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Synthesis, Evolution Anticancer and Microbial Activity of Some 1,3,4-Oxadiazoles Analogues

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ABSTRACT:
Introduction: The need to develop anti-cancer drugs and the increasing bacteria resistance towered many antibiotics have made researchers more interested in testing the ability of synthetic derivatives to inhibit different types of microorganisms, although studying the cytotoxicity effect on various species of cancer cells.
Aim: synthesis methyl nicotinate derivatives and evolution their anti-microbial and anti-cancer ability.
Material and methods: a series of 1,3,4-oxadiazole heterocyclic compounds were synthesized, a nicotinoyl hydrazine was synthesized by refluxed methyl nicotinate and hydrazine hydrate. a good yield of Schiff bases was isolated by a reaction equimolar quantity of nicotinoyl hydrazine with several aromatic aldehydes. Up on Schiff bases acylation, heterocyclic compounds were collected, and synthetic derivatives were identified using Infra-Red (IR), Hydrogen Nuclear Magnetic Resonance (^{1}HNMR). The methyl thiazolyl tetrazolium (MTT) Method was used for the Evolution of anti-cancer activity. Well diffusion method over agar was used to determine antibacterial activity, gram-positive and gram-negative bacteria were used to test the strength of inhibition for synthesis derivatives.
Results: a white solid of nicotinoyl hydrazine was isolated, different Schiff bases were separated in a high yield, and 1,3,4-oxadiazole analogues were collected. The inhibition values of the prepared derivatives towards bacterial growth were measured. Cytotoxicity was an accounted for Schiff bases and 1,3,4-oxadiazole analogues.
Conclusion: Three oxadiazole heterocyclic compounds show moderate activity. In contrast, two showed excellent inhibition toward cancer cell and bacteria growth greatly depending on the type and position of substituent groups in different hetero cyclic compounds.
Keywords: 1,3,4-Oxadiazole, Schiff bases acylation, Methyl nicotinoyl hydrazine.

INTRODUCTION

From many decades 1,3,4-oxadiazoles have been proven their synthetic and biological importance against various species of microorganisms and introduced hundreds of products as antibacterial, anti-fungi, anti-tuberculosis, antitumor, anti-virus, anti-septic, and anti-Arteriosclerosis[1,2,3,4,5]. It has well known the wide ring application of methyl nicotinate in synthesis. It has been used as a starting material in nucleophilic displacement reactions [6,7]; hydrazine hydrate shows excellent activity in the synthesis of isoniazid derivatives (Ar-NH-NH2), which have many implementations in therapeutic, especially inhibition of a wide series of tuberculosis [8]. The direct cyclisation of methyl nicotinoyl hydrazone to 1,3,4- heterocyclic compounds were completed by using various reagents such as acetic anhydride, phosphorus penta oxide, thionyl chloride, and polyphosphoric acid. Increasing bacterial resistance against antibiotics, leading to many drugs interaction and reducing the drug efficacy, such as local tissue irritation, narrow antimicrobial spectrum and Interference with wound healing processes [9,10]. For this reason, there is always a need to develop new types of synthetic agents and reduce the toxic effect by modifying the chemical structure. Many researchers have reported that gram positive is fewer resistances to antibiotics than gram negative due to the outer membrane of gram negative bacteria, which can alter the hydrophobic properties, and the cell wall is a barrier for different various of antibiotics which causes antibiotic resistance; on the other hand, gram positive bacteria have alack in this ability [11,12] .several 1,3,4-oxadiazole have been reported to possess good anticancer potential against various types of cancer cells. Although the anticancer activity of oxadiazoles is well documented, a comprehensive study on their putative targets and mechanism of action has not been reported so far [13].

AIMS

The work aims to synthesise methyl nicotinate derivatives as 1,3,4-oxadiazole heterocyclic in mild conditions. Evolution of their anticancer and antibacterial activity and no further reagents were used, lead to less contamination and high purity of isolate products.

MATERIAL AND METHODS

The melting points of the synthetic derivatives were measured using a capillary tube method with no further correction. The IR measurements were recorded on a Shimadzu spectrometer using a KBr pellet, and ^{1}HNMR measurements were done using the DMSO. No further purification of starting materials (Methyl nicotinate, hydrazone hydrate, absolute ethanol and aromatic aldehydes), while the purification of synthesis derivatives was achieved by using a recrystallisation

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process. the anti-cancer and microbial activity of synthesised derivatives was tested against MCF7 (breast cancer), *Escherichia coli* (gram negative) *Staphylococcus aureus* (gram positive) were supplied from Alsadar hospital (Najaf city). Culture media supplies from Sigma-Aldrich, USA.

Synthesis of Nicotinoyl hydrazine:

In a 250 ml round flask (0.01 mol, 1.37 gm), Methyl nicotinate was added to about 50 ml of absolute ethanol under stirring; after a half hour, a hydrazine hydrate solution (0.01mol) was added dropwise. The mixture was refluxed for 6 hours at 80°C. Then upon cooling, the resulting white solid crystals of methyl nicotine hydrazone separated and were recrystallised for further purification with absolute ethanol.) [14,15].

Synthesis of nicotine hydrazine Schiff bases:

In a 100 ml round flask (0.001 ml, 0.136 gm), Nicotinoyl hydrazine was dissolved in a stirred solution of absolute ethanol (25 ml), and the appropriate aldehydes were added after half an hour (0.001 ml) at 90°C and for 6 hours a mixture of reaction was refluxed. Then, at room temperature, a mixture was cooled and recrystallised with hot ethanol, a different Schiff bases (2a-2e) were separated [16]. The identification of the synthesised derivative was achieved using the IR spectrum and ^{1}HNMR, milting point and some of their physical properties were also determined.

Synthesis 1,3,4-oxadiazole derivatives:

In a 100ml round flask, dissolve 0.01 mol of Schiff bases derivatives (2a-2e) in 20 ml of absolute ethanol. After about a half hour of continuous stirring, add 5 ml of acetic anhydride dropwise. The reaction mixture refluxed for 3 hours at 100°C and stood overnight at room temperature; a different 1,3,4-oxadiazole derivative was isolated and recrystallised using CCL_4. A per cent yield of 50-60% [17]

Antimicrobial Evolution:

The antibacterial activity of whole synthesised compounds was evaluated using the well diffusion method on Mueller-Hinton agar. *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) were used to determine the antibacterial activity of prepared compounds, and inhibition zones were reported in millimeters. MHA agar was cultured with bacterial strain under aseptic conditions, and wells (diameter =6mm) were filled with 50µl of tested samples and incubated at 37°c for 24 hours. After the incubation time, the diameter of the inhibition zone was measured. The antimicrobial Evolution of synthesised derivatives was tested at 200µg, 150µg, and 100µg. 2mg of each derivative dissolved in dimethyl sulfoxide (DMSO, 1ml), then made up to 10ml with sterile water to give appropriate concentration of 200 µg/ml. (use the same mothed to prepare other concentrations). Antibacterial activity results showed that most oxadiazole compounds have moderate to strong activity. each sample was examined in a separate petri dish and at three different concentrations, and the inhibition diameter was measured. Noting that increased concentration leads to an increase in the inhibition zones [18].

Cytotoxicity assay:

Methyl thiazolyl tetrazolium (MTT) cell viability was done using 96-well plats. MCF-7, Cell line (breast cancer) was seeded at 1×10^4 cells/well. After 24 hrs., a confluent monolayer was formed, and cells were treated with tested derivatives at 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µl of 2mg/ml solution of (MTT) stain and incubating the cells for 2.5 hrs. at 37°C. after removing the MTT solution, a crystal remaining in the wells solubilised by addithion130 µl of 1% dimethyl sulphoxide followed by 37°C incubation for 15 min with shaking. The absorbency was determined on the microplate reader at 492nm, and the inhibition rate of cell growth (percentage of cytotoxicity) was calculated as the following equation:

Cytotoxicity=A-B/A× 100, where A is the optical density of control, and B is the optical density of the sample [19].

The cytotoxicity of synthesised compounds was evaluated against the MCF-7 tumor cell line. 3c exhibited significant inhibitory activities (77%) against MCF-7 at 100µg concentration, and 3d exhibited good inhibitory activation (63.3%); however, when compared with synthesised Schiff bases, the inhibition activation increased in oxadiazole compounds. 3a,3b,3e exhibit moderate activation (55%,49%, 44%) at 100µg/ml.

Ethical Approve: The study was approved by the Ethics Board of the University of Kufa. The ethics approval and written agreement to participate in the study had been signed by all patients and controls.

RESULTS

Below are the results obtained, including the names of the prepared products, their physical properties, the identification of the active groups using the ^{1}HNMR and IR technique, tables of the values obtained from the study of biological activity and its study as anti-cancer agents, as well as figures of inhibition in a bacteria growth for 3c and 3d.

Nicotinoyl hydrazine (1), White solid, yield 98%, 1.23gm, m.p 159-161°C. IR KBr cm^{-1}: C=O 1690, NH_2 3214, CH aromatic 3025, ^{1}HNMR (δ ppm): 4.6 (s,NH2 moiety), 9.8(s,CONH), 7-9(m, 4H, Ar-H), scheme (1).

2a -(4- hydroxy benzylidene) nicotine hydrazine.

$C_{13}H_{11}N_3O_2$ Yellow precipitate, white 221 mg, yield 92%, m.p 241-243°C, IR (KBr, $cm^{-1)}$, 1685(C=O), 1660 (HC=N), 1600 (C=C aromatic), 3030 cm (C-H aromatic), 3350 cm (OH aromatic), ^{1}HNMR (δ, ppm): 11.8 (s, 1H, C=NH), 10.95(s,1H, OH), 7-10 (m, 8H, Ar-H)

2b -(3-hydroxy benzylidene) nicotine hydrazine $C_{13}H_{11}N_3O_2$ white precipitate, 200 mg, yield 84% m.p 235- 238°C, IR (KBr, cm^{-1}), 1695(C=O),1645 (HC=N), 1585 (C=C), 3010cm (C-H, Aromatic), 3340cm (OH), HNMR (δ ppm):12.1 (s,1H, C=NH), 10,1 (s,1H, OH), 6.8-9.5(m,8H, Ar-H).

2-c (3,4,5-trimethoxy benzylidene) nicotine hydrazine $C_{16}H_{17}N_3O_3$ White precipitate, 269mg 90% yield, m.p 245-248 °C, IR (KBr, cm^{-1}) 1683 (C=O), 1659 (CH=N), 1598 (C=C aromatic), 3025 (C-H aromatic), HNMR (δ ppm):11.9 (s,1H, C=NH), 3.7 (t, OCH3), 6.8-9.5 (m,7H, Ar-H).

2-d (4-Nitro benzylidene) nicotine hydrazine: $C_{13}H_{10}N_4O_3$, light yellow solid, 256 mg 95% yield, m.p 215- 218°C (KBr, cm^{-1}), 1695(C=O 1662 (HC=N), 1610 (C=C), 3015 (C-H, Aromatic), 1475 (N-O),), HNMR (δ ppm):12.5 (s,1H, C=NH), 10,1 (s,1H, NH), 7-10.8(m,8H, Ar-H).

2-e (4-(N- dimethyl) benzylidene) nicotine hydrazine: $C_{15}H_{16}N_4O$, white precipitate, 225mg 80% yield, m.p 232-235°C, (KBr, cm^{-1}), 1690(C=O), 1658 (HC=N), 1645 (C=C), 3020 (C-H, Aromatic), HNMR (δ ppm):11.5 (s,1H, C=NH), 2.5 (s,6H), 6.9-9.8(m, 8H, Ar-H).

3a-(3-Acetyl-2-(4- hydroxy phenyl)-5-(pyridyl-3-yl))-1,3,4-oxadiazole: $C_{16}H_{14}O_3N_3$, yellow precipitate, 50% yield,150mg, m. p154-157°C IR (KBr, cm-1) 3385 (OH), (C=O) 1715, (C=N) 1590, 1275(C-O-C), 3004 (C-H aromatic), HNMR (δ, ppm): 9.8 (s,1H, OH), 7-8 (m, 8Haromatic), 3.2(s, $COCH_3$).

3b-(3-Acetyl-2-(3-hydroxy phenyl)-5-(pyridyl-3-yl))-1,3,4-oxadiazole $C_{15}H_{14}O_3N_3$, yellow precipitate, 58% yield, 163mg, m.p159-162°C C°IR (KBr, cm-1) 3365 (OH), 1705 (C=O), 1582 C=N, 1278 C-O-C, 3015 C-H aromatic, HNMR (δ, ppm): 9.5 (s,1H, OH), 7-8 (m, 8Haromatic), 2.9(s, $COCH_3$).

3c-(3-Acetyl-2-(3,4,5-trimethoxy phenyl)-5-(pyridyl-3-yl))-1,3,4-oxadiazole $C_{18}H_{19}O_5N_3$, white precipitate, 58% yield, 207mg, 145-148°C, IR: KBr, C=O,1715cm, C=N,1995, 1285 C-O-C, C-H aromatic 3005 cm, HNMR (δ, ppm): 7-8 (m, 8Haromatic), 3(s, $COCH_3$).

3d-(3-Acetyl-2-(4-nitro phenyl)-5-(pyridyl-3-yl))-1,3,4-oxadiazole $C_{15}H_{13}O_4N_4$, yellow precipitate, 65% yield, 174mg, 142-145°C IR: KBr, C=O,1720, C=N,1610, 1278 C-O-C, C-H aromatic 3025 cm, HNMR (δ, ppm): 7-8 (m, 8Haromatic), 2.7(s, $COCH_3$).

3e-(3-Acetyl-2-(4-N-dimethyl phenyl)-5-(pyridyl-3-yl))-1,3,4-oxadiazole $C_{17}H_{18}O_2N_4$, white precipitate, 54% yield,168mg, 168-170°C, IR (KBr,cm^{-1}) C=O,1710, C=N,1605, 1274C-O-C, C-H aromatic 3015 cm^{-1},^{1} HNMR (δ, ppm): 7-8 (m, 8Haromatic), 2.4 (S,3H), 2.7(s.6H).

Table (1): Physical Properties of Synthesised Compounds.

Compounds. No.	Chemical Formula	Molecular weights mg/mol	Yields	Melting points	Colour
1	$C_6H_7N_3O$	137	1.32gm (98%)	159-161C°	White
2a	$C_{13}H_{11}N_3O_2$	241	221mg (92%)	241-243 C°	Yellow
2b	$C_{13}H_{11}N_3O_2$	241	200 mg (84%)	235- 238 C°	Yellow
2c	$C_{16}H_{17}N_3O_3$	299	269mg (90%)	245-248 C°	Light yellow
2d	$C_{13}H_{10}N_4O_3$	261	256 mg (95%)	215- 218 C°	White
2e	$C_{15}H_{16}N_4O$	268	225mg (80%)	232-235 C°	White
3a	$C_{16}H_{14}N_3O_3$	296	150mg (50%)	154-157 C°	Yellow
3b	$C_{16}H_{14}N_3O_3$	296	163mg (58%)	159-162 C°	Yellow
3c	$C_{18}H_{19}N_3O_5$	313	174mg (65%)	145-148 C°	Yellow
3d	$C_{15}H_{13}N_4O_4$	371	207mg (58%)	142-145 C°	White
3e	$C_{17}H_{18}N_4O_2$	310	168mg (54%)	168-170 C°	White

Table (2): The antibacterial activity of synthesised compounds was measured in mm (inhibition zones) and at concentrations of 200, 150, and100 µg /ml. ≤15 strong, ≤7 moderate, ≤ 5 weak

Type of bacteria	***E. coli***			***Staphylococcus.***		
Conc. / Comp.	200	150	100	200	150	100
2a	12	12	9	15	13	11
2b	11	10	8	13	11	9
2c	19	16	10	21	18	17
2d	15	13	11	17	15	14
2e	10	10	8	11	9	7
3a	15	14	11	16	15	13
3b	12	11	9	15	14	13
3c	24	17	11	22	21	15
3d	21	14	13	23	18	17
3e	11	11	9	13	12	9

Table (3): The cytotoxicity of breast cancer cell line using MTT assay according to different concentrations of prepared compounds.

Conc.	2a	3a	2b	3b	2c	3c	2d	3d	2e	3e
100µg	42.2%	55.3%	58.9%	63.3%	55.4%	77%	40.5%	49%	33%	44%
50µg	41.3%	47.2%	57.1%	58.3%	49.55%	54%	33.8%	43.4%	28%	32%
25µg	32.1%	38.6%	44.6%	46.1%	42.5%	47.6%	28.2%	33.2%	22%	26.8%
12.5µg	22.9%	29.5%	28.9%	35.2%	33.0%	38%	17.2%	18%	15%	22.3%
6.25µg	19.5%	21.2%	25.6%	28.2%	26.0%	32%	4.5%	9.8%	6.5%	9.8%

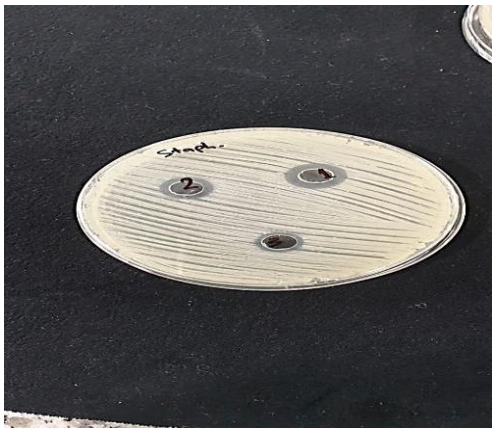


Fig (1): The inhibition zones of 3c at three different concentration 200, 150,100 µg

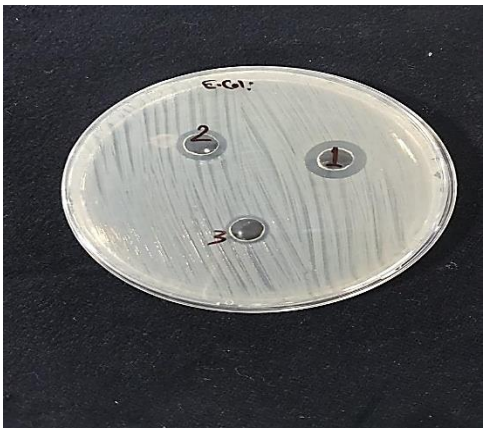


Fig (2): The inhibition zones of 3d at three different concentrations, 200, 150,100 µg

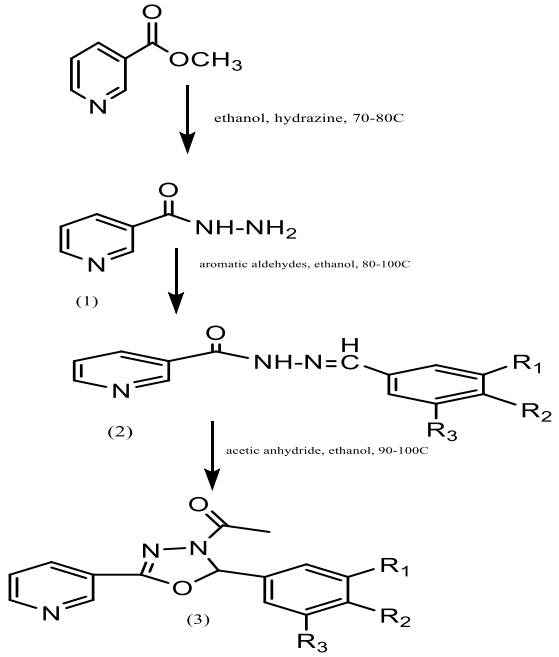
DISCUSSION

Methyl nicotinate is a good versatile starting material in many reactions the efficiency of removing methyl group and easy replacement with hydrazine hydrate with no more catalysts leading to a high yield of nicotinoyl hydrazine. the acyl group was removed and a new derivative was formed, the IR spectrum was appeared moderate band of NH_2 near 3240 and 3214 cm^{-1} and disappear the sharp band of ester in the region 1750 -1730 cm^{-1}, C=O band at1695cm^{-1}, C=C at 1595cm^{-1}, also appeared.^{1}HNMR spectrum appear strong single beak of NH_2 at 4.8 ppm, the single peak of CO-NH at 9.8, also multi peaks due to aromatic H at 6.5-9ppm. A Schiff bases synthesis was achieved in the next step by directly refluxing nicotinoyl hydrazone and aromatic aldehydes with a temperature ring of 75-100°C. The chemical equation of Schiff bases synthesis involved the reaction of nicotinoyl hydrazine with aromatic aldehydes under refluxed and using absolute ethanol as a solvent; no acid catalyst was added, and the time of reflux in about 5 hours. The HC=N bands of the synthesised derivatives appear in the region 1650, 1660cm^{-1}, for derivatives 2a-2e, and this appear the moderate bands of NH_2 in the region 3200-3400cm^{-1}. ^{1}HNMR of the synthetic compounds show a strong single peak in shift value 11.8-12.5 ppm of HC=N group, and disappear the single peak of NH_2 in region 4.2-4.6 ppm. Also, m.w and m.p were determined, and the purification of prepared compounds was achieved by using absolute ethanol and a high yield of imines (95-84%) were produced. the physical properties of synthesised compounds were determined, a processes of Schiff bases cyclisation to prepare the type of derivatives 1,3,4-oxadiazoles, were used a high purity acetic anhydride. The closing processes were formed in a good yield (50%-60%), the IR spectrum shows a strong and sharp new band of C=O of acyl substituent at 1710-1800 cm^{-1} else well C-O-C peaks at 1270-1280cm^{-1}, and disappear the HC=N peak at the 1650-1660cm^{-1}. ^{1}HNMR of 1,3,4-oxadiazole derivatives show disappear the single peak of HC=N in a region near 12 ppm, and appear the single peak of CH_3C=O in the region near 2.7ppm. The aromatic protons for all synthesised derivatives appear in the region range of 7-10 ppm as a double and triple peak [20,21].

The inhibition zones of synthesised derivatives towered *E. coli* and staphylococcus were measured. both 3d and 3c have a strong antibacterial activity against *E. coli* and *staphylococcus*, the inhibition increases with an increase in the concentration. in general, all compounds have moderate to good ability to inhibit microbial growth and the inhibition increase in 1,3,4-oxadizole analogues. 3b and 3c have excellent inhibition in comparison with another compound. 3a, 3d and 3e also show good activity. Some derivatives showed a weak inhibition at a lower concentration. This phenomenon can be explained by the type and position of the substituent in different aldehydes. MTT assay was used to Evolution the cytotoxicity of synthesised compounds. 3c exhibited strength inhibition in MCF-7cells growth (77%), 3d showed good inhibition (63%), and other derivatives 3e, 3d, and 3a appeared to have moderate inhibition.

CONCLUSION

Conclusions: methyl nicotinate is an excellent ester have the ability to undergo nucleophilic replacement with less contamination. No more catalysts were needed. Therefore, the next condensation reaction was done in mild conditions, high purity solids and considerable yields. Synthesis of 1,3,4-oxadiazole heterocyclic compounds has many of approaches. One of them is Schiff bases acylation. The derivatives were isolated in solid form. The microbial Evolution appears to increase in inhibition ability to microbe growth in 1,3,4-oxadiazole analogues in comparison with nicotinoyl hydrazine Schiff bases. Well diffusion method is a good procedure to determine the strength of inhibition in various derivatives. Greatly depending on the type of substitutes. MTT assay protocol proved the cytotoxicity effect of synthesised derivatives towered MCF-7 cells, which is proportional with concentration.



Scheme (1): The general Scheme of synthesizes.

Conflict of Interests: The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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