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Synthesis, Characterization and Antimicrobial Evaluation for New Esters Derivatives Containing Two 1, 3, 4-Oxadiazole Units

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Abstract

This work includes the synthesis of new ester compounds containing two 1,3,4-oxadiazole rings, **15$_{a-c}$ and 16$_{a-c}$**. This was done over seven steps, starting with *p*-acetamido-phenol **1** and 2-mercaptobenzoimidazole **2**. The structure of the products was determined using FT-IR, ^{1}H NMR, and mass spectroscopy. The evaluation of the antimicrobial activities of some prepared compounds was achieved against four types of bacteria (two types of gram-positive bacteria; *Staphylococcus aureus and Bacillus subtilis*, and two types of gram-negative bacteria, *Pseudomonas aeruginosa and E. Coli*), as well as against one types of *fungus* (C. albino). The results show moderate activit against the study bacteria, and the theoretical analysis of the toxicity demonstrates non-toxicity with acute oral human toxicity. Also, the toxicity of these compounds in rats was weak. A molecular modelling study explains that these compounds possess good binding with lung cancer proteins.

Keywords: 1,3,4-Oxadiazole unite, Oral human toxicity, Molecular modelling, LD_{50}.

تحضير و تشخيص و تقييم الفعالية البيولوجية لمشتقات استرية جديدة حاوية على وحدتين 1,3,4- اوكساديازول

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الخلاصة

يتضمن هذا العمل تحضير مركبات استر جديدة تحتوي على حلقتين 1،3،4-أوكساديازول . تم إجراء ذلك من خلال سبع خطوات ، بدءًا من (p-acetamido-phenol) و(2-mercaptobenzoimidazole) تم تشخيص المركبات المحضرة باستخدام الطرق الطيفية FT-IR و NMR-H ^{1} و Mass. تم تقيم الفعالية البايلوجية لبعض المركبات المحضرة ضد أربعة أنواع من البكتيريا (نوعان من البكتيريا موجبة الجرام ؛ المكورات العنقودية الذهبية و *Bacillus subtilis* ، ونوعين من البكتيريا سالبة الجرام؛ *Pseudomonas aeruginosa* و *E. Coli*) أيضًا. مقابل نوع واحد من الفطريات (*C. albino*). تظهر النتائج أنشطة معتدلة ضد بكتيريا المدروسة ، والتحليل النظري للسمية يظهر عدم سمية المركبات المحضرة للإنسان. كما أن سمية

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هذه المركبات في الفئران كانت ضعيفة كما تم تحديد نصف الجرعة القاتلة . توضح دراسة النمذجة الجزيئية أن هذه المركبات تمتلك ارتباطًا جيدًا ببروتينات سرطان الرئة.

1. Introduction

The synthesis of heterocyclic compounds is one of the important fields in organic synthesis that attracts the interest of many researchers. This is due to the fact that it contains atoms like nitrogen, oxygen, and sulphur in its composition, which gives it biological and medicinal importance. Oxadiazole is a pentacyclic ring that contains an oxygen atom and two nitrogen atoms. A number of its isomers can be distinguished in the composition of some drugs, and this is due to It has a wide pharmacological spectrum, including antibacterial [1], anti-tubercular agents [2], antiviral agents [3], inhibition of cyclooxygenase (enzyme COX) [4], anti-inflammatory [5], antidepressant [6], anticonvulsant [7], and anticancer [8]. 1,3,4-Oxadiazoles are a purposive ring structure in the field of medicinal chemistry that provides diversity in structural composition and diversity in the atoms that contain them in their composition for the synthesis of molecules. The oxadiazole nucleus sometimes acts as a bridge connecting the aromatic ring to improve molecular stereotypes or a bio-isostearate with compounds containing a carbonyl group, such as esters, amides, and carbamates. The ability of the oxadiazole ring to overlap and its stability with vital goals through hydrogen bonding and interactions justify interest in the preparation of bioactive particles that contain these nuclei [9,10]. Our work will include the synthesis of new derivatives containing two 1,3,4-oxadiazole rings. These compounds will be tested for their biological activity against microorganisms. The theoretical toxicity will be predicted using computational methods, and molecular modeling has been studied to verify the susceptibility of the anti-cancer activity of some of these compounds to binding with the protein 7r7k in lung cancer.

2. Experimental part

2.1. Materials and apparatus

All chemicals were obtained from commercial sources and used directly, without further purification. The melting point was measured using Gallen Kamp uncorrected melting point with open capillaries, FT-IR spectral data were recorded using a Shimadzu 1800 FT-IR spectrometer (KBr disk in cm^{-1}), ^1H NMR spectral data were measured using a Burker NMR 400 MH_Z spectrometer. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal standard or deuterated DMSO in ^1H NMR as a reference. Mass spectrometry data were recorded using an Agilent G1956LC MSD spectrometer in Iran.

2.2. Synthesis of ethyl 2-(4-acetamidophenoxy)acetate (**3**)

To a solution of *p*-acetamidophenol 1 (0.01 mol) and ethyl α-chloroacetate (0.01 mol) in dry acetone (15 mL), K_2CO_3 (0.01 mol) was added before refluxing for 6 hours. When the reaction had completed, the mixture was cooled and placed on crushed ice. The white solid product was filtrated and recrystallized from EtOH. Off-white powder, m.p. 78 ^oC [11], yield 85%. FT-IR (υ, cm^{-1}): 3387 (N-H), 3002 (C-$H_{aro.}$), 2939, 2866 (C-H_{ali}), 1743 (C=O_{ester}), 1681 (C=O_{amide}), 1523 (C=C_{aro}), 1242 and 1211 (C-O-C), 1315 (C-N), 829 (*p*-substitution).

2.3. Synthesis of ethyl-2-(1H-benzo[d]imidazole-2-ylthio)acetate (**4**)

A mixture of 2-mercaptobenzoimidazol **2** (0.001 mol), ethyl α-chloroacetate (0.001 mol) and fused sodium acetate (0.003 mol) in absolute ethanol (5 mL) was stirred for 4 hours. The reaction mixture was then cooled, poured onto ice water, and the resulting solid product was filtered, dried, and recrystallized from EtOH [12,13]. Light yellow powder, m.p. 86-88 °C,

yield 93%. FT-IR (υ, cm^{-1}): 3125 (N-H), 3080 (C-H_{aro}), 2954, 2866 (C-H_{ali}), 1735 (C=O_{ester}), 1601 (C=N), 1504 (C=C_{aro}), 1215 (C-O), 759 (C-S-C).

2.4. General procedure for the synthesis of the hydrazide ester derivatives (**5**,**6**)

Hydrazine hydrate (8 mL, 80%) was added to a solution of ester compounds 3, 4 (0.03 mol) in ethanol absolute (12.5 mL) and refluxed for 5-6 hours. After that, the mixture was cooled by adding crushed ice to it. The solid product was filtered (off-white), dried, and recrystallized from ethanol [11,14].

2.4.1. N-(4-(2-Hydrazinyl-2-oxoethoxy)phenyl)acetamide (**5**)

Light yellow powder, m.p. 155-157 °C, yield 89%, FT-IR (*v*, cm^{-1}), 3340-3190 (NH_2, N-$H_{sec.\ amide}$), 3055 (C-$H_{aro.}$), 2924, 2830 (C-$H_{ali.}$), 1643 (C=O), 1570 (C=$C_{aro.}$), 1357 (C-N), 1230 (C-O-C).

2.4.2. 2-(1H-Benzo[d]imidazol-2-ylthio)acetohydrazide (**6**)

Pale yellow powder, m.p. 78-80 °C, yield 89%, FT-IR (*v*, cm^{-1}), 3345-3184 (NH_2, N-H), 3020 (C-$H_{aro.}$), 2862, 2820 (C-$H_{ali.}$), 1651 (C=O), 1616 (C=N), 1554 (C=$C_{aro.}$), 732 (S-C).

2.5. General procedure for the preparation of the 1,3,4-oxadiazole derivatives (**7**,**8**)

A mixture of hydrazine 5, 6 (0.01 mol) and 4-aminobenzoic acid (0.01 mol) in phosphorus oxychloride (5 mL) was refluxed for 6 hours. When the reaction had completed, the reaction mixture was poured onto crushed ice and neutralized with a solution of sodium bicarbonate. The solid crude material was filtrated (yellow color), washed, dried, and recrystallized from ethanol [15].

2.5.1. N-(4-((5-(4-Aminophenyl)-1,3,4-oxadiazol-2-yl)methoxy)phenyl)acetamide (**7**)

Yellow powder, m.p. 258-260 °C, yield 75%, FT-IR (*v*, cm^{-1}), 3383-3200 (NH_2, N-H), 3070 (C-$H_{aro.}$), 2839 (C-H $_{ali.}$), 1678 (C=$O_{sec.amide}$), 1647 (C=N), 1600 (C=$C_{aro.}$), 1323 (C-N), 1249(C-O)., 846 (*p*-substitution**)** [16].

2.5.2. 4-(5-((1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,4-oxadiazol-2-yl)aniline (**8)**

Greenish brown powder, m.p. 190-192 °C, yield 75%, FT-IR (*v*, cm^{-1}), 3340-3221 (NH_2, N-$H_{sec.\ amine}$), 3020 (C-H_{aro}.), 2943, 2831 (C-$H_{ali.}$), 1645 (C=N), 1600 (C=$C_{aro.}$), 1257 (C-O), 744 (C-S), 837 (*p*-substitution**)** [17].

2.6. General procedure for the synthesis of the carboxylic acids (**9**,**10**)

A solution of 1,3,4-oxadiazole derivative (75 mmol) in water (120 mL) and concentrated HCl (40 mL) was cooled to 5 °C and diazotized with a solution of $NaNO_2$ (91 mmol) in H_2O (35 mL). After 20 minutes, a solution of benzoic acid (75 mmol) in acetone (50 mL) and a solution of $CuCl_2.2H_2O$ (23 mmol) in water (25 mL) were added to the reaction mixture and stirred at room temperature for 48 hours [18]. After dilution with water (500 mL), the pale precipitate was filtered, washed with cooled water, and recrystallized from ethylacetate to give the desired products **9** and **10**.

2.6.1. 4'-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-yl)biphenyl-4-carboxylic acid (**9**)

Brown powder, m.p. 118-120 °C, yield 90%, FT-IR (υ, cm^{-1}): 3500-2650 (N-H overlap with O-H), 3056 (C-H_{aro}), 2835 (C-H_{ali}), 1699 (C=O_{acid}), 1670 (C=O_{amide}), 1641 (C=N), 1335 (C-N), 1249 (C-O). ^{1}H NMR (400 MHz, DMSO-d_6, δ_H, ppm), 10.71 (s, O-H), 8.19-7.15 (m,

12H, Ar-H), 4.67 (s, 2H, O-CH_2), 2.1 (s, 3H, CH_3). Mass spectrum *m/z*, 385, 309, 233, 239,266, 105, 77, 51.

2.6.2.*4'-(5-((1H-Benzo[d]imidazole-2-ylthio)methy)-1,3,4-oxadiazol-2-yl)biphenyl-4-carboxylic acid* (**10**)

Dark brown powder, m.p. 180 -182 ^oC, yield 90%, FT- IR (*v*, cm^{-1}), 3448-2500 (O-H_{acid}), 3421 (N-H), 3058 (C-$H_{aro.}$), 2979, 2933 (C-$H_{ali.}$), 1691 (C=O_{acid}), 1625 (C=N), 1600 (C=$C_{aro.}$), 1245 (C-O), 745 (C-S). ^{1}H NMR (400 MHz, DMSO), δ 10.8 (s, O-H), 8.24-6.9 (m, 12H, Ar-H), 4.78 (s, 2H, S-CH_2). Mass spectrum *m/z* 313, 163, 122, 105, 77, 51.

*2.7. General procedure for the synthesis of the esters (**11**, **12**)*

A solution of acid 9, and 10 (0.246 mol) in absolute methanol (101 mL) with concentrated sulphuric acid (2.6 mL) was refluxed for 6 hours. The resulting solution was cooled and placed on crushed ice, neutralized with sodium bicarbonate solution (10%), washed several times with water, dried, and recrystallized from ethylacetate.

*2.7.1. Methyl-4'-(5-((4-acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-carboxylate (**11**)*

Brown powder, m.p. 120-122 ^oC, yield 75%, FT-IR (*v*, cm^{-1}), 3340-3217 (N-$H_{sec.\ amide}$), 3062 (C-$H_{aro.}$), 2947, 2898 (C-$H_{ali.}$), 1716 (C=O_{ester}), 1654 (C=$O_{sec.\ amide}$), 1632 (C=$N_{oxadiazole}$), 1602 (C=C), 1276 and 1236 (C-O). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm) 9.01 (1, H, $NH_{sec.amide}$), 8.12-7.08 (m, 12H, Ar-H), 5.10 (2H, CH_2), 3.95 (3H, $COOCH_3$), 2.22 (3H, N-CH_3).

*2.7.2. Methyl 4'(5-(((1H-benzo[d]imidazole-2-yl)thio)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-carboxylate (**12**)*

Brown powder, m.p. 136-138C^o, yield 75%, FT-IR (*v*, cm^{-1}), 3433 ($NH_{sec.\ amine}$), 3371 ($CH_{aro.}$), 2947 (C-$H_{ali.}$), 1716 (C=O_{ester}), 1631 (C=N), 1600 (C=$C_{aro.}$), 1276 and 1244 (C-O), 740 (S-C). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm), 8.01 (s, 1H, NH), 6.56- 7.74 (m, 12H, Ar-H), 4.23 (s, 2H, CH_2), 3.73 (s, 3H, CO_2CH_3).

*2.8. General procedure for the synthesis of the hydrazide derivatives (**13**,**14**)*

Hydrazine hydrate (8 mL, 80%) was added to the solution of ester **11** and **12** (0.03 mol) in ethanol (12.5 mL) and refluxed for 5-6 hours. The resulting mixture was then cooled and poured over crushed ice. The solid crude material (of an off-white color) was filtered, dried, and recrystallized from EtOH [11,14].

*2.8.1. N-(4-((5-(4'-(Hydrazinecarbonyl)-[1,1'biphenyl]-4-yl)-1,3,4-oxadiazol-2-yl)methoxy) phenyl)acetamide (**13**)*

Shiny brown crystals, m.p. 84-86 ^oC, yield 87%, FT- IR (*v*, cm^{-1}), 3302-3205 (NH_{2amine} and N-H_{amide}), 3048 (C-$H_{aro.}$), 2951, 2893 (C-$H_{ali.}$), 1650 (C=$O_{sec.\ amide}$), 1635 (C=$N_{oxadiazole}$),1597 (C=$C_{aro.}$), 1280 and 1234 (C-O). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm) 8.01 (s, 1H, N-H), 7.92 (s, 1H, NHCO), 7.90-6.45 (m, 12H, Ar-H), 5.63 (s, 2H, CH_2), 2.29 (s, 2H, CH_2).

*2.8.2. 4'-(5-(((1H-benzo[d]imidazole-2-yl)thio) methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-carbohyd razide (**14**)*

Shiny brown crystals , m.p. 76-78 ^oC, yield 90%, FT-IR (*v*, cm^{-1}), 3309-3194 (NH_{2amine} and N-H_{amide}), 3062 (C-$H_{aro.}$), 2947 (C-$H_{ali.}$), 1695(C=O), 1654 (C=$N_{imidazole}$), 1635 (C=$N_{oxadiazole}$), 1566 (C=$C_{aro.}$), 1276 and 1225 (C-O). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm) 10.1 (s, 1H,

CONH), and 9.4 (s, 1H, NH), 6.6-7.83 (m, 12H, Ar-H), 5.63 (s, 2H, NH_2), 4.43 (s, 2H, CH_2), 2.17 (s, 3H, N-CH_3).

2.9. General procedure for the preparation of the di-1,3,4-oxadiazol derivatives ***15$_{a-c}$*** *and* ***16$_{a-c}$***

A mixture of hydrazine 13, 14**,** (0.01 mol) and carboxylic acids 4-(benzoyloxy)benzoic acid, 4-(4-methoxybenzoyl)oxy)benzoic acid, and 4-(4-chlorobenzoyl)oxy)benzoic acid (0.01 mol) in $POCl_3$ phosphorus oxychloride (5 mL) was refluxed for 6 hours. When the reaction had completed, the reaction mixture was poured onto crushed ice and neutralized with a solution of sodium bicarbonate (10%). The solid crude material (yellow color) was filtered, dried, and recrystallized from ethanol [15].

*2.9.1. 4-(5-(4'-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-yl)-1,3, 4-oxadiazol-2-yl)phenyl benzoate, (****15$_a$****)*

Pale yellow powder, m.p. >300 ^oC, yield 92%, FT-IR (ν, cm^{-1}, 3367-3244 (N-$H_{sec.\ amide}$), 3074 (C-$H_{aro.}$), 2927-2858 (C-H $_{ali.}$), 1735 (C=O_{ester}), 1643 (C=$N_{oxadiazole}$), 1508 (C=$C_{aro.}$), 1261 and 1203 (C-O).

*2.9.2. 4-(5-(4'-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-yl)-1, 3,4-oxadiazol-2-yl)phenyl-4-methoxybenzoate (****15$_b$*** *)*

Pale yellow powder, Light beige powder, m.p >300C^o, yield 95%, FT-IR (ν, cm^{-1}), 3352-3240 (N-$H_{sec.\ amide}$), 3070 (C-$H_{aro.}$), 2927-2850 (C-H $_{ali.}$), 1732 (C=O_{ester}), 1659 (C=$N_{oxadiazole}$), 1508 (C=$C_{aro.}$), 1257 and 1203 (C-O). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm) 8.28 (s, 1H, NH), 8.11- 6.48 (m, 12H, Ar-H), 5.12 (s, 2H, CH_2), 3.86 (m, 3H, OCH_3), 2.56 (s, 3H, $COCH_3$).

*2.9.3. 4-(5-(4'-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-yl)1,3, 4-oxadiazol-2-yl) phenyl-4-chlorobenzoate (****15$_c$****)*

Off-white powder, m.p. >300 ^oC, yield 93%, FT- IR (ν, cm^{-1}), 3340 (N-$H_{sec.\ amide}$), 3005 (C-$H_{aro.}$), 2870 (C-$H_{ali.}$), 1739 (C=O_{ester}), 1643 (C=$N_{oxadiazole}$), 1560 (C=$O_{sec.\ amide}$), 1450 (C=$C_{aro.}$), 1291 and 1230 (C-O), 744 (C-Cl).

*2.9.4.4-(5-(4'-(5-(((1H-benzo[d]imidazole-2-yl)thio)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4- yl)-1,3,4-oxadiazol-2-yl)phenyl benzoate (****16$_a$****)*

Pale brown powder, m.p.>300, yield 90%, FT-IR (ν, cm^{-1}), 3313-3213 (N-$H_{sec.\ amine}$), 3062 (C-$H_{aro.}$), 2870 (C-$H_{ali.}$), 1724 (C=O_{ester}), 1658 (C=N), 1604 (C=C), 1265 and 1254 (C-O), 744 (S-C). ^{1}H NMR (400 MHZ, DMSO-d_6, δ_H, ppm), 8.5 (s, 1H, N-H), 8.19-6.99 (m, 17H, Ar-H), 5.53 (s, 2H, CH_2).

*2.9.5.4-(5-(4'-(5-(((1H-benzo[d]imidazole-2-yl)thio)methyl-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl] -4 yl)-1,3,4-oxadiazol-2-yl) phenyl-4-methoxybenzoate (****16$_b$*** *)*

Light pal yellow powder, m.p.>300C^o, yield 90%, FT-IR (ν, cm^{-1}), 3429 (N-$H_{sec.\ amine}$), 2974 (C-$H_{aro.}$), 2880 (C-$H_{ali.}$), 1739 (C=O_{ester}), 1631 (C=N), 1570 (C=$C_{aro.}$), 1257 and 1207 (C-O), 748 (C-S).

*2.9.6.4-(5-(4'-(5-(((1H-benzo[d]imidazole-2-yl)thio)methyl)-1,3,4-oxadiazol-2-yl)-[1,1'-biphenyl]-4-yl)-1,3,4-oxadiazol-2-yl) phenyl-4-chlorobenzoate (****16$_c$*** *)*

Off-white powder, m.p. >300 ^oC yield 90%, FT- IR (ν, cm^{-1}), 3402 (N-$H_{sec.\ amine}$), 2985 (C-$H_{aro.}$), 2860 (C-$H_{ali.}$), 1739 (C=O $_{ester}$), 1647 (C=N), 1597 (C=$C_{aro.}$), 1319 and 1253 (C-O), 744 (C-S), 771 (C-Cl).

2.10. Evaluation of anti-microorganism activity

The disc spread method was used for the examination of bioactivity for synthesis compounds against two types of gram-positive bacteria (*Staphylococcus aureus and Basillus subtilis*) and two types of gram-negative bacteria (*Pseudomonas aeruginosa and E. Coli*), as well as against *C. albino* fungi [19].

2.11. Toxicity prediction

To predict the toxicity of the prepared compounds, the following program was used: Pro-Tox-II: Prediction of Toxicities of Chemicals Online https://tox-new.charite.de, , [20,21,22], and the prediction of the toxicity of a synthetic compound with rats and the determination of LD50 online http://www.way2drug.com/passonline, [23,24]

2.12. The molecular docking

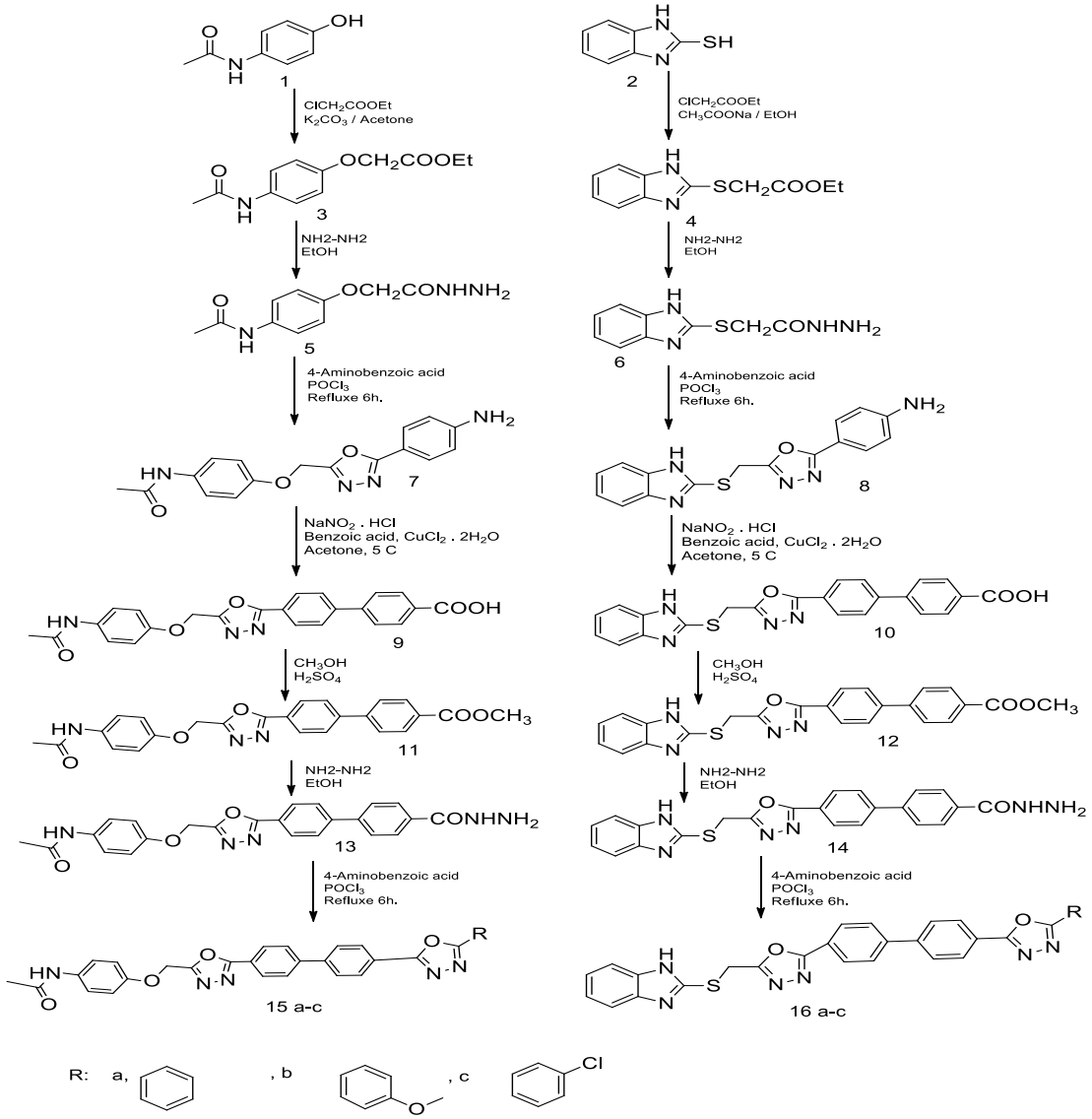
The X-ray crystallographic structure for the protein that was used in this research (ID: 7r7k) was taken from the Protein Data Bank at www.rcsb.org. The most stable positions of the prepared compounds were calculated (energy minimization), water and ligand molecules have been removed from the protein. Docking has been carried out using Molegro 6. The docking score and some important parameters were evaluated.

3. **Results and discussion**

3.1. Chemistry

In this work, a new series of esters containing a 1,3,4-oxadiazole ring was prepared as shown in Scheme 1. This was done using *p*-acetamidophenol **1** and 2-mercaptobenzoimidazole **2** as starting materials. The reaction of compounds **1** and **2** with ethyl α-chloroacetate afforded the desired esters **3** and **4** in 85-93% yields. The hydrazide derivatives were then obtained when the compounds **3** and **4** were treated with hydrazine hydrate, which was used to prepare 1,3,4-oxadiazole derivatives bearing an amino benzene moiety **7** and **8**. The reaction of compounds **7** and **8** with benzoic acid to produce **9** and **10** provided the corresponding carboxylic acid **9** and **10** in 90% yields. Esterification of these compounds (**9** and **10**) led to the formation of new esters **11** and **12**, which converted to hydrazides **13** and **14**. The hydrazides 13 and 14 were treated with three carboxylic acids, 4-(benzoyloxy)benzoic acid, 4-((4-methoxybenzoyl)oxy)benzoic acid, and 4-((4-chlorobenzoyl)oxy)benzoic acid, to give new ester derivatives **15$_{a-c}$** and **16$_{a-c}$**. The structure of these derivatives was confirmed by spectrophotometric methods, such as FT-IR, ^{1}H NMR, and mass spectroscopy. The FT-IR spectral data for the prepared compounds **3** and **4** show the appearance of bands at 1743 and 1735 cm^{-1} due to the carbonyl of the ester group. In hydrazide compounds **5** and **6**, two bands between 1651 and 1643 cm^{-1}, and between 3309 and 3194 cm^{-1} to the C=O and N-H NH_2) for **5** and **6** were appeared. The bands at 3340-3220 cm^{-1} are due to the NH_2 group for compounds 7 and 8. New bands at 3500-2500 cm^{-1} due to O-H absorption, and bands at 1699-1691 cm^{-1} belong to the C=O absorption of the carboxyl group. The ^{1}H NMR spectral data for compound **9** showed a singlet signal at 10.71 ppm due to O-H bond for carboxyl group, multiple singles from 8.19 to 7.15 ppm attributed to the aromatic protons, and a singlet signal at 4.76 ppm due to methylene protons. While the ^{1}H NMR spectral data of compound **10** showed a singlet signal at 10.8 ppm for O-H absorption of the carboxyl group, multiple singles at between 8.24 and 6.9 ppm belong to the aromatic protons and a singlet signal at 4.78 ppm for SCH_2. The FT-IR spectral data of the ester derivatives **11** and **12** showed a band at 1716 cm^{-1} due to C=O for the ester group of the compounds **11** and **12**. The ^{1}H NMR spectrum for 11 showed a singlet signal at 9.01 ppm due to the N-H proton of the imidazole ring, multiple signals at 8.12-7.08 ppm attributed to the

aromatic ring protons, 5.10 ppm for the methylene group, 3.95 ppm due to the methyl protons of the ester group, and 2.22 ppm for N-CH_3 protons. The ^{1}H NMR spectrum for compound **12** showed a singlet signal at 8.01 ppm due to the N-H proton, multiple signals between 7.74 and 6.56 ppm for aromatic ring protons, a singlet signal at 4.23 ppm due to the methylene group, and a singlet signal at 3.73 ppm due to CH_3 protons of the ester group. The FT-IR spectral data of hydrazide compounds **13** and **14** showed two bands at 1695-1650 cm^{-1}, and 3309-3194 cm^{-1} belong to the C=O and NH-NH_2 absorptions, respectively. The ^{1}H NMR spectrum for 13 showed a singlet signal at 8.05 ppm due to the N-H proton, 7.92 ppm for the N-H of the hydrazide group, 7.90-6.45 ppm for aromatic ring protons, 5.63 ppm due to the methylene group, and 2.29 ppm for the methyl group. The ^{1}H NMR spectrum for compound **14** showed a singlet signal at 10.1 ppm due to the N-H of the hydrazide group, 9.4 ppm for the N-H of the secondary amide, 7.83-6.6 ppm for the aromatic protons, 5.63 ppm for NH_2 protons, 4.43 ppm for the methylene group, and 2.17 ppm for the N-CH_3 protons. The FT-IR spectra of compounds **15$_{a-c}$** and **16$_{a-c}$** showed bands at 1724-1739 cm^{-1} are attributed to the C=O of the ester group. The ^{1}H NMR spectrum for 15_b displayed a peak at 8.28 ppm due to the N-H proton of the secondary amide, 8.11-6.48 ppm attributed to the aromatic ring protons, 3.86 ppm for the OCH_3 group, and 2.56 ppm for the CH_3 of the acetyl group. The ^{1}H NMR spectrum for **16$_a$** showed a singlet signal at 8.5 ppm for the N-H of the imidazole ring, 8.19-6.99 ppm due to aromatic protons, and 5.53 ppm attributed to the CH_2 methylene group.



Scheme 1: Synthetic route for compounds **15$_{a-c}$** and **16$_{a-c}$**

3.2. Evaluation of the antimicrobial and antifungal activities

The antimicrobial activity of the synthesized derivatives was studied against two types of gram-negative bacteria, two types of gram-positive bacteria, and the *Candida fungus*. Table 1 summarizes the results obtained. The results showed that the prepared compounds had medium- to low-level activities against both types of bacteria. Likewise, for *C. albinos*, the effectiveness was of moderate intensity [1].

Table 1: The antimicrobial results for the synthesized compounds

Compound number	E.Coli	Pesudomonas aeruginosa	Bacillus subtilis	Staphylococcus aureus	C. albinos
15_a	11	13	15	20	13
15_b	18	14	12	17	12
15_c	13	11	10	11	11
16_a	10	15	18	14	19
16_b	12	17	13	19	16
16_c	10	11	16	12	15
DMSO	-	-	-	-	-
Strptomycin	21	23	17	23	11

Zone inhibition in mm

3.3. Toxicity prediction

The toxicity of the new compounds was predicted online with Pro-Tox-II-predication toxicity of chemicals to test the acute toxicity of compounds to humans. The results showed that the compounds are of the 15_{a-c} and 16_{a-c} type, which can be harmful in the event of ingestion of the substance in a high concentration; the LD_{50} was also estimated at 2300-2604mg/kg. Table 2 explains the results, the toxicity of compounds in rats. Most of the compounds fall within the scope of application; compounds 15_c and 16_a were nontoxic according to the OECD classification, while 15_b and 16_b were outside the oral route of administration.

Table 2: The prediction of human toxicity and rat acute toxicity

code	LD_{50} (mg/kg) human	IP (mg/kg) rat	IV(mg/kg) rat	Oral LD_{50} rat	Sc LD_{50}
15_a	2300	1082in A.D.	365,400in A.D	3579in A.D in AD	2969in A.D
15_b	2300	929,200in A.D.	233,000in A.D	1640,00out of A.D	2533in A.D
15_c	2604	2012,00	2012,00	2012,00	2012,00
16_a	2412	1327	1327	1327	1327
16_b	2412	1019 in AD	237,500 in AD	711,600 out of AD	2754,00 in AD
16_c	2604	820,00in A.D	224,200in A.D	234,400out of A.D	344600out of A.D

IP: interpretational route of administration; IV: intravenous route of administration; Oral: oral route of administration; S.c.: subcutaneous route of administration; in A.D.: compounds fall into applicability domain models; out of A.D.: compound is out of applicability domain models.

3.4. Molecular docking study

The molecular modelling of some prepared compounds has been studied using MOL.V. D.6, and minimizing energy for compounds by Chem. Draw software 15. Through studies, it was found that the prepared compounds containing the oxadiazole ring have good binding with FAK, EGFR, and VEGFR-2 [25]. However, according to the study, it was found that the prepared compounds had weak binding with these proteins, but they had good efficacy by binding to the lung cancer protein 7R7K. Table 4 summarizes the docking score (points), re-rank score, H-bond energy values, interactions of H-bonds, and steric interaction. The best docking score is the lowest energy [26]. Figures 1 and 2 show the energy map for the complexes (ligand-proteins) of **16_a** and **16_c**. Compound **16_c** has a good dock score of -150 kcal/mol and a -3.85 H-bond interaction. Because of their high molecular weight, they have weak binding points for pharmacokinetic activity (bioactivity), and they have low drug-likeness properties.

Table 3: Docking score and interactions for the prepared compounds

Compound number	Dock score kcal/mol	Rerank score kcal/mol	H-bond kcal/mol	Interactions H-bond H-bond interaction	steric interactions
15_a	-147	-105	-1.4	Glu1197	Arg1253 Leu1122 Gly1202 Leu1256 Glu1197 Leu1196
15_c	-152	-66	-0.97	Arg1253 Asp1203	Asp1203 Leu1256 Val1130 Arg1253 Met1290
16_a	-148	-105.7	-3.19	Met199 Arg1253 Pro1292	Met1290 Leu1291 Pro1292 Leu1122 Leu1256 Ley1198 Gly1202 Met1199
16_c	-150	-113	-3.85	Met119 Asp1203	Leu1196 Glu1197 Gly1202 Asp1203 Arg1253 met 1290 Trp1295 Lys1294 Met1328

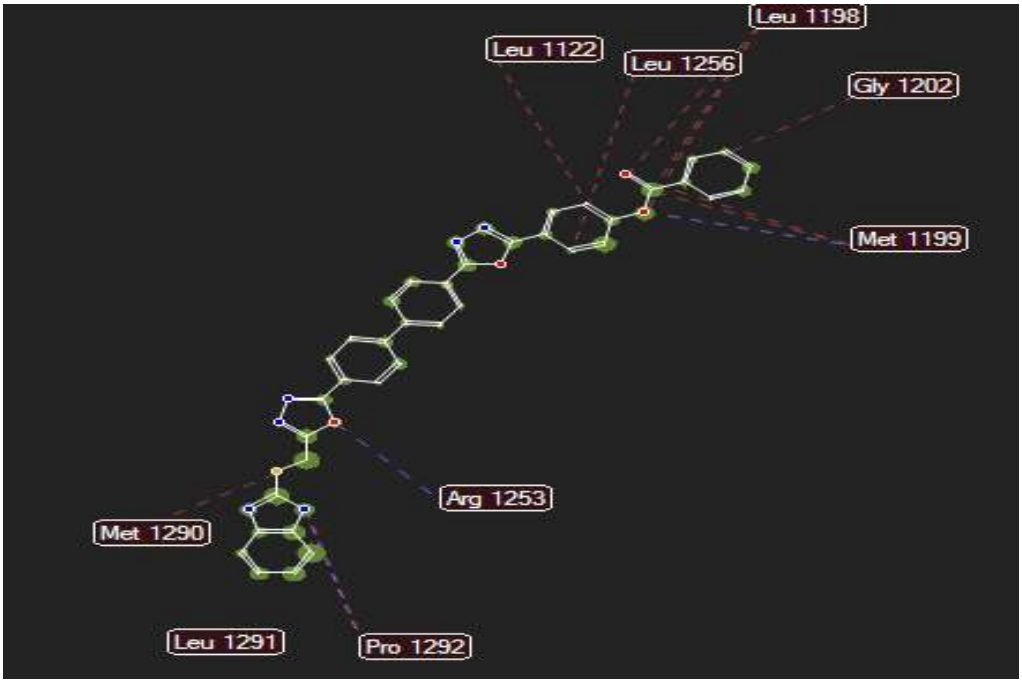


Figure 1: Energy map for complex (ligand protein) **16_a**

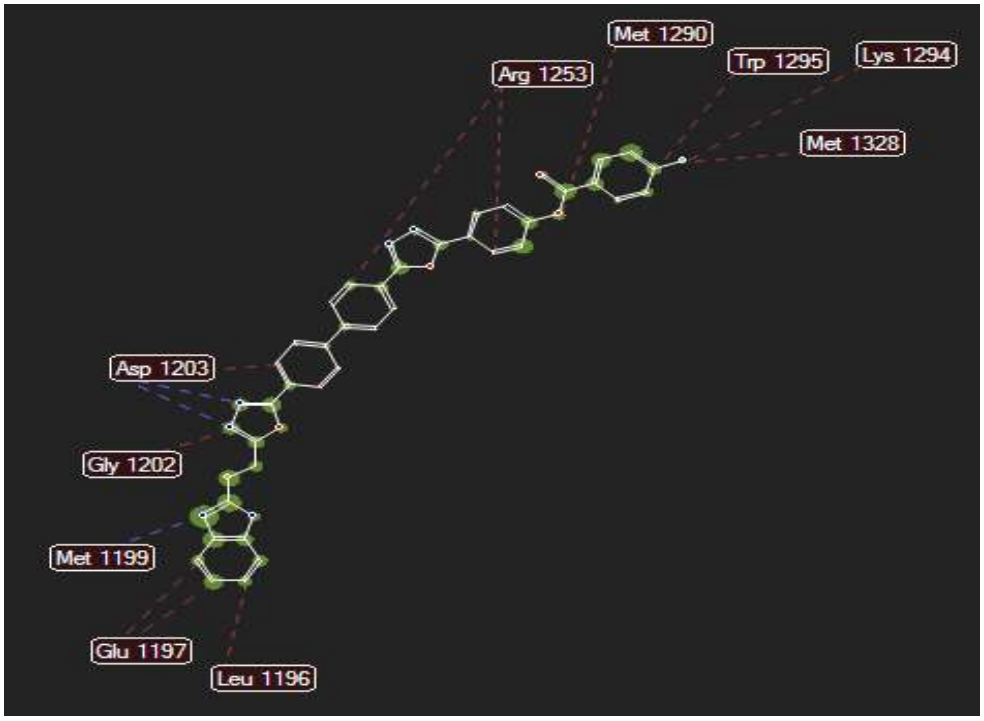


Figure 2: Energy map for complex (ligand protein) **16c**

4. Conclusion

New heterocyclic compounds bearing two 1,3,4-oxadiazole rings have been successfully prepared, characterized, and evaluated for their biological activity. It was found that these compounds possess a moderate level of biological activity against the studied microorganisms. Theoretical tests showed that these compounds are non-toxic to humans as well as mice, and the LD_{50} dose was determined to be between 2300 and 2604 mg/kg. A molecular modelling study showed that the compounds had activity against the lung cancer protein 7R7K.

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