Synthesis, Antibacterial study and ADME Evaluation of Novel Isonicotinoyl Hydrazide Derivative Containing 1,3,4-Oxadiazole Moiety

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Abstract:

Novel derivative of isoniazid containing 1,3,4-oxadiazole pharmacophore has been synthesized. The chemical structure of the compound was characterized and confirmed by using FT-IR and ^{1}H-NMR spectroscopy.

The desired compound was tested against gram-positive and gram-negative bacteria using agar well-diffusion technique for their ability as antibacterial agent and showed good antibacterial activity against gram-negative bacteria and gram-positive bacteria. In addition, ADME evaluations were performed using Swiss ADME to predict if the synthesized compound can be given orally, the bioavailability, topological polar surface area, and drug-likeness. The result showed that all tested compounds absorbed orally and fulfilled the Lipinski rule.
Key words: Isoniazid, 1,3,4-Oxadiazole, Antibacterial, ADME evaluation.

تحضير ودراسة مضادات البكتريا وتقييم ADME لمشتق ايزونيكوتينيل هايدرازيد يحتوي على مجموعة اوكساديازول

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الخلاصة:

تم تصنيع مشتق من الايزونيازيد يحتوي على مجموعة الاوكساديازول .تم توصيف المركب عن طريق قياس اطياف الاشعة تحت الحمراء وطيف الرنين المغناطيسي.
تم فحص النشاط المضادة للبكتريا في المركب عن طريق تقنية اكار المنتشر جيدا على البكتريا الموجبة للجرام والبكتيريا سلبية الغرام وقد اظهرت النتائج نشاطا جيدا مضادا للبكتريا.
اضافة الى ذلك تم اجراء دراسات ADME للتنبؤ هل المركب المصنع يمكن اعطائه عن طريق الفم, وموقع الامتصاص , والتوافر البايولوجي, ومساحة سطح القطب القطبية, شبه الدوائية. اظهرت نتائج ADME ان جميع المركبات يمكن امتصاصها عن طريق الجهاز الهضمي ومستوفية لشروط قاعدة لبنسكي

الكلمات المفتاحية: ايزونيازيد , اوكساديازول , مضاد بكتيري , دراسات ADMEIR

Introduction

For many years, the existence of microbial pathogens has been recognized. [1] Thus, throughout history, there has been an ongoing desire to eradicate the multitude already existing and emerging these microorganisms which cause infectious illnesses. Huge numbers of antibiotics have been advanced since the first introduction of sulfa and penicillin in clinical trials, which contributed positively to human health and thus control infection and prevent it from spreading. However intensive use of antibiotics can cause serious health problem caused by bacterial resistance to the antibiotics, so the development of new and numerous Antibacterials provided the means for

curing these resistant strains of organisms that previously had been susceptible to another Antibacterial. [2]

Oxadiazole heterocycle represents an important moiety in medicinal chemistry, the 1, 3, 4-Oxadiazole derivatives are the heterocyclic that have received considerable attention during the last two decades as they possess wide range of biological properties include anti-bacterial[3], antituberculosis[4], anti-inflammatory[5], antifungal[6], analgesic[7], anticancer[8], immunosuppressive[9], anti-convulsant [10], and tyrosinase inhibitor.[11]

Oxadiazole is important in drug discovery as many studies documented that 1,3,4-oxadiazole ring enhances ligand binding, polarity, flexibility, pharmacokinetics, in vivo metabolic profile, and receptor interaction. [12,13] So, the goal of present work is to synthesize compound bearing this core starting from isoniazid molecule. Isoniazid, (fig. 1) also called isonicotinoyl hydrazide (INH), is a hydrazide derived from isonicotinic acid compound (pyridine-4-carbohydrate), which is one of the first-line tuberculosis therapeutic drugs.

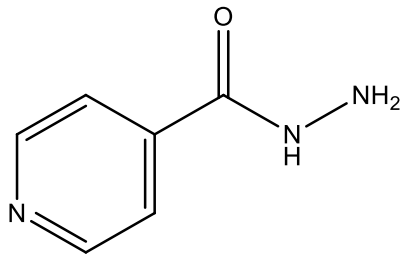
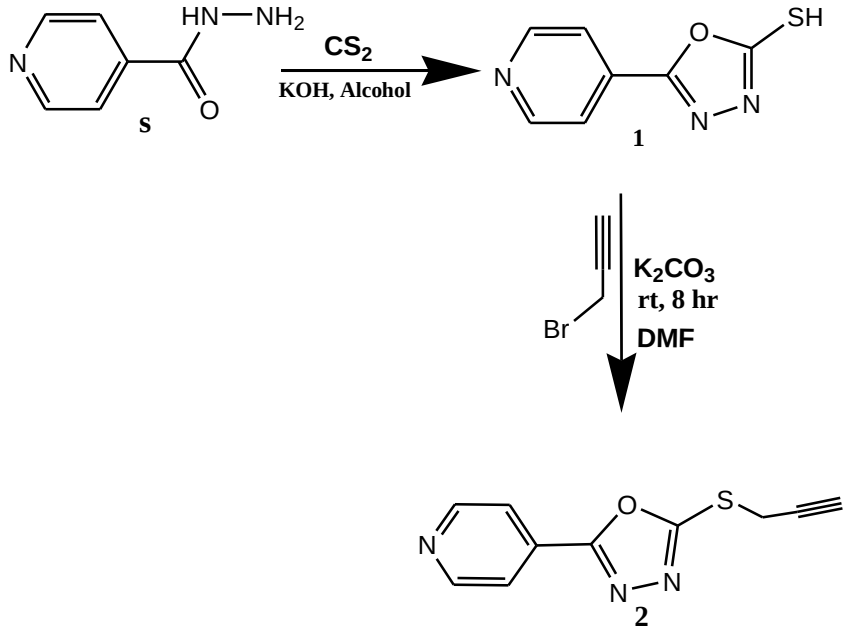


Figure (1): Structure of isonicotinoyl hydrazide [14]

Materials and Methods:

The chemicals that were utilized in this work were of analytical grade. Determination of melting points (M. Ps) of the synthesized chemicals were done by using Electro thermal M.P. Apparatus and open capillary tubes and they are uncorrected. Determinations of infrared spectra were performed in KBr disc using FT-IR spectrophotometer in the College of Pharmacy/Al-Mustansiriyah University, Iraq. The ^{1}H-NMR spectra were performed at Tehran University/ Collage of Science/Department of Chemistry in Iran, ^{1}H-NMR Spectrometer frequency (SF): 300MHz and 500MHz.

Chemical synthesis:



Scheme (1): synthesis of the intermediate and final compound

General procedure for the synthesis of 1,3,4-oxadiazole derivative (compound 1):

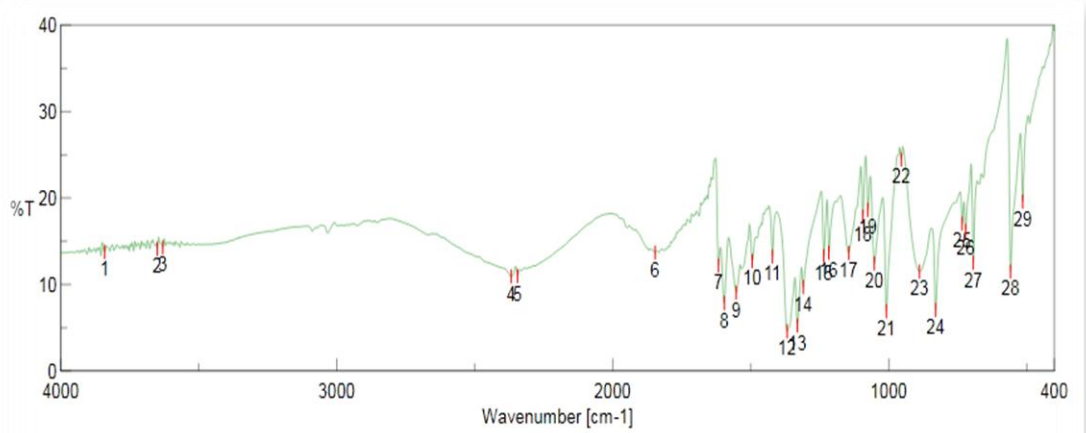
Isoniazid (0.01 mol, 1.37g) was dissolved in 100ml ethanol then potassium hydroxide and carbon disulfide were added in equimolar amounts to this solution. After that, the mixture was refluxed at 78°C for 24 hr. Distilled water was then added, followed by neutralization with dilute HCl(**1N**). A solid mass appeared was filtered and recrystallized from methanol to give bright yellow crystals. [15]

Bright yellow crystal, yield= 82%, M.P.= 263-265°C. **IR KBr** (cm^{-1}): 2368(S-H str), 1595(C=N str of 1,3,4-oxadiazole), 1008(C-O str of oxadiazole nucleus).

Synthesis of 2-(prop-2-yn-1-ylthio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (compound 2):

8.74 mmol(1.56g) of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (Compound **1**) was added to a mixture of 4.91 mmol (0.37 mL)propargyl bromide and 10.5 mmol (1.45g) K_2CO_3 in 30 ml DMF at room temperature ,and the resulted mixture stirred for eight hours. The resulted combination was dissolved in distilled water, then extracted by diethyl ether, the organic layer was dried by using Na_2SO_4 and then was purified. After that, evaporation of filtrate was done to obtain the desired compound. [16]

Yellow powder, yield= 66%, M. P= 96-98°C. **IR KBr** (cm^{-1}): 3151(≡C-H str), 2108(C≡C str). **^{1}H-NMR**: δ 2.50 (DMSO), δ 3.41(HDO), δ 4.14-4.44 (s, 3H as overlapped two signals; s, 2H, S-CH_2 and s, 1H, ≡C-H), δ 7.75-8.00(d, 2H, CH of pyridine close to the oxadiazole ring), δ 8.65-8.92(d, 2H, CH of pyridine close to N).



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3840.54	13.7511	2	3650.59	14.0923	3	3630.34	14.2628
4	2368.16	10.9166	5	2345.02	10.9416	6	1846.51	13.6465
7	1617.02	12.1634	8	1595.81	7.83526	9	1552.42	9.04741
10	1494.56	12.6826	11	1421.28	13.2101	12	1368.25	4.57285
13	1330.64	5.23223	14	1309.43	9.68333	15	1235.18	13.2589
16	1216.86	13.6606	17	1145.51	13.6234	18	1093.44	17.8304
19	1075.12	18.6056	20	1051.98	12.41	21	1008.59	6.88434
22	954.591	24.4469	23	888.059	11.4644	24	830.205	7.01348
25	733.782	17.0224	26	721.247	16.1794	27	693.284	12.4906
28	558.291	11.4791	29	513.936	19.5638			

Figure (2): FT-IR Spectrum of Compound 1 Using KBr Disc

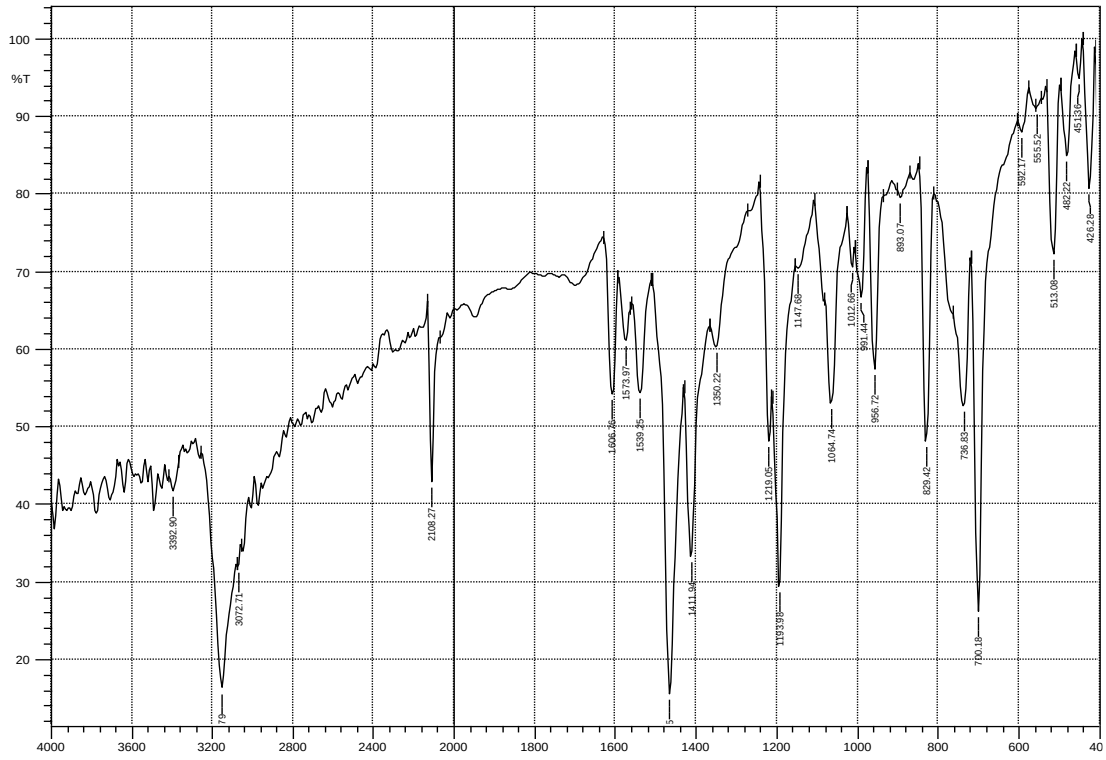


Figure (3): FT-IR Spectrum of Compound 2 Using KBr Disc

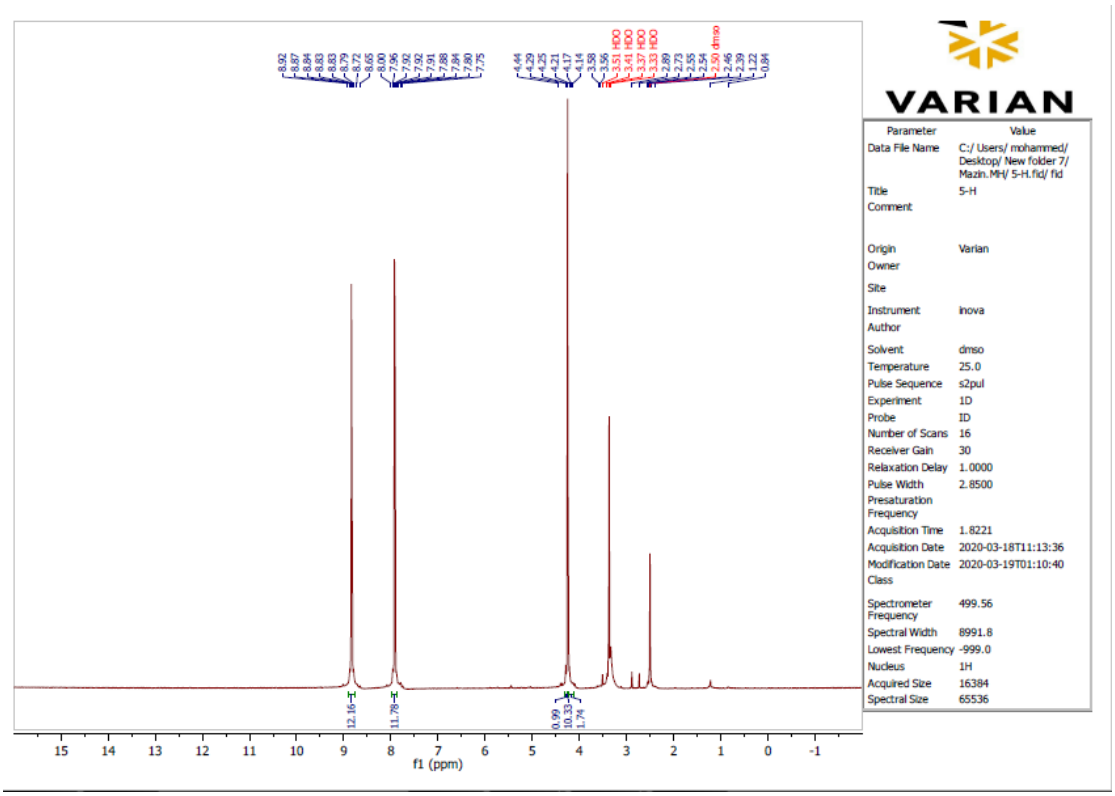


Figure (4): ^{1}H-NMR spectrum of compound (2)

Antibacterial screening:

The preliminary antimicrobial activity of the compound tested was carried out at the College of Pharmacy/Al-Mustansiriyah University. This antibacterial test was performed using the Well Diffusion Method: The synthesized compound was tested against four bacterial species for

their antimicrobial activity in vitro: two gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and the other two were gram negative (*Escherichia coli* and *Klebsiella pneumoniae*). The isolates were collected from different clinical sources. Ciprofloxacin drug was the reference drug for antibacterial activity. DMSO was used as negative control in 0.5% concentration.

Serial dilutions Preparation of the newly synthesized compound:

- 5mg from each compound dissolved in 5mL DMSO (5%) to prepare the stock solution (1000µg/mL).
- The first dilution prepared by addition of 2.5 mL of DMSO (5%) to 2.5 mL of the stock solution (500µg/mL).
- The second dilution prepared by addition of 2.5 mL of DMSO (5%) to 2.5 mL of the 1st dilution (250µg/mL).
- The third dilution prepared by addition of 2.5 mL of DMSO (5%) to 2.5 mL of the 2nd dilution (125µg/mL).
- The fourth dilution prepared by addition of 2.5 mL of DMSO (5%) to 2.5 mL of the 3rd dilution (62.5µg/mL).

This procedure performed for compound **2** in addition to the standard drug Ciprofloxacin.

Sensitivity Assay:

The antibacterial activity of the tested compounds was measured by an agar well diffusion assay and involves the use of pure culture for all bacterial species. The concentration of 1.5×108 CFU/ml has been formed by utilizing number 0.5 McFarland turbidity standard for every bacterium that inoculated with glass spreader on the surface of previously prepared plates of Mueller Hinton Agar (MHA). Then, these were kept away to dry and punch wells (five) in diameter of 6 mm into agar. Consequently, five wells were made in each agar plate. of bacteria and (100µl) of compounds' dilutions (500,250,125 and 62.5) were inserted into wells. on MHA plate. The negative controller was DMSO (5%). For one day, the plates kept heated at 37 ° C and the antibacterial. activity was assessed by calculating the distance from the inhibition zone. [17] The antimicrobial assessment was based on the extent of the inhibition zone diameter developed all over the place of the well.

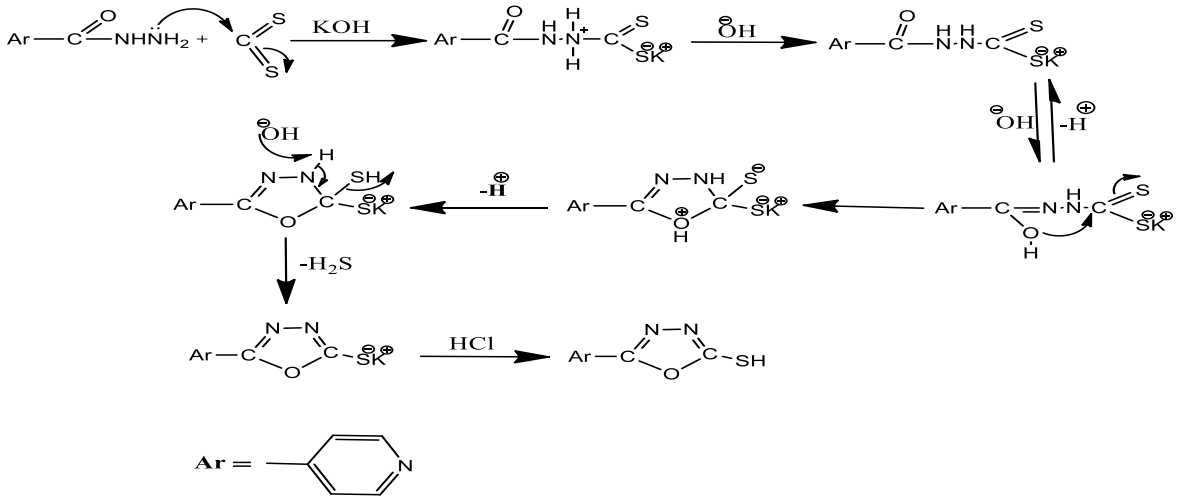
ADME evaluation studies:

The pharmacokinetic profile (ADME) Study was performed by using Swiss ADME (Server). The ligands (**S, 1** and **2**) were drawn by using Chem. Sketch (v. 12) and then converted by Swiss ADME tool to SMILE name to predict pharmacokinetic and physicochemical properties. The polarity and lipophilicity of the small molecule were determined by BOILED EGG. [18]

Results and discussion

Mechanism of the 1,3,4-oxadiazole formation:

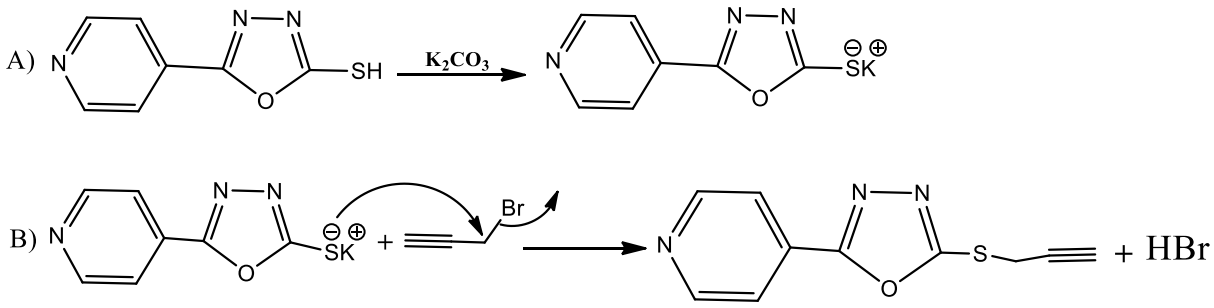
The reaction of hydrazide moiety with CS_2 in the presence of KOH is the most common reaction to prepare 1,3,4-oxadiazole derivative (compound **1**). Firstly, an acyl hydrazide reacts with carbon disulfide in a solution of basic alcohol, then acidification of the mixture occurred. The mechanism of this ring closure illustrated in the scheme (2).



Scheme (2): Mechanism of 1,3,4-oxadiazole synthesis. [19]

Synthesis of S-propagylated derivative (compound 2):

2-(prop-2-yn-1-ylthio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (**2**) was prepared by nucleophilic substitution reaction (SN_2). Mechanism involved replacement of hydrogen from thiol group by the K^+ from the base (K_2CO_3), then nucleophilic substitution take place as shown in scheme (3). Since the SN_2 reaction mechanism occurs by a single step that involves formation of reaction intermediate. This reaction is affected strongly by the type of solvent.



scheme (3): Mechanism of alkyne synthesis. [20]

Antibacterial assay:

DMSO(5%) was the control, the reference drug was Ciprofloxacin and the antibacterial activity of the synthesized compound (2) was tested by using gram negative bacteria: Escherichia coli and Klebsiella pneumoniae and gram positive bacteria: Staphylococcus aureus and Streptococcus pyogenes at concentrations of (62.5,125, 250 and 500 µg/mL), while the control was used in 0.5% concentration to eliminate the Antibacterial activity of it.[21]

The inhibition zone in (mm) for each concentration of the tested compound illustrated in table (1). The tested compound gave an interesting activity against staphylococcus aureus and the gram negative bacteria (Escherichia coli and Klebsiella pneumoniae), this tested derivative exert significant antibacterial activity in comparison to DMSO as a control group, and these results are in agreement with many studies showed that 1,3,4-oxadiazole derivatives have good antibacterial action.[22]

Table (1): Antibacterial activity of compound 2 and ciprofloxacin against tested bacteria

Compound	Conc. (µg/ml)	Inhibition zone(mm)			
		Gram positive		Gram negative	
		Staphylococcus aureus	***Streptococcus pyougenes***	***Klebsiella pneumoniae***	***E. coli***
Ciprofloxacin	500	34	40	32	35
	250	32	34	30	33
	125	28	30	28	30
	62.5	27	27	26	28
DMSO	5%	-	-	-	-
2	500	26	0	17	9
	250	22	0	11	10
	125	0	0	0	7
	62.5	0	0	0	0

Interpretation ADME results:

The ADME properties of the desired compounds (**S, 1** and **2**) were studied by Swiss ADME**.** We assessed the compounds ADME (adsorption, distribution, metabolism and excretion) method. In addition, the topological polar surface area (TPSA) was calculated, as it is another critical property that has been linked to the bioavailability of drugs. Thus, passively absorbed compounds with a TPSA > 140 Å are thought to have low oral bioavailability. [23]

The ADME prediction data obtained showed that all the ligands were within the range of accepted values, and have TPSA below 140, which are in the range (68.01 to 77.11 and the bioavailability was 0.55 for all tested compounds, meaning that they reach systemic circulation. Additionally, the ligands have no violation from Lipinski's rule of five (RO5) and fulfilled the topological descriptors and fingerprints of molecular drug-likeness structure keys as LogP and LogS (table 2). Also, the absorption of all compounds was high, expecting them to have good Intestinal absorption.

Table (2): ADME result of the final derivatives

Comp	Formula	MWT	H-bond acceptor	H-bond donor	MR	TPSA	GI Abs	BBB permeant	Bioavail ability	Lipiniski violation
S	$C_6H_7N_3O$	137.14	3	2	35.13	68.01	High	NO	0.55	0 violation
1	$C_7H_5N_3OS$	179.20	4	0	44.78	90.61	High	NO	0.55	0 violation
2	$C_{10}H_7N_3OS$	217.25	4	0	53.03	77.11	High	NO	0.55	0 violation

Conclusion:

The 1,3,4-Oxadiazole moiety continues to be one of the commonly researched area in medicinal chemistry, the synthetic procedure for the designed oxadiazole derivative was successfully achieved, the structural formula for the synthesized compound was characterized using IR spectroscopy, ^{1}H-NMR and melting points. The anti-bacterial assessment of the product indicates that the 1,3,4-oxadiazole pharmacophore gives excellent anti-bacterial action, and the ADME evaluations showed that the newly synthesized compound 2 fulfilled the

Lipinski rule, and this compound absorbed from GIT.

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