Article

Synthesis of novel 1, 3, 4-oxadiazole derivatives as anti-esophageal cancer: *In-vitro* cytotoxic evaluation

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Abstract

In this work, an efficient protocol address a facile procedure employing safer and commercially available starting materials to afford the target products that are 1, 3, 4-oxadiazole derivatives (**5a-c**) in satisfactory yields with elevated purities. To elucidate these derivatives, their chemical structures were unambiguously confirmed by using different spectroscopic tools including FT-IR, NMR (^{1}H and ^{13}C), Mass spectra, and melting points. The enantiomers of the synthesized derivatives were checked by enantioselective HPLC analysis and NMR study which showed these derivatives obtained as racemates. With the aim to identify *in vitro* potency of these derivatives, MTT method has been applied to explore and evaluate their antiproliferative efficacy against esophageal cells (SKGT-4). The cell viability testing *via* MTT assay revealed that these derivatives displayed potent *in vitro* activity, showing IC_{50} values with a range of potential inhibition in comparison to the selected drug as a standard reference. The biological activity present in this work showed the synthesized derivatives offer significant antiproliferative efficacy with safer chemotherapeutic leads in medicinal chemistry to explorations for esophageal cancer treatment.

Keywords: 1, 3, 4-Oxadiazole, Esophageal cancer, *In-vitro* cytotoxicity.

1. Introduction

Cancer has been the most formidable diseases as it is considered as a biggest reason of death throughout the world [1]. According to health reports declared by WHO, the second potential death rate is caused by cancer and this rate is diagnosed in terms of the cardiac diseases in humans [2]. Globally, the account of cancer deaths escalated to 8.8 million deaths in the year 2015 [3]. In 2020, the number of death cases by cancer was found to be 19.3 million deaths and these cases are primarily influenced by cancers of prostrate, liver, stomach, lung, colon, breast, and etc. [4]. It is expected that the annual account of cancerous cases is diagnosed to be 25-30

million, with 17 million death cases every year and approximately 75 million patients living with symptoms of cancers [5]. Esophageal cancer is the sixth characterized cancer and considered as the major cause of death cases, numbering for about 150,500 cancerous deaths in both man and women around the world [6]. By investigating the esophageal cancer incidences reported by health records, it is concluded that this cancer type is the most common in men compared with women. In this cancer type, the appearance of malignant tumor symptoms is due to the mucosa of the esophagus cells occur in inner layers (lining). The progression of this cancer type starts with an accumulation of epigenetic and genetic alterations under a multistep process that causes he activation of oncogenes [7]. At the most esophageal cancer cases, two common types include adenocarcinoma and squamous cell carcinoma have been reported. The second one has a real and serious threat to public health as is almost associated with several reasons including advanced age, tobacco excess use, alcohol abuse, achalasia, and high-starch diets [8, 9].

Despite fascinating advances in cancer treatment in both diagnostic and therapeutic approaches, these approaches have been recently an entirely new field of research in pharmaceutical industry [10]. Although, the rapid and significant advancements in drug accessibility for potential treatment and diagnosis, esophageal cancer as like an type of cancer remains a common malignancy around the world because of there is drug resistance before or after chemotherapeutic process and inadequacy of current therapeutic alternates. In overall survival rate for esophageal cancer patients remains poor [11, 12]. Present methodologies in both diagnosis and treatments of cancer diseases involve using different forms of radiation therapy, surgery, hormone therapy, and chemotherapy. Chemotherapy is undergone to be the most standard approach for eliminating cancer cells. In spite of several advantages shown by this approach, some provided anticancer drugs accompany with relative cytotoxicity, lower efficiency, and low permeability [13]. Furthermore, these selected drugs also accompany with a range of undesirable or serious side effects that cause environmental organ failure [14]. Thus, the search for new anticancer agents or design and synthesis of new anticancer drugs are become nowadays as the most urgent field of research in recent times [15, 16].

Heterocyclic compounds bearing an oxadiazole moiety have been undergone to biological interest since their wide broad of the pronounced biological activities such as antimicrobial, anti-leishmanial, HIV remedy, anticancer, etc. [17-20]. Additionally, oxadiazole nucleus are found to contribute as bioisosteres of esters and amides accompany with several ecological benefits such as metabolic stability, superior hydrolytic, enzyme inhibitors, and *in vivo* performance to improve their pharmacokinetics [12, 21]. Among oxadiazole nucleus, 1, 3, 4-oxadiazole which refers to five-membered furan molecule substituted with two types of pyridine nitrogens (-N=) instead of two groups of methine (= CH). 1, 3, 4-Oxadiazole and their derivatives are found to be prospective bioactive compounds in the treatment of a wide broad of diseases in medical chemistry [22]. Many marketed drugs containing oxadiazole moieties are introduced in **Fig. 1**. Raltegravir (**I**) is synthesized and conducted to be used as an antiretroviral drug towards HIV infection [23]. Nesadipil (**II**) is in late-stage of clinical trials to improve as an antibiotic compound for the hypertension remedy [24]. In addition, Zibotentan (ZD4054)

(**III**) is marketed as recommended agent for breast, colorectal, ovarian, and particularly prostate cancers [25].

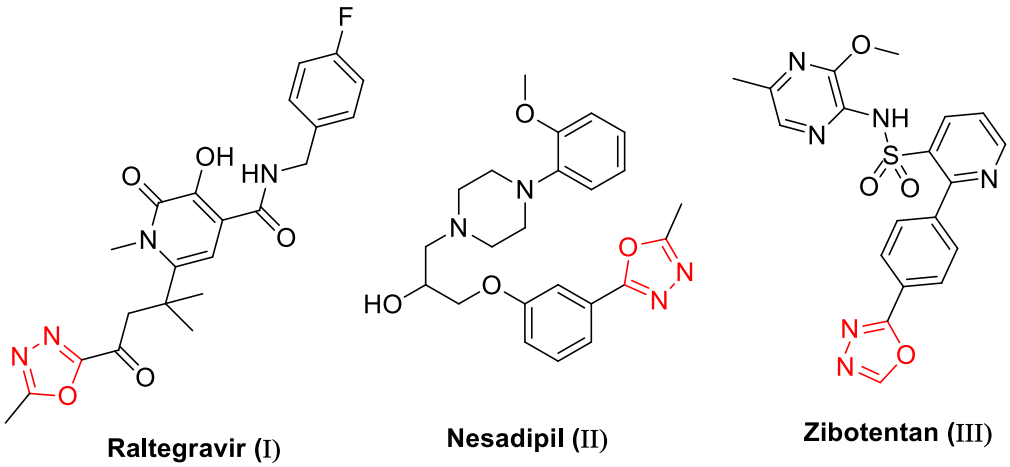
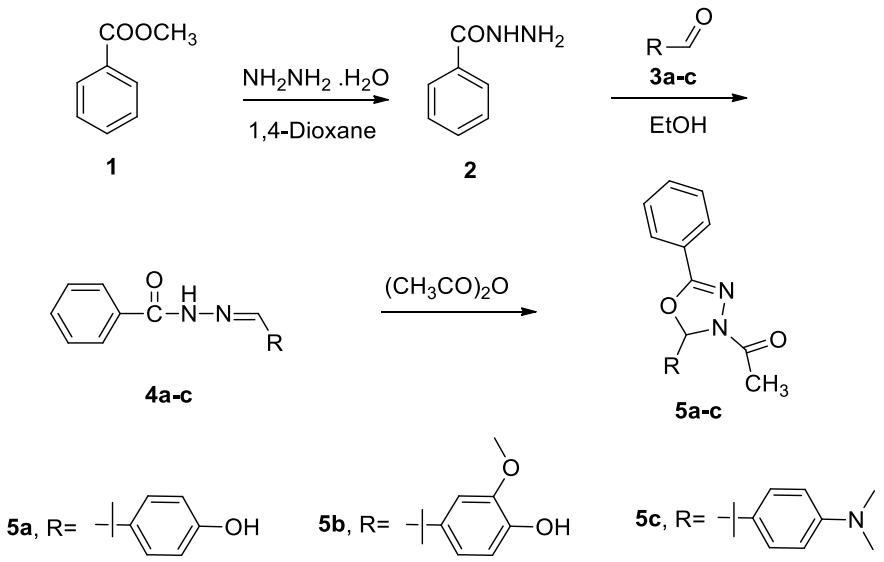


Fig. 1. Marketed drugs containing an oxadiazole moiety.

Due to 1, 3, 4-oxadiazole derivatives have been gotten significant attention in medicinal and organic chemistry, several promising strategies are addressed for synthesizing these derivatives. Two traditional strategies are reported to 1, 3, 4-oxadiazoles synthesis. The first one includes using an acid catalyzed cyclization of 1, 2-diacylhydrazine derivatives in the presence of a common cyclizing agent (exp. polyphosohoric acid, phosphorous oxy chloride, and $BF_3.OEt_2$) [26, 27], while the second one includes using an oxidative cyclization of hydrazine/*N*-acylhydrazones in the presence of an oxidant agent [28]. *N*-arylhydrazone derivatives are used as an efficient intermediate which is produced by condensation aldehydes or ketones with hydrazide derivatives. *N*-Acylhydrazone derivatives are subjected to intramolecular cyclization-condensation followed by oxidation process to afford the target 1, 3, 4-oxadiazole derivatives. The common oxidizing agents are used involving Fe(III)/TEMPO, I_2, $Cu(OTf)_2$ [29-31]. Such above approaches show a significant way to 1, 3, 4-oxadiazole derivatives, some of these approaches involve the use of toxic and harmful reagents, difficult handling control, produce undesirable byproducts, and no satisfactory in product yield. Thus, new or modified synthetic approaches toward 1, 3, 4-oxadiazole derivatives are still important.

On the other hand, 2, 3-dihydro-1, 3, 4-oxadiazole derivatives are an important structural isomer that is obtained *via* intramoleculer cyclization-dehydration of hydrazide or hydrazones by acyl chlorides or acid anhydrides. There are only a few reported methods towards the synthesis of 2, 3-dihydro-1, 3, 4-oxadiazole derivatives which are basis on using hydrazide/hydrazones with other cyclization agents [32]. Pursuing this field of search and encouraged by the above findings as well as in continuation of our devoted efforts to synthesize 1, 3, 4-oxadiazol derivatives for biological importance, our work addresses an efficient synthesis of 2, 3-dihydro-1,3,4-oxadiazole derivatives with the aim of improving their potential their cytotoxic activity towards esophageal cancer. As given in Scheme 1, initially the commercially available methyl benzoate (**1**) was reacted with hydrazine hydrate in the presence

of 1,4-dioxane to afford the benzohydrazide compound (**2**). The obtained benzohydrazide (**2**) thus was reacted with appropriate aldehydes (**3a-c**) in presence of a few drops of acetic acid in ethanol as solvent under reflux for 3-4 h to give the key intermediate (imines compounds, **4a-c**). The prepared imines were subsequently condensed with absolute acid anhydride under dry conditions to complete the desired 2, 3-dihydro-1, 3, 4-oxadiazole derivatives (**5a-c**) in good total yields (70–75%).



Scheme 1. Synthesis of novel 2, 3-dihydro-1, 3, 4-oxadiazole derivatives (**5a-c**).

2. Chemicals and Methods

Solvents used with analytical grade and the selected chemicals (ACS grade) were purchased from Sigma-Aldrich. Analytical properties to monitor the progress of the reactions were carried out by using TLC plates precoated with a silica gel 60 UV 254. UV light or alkaline potassium permanganate solutions was employed to determine the spots of the obtained compounds. FT-IR spectra were collected on Shimadzu FTIR-8300 infrared spectrophotometer (Iraq, University of Basrah) and the absorbance were acquired between 4000-400 cm-1. At room temperature, NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker Anovo AV-400 spectrometer (Iraq, University of Basrah) in DMSO-d6 as solvent associated with a common signal of 1H spectra at δ 2.50 ppm and 3.40 ppm for its water molecule as well as other signal of ^{13}C spectra at δ 49.5 ppm.. Decoupling values (J) for integrated protons are given in Hz. a Gallenkamp melting device are employed to determine melting points depends on capillary tubes.

2.1. Synthesis of benzohydrazide compound (2)

Benzohydrazide (**2**) was synthesized according to the method in the reference [33]. To purchased methyl benzoate **1** (4.2 g, 27 mmol) in ethanol (40 mL), fresh hydrazine hydrate (40 mL) was slowly added. The above mixture was further refluxed for 8-10 h as followed by TLC analysis. Resulting mixture was cooled to room temperature and concentrated until the precipitate is formed. The solid precipitate then thoroughly washed with absolute ether, filtered,

and conducted to recrystallization from ethanol to offer the target benzohydrazide **2** as yellow crystals. Yield: (85%); m.p. 112–114 ºC. ^{1}H NMR spectrum, δ, ppm: 7.75 (d, 2H, *J* = 2.6 Hz, ArC-H), 7.53 (t, 1H, *J* = 2.9 Hz, ArC-H), 7.45 (d, 2H, J = 2.6 Hz, ArC-H), 3.84 (s, 2H, NH_2).

2.2. Synthesis of imine compounds (4a-c)

To a stirred solution of the selected aldehydes (**3a-c)** (6.0 mmol) dissolved in ethanol (40 mL) with a few drops of acetic acid, the obtained benzohydrazide (**3**) (1.0 g, 6.0 mmol) was added. After refluxing step for 4-6 h (as indicated *via* TLC), the obtained mixture was collected and filtered. The collected precipitate was conducted to purification step *via* recrystallization process from ethanol to offer the desired imine compound (**4a-c**).

***N*-(4-Hydroxybenzylidene)benzohydrazide (4a)**

Yellow solid, yield: (90%); m.p. 210-211 ºC. FT-IR, ν, cm −1 (KBr disk): 3349 (O-H), 3059, 3030 (C-H, Ar), 1643 (C=O, amide), 1602 (-N=CH), 1579 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 11.57 (s, 1H, NH), 8.32 (s, 1H, -N=CH), 7.89 (d, 2H, *J* = 2.7 Hz, ArC-H), 7.58-7.50 (m, 5H, ArC-H), 6.76 (d, 2H, *J* = 2.2 Hz, ArC-H), 2.98 (s, 1H, OH). ^{13}C NMR, *δ*, ppm: 163.2 (C=O amide), 151.9 (-N=CH), 149.5 (Ar-C), 134.3 (Ar-C), 131.9 (Ar-C), 128.9 (ArC-H), 128.7 (ArC-H), 127.8 (ArC-H), 122.1 (ArC-H), 112.3 (ArC-H). EI-MS: *m/z* 240 $[M]^{+}$ observed for $C_{14}H_{12}N_2O_2$.

***N*-(4-hydroxy-3-methoxybenzylidene)benzohydrazide (4b)**

Yellow solid, yield: (85%); m.p. 215-216 ºC. FT-IR, ν, cm −1 (KBr disk): 3487 (O-H), 3240 (N-H), 3059 (C-H, Ar), 2851 (C-H, aliphatic), 1639 (C=O, amide), 1608 (-N=CH), 1577 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 11.69 (s, 1H, NH), 8.34 (s, 1H, -N=CH), 7.90 (d, 2H, *J* = 2.7 Hz, ArC-H), 7.58 (t, 1H, *J* = 2.3 Hz, ArC-H), 7.52 (t, 2H, *J* = 2.2 Hz, ArC-H), 7.32 (s, 1H, ArC-H), 7.09 (d, 2H, *J* = 1.9 Hz, ArC-H), 6.85 (d, 2H, *J* = 2.1 Hz, ArC-H), 3.84 (s, 3H, OCH_3). ^{13}C NMR, *δ*, ppm: 163.7 (C=O amide), 149.5 (-N=CH), 148.5 (Ar-C), 147.3 (Ar-C), 134.9 (Ar-C), 132.4 (Ar-C), 128.9 (ArC-H), 126.7 (ArC-H), 124.8 (ArC-H), 122.6 (ArC-H), 116.3 (ArC-H), 56.2 (C, OCH_3). EI-MS: *m/z* 270 $[M]^{+}$ observed for $C_{15}H_{14}N_2O_3$.

***N*-(4-(dimethylamino)benzylidene)benzohydrazide (4c)**

Yellow solid, yield: (90%); m.p. 196-198 ºC. FT-IR, ν, cm −1 (KBr disk): 3255 (N-H), 3059 (C-H, Ar), 2879 (C-H, aliphatic), 1651 (C=O, amide), 1602 (-N=CH), 1545 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 11.57 (s, 1H, NH), 8.31 (s, 1H, -N=CH), 7.89 (d, 2H, *J* = 2.1 Hz, ArC-H), 7.57-7.49 (m, 5H, ArC-H), 6.76 (d, 2H, *J* = 2.2 Hz, ArC-H), 2.98 (s, 6H, $N(CH_3)_2$). ^{13}C NMR, *δ*, ppm: 163.1 (C=O amide), 151.5 (-N=CH), 149.6 (Ar-C), 134.3 (Ar-C), 133.2 (Ar-C), 132.5 (Ar-C), 128.4 (ArC-H), 128.1 (ArC-H), 123.8 (ArC-H), 122.1 (ArC-H), 113.3 (ArC-H), 40.3 (C, $N(CH_3)_2$). EI-MS: *m/z* 267 $[M]^{+}$ observed for $C_{16}H_{17}N_3O$.

2.3. Synthesis of the target 1, 3, 4-oxadiazol derivatives (5a-c)

The synthesized imine compounds (**4a-c**) (1.5 mmol) were conducted to reflux in the presence of fresh acetic anhydride (1.0 mL) under stirring for 6-8 h. The obtained solution with suspension behavior was progressed using TLC in order to determine the end of reaction as an intense orange color appeared. The suspension orange solution was then washed with ice water under vigorously stirring. The precipitate that was collected and washed with three time with water and two time with $NaHCO_3$ (10%) to afford neutral medium. The collected precipitate

was subjected to chromatography purification with an eluent contains chloroform: methanol (20:1) to afford the pure 1, 3, 4-oxadiazol derivatives (**5a-c**).

1-(2-(4-Hydroxyaryl)-5-aryl-1, 3, 4-oxadiazol-3(*2H*)-yl)ethanone (5a)

Orange solid, yield: (70%); m.p. 154-155 ºC. FT-IR, ν, cm −1 (KBr disk): 3228 (O-H), 3012, (C-H, Ar), 2924 (C-H, aliphatic), 1649 (C=O, amide), 1608 (-N=CH), 1541 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 7.86 (d, 1H, *J* = 1.7 Hz, ArC-H), 7.81 (d, 2H, J = 2.3 Hz, ArC-H), 7.52 (t, 2H, *J* = 2.9 Hz, ArC-H), 7.24 (d, 2H, *J* = 2.3 Hz, ArC-H), 7.06 (s, 1H, oxadiazole ring), 6.71 (d, 2H, *J* = 2.3 Hz, ArC-H), 2.25 (s, 3H, CH_3- acetyl). ^{13}C NMR, *δ*, ppm: 167.2 (C=O acetyl), 155.2 (-N=C-, oxadiazole ring), 151.5 (Ar-C), 132.3 (Ar-C), 129.9 (Ar-C), 128.9 (ArC-H), 127.7 (ArC-H), 124.8 (ArC-H), 122.1 (ArC-H), 112.4 (ArC-H), 92.3 (CH, oxadiazole ring), 21.8 (C, CH_3-acetyl). EI-MS: *m/z* 282 $[M]^+$ observed for $C_{16}H_{14}N_2O_3$.

1-(2-(4-Hydroxy-3-methoxyaryl)-5-aryl-1, 3, 4-oxadiazol-3(*2H*)-yl)ethanone (5b)

Yellow solid, yield: (70%); m.p. 144-146 ºC. FT-IR, ν, cm −1 (KBr disk): 3421 (O-H), 3052 (C-H, Ar), 2862 (C-H, aliphatic), 1718 (C=O, amide), 1670 (-N=CH), 1554 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 8.18 (d, 2H, *J* = 1.7 Hz, ArC-H), 7.83 (t, 2H, *J* = 1.3 Hz, ArC-H), 7.75 (t, 3H, *J* = 1.2 Hz, ArC-H), 7.18 (s, 1H, ArC-H), 7.12 (s, 1H, oxadiazole ring), 3.93 (s, 3H, OCH_3), 3.78 (s, 1H, OH), 2.31 (s, 3H, CH_3-acetyl). ^{13}C NMR, *δ*, ppm: 168.9 (C=O amide), 164.5 (-N=C-, oxadiazole ring), 152.5 (Ar-C), 142.4(Ar-C), 132.9 (Ar-C), 129.5 (Ar-C), 127.9 (ArC-H), 126.4 (ArC-H), 123.8 (ArC-H), 122.6 (ArC-H), 111.3 (ArC-H), 92.3 (CH, oxadiazole ring), 56.7 (C, OCH_3), 20.7 (C, CH_3-acetyl). EI-MS: *m/z* 312 $[M]^+$ observed for $C_{17}H_{16}N_2O_4$.

1-(2-(4-(Dimethylamino)aryl)-5-aryl-1, 3, 4-oxadiazol-3(*2H*)-yl)ethanone (5c)

Orange solid, yield: (75%); m.p. 181-183 ºC. FT-IR, ν, cm −1 (KBr disk): 3111, 3059 (C-H, Ar), 2920 (C-H, aliphatic), 1722 (C=O, amide), 1668 (-N=CH), 1552 (C=C, Ar). ^{1}H NMR spectrum, *δ*, ppm: 7.81 (d, 2H, *J* = 3.1 Hz, ArC-H), 7.60-7.53 (m, 3H, ArC-H), 7.24 (d, 2H, *J* = 2.2 Hz, ArC-H), 7.06 (s, 1H, oxadiazole ring), 6.71 (d, 2H, *J* = 2.2 Hz, ArC-H), 2.90 (s, 6H, $N(CH_3)_2$), 2.25 (s, 3H, CH_3-acetyl). ^{13}C NMR, *δ*, ppm: 166.5 (C=O amide), 155.5 (-N=C-, oxadiazole ring), 151.7 (Ar-C), 132.3 (Ar-C), 129.2 (Ar-C), 127.5 (Ar-C), 126.4 (ArC-H), 124.8 (ArC-H), 124.2 (ArC-H), 122.1 (ArC-H), 112.3 (ArC-H), 92.5 (CH, oxadiazole ring), 40.4 (C, $N(CH_3)_2$), 21.8 (C, CH_3-acetyl). EI-MS: *m/z* 309 $[M]^+$ observed for $C_{18}H_{19}N_3O_2$.

2.4. Preparation of cell cultures

Human cancerous cells (SKGT-4) of esophageal tissue and Vero as normal cells were obtained from the Cultured Biotech Cell Bank Unit in Basrah, IRAQ. The cultured cells were maintained in in plate with a volume of 10 cm containing 10% Fetal bovine and RPMI-1640 as solution. The cellar solution was treated with 100 units/mL penicillin, and 100 µg/mL streptomycin. All cultured cells were conducted to treatment with Trypsin-EDTA, and further reseeded confluence (50%) for twice time in a week and ultimately incubated with a humid atmosphere (CO_2, 5%) at near room temperature (37 °C).

2.5. Dose response in cytotoxicity assays

MTT assay of cell viability was elected as efficient tool to determine the cytotoxic effect, this method thus was carried out on 96-well plates for 48 h. Both SKGT-4 and Vero were reseeded at cells/well (1×10^4). After 24 h of seeded treatment, a collected confluent monolayers was provided and the treated cells were conducted to the selected compounds (for each 1000

μg/mL). After 72 h of incubated process, the cell viability has been undergone through removing the medium, and 28 μL of MTT solution (2 mg/mL,) was added to above and incubated at 37 °C for 2 h. After treatment with MTT solution, the residual solution was removed to leave crystals in the wells. These well were further solubilized by an addition of DMSO (100 μL) and followed by shaking at 37 °C for 15 min. A micro-plate reader (Thermo Fisher Scientific) is used to collect the absorbance of cell viability at 520 or 620 nm as a standard wavelength. All experiments were carefully repeated in triplicate models. The inhibition rate of cell growth (the percentage of cytotoxicity) was expressed according to the following equation **1** [34].

Proliferation Rate (PR) = B/A*100……..Eq (**1**)

Where, A = an optical density of untreated wells, and B = an optical density of treated wells.

2.6. Analysis of static data

Mean ± SEM is utilized to express the obtained data. GraphPad (Prism 6.0 software) is used to analyze the data. Furthermore, the values of $*p < 0.05$ were aquired as a statistical significant.

3. Results and discussion

3.1. Chemistry

The conversion step of the synthesized imines (**4a-c**) to 1, 3, 4-oxadiazole molecules offer an efficient route in organic chemistry towards the synthesis of novel 1, 3, 4-oxadiazole systems bearing different groups with pronounced significant biological properties. In general, the common method to synthesize imine compounds as an essential key focuses on using a condensation reaction of both appropriate aldehydes or ketones and amines is gone with Schiff reaction. Thus, we introduce the synthesis of three novel 1, 3, 4-oxadiazole derivatives (**5a-c**) *via* a cyclization reaction using a cyclized agent. The synthesized imines (**4a-c**) were treated with acetic anhydride to give the target compounds. Furthermore, given the presence of a chiral center at the 2 position ring of 1, 3, 4-oxadiazole derivatives (**5a-c**) confirmed these are racemic as indicated using ^{1}HNMR and HPLC analyses.

In terms of the identification of chemical structures for the synthesized 1, 3, 4-oxadiazole derivatives (**5a-c**), this can be based on using FT-IR and ^{1}H, ^{13}C NMR, and Mass spectra. In the FT-IR spectra (**Figs. 2-4**), the essential vibrational frequencies are observed in the regions 3421-3228 cm^{-1} assigned to O-H. C-H Aromatic stretching are found in the regions 3012-311 cm^{-1}, while the C-H aliphatic stretching are in the regions 2862-2924 cm^{-1}. The strong bands relate to vibrational stretching at the regions 1694–1722 cm^{-1} are corresponded to the carbon of carbonyl groups (C=O). In the ^{1}H NMR spectra (**Figs. 5-7**), the synthesized 1, 3, 4-oxadiazole derivatives (**5a-c**) showed two distinctive signals that confirm the formation of oxadiazole ring. The first one at chemical shifts δ 2.25–2.31 ppm assigned to the methyl of acetyl groups connected with the oxadiazole uncle. The second on chemical shifts δ 7.06–712 ppm assigned to proton of a chiral center at the 2 position ring of oxadiazole uncle. Singlet to triplet singles at the chemical shifts δ 6.71–8.18 ppm returned to the aromatic protons resonance (Aromatic-CH). Furthermore, the essential chemical shifts δ that confirmed the formation of oxadiazole

ring can be seen in the ^{13}C NMR spectra (**Figs. 8-10**). For the 1, 3, 4-oxadiazole derivatives (**5a-c**), the chemical shifts δ are noted at 20.7–21.8 ppm (C, CH_3-acetyl), 92.3-92.5 (CH, oxadiazole ring), 113.4–141.7 ppm (aromatic carbons), and 166.5–168.9 ppm (C=O acetyl groups). In addition, EI-Mass spectra for the synthesized derivatives show that the combined with the corrected values of *m/z* (%) are identical with their proposed structures.

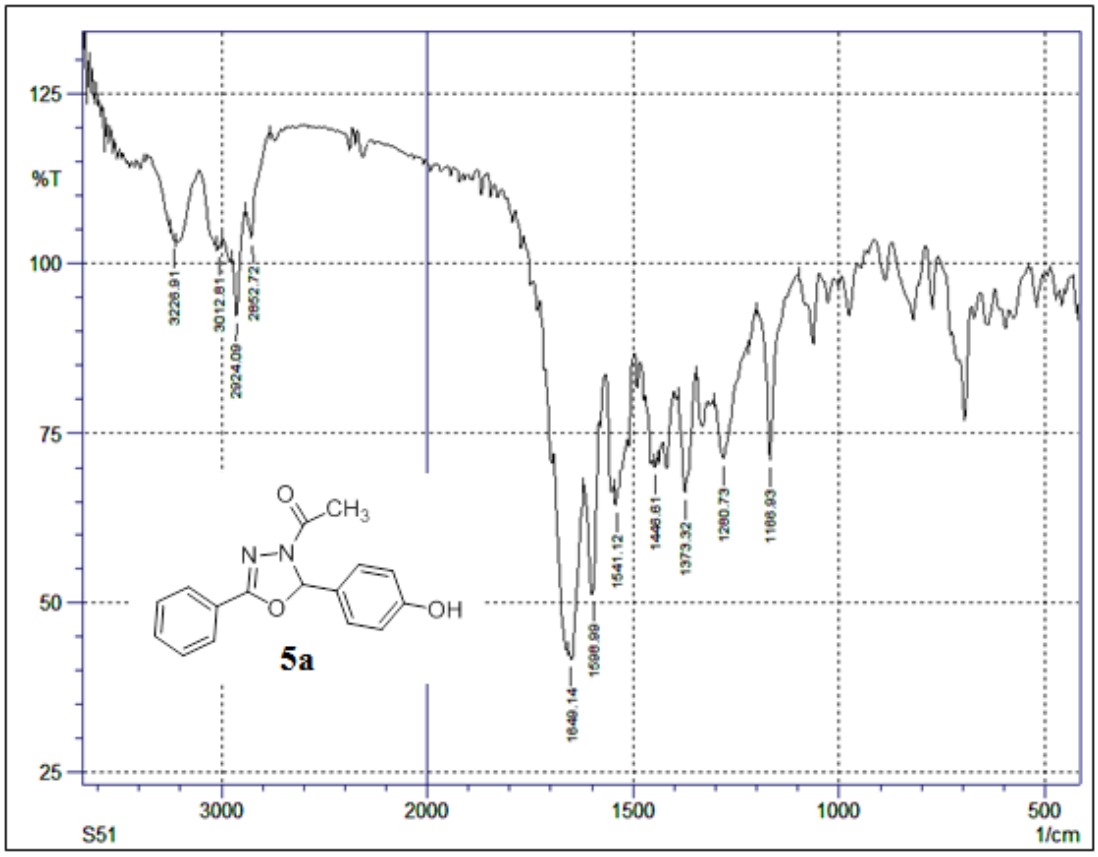


Fig. 2. FT-IR spectrum of the derivative (**5a**).

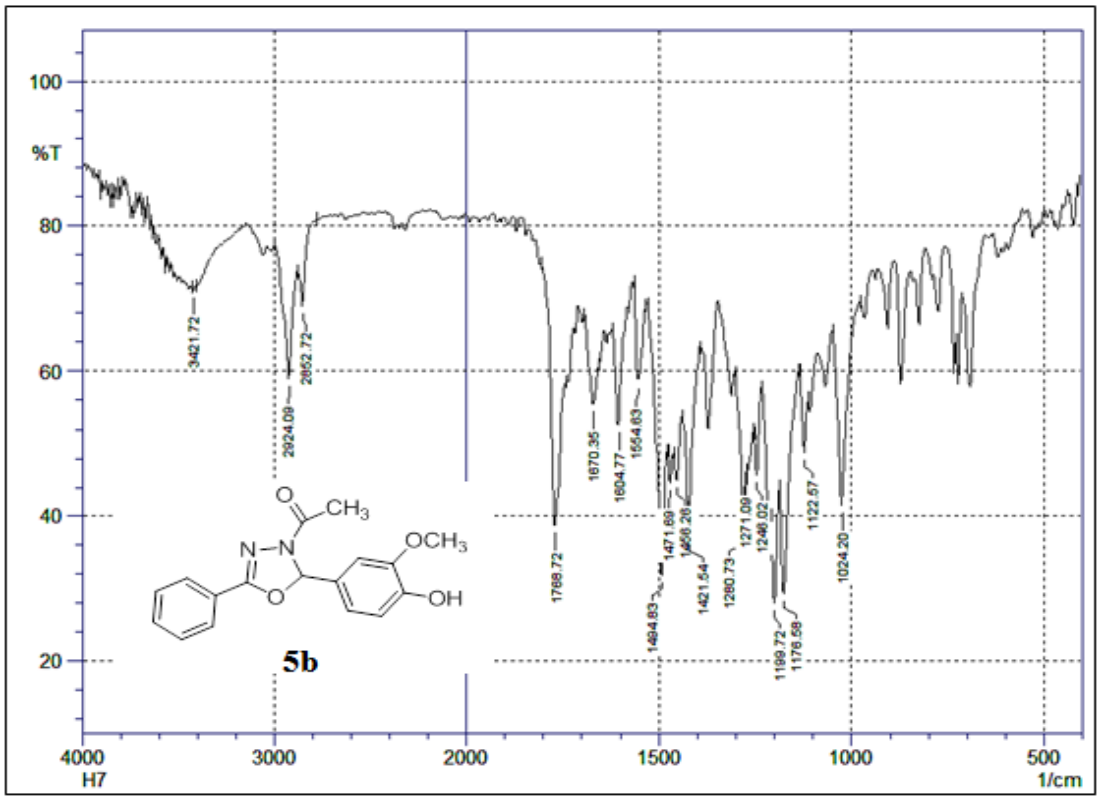


Fig. 3. FT-IR spectrum of the derivative (**5b**).

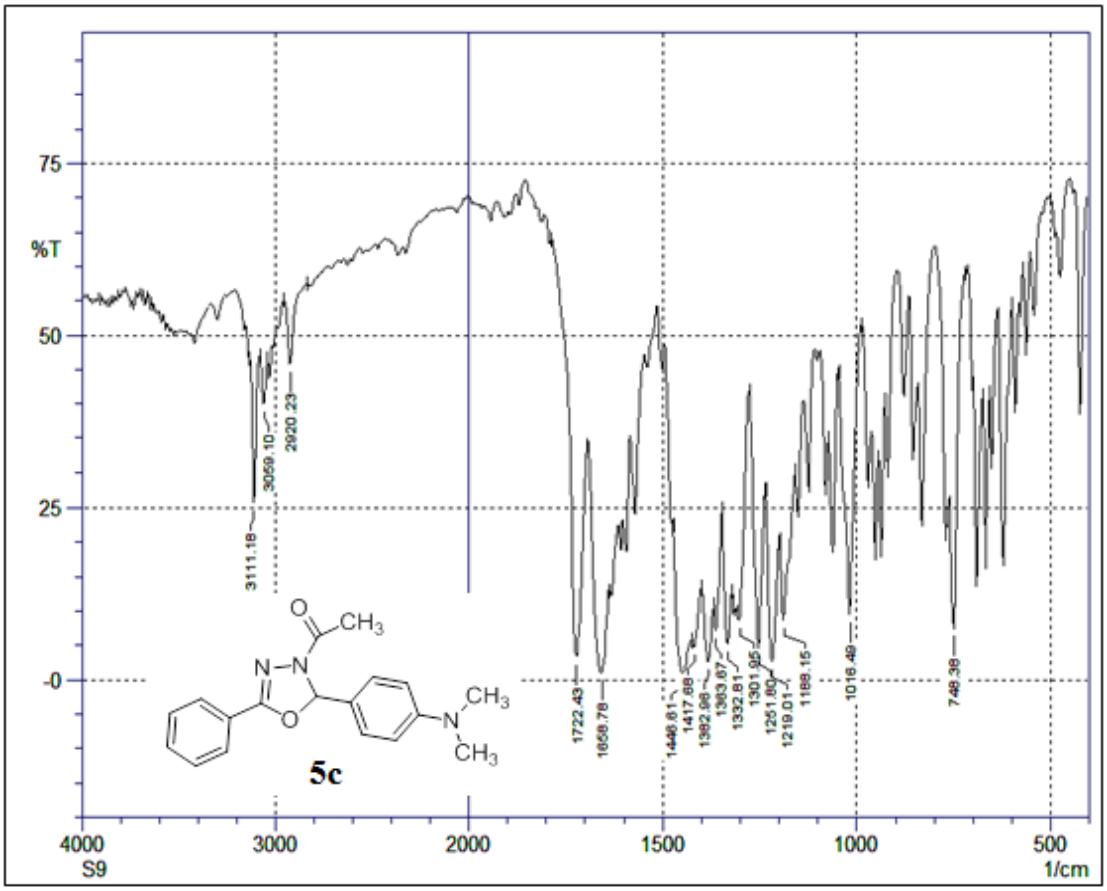


Fig. 4. FT-IR spectrum of the derivative (**5c**).

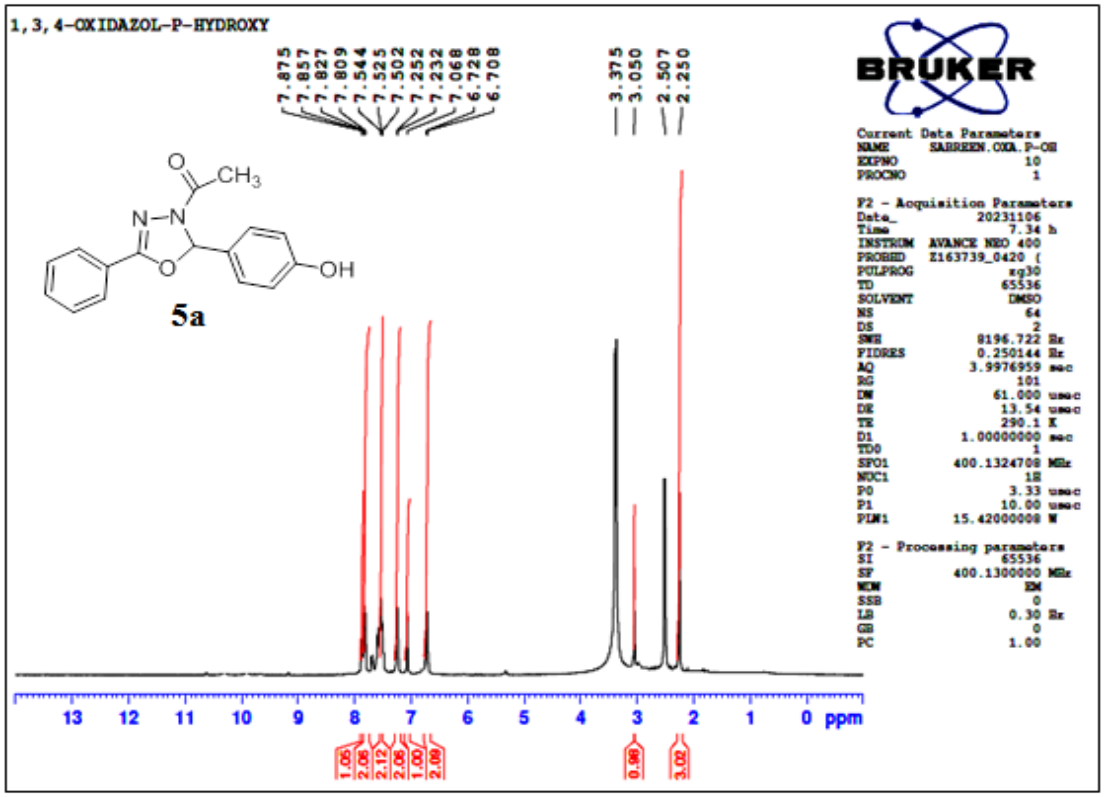


Fig. 5. ^{1}HNMR spectrum of the derivative (**5a**).

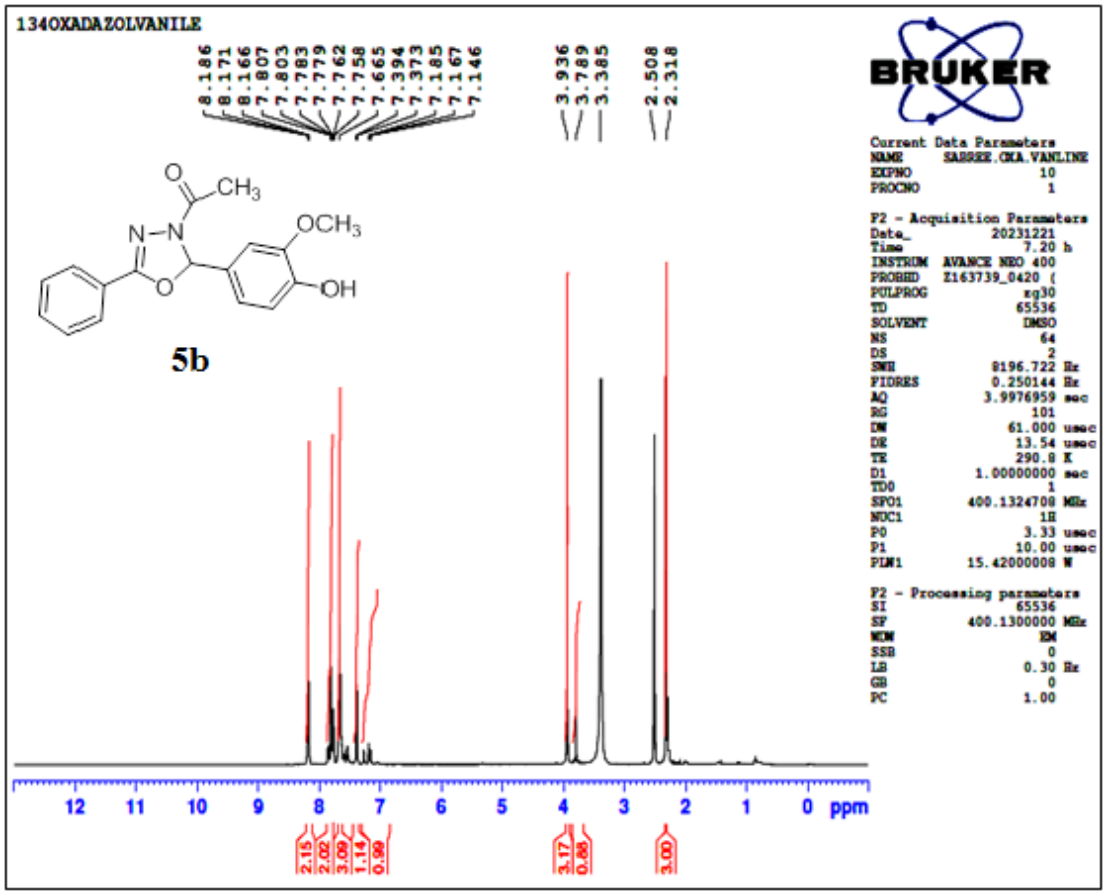


Fig. 6. ^{1}HNMR spectrum of the derivative (**5b**).

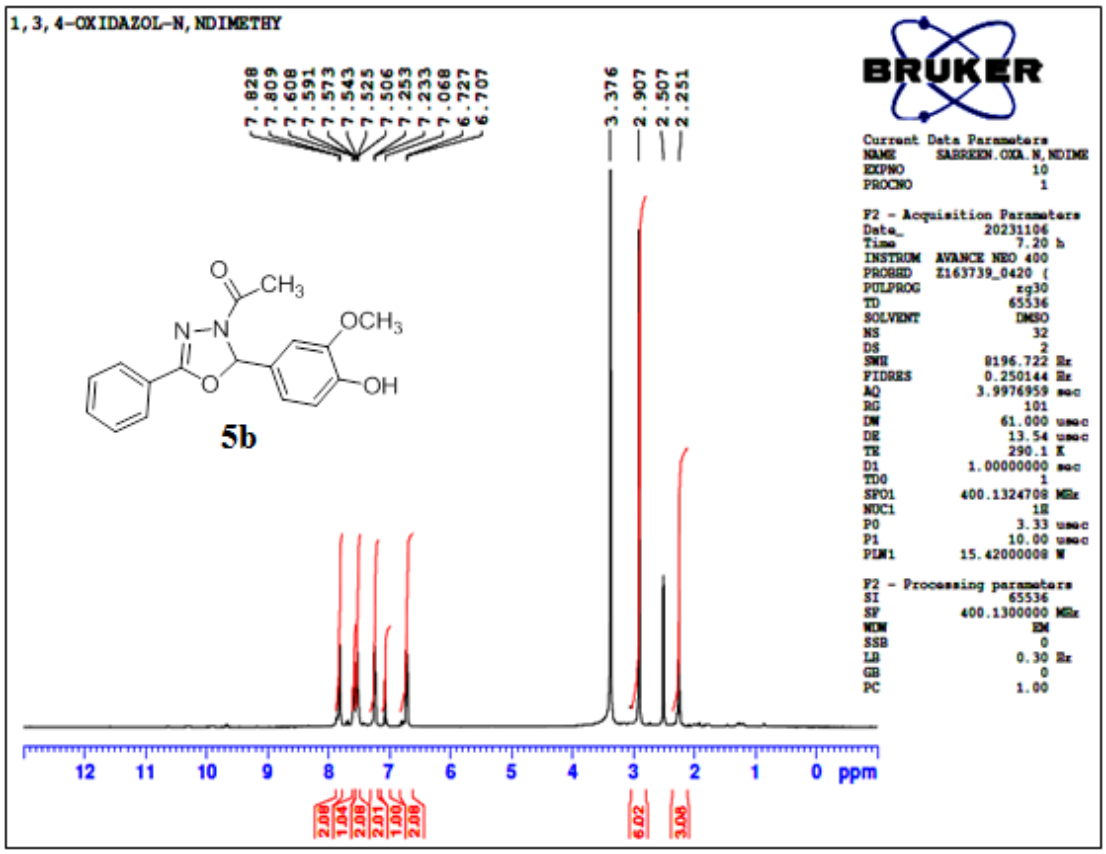


Fig. 7. ^{1}HNMR spectrum of the derivative (**5c**).

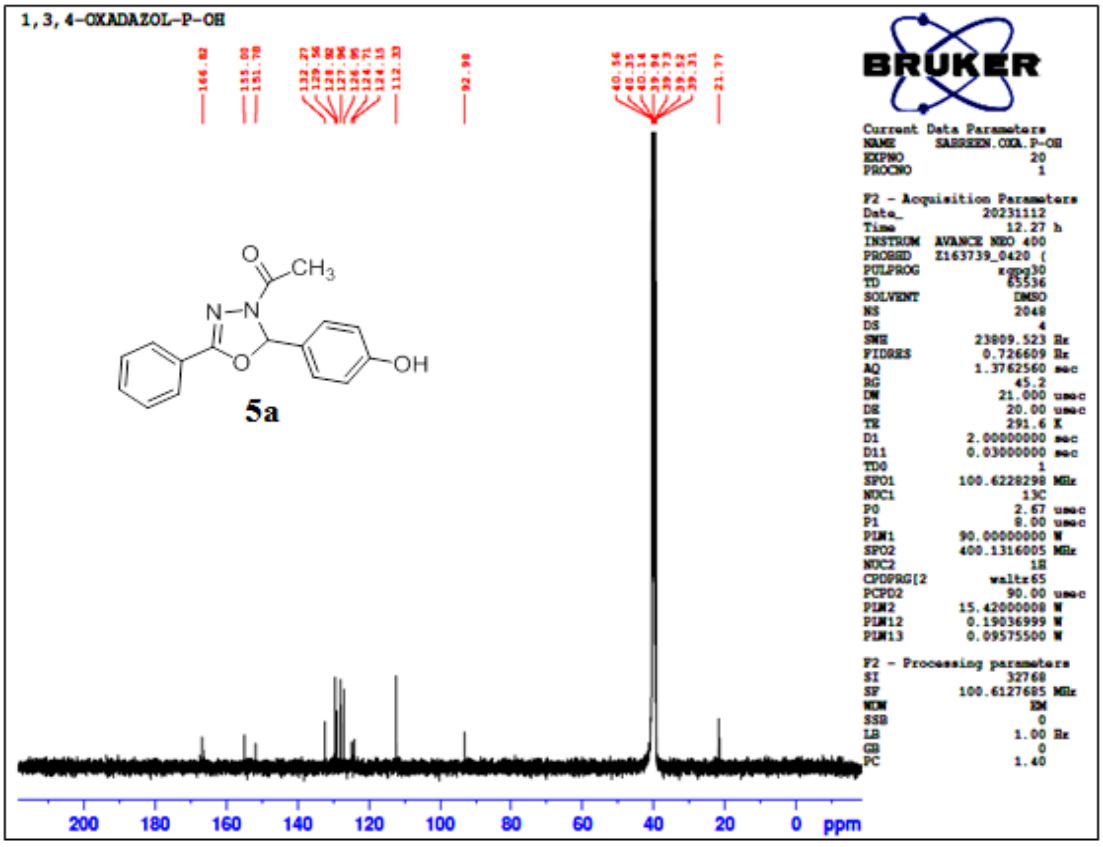


Fig. 8. ^{13}CNMR spectrum of the derivative (**5a**).

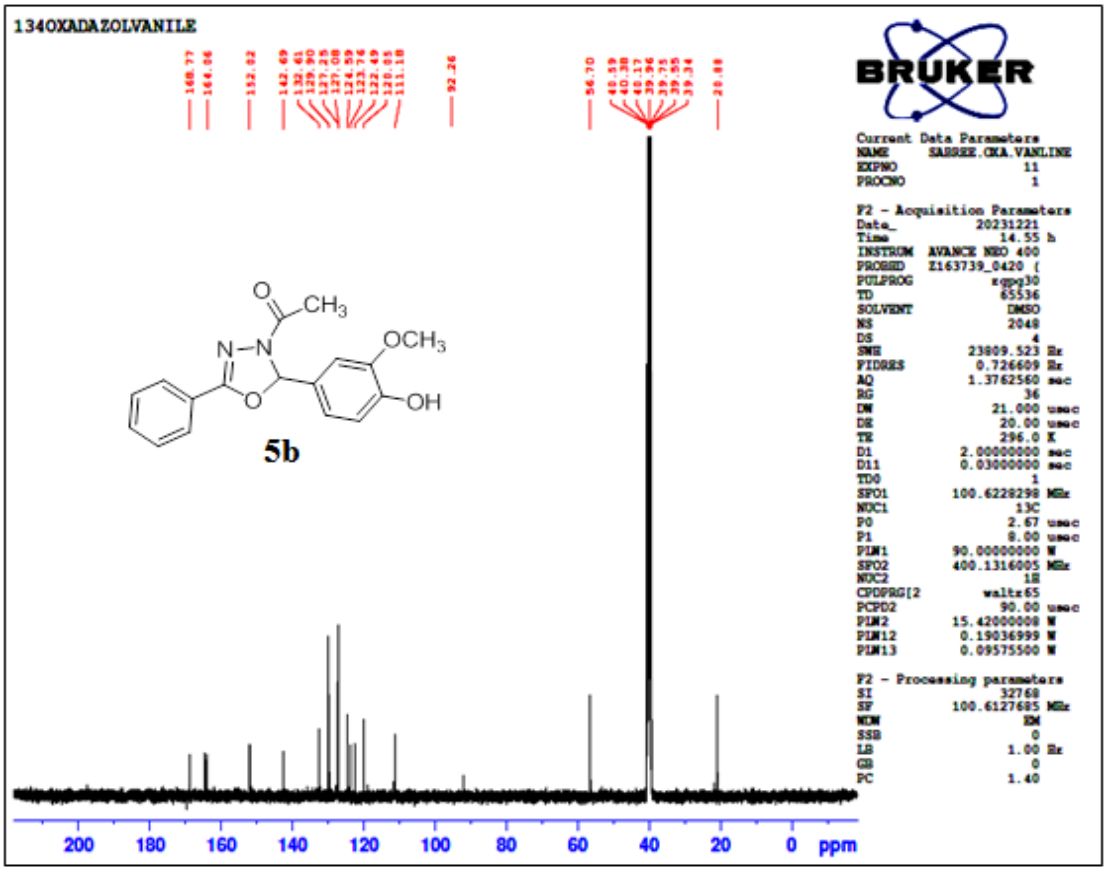


Fig. 9. ^{13}CNMR spectrum of the derivative (**5b**).

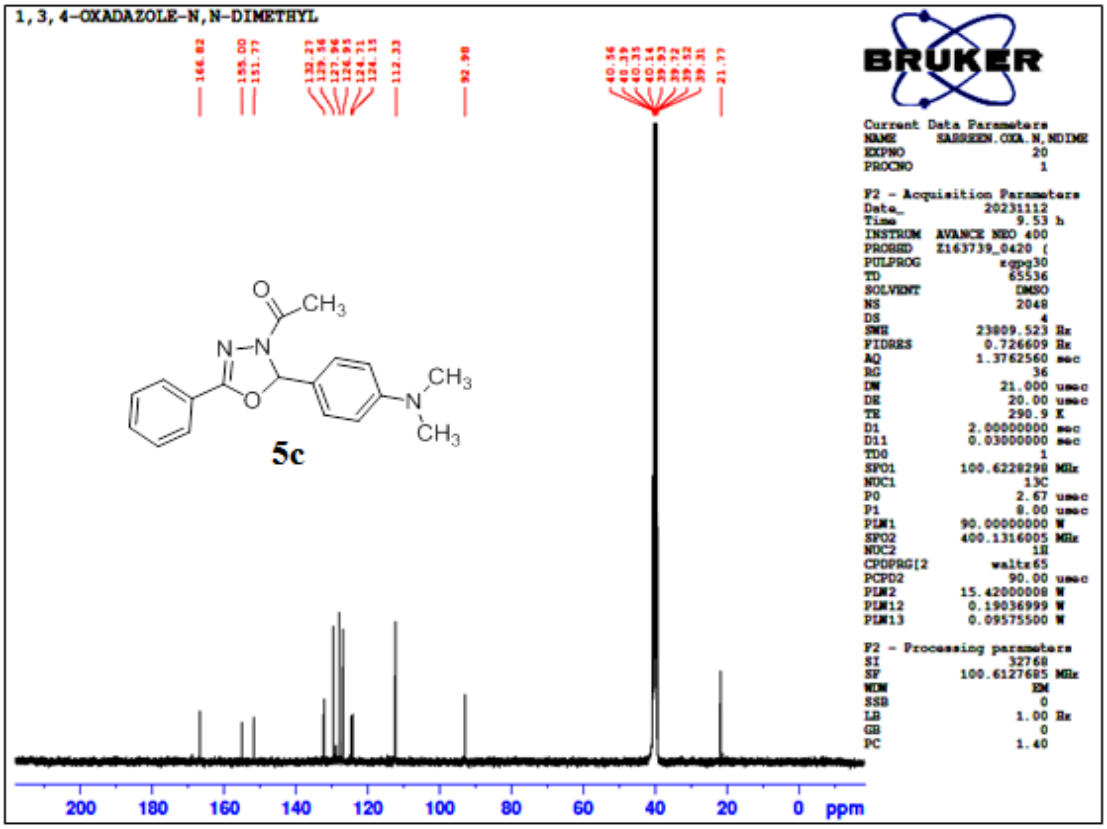


Fig. 10. ^{13}CNMR spectrum of the derivative (**5c**).

3.2. Biological and cytotoxic evaluation

1, 3, 4-Oxadiazoles and their derivative are found to be as important class of heterocyclic compounds to show large diversified activities due to the oxadiazole molecule offers fascinating properties as it possesses a –N=C–O– linkage with capability to interact with bio-targets, ability to form hydrogen bonding, and stability in aqueous medium, enhancing charge transfer interaction through π-π stacking [35, 36]. Thus, this molecule is used as a pharmacophore due to the above favorable precedents. In sights of these findings, we envisage that the modification of 1, 3, 4-oxadiazole with some diverse moiety in order to investigate potential increase of their biological profiles to a large extent [37, 38].

In the first step of cytotoxic test, the purity of the 1, 3, 4-oxadiazole derivatives (**5a-c**) was confirmed using NMR study. Then, primary cancer assays were performed on a test panel of SKGT-4 cells (cancerous cells) and esophageal cells (Vero cells). The data are collected for each tested oxadiazole derivative as a percentage of treated cells compared to untreated cells. Then the results of each oxadiazole derivative were collected and examined as a percentage in terms of the number of treated cells compared to untreated cells. After that, the evaluation of cell viability of SKGT-4 cells was done by the MTT test for the treated 1, 3, 4-oxadiazole derivatives (**5a-c**). The cell viability as a percentage was evaluated by spectrophotometry against the standard solution that was not treated as shown in **Fig. 11**.

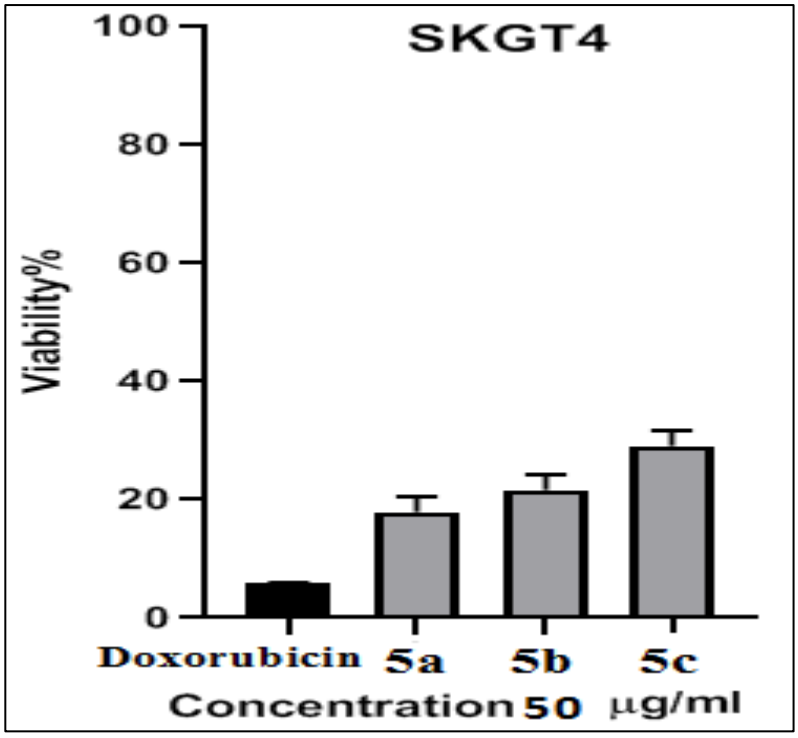


Fig. 11 shows the toxic effect of the prepared 1, 3, 4-oxadiazole derivatives (**5a-c**) on the number of esophageal SKGT-4 cells (cancer cells). At conditions of 37 °C and 5% humidity in the presence of CO_2 for 48 hours and at a concentration of 50 µg/mL.

After calculation of the live cells percentage and the IC_{50} values (concentration of compounds to inhibit 50% of the total cells), the Graph-Pad (8.1) program was used to calculate the IC_{50} values for the 1, 3, 4-oxadiazole derivatives. It was noted that the good effectiveness of these derivatives against. Inhibition of live growth of SKGT-4 cells. After 48 hours of incubation with 50 µg/mL of each derivative, the results demonstrated that the 1, 3, 4-oxadiazole derivatives (**5a-c**) provide high significant cytotoxicity and a high inhibitory ability towards the growth of esophageal cancerous cells. By comparing the IC_{50} values of the effective 1, 3, 4-oxadiazole derivatives (**5a-c**) after evaluating their toxic activity against SKGT-4 cancer cells, it was noted that most of these derivatives have an important role in inhibiting the growth of live cancer cells (SKGt-4). After 48 hours of treating the cells with 50 µg/mL of each derivative, the results indicated that most of the derivatives showed the best effective activity and offered significant inhibitory activity against the growth of cancer cells (SKGt-4), as shown in **Table 1**.

Table 1. Toxic effectiveness values for 1, 3, 4-oxadiazole derivatives (**5a-c**) on both esophageal Vero and SKGT-4 cells. The toxic activity of the cells was evaluated by exposure for 48 hours. And that all data express the average deviation values obtained from the dose response curves in three separate experiments

Compounds	IC_{50} (µg/mL) on cancer cells	IC_{50} (µg/mL) on normal cells
	SKGT-4	Vero

5a	18.2 ± 0.13	>120
5b	24.2 ± 0.13	>120
5c	52.2 ± 0.13	98.2± 0.12
Doxorubicin	2.1 ±0.02	ND

From the above **Table 1**, 1, 3, 4-oxadiazole derivatives (**5a-c**) showed a significant cellular toxicity for both the derivatives (**5a** and **5b**), which showed good toxic activity against cancer cells (SKGT-4). Compared to the standard reference (Doxorubicin, at the value of IC_{50} = 2.1 ± 0.02 µg/mL, the derivative (**5a**) showed higher effective in terms of cellular toxicity with IC_{50} value 18.2 ± 0.13 µg/mL while the derivative (**5b**) with IC_{50} value 24.2 ± 0.13 µg/mL. Also the moderate toxic effect with IC_{50} value of 52.2 ± 0.13 µg/mL shown by the derivative (**5c**). This can be explained on dependence on its natural structures and environmental electronic mediums which affects either compound protein reactions or biological availability.

Conclusion

In this research project, an efficient procedure for the preparation of novel 1, 3, 4-oxadiazole derivatives (**5a-c**) has been developed. These derivatives were synthesized through reaction of investigated molar ratios of the synthesized imines as a key intermediate with acetic anhydride to afford the target derivatives in satisfactory yields. By applying MTT assay to evaluate their antiproliferative efficacy against esophageal cells (SKGT-4). The results revealed that these derivatives displayed potent *in vitro* activity with potential inhibition, showing IC_{50} values ranging from 18.2 ± 0.13 to 52.2 ± 0.13 µg/mL in comparison to Doxorubicin (IC_{50}= 2.1±0.02 µg/mL) as a standard drug. Our work holds promising anti-esophageal cancer compounds with the devleopment of further clinical investigations in drug discovery.

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