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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2018, Vol. 34, No.(5):
Pg. 2477-2486

Synthesis and Evaluation of Antimicrobial Activity of New Imides and Schiff Bases Derived From Ethyl-4-Amino Benzoate

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http://dx.doi.org/10.13005/ojc/340533

(Received: August 06, 2018; Accepted: September 29, 2018)

ABSTRACT

A series of disubstituted 1,3,4-oxadiazole derivatives, including: imides and Schiff bases, was achieved from the starting material, ethyl-4-aminobenzoate, which was converted to the corresponding 4-aminobenzohydrazide (1), by its reaction with hydrazine hydrate in absolute ethanol. Two oxadiazole parent nuclei had been synthesized from (1), the first nucleus 5-(4-aminophenyl)-1,3,4-oxadiazol-2-amine(2), and the second is 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thione (3). Compound (2) obtained from stirring methanolic solution of (1) with cyanogen bromide(CNBr) and sodium bicarbonate ($NaHCO_3$) at RT. While compound (3) was synthesized by refluxing of (1) with CS_2 in the presence of (KOH), the produced potassium salt of hydrazide underwent cyclization by acidification with 10% HCl. Meanwhile, the cyclic imides derivatives (4-6) and (10-12) were synthesized by thermal fusion of (2) or (3) with acid anhydrides, while Schiffs bases derivatives (7-9) and (13-15) were synthesized by a conventional method involved refluxing of (2) or (3) with different aromatic aldehydes, in acidic medium (using glacial acetic acid). The new derivatives had been tested against three *Gram-positive* bacteria (*Staphylococcus aureus, Micrococcus luteus*, and *Bacillus pumilus*) and two *Gram-negative* bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal species: (*Saccharomyces cerevisiae* and *Candida albicans*). Among the synthesized derivatives, compound (15) displayed a moderate to potent antibacterial activity, against different (*Gram – positive* and *Gram–negative*) bacteria, and also showed a slight to moderate antifungal activity.

Keywords: Antimicrobial, Imides, Schiff base, Synthesis, 1,3,4-Oxadiazole derivatives.

INTRODUCTION

1,3,4-Oxadiazole is a heterocyclic five–member ring possessing one oxygen atom and two nitrogen atoms. It is originated from a furan ring, where two methylene groups (=CH) substituted with two pyridine type nitrogen atoms (-N=)[1,2]. The 1,3,4-oxadiazole nucleus and their 2,5-disubstituted

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derivatives have a significant pharmacological activity including, anti inflammatory[3,4], anticancer[5,6,] antibacterial[7], antifungal[8], anti-HIV[9] and antioxidant activities[10].

1,3,4-oxadiazole derivatives can go through different chemical reactions, which made them essential for new molecule preparation, due to their distinct chemical structure, that has vast pharmacological importance[11].

Many methods in the literature document the synthesis of 1,3,4-oxadiazoles[1,12,13]. The more commonly used pathway for 1,3,4-oxadiazoles preparation, includes reactions of acid hydrazides with carboxylic acids/acid chlorides then after, cyclization or ring closure of diacylhydrazines via some of dehydrating agents, for example, phosphorous oxychloride[14-18].

It has been documented that *Gram-negative* bacteria are much more resistant to different antimicrobial agents, as compared to *Gram-positive* bacteria[19]. The differences may be due to, the constituent of the cell wall in *Gram-positive* bacteria is of a simple and single layer, while the cell wall in Gram-negative bacteria possesses an "outer membrane" with high lipid and lipoprotein content, which is not found in *Gram-positive* bacteria[20].Therefore the lipophilic nature of the cell membrane of *Gram-negative* bacteria is more resistant towards antibacterial agents, which functions as a strong barrier for a variety of antimicrobial agents. Therefore, compounds possessing hydrophilic properties, will not be able to penetrate the cell membranes of *Gram-negative* bacteria. Meanwhile, the cell wall of *Gram- positive* bacteria is not a complex structure like *Gram- negative* bacteria. Antibacterial agents can quickly collapse the bacterial cell wall of *Gram- positive* bacteria, which leads to disruption of the cytoplasm[21].

This work targets to synthesize of new derivatives of cyclic imides and Schiff bases derived from two parent nuclei: 5-(4-aminophenyl)-1,3,4-oxadiazol-2-amine (2), and 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thione(3), respectively. Imides are essential to lead components for natural products and drugs[22]. While, Schiff bases have been widely studied and the focus of different researches, due to their extensive use and distinct biological activities[23].

MATERIALS AND METHODS

The starting material for the synthesis of 1,3,4-oxadiazole derivatives, is ethyl-4-amino benzoate, and the first step of the reaction involves the formation of the corresponding hydrazide then after, preparation of two- parent nuclei (1) and(2).

All chemicals and solvents used during synthesis were of AR grade and used without further purification. Reaction monitoring was ascertained by thin-layer chromatography (TLC), using Silica gel GF_{254} (type 60) pre-coated Aluminium sheets, Merck (Germany) exposed to UV-254nm light, and the eluent used for TLC as follows: ethyl acetate: n-hexane 9:1 for compounds (1),(4) (5), (6), (8), (9), (11)and (12); ethyl acetate: n-hexane 9.5:0.5 for compound(2); ethyl acetate: n-hexane 6:4, for compounds (3), (10), (14) and (15); ethyl acetate: n-hexane 5:5, for compounds (7) and (13). All synthesized derivatives were characterized by spectroscopic analysis (FTIR and ^{1}HNMR).

Melting points were measured using melting point apparatus in open capillary tubes, and are uncorrected. The IR spectra, were recorded, on(FTIR-600, UK) spectrophotometer, using (KBr disc), (υ,cm^{-1}).

Furthermore, ^{1}HNMR spectra were recorded on Bruker model Ultra shield 300 MHz, Avance II- at (Al-Bayt University-Jordan), using tetramethylsilane (TMS) as an internal standard, the chemical shift was expressed as (δ=ppm), DMSO-$_{d6}$ and acetone-$_{d6}$ were used as solvents.

Chemical Synthesis

Chemical synthesis of all new derivatives is depicted in (scheme 1).

Synthesis of 4-aminobenzohydrazide(1)[24]

Ethyl-4-aminobenzoate (0.06 mol, 9.9 g) was dissolved in (30 mL) of hydrazine hydrate 80%, then absolute ethanol (25 mL) was added in a rounded bottom flask and refluxed for 8 h. At the end of the reaction, as monitored by TLC, the mixture

was cooled to room temperature (RT), the crystals formed, filtered and recrystallized from absolute EtOH.

White crystals, yield 77% , m.p. 222-225^{O}C, IR(KBr), (υ, cm^{-1}): 3271 and 3234 prim. (NH_2) str, 3033 Ar(CH) str, 1626 (C=O) amide str, 1604(NH) bend , 1545 (C=C) str, 843 out of plain (C-Hbenz.) bend.

Synthesis of 5-(4-aminophenyl)-1,3,4-oxadiazol-2-amine (2)[25]

4-Aminobenzohydrazide(1), (0.013 mol, 2.0 g) was dissolved in (40 mL) of MeOH with a solution of $NaHCO_3$ (0.013 mol, 1.0 g) in (20 mL) of distilled water, then addition of cyanogen bromide (CNBr), (0.013 mol,1.4 g), and stirring the mixture in a round flask overnight at RT, then methanol is evaporated, (30 mL) of cold distilled water was added, the precipitate formed, obtained by filtration. Off-white to pink powder, yield 61%, m.p 280-282^{O}C, IR(KBr), (υ, cm^{-1}): 3327 & 3224 prim (NH_2) str, 3060 (Ar-CH)str, 1608 (C=N) str, 1585,1570 &1506 (C=C) str,1290 asym (C-O-C) str, 1068 sym (C-O-C) str, 837 out of plain(C-Hbenz.)bend.; ^{1}HNMR(300 MHz,DMSO-$_{d6}$, δ= ppm): 7.40(s,2H,NH_2); 6.85-6.50 (m,4H, 4Ar-H); 5.60 (s,2H, NH_2-oxadiaz).

Synthesis of 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thione (3)[24]

4-Aminobenzohydrazide (1) (0.02 mol, 3.0 g) dissolved in absolute EtOH (20 mL) and cooled to 20°C in the ice bath. Then after, addition of potassium hydroxide (KOH) to the mixture, (0.027 mol, 1.5 g) in an absolute EtOH (10 mL), and stirred for 15 min. Then CS_2 (0.024 mol, 1.5 mL) was added gradually. The reaction was refluxed for 12 h, The solvent was reduced by rotary evaporator, and the produced solid was dissolved in (25 mL) of cold D.W and acidified with 10% HCl, until precipitation of light yellow crystals of (3) obtained and recrystallized from absolute EtOH.

Light yellow crystals, yield 64%, m.p 243-245^{O}C, IR(KBr), (υ, cm^{-1}): 3315and 3220 prim (NH_2) str, 3059 Ar(CH) str, 2557 str of (SH) thiol gr,1618 (NH) bend, 1603 cm^{-1} (C=N) str, 1570, 1512 and 1485 (C=C)str, 1271 asym (C-O-C) str, 1065 sym (C-O-C) str, 829 (out of plain C-Hbenz.) bend; ^{1}HNMR(300 MHz, DMSO$_{d6}$, δ=ppm): 7.48 (d, 2H, 2Ar-H); 6.60(d, 2H, 2Ar-H); 5.90(brs,1H,SH); 7.78(s,2H,NH_2).

General method for the synthesis of imide derivatives (4-6)

In a pyrex test tube, added compound(2) (0.0011mol, 0.19 g), and phthalic anhydride (0.0022 mol, 0.325 g) or of maleic anhydride(0.0022 mol, 0.215 g), or succinic anhydride (0.0022 mol, 0.22g), mixed, and heated using oil bath 290^{O}C. The mixture stirred continuously by using pyrex glass road for 30 min. then washed with diethyl ether and left to dry.

Synthesis2-((4-(5-(1,3-dioxoisoindolin-2-yl)-1,3,4-oxadiazol-2-yl)phenyl)carbamoyl)benzoic acid (4)

Light yellow powder, yield 95%, m.p 286-288^{O}C, IR(KBr), (υ, cm^{-1}): 3307 (OH of COOH) str, 3095 Ar(CH) str, 1749 (C=O) acid str, 1685 and 1651 asym.& sym. (C=O) amide str, 1604 (C=N) str, 1557 (C=C) str, 1257 asym (C-O-C) str, 1043 sym (C-O-C) str, 835 out of plain (C-H benz.) bend;^{1}HNMR(300 MHz, DMSO-$_{d6}$, δ=ppm): 8.18-7.18(m,12H,12Ar-H); 10.60(s,1H,OH).

Synthesis of 1-(4-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2,5-dione (5)

Yellow powder; yield 92% , m.p 244-246^{O}C, IR(KBr), (υ,cm^{-1}): 3012 Ar(CH) str, 1664 (C=O) amide str, 1604 (C=N) str, 1552,1510 and 1427 (C=C) str, 1238 asym (C-O-C) str, 845 out of plain (C-H benz.)bend;^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 7.72(m,4H,4Ar-H); 7.12 (d, 2H, maleimide -2H); 6.36 (d,2H, maleimide-2H); 10.55 (s,1H,OH (keto/enol form).

Synthesis of 4-((5-(4-(2,5-dioxopyrrolidin-1-yl)phenyl)-1,3,4-oxadiazol-2-yl)amino)-4-oxobutanoic acid (6)

White powder, yield 89%, m.p 252-254^{O}C, IR(KBr), (υ, cm^{-1}): 3315 (OH) str, 2935 asym (CH_2), 1725 (C=O) acid str, 1701 and 1666 (C=O amide) str,1606 (C=N) str, 1583 and 1506 (C=C) str, 1061 sym (C-O-C) str, 835 out of plain (C-H benz.) bend; ^{1}HNMR(300MHz, DMSO-$_{d6}$,δ= ppm: 12,10(s,1H,OH);10.16(s,1H,NH); 7.93-7.08 (m,4H,4Ar-H); 2.76(t,2H,CH_2).Other CH_2 & 2CH_2 groups of succinimide, were masked with DMSO-$_{d6}$ peak.

Synthesis of (E)-4-(((4-(5-amino-1,3,4-oxadiazol-2-yl)phenyl)imino)methyl)-2,6-dimethoxyphenol (7)

Glacial acetic acid (3dr) was added to 3,5-dimethoxy-4-hydroxy benzaldehyde (0.0036 mol, 0.65 g), dissolved in (20 mL) of MeOH, the entire mixture stirred in a round bottom flask for 15 min, then compound (2), (0.0017 mol, 0.3 g) in (20 mL) of MeOH was added, and refluxed for 5 h. At the end of reaction, the mixture was cooled, then poured into a crushed ice to get a precipitate, which was collected by filtration, dried and washed with ethyl acetate, and recrystallized from EtOH.

Yellow powder, yield 53% , m.p138-140^{O}C, IR(KBr), (υ, cm^{-1}) : 3573 (OH) str, 3361 and 3329 (NH_2) str, 2941 and 2841 asym & sym (CH) str,1606 and 1579 (C=N) str,1512 and 1462(C=C) str, 1252 (Ar-O-CH_3) str, 1038 sym(C-O-C) str, 839 (out of plain C-H benz.)bend; ^{1}HNMR(300MHz, DMSO$_{d6}$,δ=ppm): 9.77(s,1H,OH) ; 8.50 (s,1H,CH=N); 8.17-7.24 (m, 6H,6Ar-H); 7.80(s,2H,NH_2); 3.84 (s,6H, 2×OCH_3).

Synthesis of (E)-5-(4-((4-methoxybenzylidene) amino)phenyl)-1,3,4-oxadiazol-2-amine (8)

Glacial acetic acid (3 dr) was added to 4-methoxy benzaldehyde (0.0036 mol, 0.5 g), the mixture stirred in a round bottom flask for 15 min. then compound (2), (0.0017 mol, 0.3 g) in (30 mL) of MeOH was added, and refluxed for 6 h. After completion of the reaction, the mixture was cooled to RT, and poured into crushed ice, to produce a precipitate, then filtered, dried and recrystallized from EtOH.

Yellow powder, yield 55%, m.p 254-256^{O}C, IR(KBr), (υ, cm^{-1}): 3303 and 3240 prim (NH_2) str, 3120 (NH-tautomer) str, 2964 & 2839 asym & sym (CH) of CH_3 str,1653 and 1597 (C=N) str, 1570 and 1510 (C=C) str, 1254 (Ar-O-CH_3) str, 845 (out of plain C-H benz.) bend; ^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm: 8.59(s,1H,CH=N); 7.93-7.08(m,8H, 8Ar-H and NH_2); 3.85 (s,3H,1XOCH_3).

Synthesis of (E)-5-(4-((4-nitrobenzylidene)amino) phenyl)-1,3,4-oxadiazol-2-amine (9)

Glacial acetic acid (3dr) was added to 4-nitrobenzaldehyde (0.0036 mol, 0.54 g) in (10 mL) of absolute EtOH. The entire mixture stirred in a round bottom flask for 15 min, then compound (2), (0.0017 mol, 0.3 g) dissolved in (20 mL) of hot absolute EtOH was added, stirred and refluxed for 5 h. The precipitate which is obtained, filtered and recrystallized from EtOH.

Yellow powder, yield 58%, m.p 275-277^{O}C, IR(KBr), (υ, cm^{-1}) : 3317 and 3252 prim (NH_2) str, 3080 Ar (CH) str, 2898 (CH) str, 1676 and 1597 (C=N) str, 1516 and1344 asym &sym (NO_2) str, 865 out of plain (C-H benz.) bend; ^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 8.82(s,1H, NH); 8.42 (s,1H,CH=N); 8.24 (d, 2H, 2Ar-H); 7.98(d, 2H, 2Ar-H); 7.76(d, 2H, 2Ar-H); 7.28(d, 2H, 2Ar-H); 7.50(s,2H,NH_2).

General method for synthesis of imide derivatives (10-12)

To a pyrex test tube added compound (3), (0.001 mol, 0.19 g) and phthalic anhydride (0.001 mol, 0.148 g), or maleic anhydride (0.001 mol, 0.1 g), or succinic anhydride (0.001 mol, 0.1 g), mixed and heated using an oil bath 290^{O}C. The mixture stirred continuously by using pyrex glass road for 30 min. then washed with diethyl ether and left to dry.

Synthesis of 2-(4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)isoindoline-1,3-dione (10)

Off-white powder, yield 89%, m.p 289-291^{O}C, IR(KBr), (υ, cm^{-1}) : 3225 (NH) str, 3078 Ar(CH) str, 1739 and1712 asym & sym (C=O) amide str, 1612 (C=N) str, 1514 and 1469 (C=C) str, 841 out of plain (C-H benz.) bend; ^{1}HNMR(300MHz, DMSO-$_{d6}$, δ=ppm): 8.06-7.69 (m, 8H,8Ar-H) .

Synthesis of 1-(4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrrole-2,5-dione(11)

Dark yellow powder, yield 95%, m.p 229-231^{O}C, IR(KBr), (υ, cm^{-1}) : 3168 (NH) str, 3030 Ar(CH) str, 1687 (C=O)str, 1626 (NH) bend, 1604 (C=N) str, 1514 (C=C) str, 847 out of plain (C-H benz.) bend; ^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 10.68(s,1H,NH); 7.87-7.81(m,4H,4Ar-H); 6.51 (d,1H, maleimide-H); 6.33(d,1H,maleimide-H).

Synthesis of 4-oxo-4-((4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)amino)butanoic acid (12)

White powder, yield 85%, m.p 233-235^{O}C, IR(KBr), (υ, cm^{-1}) : 3450 (OH)str, 3323 and 3126 (NH) amide str, 3099 Ar(CH) str, 2966 and 2890 asym & sym (CH) of CH_2 str, 2652 (SH)str, 1697 (C=O) of COOHstr,1676 (C=O) amide str, 1608 (C=N) str, 1585 ,1516&1493 (C=C) str, 847 out of

plain(C-H benz.) bend;^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 14.60(s,1H,OH); 12.10 (s,1H,NH); 10.28(s,1H,NH); 7.75(m,4H,4Ar-H); 3.24(t, 2H,CH_2). Other (CH_2) gr, masked with DMSO-$_{d6}$ peak.

Synthesis of (E)-5-(4-((4-hydroxy-3,5- dimethoxy benzylidene)amino)phenyl)-1,3,4-oxadiazole-2(3H)-thione (13)

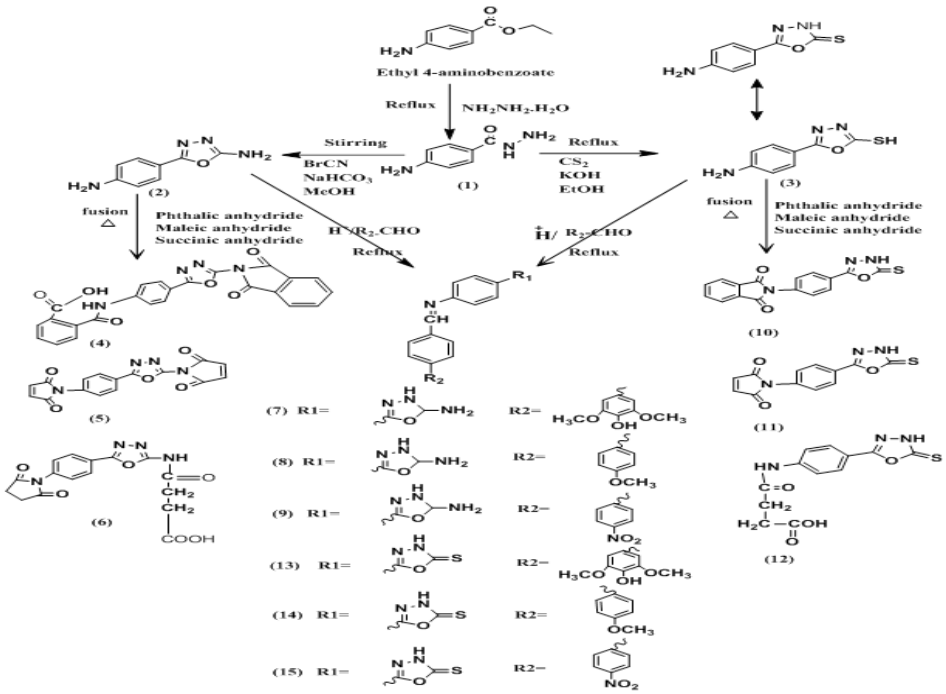
Glacial acetic acid (3^{dr}) was added to 3,5-dimethoxy -4-hydroxy benzaldehyde (0.0017 mol, 0.3 g) dissolved in absolute EtOH (20 mL), the mixture stirred in a round bottom flask for 15 min, then compound (3), (0.0016 mol, 0.3 g) dissolved in hot absolute EtOH (20 mL), was added, and the mixture refluxed for 6 h. After completion of reaction. It was cooled to RT, a precipitate formed , which was filtered and washed with ethyl acetate, then recrystallized from petroleum ether.

Orange powder, yield 62%, m.p 258-260^{O}C, IR(KBr), (υ, cm^{-1}): 3519 (OH) str, 3041 Ar(CH) str, 2945 & 2814 asym & sym (CH) of CH_3 str, 2569 (SH) str, 1651 and 1597 (C=N) str, 1512, 1489 & 1437 (C=C) str, 1234 asym (C-O-C) str, 1097 sym(C-O-C) str, 862 (out of plain CH benz.) bend; ^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 9.73 (s,1H,OH); 8.84(s,1H,NH); 8.45(s,1H,CH=N); 8.03-6.63(m,6H,6Ar-H); (2×OCH_3) masked behind H_2O(DMSO-$_{d6}$).

Synthesis of (E)-5-(4-((4-methoxybenzylidene) amino)phenyl)-1,3,4-oxadiazole-2(3H)-thione (14)

Glacial acetic acid (3dr) was added to 4-methoxybenzaldehyde (0.0017 mol, 0.23 g), the mixture stirred in a round bottom flask for 15 min, then compound (3), (0.0016 mol, 0.3 g) dissolved in hot absolute EtOH(30 mL), was added ,and the mixture refluxed for 6 h. At the end of reaction, it was cooled to RT, a precipitate formed which was filtered and recrystallized from petroleum ether.

Orange powder, yield 58%, m.p 210-212^{O}C, IR(KBr), (υ, cm^{-1}): 3221 (NH) str, 2931 and 2835 asym & sym (CH) of CH_3 str, 1610 (C=N) str,1514 and 1406 (C=C) str,1252 asym (C-O-C) str,1070 sym (C-O-C) str, 831 cm^{-1} (out of plain CH benz.)bend;^{1}HNMR(300MHz,DMSO-$_{d6}$, δ=ppm): 9.83(s,1H,NH); 8.54(s1H,CH=N); 7.94-6.53(m,8H,8Ar-H); 3.81(s, 3H,1×OCH_3).



Scheme 1. Synthesis of titled compounds (1-15)

Synthesis of (E)-5-(4-((4-nitrobenzylidene)amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione (15)

Glacial acetic acid(3^{dr}) was added to 4-nitrobenzaldehyde (0.0017 mol, 0.26 g), the mixture stirred in a round bottom flask for 15 min, then (3), (0.0016mol, 0.3 g) dissolved in hot absolute EtOH (30 mL), was added to the mixture, and refluxed for 6 h. At the end of reaction, it was

cooled to RT, the precipitate obtained, filtered, dried, washed with ethyl acetate, then recrystallized from EtOH.

Orange powder, yield 55% , m.p 258-260^{O}C, IR(KBr), (υ, cm^{-1}): 3070 cm^{-1} Ar(CH) str, 2920 and 2890 cm^{-1} asym & sym aliphatic (CH) str, 1608 cm^{-1} (C=N) str,1589 and 1489 cm^{-1} (C=C) str, 1516 and 1344 cm^{-1} *asym & sym*(NO_2) str, 858 cm^{-1} (out of plain CH benz.) bend; ^{1}HNMR(300MHz,DMSO-$_{d6}$, δ= ppm): 10.12(s,1H,NH); 8.84 (s,1H,CH=N); 8.45-6.53(m, 8H,8Ar-H).

Antibacterial Activity

The new title compounds 4-15 were tested for their preliminary antibacterial activity and measured using well diffusion technique[26] *in vitro*, against three types of tested microorganisms: *Gram-positive* (G^{+ve}) bacteria (*S. aureus, Micrococcus luteus,* and *Bacillus pumilus*), and two *Gram-negative* (G^{-ve}) bacteria (*Pseud. aeruginosa,* and *E. coli*), were clinically activated and maintained on nutrient agar medium for testing antibacterial activity, and potato dextrose agar medium for antifungal activity[27]. Cefotaxime was used as a standard drug for antibacterial activity, while miconazole was used as a standard drug for antifungal activity, using a minimum inhibitory concentration, (MIC) of 1000 and 100µg/ml of synthesized derivative in DMSO.

RESULTS AND DISCUSSION

Chemistry

The hydrazide (1) obtained by refluxing of the starting material ethyl-4-aminobenzoate with hydrazine hydrate in absolute ethanol. (1) characterized by FTIR, due to formation of two doublet peaks for the primary amine of hydrazide, at 3271 and 3234 cm^{-1}, in addition, a prominent peak for the(C=O) amide stretching, at 1626 cm^{-1}.

Two parent nuclei (2and 3), had been synthesized from (1), where (2) obtained from stirring a methanolic solution of (1) with cyanogen bromide (CNBr), and sodium hydrogen carbonate,($NaHCO_3$) at ambient temperature, while (3) obtained from refluxing an ethanolic suspension of (1) with CS_2 in the presence of KOH, the formation of potassium salt of hydrazide, which underwent cyclization by acidification with 10% HCl. Each parent nucleus (2) and (3), displayed two peaks, at 3327and 3224 cm^{-1} and 3315 and 3220 cm^{-1}, respectively, that confirmed the presence of (NH_2) group. In addition, (3) recorded a peak at 2557 cm^{-1} due to thiol group (SH), and a peak at 1290 and 1271 cm^{-1} for (2) and (3), respectively, due to asym.(C-O-C) stretching, also, other peaks at 1068 and 1065 cm^{-1}, respectively, owing to sym (C-O-C) stretching, (oxadiazole ring formation). The ^{1}HNMR spectrum for the parent nucleus (2) exhibited a singlet peak at δ=7.40 ppm, attributed to primary aromatic-NH_2 group, as well as, a peak appeared at δ=5.60 ppm, due to (NH_2)-oxadiazole.

The four aromatic protons of (2), displayed at δ=6.85-6.50 ppm, as a multiplet.

The second parent nucleus (3), displayed a peak at δ =7.78 ppm corresponds to the presence of primary –NH_2-group, while the four aromatic protons, for each (2) and (3),appeared at their expected region. See (exp. Part).

The cyclic imide derivatives (4-6) and (10-12), were synthesized in good yields, (85-95%), by thermal fusion of (2) or (3), with one of the three acid anhydrides, phthalic, succinic and maleic.

The IR spectrum of (4),displayed a distinct band at 3307 cm^{-1} owing to (OH) stretching of the carboxylic group. This indicate the opening of one imide ring, at one side of a ring fusion, besides that, a peak displayed at 1749 cm^{-1}, due to (C=O) stretching of the carboxylic acid. Other peaks assigned for (C=O) amide stretching, at 1685 and 1651 cm^{-1}, belong to the second fused imide ring. The ^{1}HNMR spectrum for (4)), exhibited a prominent peak, at δ =10.60 ppm, attributed to (OH) signal of the carboxylic acid, this confirm the opening of one of phthalic imide ring , while the aromatic rings integrating for twelve protons, appeared at their expected aromatic region. (5) showed a prominent IR band at 1664 cm^{-1}, due to conjugated (C=O) amide's stretching, and ^{1}HNMR displayed two signals, each as a doublet , appeared at δ=7.12 and 6.36 ppm, respectively, owing to the two protons of each maleimide ring.

The IR spectrum for (6) showed a characteristic carboxylic acid absorption, due to (OH) stretching at 3315 cm^{-1}, and a peak displayed at 1725 cm^{-1}, assigned for (C=O) acid's stretching.

Other peaks displayed at 1701 and 1666 cm^{-1} belong to (C=O) stretching of amide, while ^{1}HNMR analysis for (6) is similar to (4), exhibited a peak at δ= 12.10, as a singlet, due to (OH) signal of the carboxylic acid confirm the opening of one of succinimide ring), besides that, a peak integrating for two protons, belonging to (CH_2) group, displayed at δ =2.76 ppm, as a triplet. The second aliphatic (CH_2) group, and the two (CH_2)groups of succinimide ring, were masked with DMSO-d6 peak. (10) and (11) represent the product fusion, of each of phthalic anhydride, and maleic anhydride with (3), respectively, The NH-stretching of thioamide, for each, (10) and (11), displayed at 3225 and 3168 cm^{-1} respectively, also IR spectra recorded two peaks assigned for the (C=O) amide stretching, for (10) at 1739 and 1712 cm^{-1}, while (11) displayed a (C=O) amide's stretching, at 1687 cm^{-1} due to (conjugation). The ^{1}HNMR of (10) showed peaks, as multiplet, at the range of δ=8.06-7.69 ppm, due to eight aromatic protons, While (11) showed a peak at δ =10.68 ppm, due to NH-thioamide, also another two distinct signals, each attributed for one proton, appeared as a doublet, at δ=6.51 and δ=6.33ppm, respectively, (maleimide ring).

The IR spectrum for (12) is similar to (6), in which succinimide ring is opened during thermal fusion. A recorded band at 3450 cm^{-1}, assigned for (OH) stretching of a carboxylic acid,(ring opening), a new peak at 1697 cm^{-1}, due to (C=O) of the

Table 1: The antibacterial activity of tested compounds (4-15)

Compd No.	Conc. µg/ml	*Staph. aureus*	*Micrococcus luteus*	*Bacillus pumilus*	*Pseud. aeruginosa*	*E.coli*
		Inhibition zone (mm)				
4	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
5	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
6	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
7	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
8	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
9	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
10	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
11	10^3	8.3	–	8.1	–	–
	10^2	–	–	–	–	–
12	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
13	10^3	10.6	10.5	11.5	–	–
	10^2	–	–	–	–	–
14	10^3	–	–	–	–	–
	10^2	–	–	–	–	–
15	10^3	–	22.7	11.6	–	14.1
	10^2	–	18	–	–	–
Cefot.	10^3	38	68	33	35.6	39
	10^2	31.8	63	25.8	30.8	32.12
DMSO	–	–	–	–	–	–

carboxylic acid stretching, While The ^{1}HNMR showed a signal for (OH) of the carboxylic acid appeared at δ=14.60 ppm, in addition, two other signals, one displayed at δ=12.10 and 10.28 ppm, attributed to NH-thioamide and NH-amide.

Schiff's bases (7-9) and (13-15) synthesized by a conventional method involved, refluxing of an ethanolic or methanolic solution of (2) or (3), with aromatic aldehydes in an acidic medium (using glacial acetic acid).

Table 2: The antifungal activity of tested compounds (4-15)

Compd No.	Conc. µg/ml	*Saccharomyces cerevisiae*	*Candida albicans*
		Inhibition zone (mm)	
4	10^3	_	_
	10^2	_	_
5	10^3	_	_
	10^2	_	_
6	10^3	_	_
	10^2	_	_
7	10^3	_	_
	10^2	_	_
8	10^3	_	_
	10^2	_	_
9	10^3	_	_
	10^2	_	_
10	10^3	_	_
	10^2	_	_
11	10^3	_	_
	10^2	_	_
12	10^3	_	_
	10^2	_	_
13	10^3	_	_
	10^2	_	_
14	10^3	_	_
	10^2	_	_
15	10^3	14.2	8
	10^2	_	_
Miconazole	10^3	15.8	32
	10^2	14	30.8
DMSO (control)	_	_	_

(-)= No activity, slightly active (ZI =5-10 mm), moderately active (ZI= 10-15 mm), highly active (ZI= more than 15 mm).[28,29], ZI= Zone of inhibition

The characteristic imine group displayed in IR spectra at 1606, 1579 cm^{-1} for (7), and at 1653 and 1597 cm^{-1} for (8), and at 1676 and 1597 cm^{-1} for (9), respectively. Also, (7) showed characteristic (OH) stretching at 3573 cm^{-1} and (Ar-O-CH_3) absorption band, at 1252 cm^{-1}, While for (8), a distinct peak for (Ar-O-CH_3) recorded at 1254 cm^{-1}. Compound (9) exhibited a characteristic band, due to asym. (NO_2) stretching, at 1516 cm^{-1} and sym (NO_2) stretching at 1344 cm^{-1}, on the other hand, Schiff bases, are well characterized by ^{1}HNMR spectroscopy, due to the appearance of imine group (CH=N), at δ= 8.50,8.59 and 8.42 ppm, for (7,8 and 9), respectively.

All the aromatic protons for (7-9) and (13-15), are well characterized and displayed at their expected aromatic regions. For more details, for the rest of the functional groups, see (exp. part).

Antimicrobial Evaluation

It's evident from the data displayed in Tables 1 and 2, compound (11) showed slight antibacterial activity against (Staph. aureus and Bacillus pumilus). Compound (13) showed moderate activity towards tested *Gram positive* bacteria. Among all synthesized compounds, (15) showed potent antibacterial activity against (*Micrococcus luteus*), moderate antibacterial activity against (*Bacillus pumilus*), moderate antibacterial activity against (*E. coli*), moderate antifungal activity against (*Saccharomyces cerevisiae*) and slight antifungal activity against (*Candida albicans*).

CONCLUSION

A new oxadiazoles derivatives (imides and Schiff bases), derived from ethyl -4-amino benzoate, were successfully synthesized, by thermal fusion , and conventional methods, respectively, they were characterized and evaluated for their antimicrobial activities.

Compound (11) showed a slight activity against some of *Gram- positive* bacteria, while (13) showed a moderate activity against all *Gram-positive* bacteria used in this evaluation. Furthermore, (15) displayed a moderate to potent antibacterial activity, against different (*Gram-positive* and *Gram- negative*) bacteria, and also showed a slight to moderate antifungal activity.

ACKNOWLEDGMENT

We're grateful to the College of Pharmacy-Department of Pharmaceutical Chemistry-University of Baghdad, for conducting the research, and Dr. Raeid M. Al-Sayab/College Ibn -Al-Haitham, for Pure Science, for running ^{1}HNMR analysis.

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