A Facile Synthesis and Biological Study of tri-[4-(5-aryl-1,3,4-Oxadiazol-2-yl)Phenyl]Phosphate.

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Abstract

In the present investigation, nine novel 2-(aryl)-5-[4-(aroyloxy)phenyl]-1,3,4-oxadiazoles [2-10] were synthesized by a facile one step method through the reaction of 4-hydroxybenzoyl hydrazine with two equivalent of appropriate acid in the presence of phosphorus oxychloride.

Three novel phosphate esters containing three 1,3,4-oxadiazole units [14-16], namely {tri-[4-(5-phenyl-1,3,4-oxadiazol-2-yl)phenyl]phosphate [14], tri-[4-(5-(*p*-methoxy phenyl)-1,3,4-oxadiazol-2-yl)phenyl]phosphate [15] and tri-[4-(5-(*p*-nitro phenyl)-1,3,4-oxadiazol-2-yl)phenyl]phosphate [16]} were also synthesized by one step method through condensation of three equivalent of phenolic compounds [11-13] with phosphorus oxychloride.

All the synthesized compounds were characterized by their melting points and spectral (FT-IR, mass, CHN and UV) data. The newly synthesized compounds were subjected to ***in vitro*** against two strains of pathogenic microorganism viz., ***Staphylococcus arueus*** and ***Escherichia coli***. The results obtained revealed that compounds [15 and 16] showed considerable activity against ***Escherichia coli***.

الخُلاصّة

من خلال هذا البحث، تم تحضير تسع مركبات جديدة من ٢-(اريل)-٥-[٤-(اروايلوكسي) فنيل]-١،٣،٤-اوكسادايازول بخطوة واحدة من خلال تفاعل ٤-هيدروكسي بنزوايل هيدرازين مع مكافئين من حامض مناسب بوجود اوكسي كلوريد الفسفوريل.

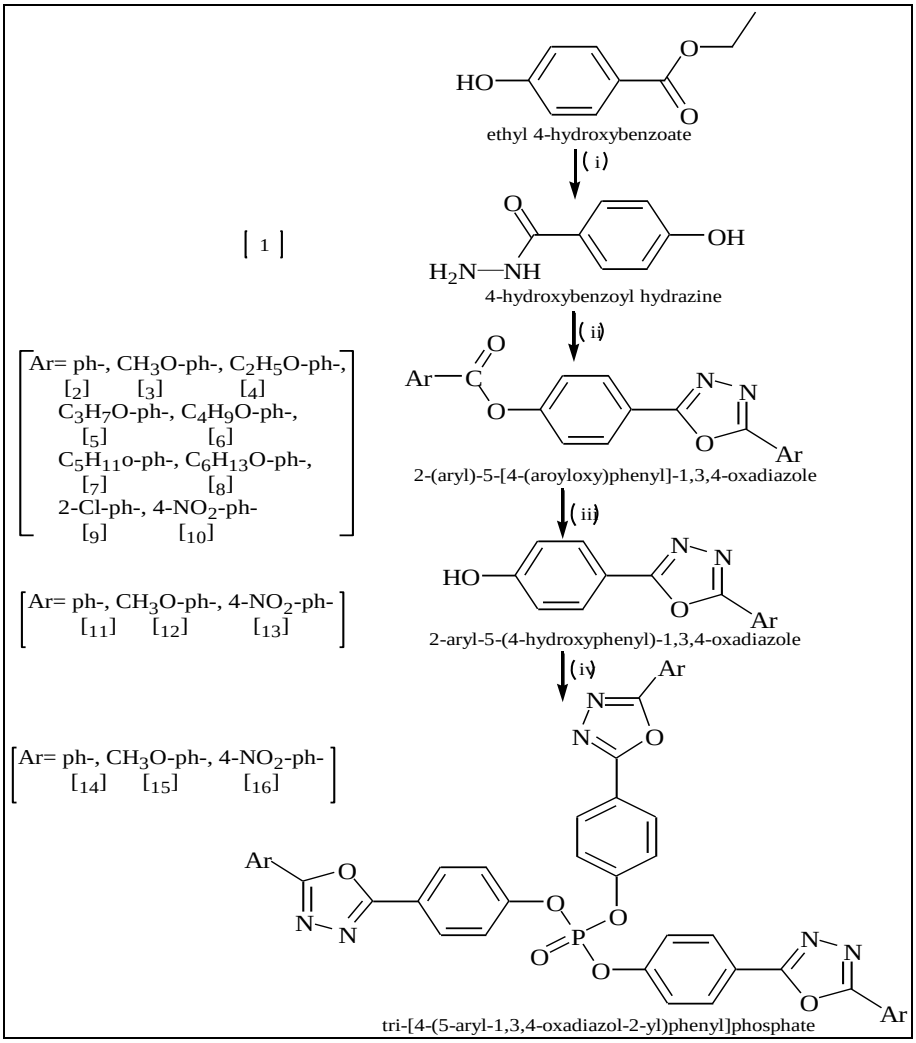
و ينتج من تكاثف اوكسي كلوريد الفسفوريل مع ثلاث مكافئات من الفينولات المناسبة [١١-١٣]، مكونة استرات الفوسفات الجديدة المحتوية على ثلاث وحدات من ١،٣،٤-اوكسادايازول [١٤-١٦] و المسماة { ثلاثي-[٤-(٥-فنيل-١،٣،٤-اوكسادايازول-٢-يل)فنيل]فوسفات [١٤]، ثلاثي-[٤-(٥-(بارا-ميثوكسي فنيل)-١،٣،٤-اوكسادايازول-٢-يل)فنيل]فوسفات [١٥] و ثلاثي-[٤-(٥-(بارا-نايتروفنيل)-١،٣،٤-اوكسادايازول-٢-يل)فنيل]فوسفات [١٦] }.

المركبات المحضرة تم تشخيصها من خلال قياس درجات إنصهارها و مطيافية الأشعة تحت الحمراء، كما تم الأستعانة بمطيافية الأشعة فوق البنفسجية–المرئية و طيف الكتلة، و التحليل الذري للعناصر في عملية التشخيص. و قد دلت النتائج المستحصلة على' صحة التراكيب المقترحة للمركبات المحضرة.

أخيراً تم دراسة الفعالية البايولوجية لهذه المركبات المحضرة كأحد التطبيقات ضد نوعين من البكتريا و هما البكتريا الموجبة الصبغة ***Staphylococcus aureus*** و البكتريا السالبة الصبغة ***Escherichia coli***. و قد دلت النتائج أن المركبات [١٥ و ١٦] أظهرت فعالية ضد ***Escherichia coli*** .

Introduction

Much attention has been paid to 1,3,4-oxadiazole derivatives in recent years because of their showing various biological activities. Reported among these activities were: nervous system depressing[1], analgesic[2,3], herbicidal[4], muscle relaxant[5] and antimicrobial agent[6]. To our knowledge organophosphorus compounds containing 1,3,4-oxadiazoles are still rare[7], therefore it was decided to synthesize some new phosphate esters containing three 1,3,4-oxadiazole unites, as shown in Scheme (1), with the hope that the incorporation of ester unit with three biological activity. 1,3,4-Oxadiazoles may enhance the biological activity of the target molecules [14-16].



Scheme (1) Reagents and conditions: - (i) NH_2NH_2, EtOH, reflux (7) hrs. (ii) Appropriate aromatic acid, $POCl_3$, reflux (24) hrs. for compound [10], and (7) hrs. for compounds [2-9]. (iii) Hydrolysis of compounds [2, 3 and 10] with 10% NaOH, reflux (2) hrs. (iv) Pyridine, $POCl_3$, chloroform.

Experimental

Instruments

Melting points were measured using **Gallen Kamp** melting point apparatus and were uncorrected. Infrared spectra were recorded on a **Perkin-Elmer 1310 infrared spectrophotometer** and **FTIR – 8300 fourier transform infrared spectrophotometer SHIMADZU** as potassium bromide disc. UV-Visible Absorbance measurements were recorded on a **Cintra 5 UV-Visible spectrophotometer.** Mass spectra were recorded on **Shimadzu QP1000A** gas chromatography spectrophotometer. Microanalysis was performed with a **CARLO-ERBA** Instrument. EA1108. ELEMENTAL Analyzer.

Synthesis

Preparation of 4-hydroxybenzoyl hydrazine [1].

A mixture of ethyl 4-methoxybenzoate (0.05 mole, 8.3 g) and hydrazine hydrate (0.1 mole, 5 ml) were refluxed for (2) hours, ethanol (15 ml) was added and refluxed for (5) hours. The precipitate which separated on cooling was filtered and washed

with cold methanol, yield (69) %, M.P.=(268-270)^{0}C.

Preparation of 2-(aryl) -5-[4-(aroyloxy) phenyl] -1,3,4-oxadiazole [2-10].

A mixture of compound [1] (0.01 mole, 1.52 g), appropriate aromatic acid (0.02 mole) and phosphorus oxychloride (7.5 ml) was refluxed overnight to prepare compound [10] and refluxed (7) hours for the preparation of compounds [2-9]. The cold reaction mixture was then poured on crushed ice and made basic by adding sodium bicarbonate solution. The resulting solid was filtered, dried and recrystallized from (chloroform-ethanol), to give the desired oxadiazoles [2-10], yield (70 – 75) %.

Preparation of 2-aryl-5-(4-hydroxyphenyl) - 1,3,4-oxadiazole [11-13].

These compounds were prepared through the hydrolysis of ester groups of compounds [2,3 and 10] in 10% NaOH and were treated according to the procedure described in the literature [8], yield (70 – 74) %.

Preparation of tri–[4-(5-aryl-1,3,4-oxadiazol-2-yl)phenyl] phosphate [14-16].

To a solution of appropriate phenolic compounds (3 mmole) in chloroform (50 ml), dry pyridine (3 mmole) and (0.1 g) NaCl were added. The mixture was stirred for (15) minutes, (1 mmole) of $POCl_3$ in (15 ml) chloroform was added drop wise to the reaction mixture at 0 ^{0}C. Stirring was continued overnight. The reaction mixture was washed with 2% NaOH solution, solvent was evaporated and the product was recrystallized to give the title compounds [14-16], yield (62 – 70) %.

Results and Discussion

The synthetic route used to prepare the target compounds is shown in Scheme (1). The convenient starting material was 4-hydroxy benzoyl hydrazine [1], Figure (1) which in turn was prepared from 4-hydroxy ethyl benzoate and hydrazine hydrate, synthon [1] on condensation with two equivalents of appropriate aromatic acid in phosphorus oxychloride give 2-(aryl)-5-[4-(aroyloxy)phenyl]-1,3,4-oxadiazoles [2-10].

A survey of the literature reveals that carrying esterfication and cyclization steps at the same time using $POCl_3$ is not reported in the literature.

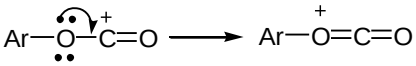
The structures of all these synthesized compounds were established on the basis of their elemental analysis (CHN, FT-IR and mass) spectral data. The elemental analysis of the compound [3], Figure (3) is consistent with its proposed structure.

The infrared absorption bands were utilized to characterize the specific structures of the synthesized compounds. The disappearance of the broad band at (3300-3100 cm^{-1}) assigned to O-H stretching and the appearance of the carbonyl stretching of ester group at (1720-1710 cm^{-1}) are good evidence to the success of the esterfication step. The appearance of a band at (1610 cm^{-1}) assignable to (C=N) band of oxadiazole moiety and a band at (1260 and 1100 cm^{-1}) assigned for (C-O-C) cyclic grouping in oxadiazole are good evidence for the presence of 1,3,4-oxadiazole ring. Besides this, the FT-IR spectra of these compounds were devoid of both amide and amino bands present in the FT-IR spectrum of compound [1]. The above data agree with the proposed structures assigned to these compounds. Figures (2) to (5) show FT-IR spectral data of compounds [2,3,9 and 10] respectively.

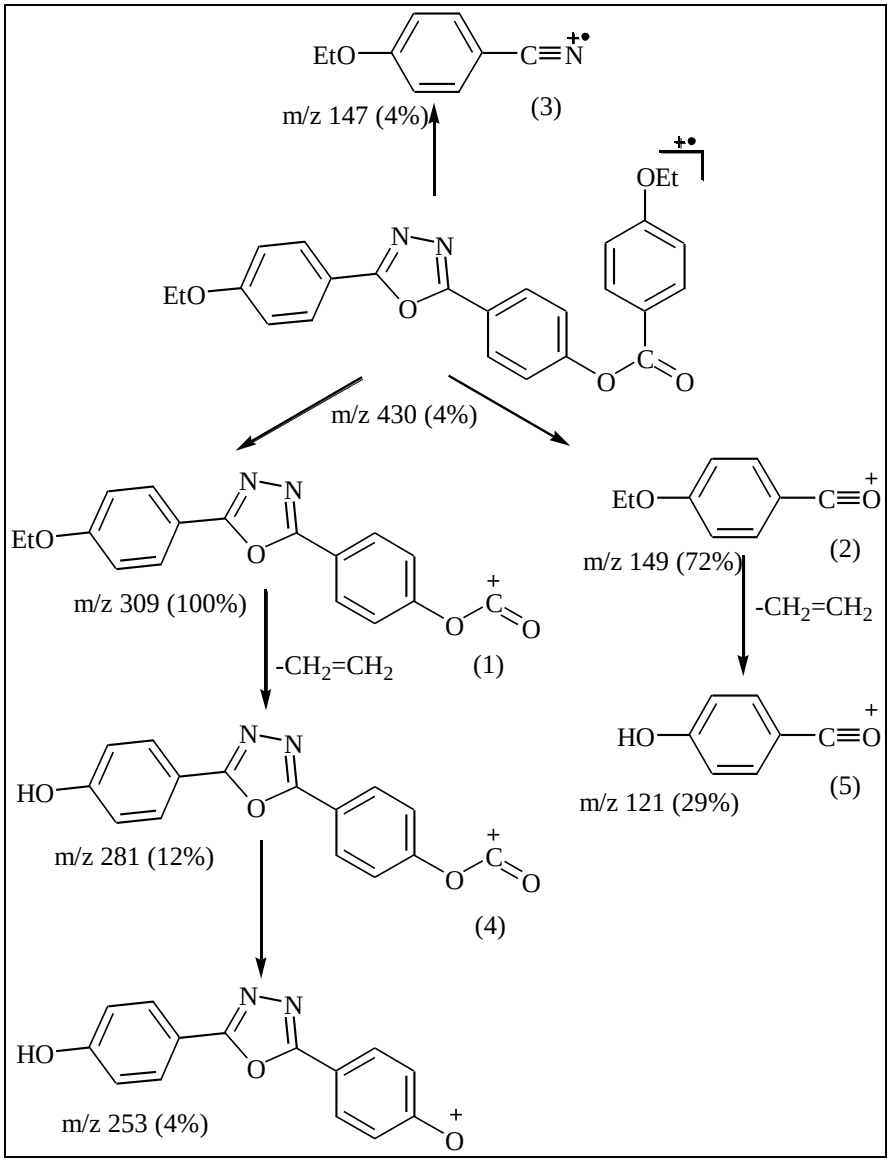
These 2-(aryl)-5-[4-(aroyloxy)-phenyl]-1,3,4-oxadiazole were

characterized by ultraviolet visible spectrophotometry. The ultraviolet visible spectra of 2-(phenyl)-5-[4-(benzoyloxy)phenyl]-1,3,4-oxadiazole [2], 2-(4-methoxyphenyl)-5-[4-(4-methoxy benzoyloxy)phenyl]-1,3,4-oxadiazole [3], 2-(2-chlorophenyl)-5-[4-(2- chloro benzoyloxy)phenyl]-1,3،4-oxadiazole [9] and 2-(4-nitrophenyl)-5-[4-(4-nitro benzoyloxy)phenyl]-1,3,4-oxadiazole [10], in ethanol solvent are shown in Figures (6) to (9), respectively. The characteristic absorption bands located at the wavelength range between (239 – 262 nm), refer to the B-band[9] originates as a result of $\pi \rightarrow \pi^*$ electronic transition. Also the appearances of K-band at the wavelength range (278– 310 nm), is assigned to the presence of $n \rightarrow \pi^*$ electronic[9] transition, Table (1).

The mass spectrum of compound [4], Figure (10) shows the molecular ion peak at m/z 430 which corresponds to the molecular weight of the structure assigned to this compound. The base peak is found at m/z 309, cation (1), Scheme (2). This is due to the formation of the highly stable cation



which is stabilized by the mesomeric effect of two oxygen atoms[9]. An important informative fragments (2) and (3) are quite familiar fragments that are present in 1,3,4-oxadiazoles. The frequently observed peaks at m/z 281 (4) and m/z at 121 (5) results from elimination of a neutral molecule (ethylene) from the cations (1) and (2), respectively. (McL-afferty rearrangement). The above features are considered strong evidence for the structure suggested to this compound.



Scheme (2) fragmentation pattern of compound [4]

The hydrolysis (or saponification) of compounds [2,3 and 10] with 10% NaOH afforded the corresponding phenolic compounds [11-13], the structures of these compounds were confirmed by (UV and FT-IR) spectral data, characterization data of all compounds are given in Figures (11) to (13).

The appearance of the broad absorption band in the range (3300 – 3000 cm^{-1}) due to hydrogen bonding (O-H) stretching and the disappearance of a stretching band around (1745 – 1695 cm^{-1}), assigned to the ester group υ(C=O) are good evidence for the structure given to the product.

The electronic spectrum of compound [11], Figure (14) displayed two bands at (269 and 324 nm) assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively.

Condensation of three equivalents of phenolic compounds [11-13] with one equivalent of phosphorus oxychloride afforded the target molecules [14-16].

The addition of phenolic compounds directly to phosphorus oxychloride is accompanied by a considerable evolution of heat.

Chloroform was therefore used as a solvent and diluent, since it permitted more careful temperature control and because the reaction product was found to be completely soluble in it.

The structures of all these newly synthesized compounds [14-16] were established on the basis of (UV and FT-IR) spectral data, characterization data of all compounds are given in Figures (15) to (17).

The FT-IR spectra of these compounds showed the disappearance of band at (3300–3000 cm^{-1}) attributed to the (O-H) stretching frequency, together with the appearance of a band at (1610–1608 cm^{-1}) and a band at (1301–1282 cm^{-1}) assigned to C=N of oxadiazole moiety and phosphoryl group (P=O), respectively are good evidence for occurring of phosphorlating step.

Compound [14], Figure (18) showed the corresponding transitions at (274 and 290 nm) assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. In addition a band was observed at (364 nm) which suggested a charge transfer transition.

Table (1): Electronic spectra for the synthesized compounds

Compound	Solvent	Melting Point ^0C	Color	Absorption Bands (nm)	Assigned Transition
[2]	EtOH	116-118	White	239	$\pi \rightarrow \pi^*$
				286	$n \rightarrow \pi^*$
[3]	EtOH	140-142	White	240	$\pi \rightarrow \pi^*$
				301	$n \rightarrow \pi^*$
[9]	EtOH	126-128	White	238	$\pi \rightarrow \pi^*$
				278	$n \rightarrow \pi^*$
[10]	EtOH	251-253	Orange	262	$\pi \rightarrow \pi^*$
				310	$n \rightarrow \pi^*$
[11]	DMSO	250-252	yellow	269	$\pi \rightarrow \pi^*$
				324	$n \rightarrow \pi^*$
[14]	DMSO	124-126	Pink	274	$\pi \rightarrow \pi^*$
				290	$n \rightarrow \pi^*$
				364	CT

Antibacterial activity

The antibacterial activity [10] of the synthesized compounds was determined ***in vitro*** using paper disc method (agar plate diffusion method) [11] against two pathogenic microorganism viz., ***staphylococcus aureus*** (Gram +ve) and ***Escherichia coli*** (Gram –ve). In this method, a standard 5mm diameter sterilized filter paper impregnated with the compound (1mg per 1ml DMSO and 1mg per 10ml DMSO for concentration (1) and (2), respectively) was placed on agar plate seeded with the test organism. The plates were incubated for 24 hours at 37 ^0C. The zones of inhibition of bacterial growth were measured in mm depending upon the diameter as shown in Table (2), and Figures (19) to (21).

Compounds [14] showed inconsiderable activity against ***staphylococcus aureus*** (G^+) and ***Escherichia coli*** (G^-), compounds [15 and 16] showed considerable activity against ***Escherichia coli*** (G^-). However, more detail investigation is required to evaluate their activities against other microorganisms.

Table (2): Antibacterial activity of the synthesized compounds.

Compound No.	Comp. No. in Figures	staphylococcus aureus		Escherichia coli	
DMSO solvent	C	Conc. (1)	Conc. (2)	Conc. (1)	Conc. (2)
		–	–	–	–
14	$7_{(1)}$, $7_{(2)}$	–	–	–	–
15	$3_{(1)}$, $3_{(2)}$	–	–	12mm	12mm
16	$8_{(1)}$, $8_{(2)}$	–	–	12mm	10mm

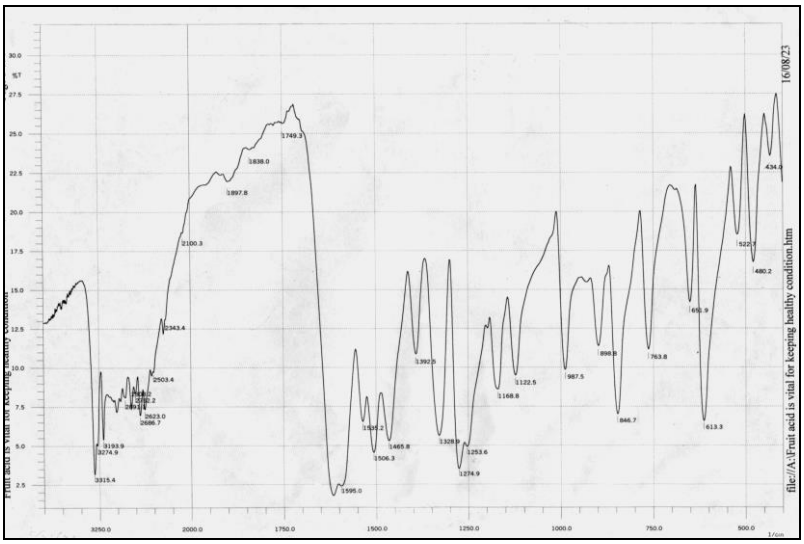


Figure (1): FT-IR spectrum of 4-hydroxybenzoyl hydrazine [1].

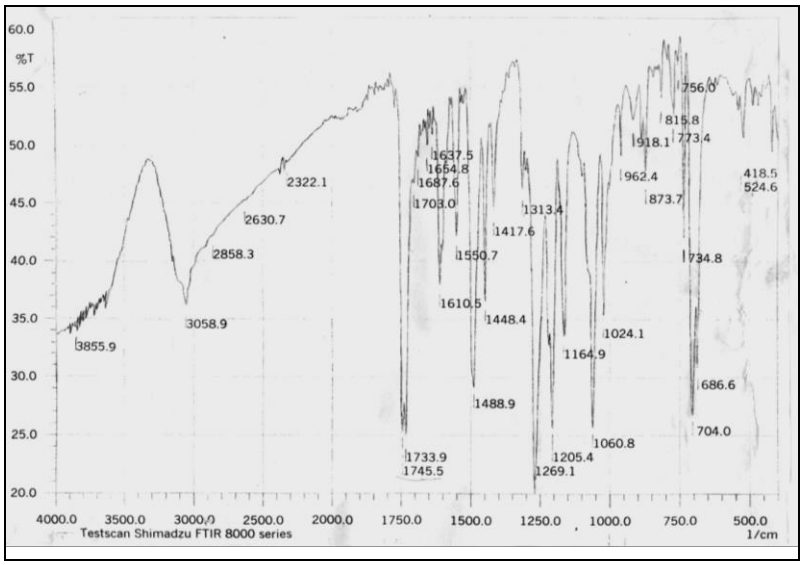
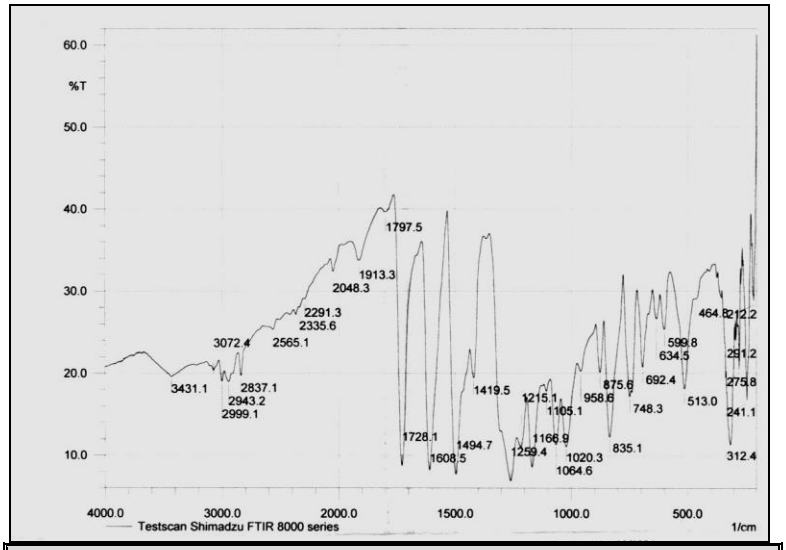


Figure (2): FT-IR spectrum of 2-phenyl-5-[4-(benzoyloxy) phenyl] -1,3,4-oxadiazole [2].



Found / Calc. %		
C	**H**	**N**
69.09	5.02	6.39
68.65	4.47	6.96

Figure (3): FT-IR spectrum of 2-(4-methoxyphenyl)-5-[4-(*p*-methoxybenzoyloxy) phenyl]-1,3,4-oxadiazole [3].

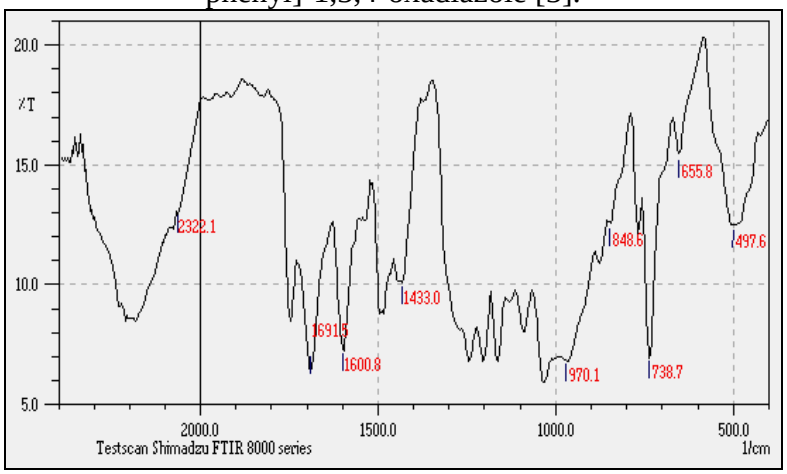
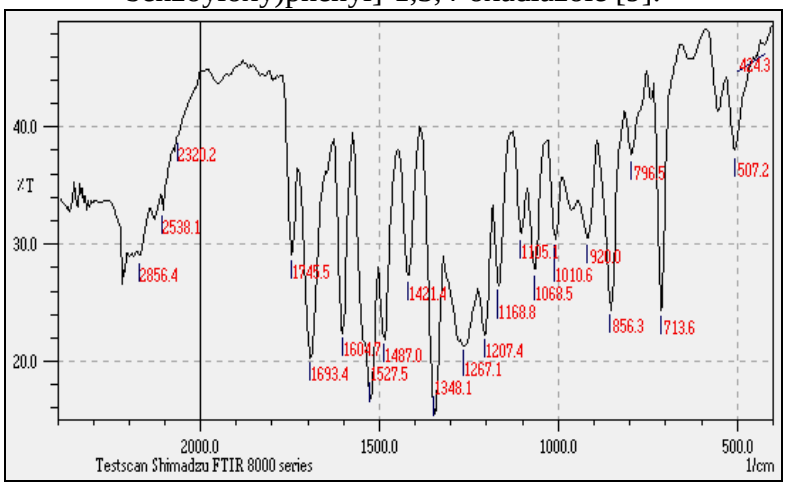


Figure (4): FT-IR spectrum of 2-(2-chloro phenyl)-5-[4-(2-chloro benzoyloxy)phenyl]-1,3,4-oxadiazole [9].



Figure(5): FT-IR spectrum of 2-(4-nitro phenyl)-5-[4-(4-nitro benzoyloxy)phenyl]-1,3,4-oxadiazole [10].

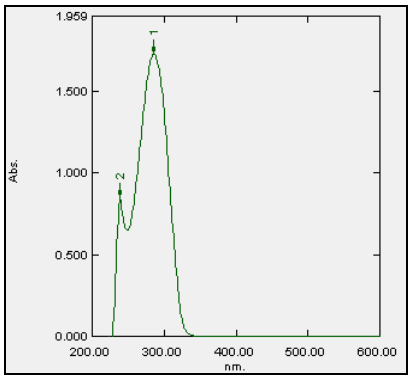


Figure (6): The ultraviolet visible spectrum for 2-(phenyl)-5-[4-(benzoyloxy)phenyl]-1,3,4-oxadiazole [2].

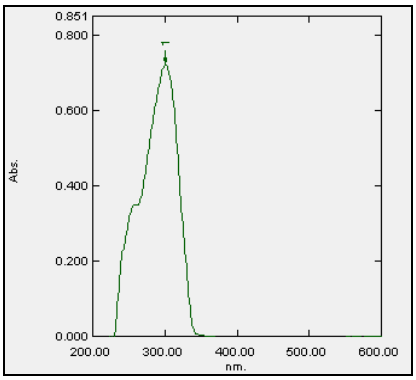


Figure (7): The ultraviolet visible spectrum for 2-(4-methoxy phenyl)-5-[4-(4-methoxy benzoyloxy)phenyl]-1,3،4-oxadiazole [3].

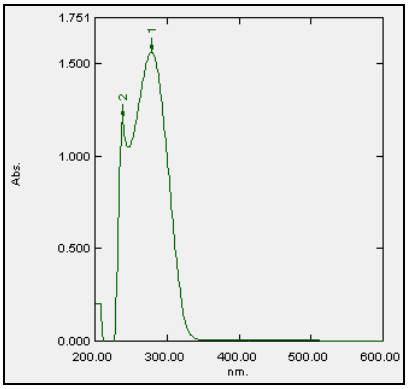


Figure (8): The ultraviolet visible spectrum for 2-(2-chloro phenyl)-5-[4-(2- chloro benzoyloxy)phenyl]-1,3،4-oxadiazole [9].

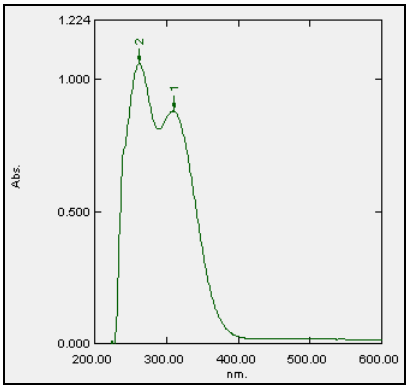


Figure (9): The ultraviolet visible spectrum for 2-(4-nitro phenyl)-5-[4-(4-nitro benzoyloxy)phenyl]-1,3,4-oxadiazole [10].

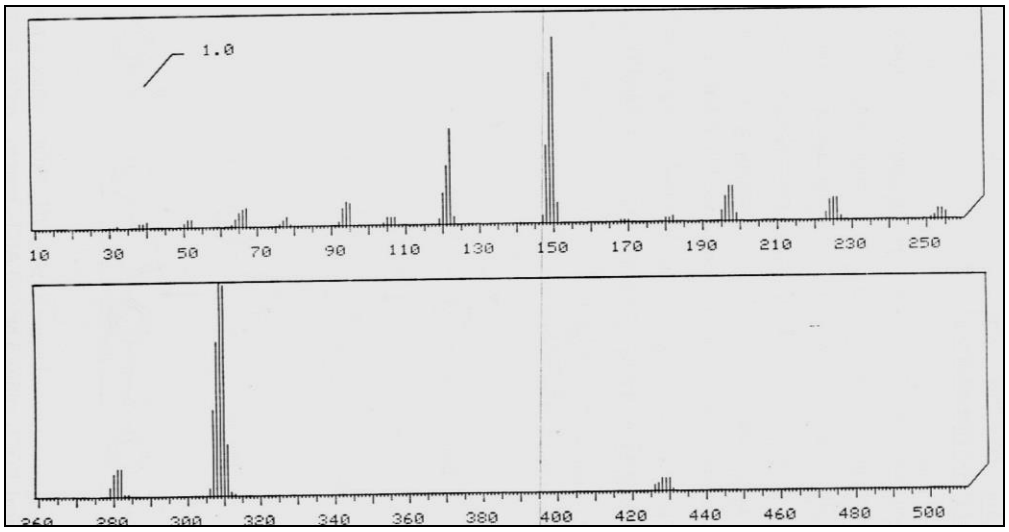


Figure (10): mass spectrum of 2-(*p*-Ethoxyphenyl)-5-[*p*-(4-ethoxybenzoyloxy)phenyl]-1,3,4-oxadiazole [4].

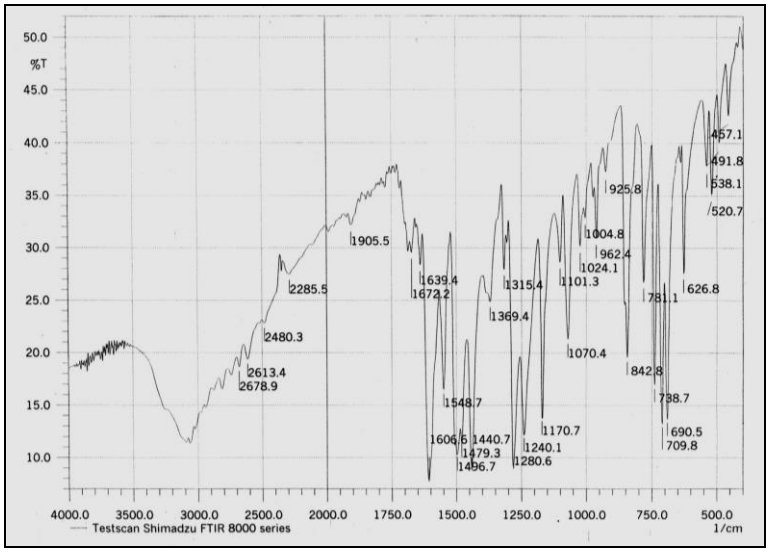


Figure (11): FT-IR spectrum of 2-phenyl-5-(4-hydroxyphenyl)-1,3,4-oxadiazole [11].

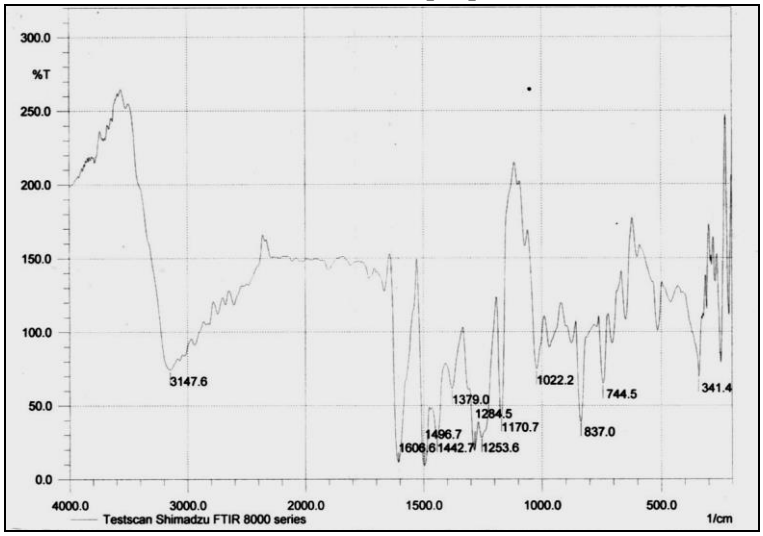


Figure (12): FT-IR spectrum of 2-(4-methoxyphenyl)-5-(4-hydroxy phenyl)-1,3,4-oxadiazole [12].

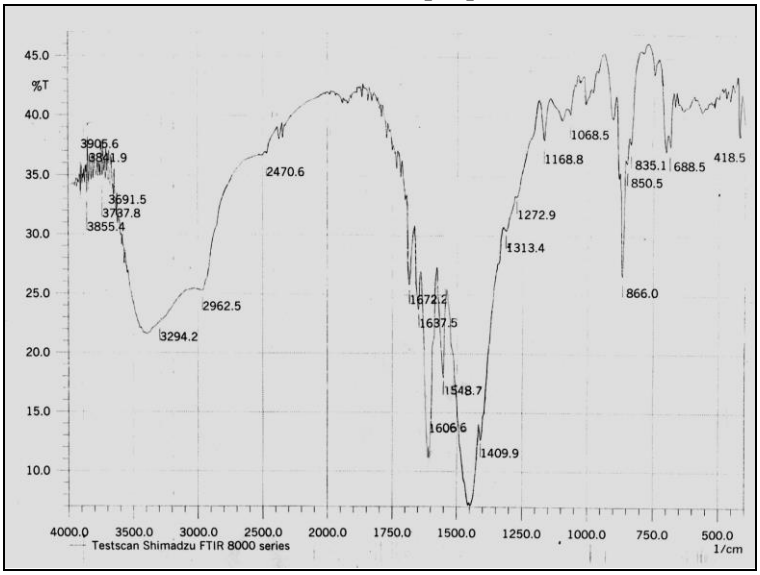


Figure (13): FT-IR spectrum of 2-(4-nitro phenyl)-5-(4-hydroxyphenyl)-1,3,4-oxadiazole [13].

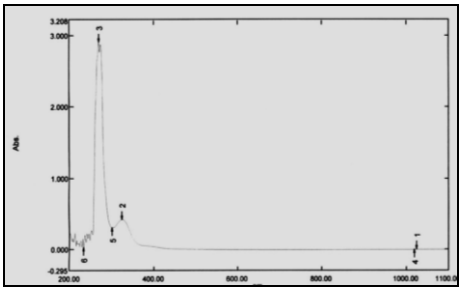


Figure (14): Ultraviolet spectrum of 2-phenyl-5-(4-hydroxyphenyl)-1,3,4-oxadiazole [11].

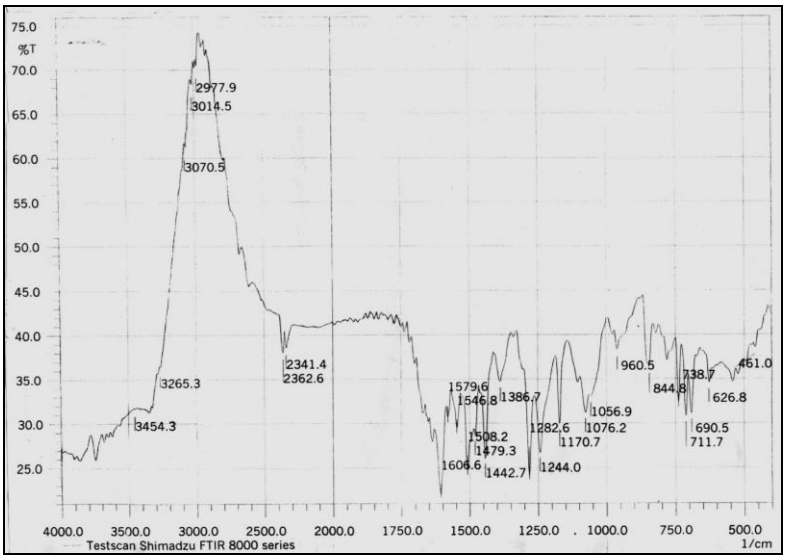


Figure (15): FT-IR spectrum of tri-[4-(5-phenyl-1,3,4-oxadiazol-2-yl) phenyl]phosphate [14].

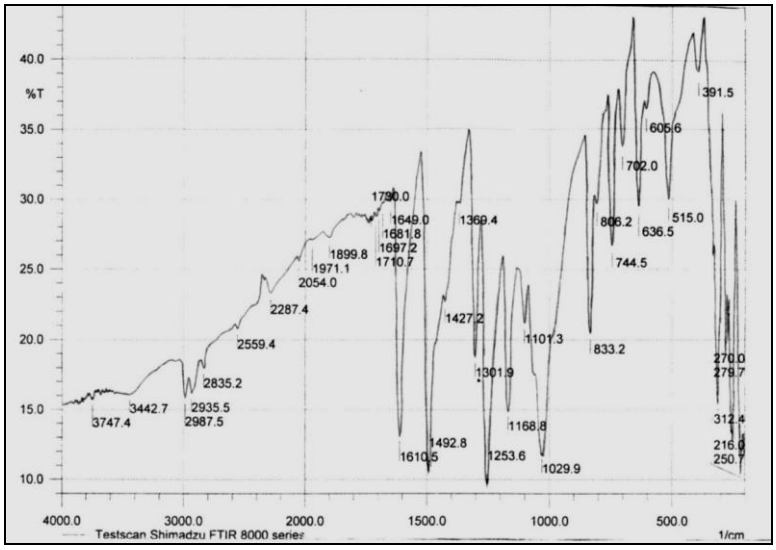


Figure (16): FT-IR spectrum of tri-[4-(5-(*p*-methoxyphenyl)-1,3,4-oxadiazol-2-yl)phenyl] phosphate [15].

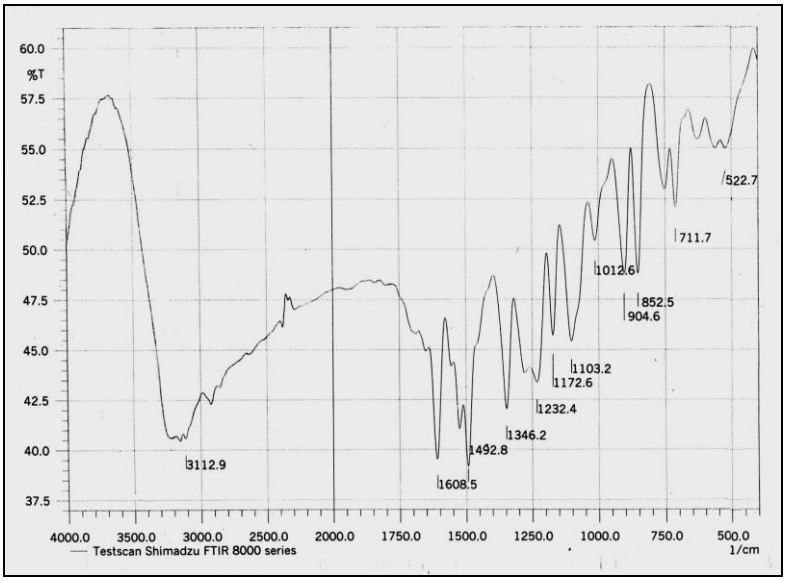


Figure (17): FT-IR spectrum of tri-[4-(5-(*p*-nitro phenyl)-1,3,4-oxadiazol-2-yl) phenyl] phosphate [16].

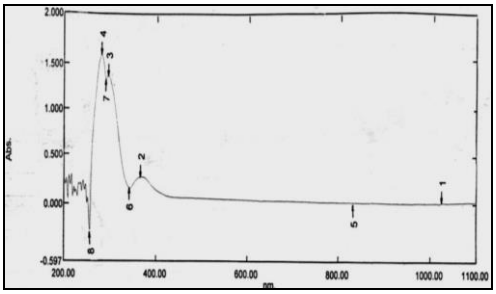


Figure (18): Ultraviolet spectrum of tri-[4-(5-phenyl-1,3,4-oxadiazol-2-yl) phenyl] phosphate [14].

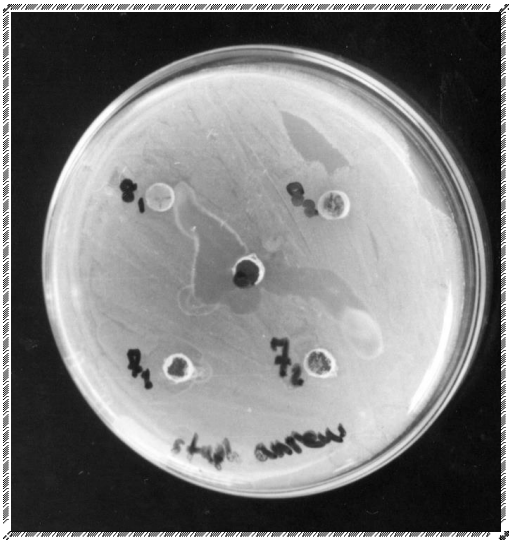


Figure (19): Effect of [14] and [16] on Staph.aureus.
$7_{(1)}$ and $7_{(2)}$ = effect of [14], $8_{(1)}$ and $8_{(2)}$ = effect of [16].

Figure (20): Effect of [14] and [16] on Esch. Coli $7_{(1)}$ and $7_{(2)}$ = effect of [14], $8_{(1)}$ and $8_{(2)}$ = effect of [16].

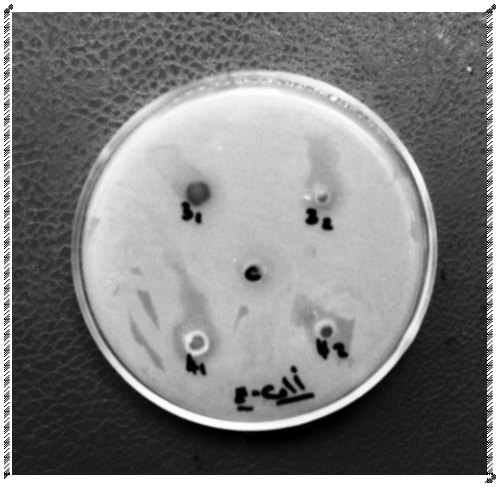


Figure (21): Effect of [15] on Esch. Coli $3_{(1)}$ and $3_{(2)}$ = effect of [15].

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