52 ± 0.5 10^{-24} and Nominal Mass (Da); 290.

Compound 3b

2, 2'- (2, 2'-bi-1,3,4-oxadiazole-5, 5'-diyl) diphenol.

IR (ν cm^{-1}, KBr): 3201.6(NH, m, stretch), 3062.7, 2368.4 (C-H, s, aromatics), 1666.4 (C=N), 1535.2 (C=C, aromatic), 1458.1, 1357.8 (C-N), 1257.5, 1218.9 (C-O-C), 1056.9, 964.3, 871.8, 825.5, 756.0, 632.6 and 532.3(C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.0 (s, 2H, OH), 6.88 – 7.31 (m, 8H, ArH), Molecular Formula; $C_{16}H_{10}N_4O_4$, Formula Weight; 322.275, Anal. Cald for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30; O, 11.02. Density (g/cm^3); 1.297 ± 0.06, Polarizability (cm^3); 30.52 ± 0.5 10^{-24} and Nominal Mass (Da); 290.

Compound 3c

5, 5'-bis (4-methoxyphenyl)-2, 2'-bi-1, 3, 4-oxadiazole.

IR (ν cm^{-1}, KBr): 3247.9 (NH, m, stretch), 2839.0(C-H,s, aromatics), 1897.8, 1658.7 (C=N), 1604.7, 1504.4 (C=C, aromatic), 1365.5 (C-N), 1303.8, 1249.8 (C-O-C), 1026.1, 964.3, 817.8, 717.5, 586.3 and 547.7 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.73 (s, 6H, CH_3), 6.83 – 7.31 (m, 8H, ArH), Molecular Formula; $C_{18}H_{14}N_4O_4$, Formula Weight; 350.32816, Anal. Cald for $C_{18}H_{14}N_4O_4$: C, 61.71; H, 4.03; N, 15.99; O, 18.27. Density (g/cm^3); 1.289 ± 0.06, Polarizability (cm^3); 35.81 ± 0.5 10^{-24} and Nominal Mass (Da); 350.

Compound 3d

4, 4'-2, 2'-bi-1, 3, 4-oxadiazole-5, 5'-diyl) bis (2-methoxy-phenol).

IR (ν cm^{-1}, KBr): 3502.5 (OH, m, stretch), 3232.5 (NH, m, stretch), 2931.6, 2800.4, 2368.4 (C-H, s, aromatics), 2044.4, 1658.7 (C=N), 1596.9, 1512.1 (C=C, aromatic), 1427.2, 1380.9 (C-N), 1272.9 (C-O-C), 1211.2, 1103.2, 1026.1, 964.3, 833.2, 740.6, 663.5, 624.9 and 532.3 (C-H,s, aromatics), ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.73 (s, 6H, CH_3), 5.0 (s, 2H, ArOH), 6.68-6.87 (m, 6H, ArH), Molecular Formula; $C_{18}H_{14}N_4O_6$, Formula Weight; 382.32696, Anal. Cald for $C_{18}H_{14}N_4O_6$: C, 56.55; H, 3.69; N, 14.65; O, 25.11. Density (g/cm^3); 1.423 ± 0.06, Polarizability (cm^3); 37.30 ± 0.5 10^{-24} and Nominal Mass (Da); 382.

Insecticidal activity

The activity was studied into three general classes as a stomach poison, contact poison and untreated check.[12] About 25 mg of synthesized compounds were dissolved in a minimum amount of acetone and 4-5 drops of Tween-80 were added as an emulsifying agent and diluted to 50 mL into a volumetric flask separately. For stomach poison, the prepared solutions were sprayed on the red gram by using a micro sprayer (i.e. atomizer). On the other hand the larvae of *Heliothis armigera* were taken. These (24 in number) were placed one in each compartment of the tray. The sprayed pods were then fed and observation of the percentage of mortality recorded in 24, 48 and 72 hr. For contact poison, the larvae of *Heliothis armigera* were placed one in each compartment of the tray which was half filled with diet. The solutions above prepared were sprayed directly on the larval body by using atomizer and the percentage of mortality was recorded after 24, 48 and 72 hr as shown in Table 2. These stomach and contact poisons are observed and verified against untreated check by placing the larvae in each compartment of the tray. The red gram pods are fed without any treatment and were kept for observation. The mortality was observed after 24, 48 and 72 hr were as same as the test compound observed. Bio-efficacy testing of semicarbazones (2a-d) and ox adiazoles (3a-d).

Anti-bacterial activity

The Cup-Plate Method given by Cruickshank *et al*.[13] Nutrient agar was poured onto the sterilized petri dishes (20-25 mL each petri dish). The poured material was allowed to set for 2h and thereafter the "CUPS" (6 mm diameter) were made by punching into the agar surface with a sterile corn borer and scooping out the punched part of the agar. To these cups the test compound solution was added with the help of sterile syringe. The plates were incubated at 37°C for overnight and the results pertaining to zone of inhibition developed were noted. The zone of inhibition developed. A (1% v/v) solution of Gentamycin was used as standard and solvent control (DMSO) was also used to know the activity of the solvent. The above said standard drugs

were also screened under similar conditions for comparison.

Anti-fungal activity

The spore suspension of each test organism was added to sterilize the sabouraud dextrose agar medium at 40-50°C by thorough shaking. The petri dishes were seeded with the mixture and the paper discs of the methanol solution of compounds and the reference antibiotic as the control was placed in the same manner as in anti-bacterial activity determination.[14,15] These petri dishes were incubated for 24 hr at room temperature. The zone of inhibition developed of any, was then accurately measured and recorded.

Anti-inflammatory activity

Albino rats of either sex weighing between 120-150g were selected; the animals were fasted for 24 hr with water *ad libitum*. Animals were marked on their hind paws (right and left) just beyond the tibio-tarsal to ensure constant dipping in the mercury column up to the fixed mark. The test doses (25 mg/kg) were prepared in Tween-80 and suspended in water. After 30 min, (0.1 mL of 1% v/v) formalin solution was injected into the plantar region of the left paw of all experimental animals. The right non-inflamed paw served as reference for comparison. At the end of 5 hr, after formalin injection the paw volume of both legs of all the experimental animals was measured with the help of the plethysmograph. In the same way test group were used for evaluating the anti-inflammatory activity of the respective test compounds. The percentage inhibitions of the inflammation by the drug treated animals were recorded using the formula. Percentage inhibition= 100 (1-T/C), Where T and C are volumes of edema of drugs treated and control group respectively.

The synthesis compounds were tested for anti-inflammatory activity *in-vitro* against albino rats and compared with that of standard drug Dichlophenac sodium.

Protein and ligand structure preparation

The X-Ray Crystallographic structures of the 1.6 Å models of crystal structure of the complex formed between Russell's Viper Phospholipase A2 and an Anti-inflammatory agent Diclofenac (PDB: 2B17) were obtained from the protein databank (www.pdb.org). Topology file and other force field parameters were generated for all Semicarbazones andoxadiazolederivatives using the PRODRG program. Flexible torsions were defined using AUTOTORS. The docking site for semicarbazones and oxadiazolederivatives on 2B17 was defined at the position of the co-crystallized ligand by using PyRX 0.8 interface with grid box size of 52 x 49 x 58 spacing of 0.375, grid center 48.098, 32.710, 6.880 and assigned 3 Degrees of Freedom. The Lamarckian Genetic Algorithm (LGA)[16] was employed with the population size of 150 individuals, maximum number of generations and energy evaluations of 27,000 and 2.5 million respectively. From the estimated free energy of ligand binding (ΔG), the inhibition constant (K_i) for each ligand was calculated. Only the best pose (the one with the lowest binding energy) was considered for the ligand. The best conformation was analyzed for protein, semicarbazones and oxadiazole derivatives interaction using Ligplot+. PyMOL[17,18] was used for docking conformation representation.

RESULTS AND DISCUSSION

The compounds N'^{1}, N'^{2}-bis [phenylmethylidene]ethanedihydrazide (2a), N'^{1},N'^{2}-bis[(2-hydroxyphenyl) methylidene] ethanedihydrazide (2b), N'^{1}, N'^{2}- bis [(4-methoxyphenyl) methylidene] ethanedihydrazide (2c), N'^{1}, N'^{2}- bis [(4-hydroxy-3-methoxyphenyl) methylidene] ethanedihydrazide (2d), 5,5'-diphenyl-2,2'-bi-1,3,4-oxadiazole (3a),2,2'-(2, 2'-bi-1,3,4-oxadiazole-5, 5'-diyl) diphenol (3b), 5,5'-bis(4-methoxyphenyl)-2,2'-bi-1,3,4-oxadiazole (3c), 4,4'-(2,2'-bi-1,3,4-oxadiazole-5,5'-diyl) bis (2-methoxyphenol) (3d) were synthesized by using reflux method. The synthetic routes of these compounds are illustrated in the Figures. The chemical structures of these were found to be in accordance with their IR and ^{1}H-NMR Spectra; detail spectral data are summarized in the material and methods section. The IR Spectrum of 2a-d and 3a-d were measured by using the powdered potassium bromide as background correction. The IR Spectrum of compound 2a showed wave numbers 3247.9cm^{-1} corresponding to the NH medium stretch, 3055.0cm^{-1} corresponding to CH strong stretch, 1959.5 and 1658.7cm^{-1} corresponding to C=O strong stretch, 1234.4 cm^{-1} corresponding to CO strong stretch and for compound 3a wave numbers 3247.9cm^{-1} corresponding to NH medium stretch, 2360.7cm^{-1} corresponding to C-H strong aromatics, 1658.7cm^{-1} corresponding to C=N, 1527.5cm^{-1} corresponding to C=C aromatic, 1450.4cm^{-1}, 1357.8cm^{-1} corresponding to the C-N, 1234.4, 1195.8 corresponding to C-O-C. The ^{1}H-NMR Spectra were measured in dimethylsulfoxide-d_6 for compounds 2a and 3a. ^{1}H-NMR Spectrum of compound 2a showed a characteristic signal at δ 7.3-7.6 (m, 10H, Rah), 8.0 (bus, 2H, NH_2), 8.1(s, 2H, CH) and the compound 3a showed a characteristic signal at δ 7.22-7.48 (m, 10H, Rah).

Screening of insecticidal activity

Insecticides are those substances which kill insects by their chemical reaction, the effect of insecticidal activity on plant, animals or products in the open area to be treated by sprays or dusts. The larvae of *Heliothis armigera* were used to study the effect of toxicity by stomach poison and contact poisons. On screening of stomach poison of N'^{1}, N'^{2}- bis [(4-hydroxy-3-methoxyphenyl) methylidene] ethanedihydrazide (2d)and 5,5'-diphenyl-2, 2'-bi-1,3,4-oxadiazole (3a) showed 33.33 and 29.17% of mortality while other compounds N'^{1},N'^{2}-bis[(2-hydroxyphenyl) methylidene] ethanedihydrazide (2b), N'^{1}, N'^{2}- bis [(4-methoxyphenyl) methylidene] ethanedihydrazide (2c), 2,2'-(2, 2'-bi-1,3,4-oxadiazole-5, 5'-diyl) diphenol (3b), 4,4'-(2,2'-bi-1,3,4-oxadiazole-5,5'-diyl) bis (2-methoxyphenol) (3d) showed moderate mortality rate of 16.67, 16.67, 20.83 and 16.67%. The compounds N'^{1}, N'^{2}-bis [phenylmethylidene] ethanedihydrazide (2a) and 5, 5'-bis (4-methoxyphenyl)-2, 2'-bi-1, 3, 4-oxadiazole (3c) exhibits low mortality of 12.50% each. In case of contact poison toxicity of 2b, 2c and 2d compounds showed high percentage of mortality i.e., 41.67, 62.50 and 70.83% while other compounds 2a, 3a, 3b, 3c and 3d showed moderated percentage of mortality i.e., 29.19, 24.17, 20.83, 20.83 and 29.17%, respectively when compare with standard and untreated control. The results are summarized in Table 2.

Anti-bacterial activity

The results of anti-bacterial activity study indicated that among the tested compounds 3(a) and 3(b) showed high activity against *Kallipsi calla,* whereas in case of *Escherichia coli* 2(d), 3(a), 3(c), 3(d) showed maximum activity, while other compounds 2(a), 2(b) and 2(c), showed moderate activity. The compounds were ineffective against *Portious vulgaris* as compared with the standard drug.

Antifungal activity

Among the synthesized compounds the anti-fungal activity of the compound 3(a) and 3(d) showed high activity against *A. nigar* 2(a), 2(c), 3(b) and 3(c) showed moderate results whereas 2(b) and 2(d) are inactive. In case of *A. flavous* 3(c) and 3(a) compounds showed maximum and average inhibition, the remaining compounds are inactive. All the compounds were moderately active against *C. albicans* while 2(c), 3(a) and 3(d) not shown any activity. The antibacterial and anti-fungal activity results are summarized in Table 3.

Anti-inflammatory activity

Among all the compounds the 2(a), 2(b) and 3(c) have shown good anti-inflammatory activity whereas 2(d)

Table 2: Bio-efficacy testing of semicarbazones (2a-d) and oxadiazoles (3a-d) [Stomach and contact poison].

Compounds	Dosage (in ppm)	Stomach poison						Contact poison					
		Mortality out of 24 larvae			% Mortality after (Stomach)			Mortality out of 24 larvae			% Mortality after (contact)		
		24 HAAPP	48 HAAPP	72 HAAPP	24 HAAPP	48 HAAPP	72 HAAPP	24 HAAPP	48 HAAPP	72 HAAPP	24 HAAPP	48 HAAPP	72 HAAPP
2a	500	1	2	0	4.17	12.50	12.50	3	2	2	12.50	20.83	29.19
2b	500	2	2	0	8.33	16.67	16.67	3	5	2	12.50	33.33	41.67
2c	500	1	3	0	4.17	16.67	16.67	4	7	4	16.67	45.83	62.50
2d	500	2	2	4	8.33	16.67	33.33	3	8	6	12.50	45.83	70.83
3a	500	3	4	0	12.50	29.17	29.17	2	2	3	8.33	16.67	24.17
3b	500	2	2	1	8.33	16.67	20.83	2	3	0	8.33	20.83	20.83
3c	500	1	1	1	4.17	8.33	12.50	4	0	1	16.67	16.67	20.83
3d	500	2	2	0	8.33	16.67	16.67	4	1	2	16.67	20.83	29.17
Acetone (Standard)	-	4	0	0	16.67	16.67	16.67	4	0	0	16.67	16.67	16.67
U.T.C.	-	0	0	0	0.00	0.00	0.00	0	0	0	0.00	0.00	0.00

and 3(d) have shown moderate activity while 2(c), 3(a) and 3(b) compounds did not possess any activity. The results of anti-inflammatory activity of standard and samples are tabulated in Table 4

Molecular Docking Studies

Molecular docking simulation of 2a-d and 3a-d with Crystal Structure of Russell's viper Phospholipase A2 was performed to gain functional and structural insight into the mechanism of inhibition. AutoDock 4.2 suites were used as molecular-docking tool. For the *in silico* docking studies, semicarbazones and oxadiazole derivatives were docked against Phospholipase A2, using the co-crystallized ligand structure of Diclofenac as reference. Diclofenac was relocked to its active site to calculate the binding energy, inhibition constant using Auto Dock. The active

Table 3: Anti-bacterial and anti-fungal activity of compounds (2a-d) and (3a-d).

Compounds	Anti-bacterial			Antifungal Activity		
	Kallipsi calla 'k' (in mm)	*Escherichia coli 'EC' (in mm)*	*Portious vulgaris 'PV' (in mm)*	*A. niger 'AN' (in mm)*	*A. flavas 'AF' (in mm)*	*C. albicans 'CA' (in mm)*
2a	10	09	08	09	-	09
2b	10	08	09	-	-	08
2c	10	10	09	07	-	-
2d	10	12	08	-	-	08
3a	14	15	09	10	08	-
3b	15	10	07	08	-	06
3c	12	15	10	09	12	07
3d	13	12	08	12	-	-
Standard	29	28	22	-	-	-
Control (DMSO)	06	06	06	-	-	-

Table 4: Anti-inflammatory activity of compounds (2a-d) and (3a-d).

Group	Dose (mg/kg)	Mean value of hind paw odema at different time of intervals in hours (mean 24 ± SE)				
		At 0	After 1/2	After 1	After 3	After 5
Control	1%	0.575 (± 0.0478)	0.6 (± 0.0408)	0.775 (± 0.0478)	0.65 (± 0.0645)	0.55 (± 0.0288)
Standard	25	0.55 (± 0.0281)	0.625 (± 0.075)	0.725 (± 0.047)	0.675 (± 0.025)	0.625 (± 0.025)
Compounds	Control Tween-80: Standard Diclophenac sodium					
2a	25	1.025 (± 0.047)	0.095 (± 0.0285)	0.095 (± 0.0285)	0.25 (± 0.2041)	0.275 (± 0.0408)
2b	25	0.925 (± 0.047)	0.975 (± 0.075)	0.625 (± 0.0853)	0.5 (± 0.0408)	0.5 (± 0.0912)
2c	25	1.075 (± 0.025)	1.275 (± 0.2462)	1.35 (± 0.1190)	1.05 (± 0.0645)	0.85 (± 0.028)
2d	25	1.025 (± 0.047)	0.975 (± 0.025)	0.9 (± 0.0408)	0.9 (± 0.0408)	0.875 (± 0.0478)
3a	25	1.625 (± 0.047)	1.325 (± 0.047)	1.275 (± 0.042)	1.3 (± 0.04082)	1.225 (± 0.0144)
3b	25	0.75 (± 0.0645)	1.05 (± 0.064)	1.05 (± 0.028)	1.15 (± 0.064)	0.45 (± 0.028)
3c	25	1.125 (± 0.047)	0.95 (± 0.064)	1.05 (± 0.028)	0.95 (± 0.0643)	0.95 (± 0.0645)
3d	25	1.375 (± 0.078)	1.225 (± 0.062)	1.225 (± 0.0625)	1.075 (± 0.0408)	0.925 (± 0.0408)

Table 5: Molecular Docking Studies between 2a-d and 3a-d with 2B17.

Protein name	Ligand structure	Binding energy (kcal/mol)	Amino acids involved in Hydrogen Bonding	Predicted IC_{50} (micro molar) from AutoDock
Phospholipase A2	2a	-7.11	Asp49	6.14
	2b	-7.69	Tyr28 Gly30 Trp31 Asp49	2.33
	2c	-7.03	Leu2 Gly30	7.04
	2d	-6.01	Leu2 Gly30 Asp49	39.2
	3a	-7.17	Gly30 Asp49	5.59
	3b	-7.54	GLY30 ASP49 LYS69	2.95
	3c	-6.2	-	28.36
	3d	-7.78	Tyr22 His48 Asp22	2.00
Phospholipase A2	*Diclofenac(DIF)*	-7.04	His 48(A) Asp 99(A)	6.9

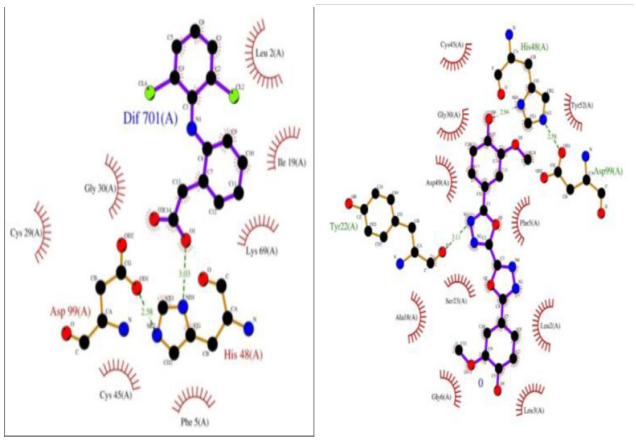


Figure 4: Molecular interaction of DIF and 3d with Phospholipase A2 from Ligplot+.

(A) Molecular interaction of DIF with Phospholipase A2 as represented in PDBsum (PDB: 2B17)

(B) Molecular docking interaction of 3d with Phospholipase A2.

site comprises of Leu2, Phe5, Ile19, Cys29, Gly30, Cys45, His48, Lys69 and Asp99. The semicarbazones and oxadiazole derivatives were allowed to dock at the active site with the complete flexibility. The best pose having least binding energy revealed the electrostatic interaction and hydrogen bonding which facilitate the binding to the active site. The experimental results of interaction of Diclofenac (PDB: 2B17) with Phospholipase A2 shows that the K_i = 6.9 μM by forming hydrogen bonds with His 48and Asp99. The interaction of 3d with PAP2 (PDB: 2B17) shows three hydrogen bonds with active site residues Tyr22, His48 and Asp99. The K_i of 2.0 μM was observed which is low as compare to standard drug Diclofenac. IC_{50} values of declofenac and semi-carbazones derivatives were predicted from AutoDock. Based on very low binding energy of -7.78kcal/mol and low IC_{50} of 2.0 μM, the 3(d) proves to be a competitive inhibitor as compared to Diclofenac and as a potential anti-inflammatory lead molecule since the ligand interact with a relatively higher affinity and lower inhibition constant than the standard diclofenac. PLA2 catalyzes the calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides. The cofactor calcium uses the metal binding residues Tyr27, Gly29, Gly31 and Asp48 (residue numbering according to PDB) via carbonyl oxygen for its activity. Compound 3d formed hydrogen bond with these metal binding residues and blocked the interaction of calcium and further disturbs the hydrolysis and inactivates the enzyme. Compound 3d formed Binding energy and inhibition constant of all the docked compounds including both the standard drugs were presented in Table 5 and supported by enclosing Figure 4.

CONCLUSION

The novel series of semicarbazones and oxadiazoles were synthesized in reasonably good yields. The outcome from this study clearly showed that the chemical

structures were found to be in accordance with their IR and ^{1}H-NMR Spectra. The synthesized compounds were assessed for insecticidal activity, anti-bacterial, anti-fungal, anti-inflammatory potentials.

The Compound 2(d) and 3(a) shows high percentage of mortality compared to standard acetone. The increased percentage of mortality of compound may be due to the very low binding energy and presence of substituent. Other compounds show moderated and less mortality because of moderately high binding energy. The Compounds 3(a) and 3(b) showed high activity against *Kallipsi calla* and *Escherichia coli* as compared to standard. The compounds 3(a) and 3(d) showed high activity against *A. nigar* and in case of *A. flavous* 3(c) and 3(a) compounds showed maximum inhibition. Compounds 2(a), 2(b) and 3(c) have shown good anti-inflammatory activity when compared with the standard diclophenac sodium. The molecular docking studies of the synthesized compounds were performed. The Compound 3(d) proves to be a competitive inhibitor as compared to Diclofenac and as a potential anti-inflammatory lead molecule since the ligand interact with a relatively higher affinity and lower inhibition constant. Compound 3(d) showed the least binding energy which is in agreement with the *in vitro* results. Hence, this study has extended the scope of developing these semicarbazones and oxadiazole derivative as positive antimicrobial agents.

ACKNOWLEDGEMENT

We extend our special thanks to Dr. M. G. Purohit. Professor of Organic Chemistry, Department of Chemistry, Gulbarga University, Gulbarga for providing facilities in animal experimentation techniques / procedures and to Dr. Suhas Yelshetty, Scientist (Entomology). Agricultural Research Station, Gulbarga for his timely help in screening the synthesized compounds for insecticidal activity at Agricultural Research Station, Gulbarga. The authors express deep sense of gratitude and indebtedness to Dr. Somanth V. Patil for valuable suggestions and encouragement throughout the project work. Authors acknowledge the funding agencies VTU Belagavi, AICTE New Delhi and VGST-KFIST (L1) / GDR-580/2016-17 for their financial support.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

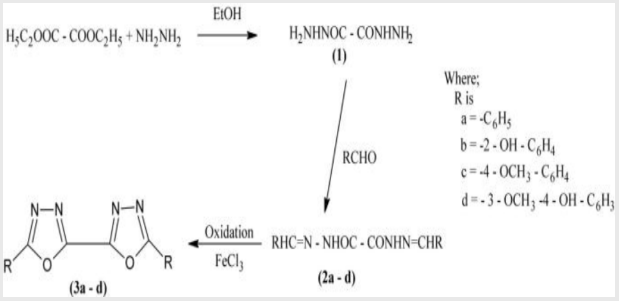
ABBREVIATIONS

HCl: Hydrochloric acid; **KBr:** Potassium bromide; **NaOH:** Sodium hydroxide; **DCC:** 1,3- dicyclohexyl-carbodimide; **Hg (OAc)$_2$:** Mercury (II) acetate; **HgO:** Mercury (II) oxide; **TMS:** Tetramethylsilane; **TLC:** Thin layer chromatography; **DMSO-d$_6$:** Dimethyl sulfoxide-d$_6$; **AR:** Analytical Reagents; **DMF:** Dimethylformamide.

REFERENCES

1. Pavithra G, Ajay KK. Design, synthesis and biological evaluation of 1, 3, 4-oxadiazoles / thiadiazoles bearing pyrazole scaffold as antimicrobial and antioxidant candidates. Current Chemistry Letters. 2016;5(3):109-22.
2. Mohamed NI, Al-Difar HA. Synthesis and anti-bacterial activity of semicarbazone derivatives of some carbonyl compounds. Pelagia Research Library: Der Chemica Sinica. 2011;2(1):171-3.
3. Rajak H. Synthesis and evaluation of some novel semicarbazones based benzimidazole derivatives as anticonvulsant agent. International Journal of Chemical Engineering and Applications. 2015;6(2):142-5.
4. Davinder P, Nisha A, Rajesh K, Mahendra N. Synthesis of novel hetroareans based [1, 2, 3]-triazoles via click chemistry and their evaluation for antibacterial activity. Indian Journal Chemistry. 2012;51:731-8.
5. Jianming X, Yongbing C, Zhang J, Shichong Y, Yan Z, Xiaoyun C, *et al*. Design synthesis and antifungal activities of novel 1, 2, 4- triazole derivatives. European J Med Chem. 2011;46(7):3142-8.
6. Zahra R, Soghra K, Keyvan P, Zahra H, Fatemeh A, Elham A, *et al*. Design, synthesis and antifungal activity of triazole and benzotriazolederivatives. European J Med Chem. 2009;44(7):3064-7.
7. Frank W, Xinli T, Torsten K, Jens P, Mavanur S. Synthesis and cyclooxygenase inhibition of various (aryl-1, 2, 3- triazole-1-yl)-methanesulfonylphenyl derivatives. Bioorganic and Medicinal Chemistry. 2009;17(3):1146-51.
8. Sanjeev K. Synthesis and biological activity of 5-substituted-2-amino-1, 3, 4oxadiazole derivatives. Turk J Chem. 2011;35(1):99-108.
9. Yogesh M, Devender P. Design, synthesis and biological evaluation of some novel 2, 5-disubstituted-1, 3, 4-oxadiazole derivatives. International Journal of Drug Design and Discovery. 2011;2:659-65.
10. Kumar S, Srivastava DP. Efficient electrochemical synthesis, antimicrobial and anti-inflammatory activity of 2–amino-5-substituted- 1, 3, 4-oxadiazole derivatives. Indian J Pharm Sci. 2010;72(4):458- 64.
11. Robert MS, Clayton BG, Terence CM. Spectrometric identification of organic compounds. Wiley Publishe. 1981.
12. Giri SK. K. M. Sc. Dissertation, Gulbarga University, Gulbarga. 2001.
13. Cruinckshank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology, London, Churchill Livingstone. 1975;196.
14. Aalten DMFV, Bywater R, Findlay JBC, Hendlich M, Hooft RWW, Vriend G, *et al*. A program for generating molecular topologies and unique molecular descriptors from coordinates of small molecules. Journal of Computer -Aided Molecular Design. 1996;10(3):255-62.
15. Wolf LK. New software and websites for the chemical enterprise. Chemical and Engineering News. 2009;87(45):31.
16. Garrett MM, David SG, Robert SH, Ruth H, William EH, Richard KB, *et al*. Automated Docking using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function. Journal of Computational Chemistry. 1998;19(14):1639-62.
17. Wallace AC, Laskowski RA. LIGPLOT: A program to generate schematic diagrams of Protein-ligand interactions. Protein Eng.1995;8(2):127-34.
18. Delano WL. The Pymol Molecular Graphics System DeLano Scientific, San Carlos, CA USA. 2002.

PICTORIAL ABSTRACT



The novel series of semicarbazones (2a-d) and oxadiazoles (3a-d) were synthesized in reasonably good yields. The synthesized compounds were in noble arrangement with elemental and spectral data. The synthesized compounds were assessed for insecticidal activity, anti-bacterial, anti-fungal, anti-inflammatory potentials and molecular docking studies.

SUMMARY

- A series of novel substituted semicarbzones and oxadiazoles were synthesized in reasonably good yield. Chemical structures were characterized and are in accordance with their IR and ^{1}H-NMR spectra. Obtained compounds were subjected for insecticidal activity and pharmacological screening viz, anti-bacterial, anti-fungal, anti-inflammatory potentials and molecular docking studies.
- Majority of the synthesized compounds exhibits potential inhibition while few of them exhibits moderate activity. The docking studies for semicarbzones and oxadiazoles derivatives were against Phospholipase A2, using the co-crystallized ligand structure Diclofenac as reference. The compound formed binding energy and inhibition constant of all the docked compounds including both the standard drugs showed the least binding energy which is in agreement with the *in vitro* results.

About Authors

Dr. Veerabhadraswamy Mruthyunjaya, Assistant Professor of Chemistry, PES University, Bengaluru, India working since 2009, is a post doctoral fellow from Queens University Belfast, Belfast, Northern Ireland UK, presently engaged in green chemistry and green engineering education promotion in India through design and develop learning model to suite Indian education system. His current research interests are conversion of biomass into bio-platform molecules, green synthesis of value added chemicals from renewable resources. He is active member steering the Green Chemistry Centre at P E S University aims to develop, execution of green and sustainable chemistry, fuse allied technologies into new products and process. Networking both with academia and industry as well as conducting awareness programs for common people.

Mr. Bhimashankar B Molkere is a Research Fellow at PES University, Bengaluru, Karnataka, India presently working in the field of green synthesis of compounds having high value products employing novel greener techniques. His focus is to design, develop and synthesis semicarbazones, oxadiazoles and triazoles.

Dr. Prashantha Karunakar is an Assistant Professor at PES University Bengaluru, Karnataka, India since 2011. He worked at synchrotron facilities like DESY, Hamburg, Germany and ESRF, Grenoble, France. His research interest involves Crystallography, Bioinformatics, Computational Biology, Computational Chemistry and Molecular Docking and Dynamics Simulations.

Cite this article: Veerabhadraswamy M, Molkere BB, Karunakar P. Synthesis and Evaluation of Insecticidal Activity of Bis-Semicarbazones and Bis-(5-Aryl Substituted-1, 3, 4-Oxadiazole). Indian J of Pharmaceutical Education and Research. 2019;53(3 Suppl 2):s423-s432.