Synthesis, characterization, antimicrobial and antifungal evaluation of novel [1,3]-Benzodiazepine derivatives incorporated with nicotinic acid under Microwave irradiation

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Abstract

A series of some newly [1,3]-benzodiazepine derivatives of Nicotinic acid hydrazide have been synthesized by treating the hydrazide-hydrazones of nicotinc acid hydrazide with two different phthalimide, glycene phthalimide and p-amino benzoic acid phthalimide using the microwave irradiation technique to give a newly seven-membered ring containing two nitrogen atoms. This method is facile and efficient and afforded 1,3-Benzodiazepine derivatives in an excellent yield. The structures of synthesized compounds were established on the basis of IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The microbial inhibitory effect of the new agents has been evaluated in vitro against Gram-positive and Gram-negative bacteria as well as antifungal activity. The most significant antimicrobial activity was obtained with compound 18 in addition to the antifungal activity.

key words: Nicotinic acid hydrazide, Benzodiazepine, seven membered ring, microwave irradiation

Introduction

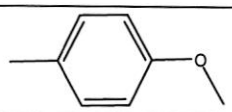
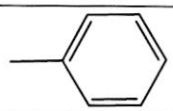
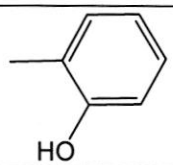
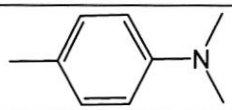
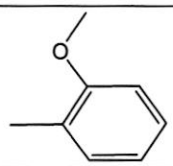
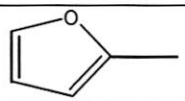
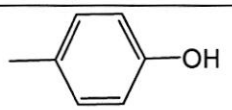
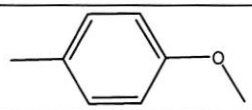
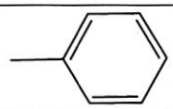
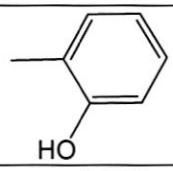
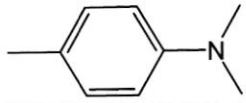
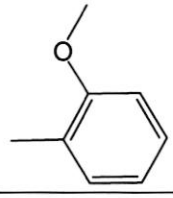
Nicotinic acid derivatives have anti-bacterial, anti-oxidant, anti-inflammatory and anti-carcinogenic activities, and have putative activity against osteoarthritis and granuloma annulare[(1)]. In addition, It can be mentioned the importance of anti-tuberculosis first-line drug isoniazid[(2)], which is an analogue of isonicotinic acid (an isomer of nicotinic acid). Nicotinic acid derivatives are also an important starting material for the preparation of other biological activity compounds[(3)]. Benzodiazepines are bicyclic heterocyclic compounds possessing various types of biological activities over varied N,N-positioned skeletal types [(4-6)].The Benzodiazepine skeleton is known in medicinal chemistry as a "privileged structure" [(7)], i.e. a type of structure that is likely to bind to many types of targets. In addition, benzodiazepines are valuable intermediates for the synthesis of fused ring compounds such as triazolo-[(8-9)], oxadiazolo - oxazino- and furanobenzodiazepines[(10-13)].

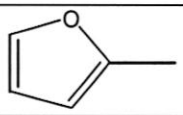
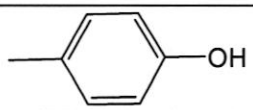
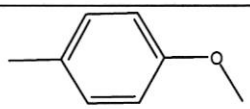
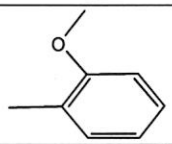
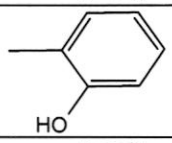
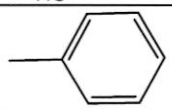
The promotion by these observations and as a continuation of another efforts on synthesis of seven membered ring derivatives with one nitrogen atom such as oxazepine derivatives[(14)], it looks worthy to synthesize a new derivatives of nicotinic acid hydrazide incorporated with 1,3-Benzodiazepine nucleus.So, we reported in this investigation a streamlined and highly efficacious entry into the benzodiazepine derivatives via a novel condensation between the hydrazide-hydrazone and phthalimide under microwave irradition.

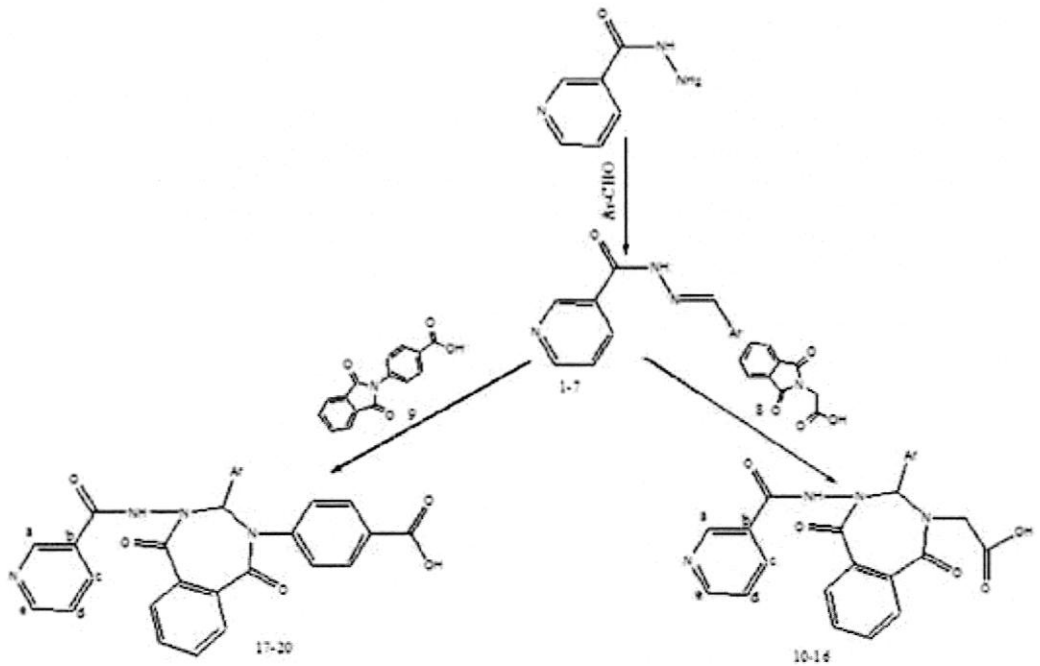
Experimental:

Melting points are uncorrected and determined by the open capillary method using Gallen Kamp melting point apparatus. The IR spectra were recorded with Perkin-Elmer FT-IR instrument using Potassium bromide pellets. ^{1}HNMR· ^{13}CNMR Spectra were recorded in deuterated chloroform($CDCl_3$), Acetone CD_3COCD_3 or Dimethyl sulphoxide(DMSO-d_6) with TMS as an internal standard on a Joel 400MH_z instrument at Manchester Metropolitan University-United Kingdom and at Aal-Al-Bayt University-Jordan (300MH_z) using d_6 DMSO as a solvent. Chemical shifts are expressed as [ppm], s for singlet, d for doublet, t for triplet, q for quartet, m for multiplet. Mass spectra were recorded at EPSRC National centre Swansea-United Kingdom and at Micro Analytical center-Cairo University-Egypt. The evaluation of biological and anti-fungal activity was carried out in Faculty of life Science, University of Manchester-United Kingdom.

Table(1): Physical properties of synthesized compounds

Comp. No.	Ar	Molecular formula	M.PC^0	colour	Yield %	Solvent recryst.
1-		$C_{14}H_{13}N_3O_2$	162-164	White	87	Ethanol
2-		$C_{13}H_{11}N_3O$	138-139	White	89	Ethanol
3-		$C_{13}H_{11}N_3O_2$	181-183	Yellow	83	Ethanol
4-		$C_{15}H_{16}N_4O$	108-111	Yellow	81	Ethanol
5-		$C_{14}H_{13}N_3O_2$	177-179	Brown	86	Ethanol
6-		$C_{11}H_9N_3O_2$	157-158	White	86	Ethanol
7-		$C_{13}H_{11}N_3O_2$	240-242	Brown	90	Ethanol/Dioxan
8-	--------	$C_{10}H_7NO_4$	192-193	White	91	ethanol
9-	--------	$C_{15}H_9NO_4$	290-293	Light yellow	88	Ethanol/DMF
10-		$C_{24}H_{20}N_4O_6$	191-193	White	88	ethanol
11-		$C_{23}H_{18}N_4O_5$	180-181	White	91	ethanol
12-		$C_{23}H_{18}N_4O_6$	205-206	Yellow	90	ethanol
13-		$C_{25}H_{23}N_5O_5$	133-135	Orange	91	ethanol
14-		$C_{24}H_{20}N_4O_6$	215-217	brown	89	ethanol

15-		$C_{21}H_{16}N_4O_6$	194-196	White	83	ethanol
16-		$C_{23}H_{18}N_4O_6$	224-225	yellow	90	Ethanol/Dioxan
17-		$C_{29}H_{22}N_4O_6$	233-235	Yellow	81	Dioxan
18-		$C_{29}H_{22}N_4O_6$	259-262	yellow	88	Dioxan
19-		$C_{28}H_{20}N_4O_6$	273-275	yellow	86	Ethanol/DMF
20-		$C_{28}H_{20}N_4O_5$	244-247	White	89	Ethanol/Dioxan



Scheme (1)

General procedure of Synthesis N'-{(Z)-Arylphenyl]methylidene}pyridine-3-carbohydrazide(1-7)[15]:A mixture of an equimolar ratio of different aldehydes (0.001mole) and nicotinic hydrazide (0.001mole) along with few drops of catalytic glacial acid was transferred to a conical flask and subjected to microwave irradiation for 2 minutes. The solid mass that obtained was collected and recrystallized from ethanol.

N-[(*Z*)-(4-methoxyphenyl) methylidene] pyridine-3-carbohydrazide **(1)**: IR(KBr ,cm^{-1}): (νC=N 1604), (νC=O amide 1660), (νN-H 3260), (=C-H Ar 3029), (C-O-C 1036, 1184).

N-[(*Z*)-phenylmethylidene]pyridine-3-carbohydrazide **(2)**: IR(KBr,cm^{-1}): (νC=N 1605), (νC=O amide 1671), (νN-H 3189), (=C-H Ar 3039).

N-[(*Z*)-(2-hydroxyphenyl)methylidene]pyridine-3-carbohydrazide **(3)**: IR(KBr cm^{-1}):(νC=N 1612), (νC=O amide 1665), (νN-H 3235), (=C-H Ar 3044).

N'-{(*Z*)-[4-(dimethylamino) phenyl] methylidene} pyridine-3-carbohydrazide **(4)**: IR(KBr cm^{-1}): (νC=N 1601), (νC=O amide 1663), (νN-H 3190), (=C-H Ar 3034).

N'-[(*Z*)-(2-methoxyphenyl) methylidene]pyridine-3-carbohydrazide **(5)**: IR(KBr, cm^{-1}):(νC=N 1602), (νC=O amide 1653), (νN-H 3195), (=C-H Ar 3041).

N- [(*Z*) – furan – 2 - ylmethylidene] pyridine-3-carbohydrazide **(6)**: IR(KBr, cm^{-1}):(νC=N 1619), (νC=O amide 1649), (νN-H 3242), (=C-H Ar 3041).

N'-[(*Z*)-(4-hydroxyphenyl) methylidene]pyridine-3-carbohydrazide **(7)**: IR(KBr cm^{-1}): (νC=N 1613), (νC=O amide 1651), (νN-H 3211), (=C-H Ar 3045).

General procedure of synthesis phthalimides (8-9)[16]**:** A mixture of an equimolar ratio of phthalic anhydride (0.001mole) and primary amines (0.001mole) was dissolved in 25ml of glacial acetic acid and refluxed for 2hr. The mixture was cooled and poured on ice water. The precipitate then collected and recrystallized from the appropriate solvent.

(1,3-dioxo-1,3-dihydro-2*H* -isoindol-2-yl)acetic acid **(8)**: IR(KBr ,cm^{-1}): (ν C=O 1724 cyclic amide), (ν C=O carboxylic 1739), (ν =C-H Ar 3045), (ν C-H aliphatic 2931).

4-(1,3-dioxo-1,3-dihydro-2*H* -isoindol-2-yl)benzoic acid **(9)**:(IR(KBr,cm^{-1}):(ν C=O 1698 cyclic amide), (ν C=O carboxylic 1724), (ν =C-H Ar 3049).

General procedure of synthesis {3-(Aryl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-acetic acid (10-16): A mixture of an equimolar ratio of compound 8 and compounds (1-7) respectively was transferred into a mortor and ground perfectly then transferred to a conical flask and subjected to microwave irradiation for 2-3 minutes. The mixture was cooled and recrystallized from appropriate solvent.

{3-(4-Methoxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-acetic acid **(10): IR (KBr,cm^{-1})**: (νC=O amide 1660),(νC=O cyclic amide 1716), (νC=O carboxylic 1770), (νN-H 3222), (ν=C-H Ar 3076). **MS , negative ion nanoelectrospray technique m/z (Irel, %):** The molecular ion peak for compound 10 is observed at m/z= 459.1310 [M-H]-, The observed data were quite identical to the theoretical data. **^{1}H-NMR:** (Chloroform-d_6,ppm)δ at 3.8(s, 3H aliphatic), 4.4(s, 2H aliphatic), 9.9(s, 1H carboxylic OH), 7.8(dd, 4H, Ar J=8.24, in 4-methoxy phenyl nuclei), 9.7(s, 1H , -NH), 8.7(s, 1Ha, Ar-H, in nicotinic acid nuclei),6.9(t,2H,Ar-H in benzene ring of phthalimide),7.5(d,2H,Ar-H in benzene ring of phthalimide),8.3(t,1Hd,Ar-H in nicotinic acid nuclei). **^{13}C-NMR**: 168,167,169(amide, cyclic amide and carboxylic), 158(C-O Ar of methoxy group), 38(CH2 aliphatic), 55(CH_3 of methoxy). The signals of Aromatic carbon atoms appeared in the range of (111-151ppm).

{1,5-Dioxo-3-phenyl-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl{-acetic acid **(11)**:IR(KBr,cm^{-1}): (νC=O amide 1651),(νC=O cyclic amide 1711), (νC=O carboxylic 1767), (νN-H 3297), (ν=C-H Ar 3063). **^{1}H-NMR:**(Chloroform-d_6, ppm)δ at 4.3(s, 2H aliphatic), 10.2(s, 1H carboxylic OH), 8.6(s, 1Ha, Ar-H in nicotinic acid nuclei),7.5(t,1Hd, Ar-H in nicotinic acid nuclei),8.1(d,1Hc, Ar-H in nicotinic acid nuclei),8.6(d,1He, Ar-H in nicotinic acid nuclei) 9.8(s, 1H, NH), 8.2(d, 2H, Ar-H), 7.5(t, 2H, Ar-H), 6.9(t, 1H, Ar-H in benzene ring of benzaldehyde), 4.1(s, 1H in seven membered ring). **^{13}C-NMR**: 166,167,170,172 (amide, cyclic amide and carboxylic), 38 (CH2 aliphatic), 67 (C-H in seven membered ring). The signals of Aromatic carbon atoms appeared in the range of (111-151ppm).

Hydroxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-acetic acid **(12)**:IR(KBr,cm^{-1}): (νC=O amide 1677),(νC=O cyclic amide 1716), (νC=O carboxylic 1766), (νN-H 3175), (ν=C-H Ar 3059). **^{1}H-NMR:**(DMSO-d_6,ppm)δ at 4.2(s, 2H aliphatic), 12.1(s, 1H carboxylic OH), 11.0(s, 1H phenolic OH), 4.6(s, 1H in seven membered ring), 8.5(s, 1Ha, Ar-H in nicotinic acid), 8.6(s,1H, NH), The aromatic protons were appeared in the range of (6.7-8.0ppm) . **^{13}C-NMR**: 161,169,170,172(amide, cyclic amide and carboxylic), 31(CH2 aliphatic),69(C-H in seven membered ring). The signals of Aromatic carbon atoms were appeared in the range of (116-157ppm).

{3-(4-Dimethylamino-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-acetic acid**(13)**:IR(KB,rcm^{-1}): (νC=O amide 1652),(νC=O cyclic amide 1712), (νC=O carboxylic 1778), (νN-H 3201), (ν=C-H Ar 3036). **MS (EI, 70 eV), m/z (Irel, %):**a molecular ion peak (M.+) at m/z =475, 4.6%, base peak at m/z=160.0 100% , total number of peaks 105. **^{1}H-NMR:**(chloroform-d_6,ppm)δ at 4.4(s, 1H in seven membered ring), 3.0(s, 6H , 2CH3), 3.02(s, 2H, CH_2), 6.6(t, 1Hd, Ar-H in nicotinic acid nuclei), 6.9(t, 2H, Ar in benzene ring of phthalimide), 7.4(d, 2H, Ar in benzene ring of phthalimide), 9.0(s, 1Ha, Ar-H in nicotinic acid nuclei), 7.8(dd, 4H, Ar in 4-N,N-dimethyl phenyl nuclei, J=8.2),7.6(d, 1Hc, Ar-H in nicotinic acid nuclei), 8.3(d, 1He, Ar-H in nicotinic acid nuclei), 9.4 (s, 1H, -NH), 9.6 (s, 1H, carboxylic OH). **^{13}C-NMR**: 162,167,169,170(amide, cyclic amide and carboxylic), 40(CH_3 aliphatic), 46(CH_2), 65(C-H in seven membered ring) . The signals of Aromatic carbon atoms appeared in the range of (112-152ppm).

{3-(2-Methoxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl) -amino] -1,3,4,5- tetrahydro-benzo[e][1,3] diazepin-2-yl} - acetic acid**(14)**: (IR(KBrcm^{-1}): (νC=O amide 1655),(νC=O cyclic amide 1717), (νC=O carboxylic 1769), (νN-H 3180), (ν=C-H Ar 3033). **MS (EI, 70 eV), m/z (Irel, %):**a molecular ion peak (M.+) at m/z =460.10, 13.4%, base peak at

m/z=268.1, 100% , total number of peaks 59. **^{1}H-NMR:**(Aceton-d_6,ppm)δ at 3.8(s, 3H, CH_3), 4.4(s, 2H, CH_2), 11.2(s, 1H, carboxylic OH),4.9(s, 1H in seven membered ring),7.3(t,1Hd, Ar-H in nicotinic acid nuclei) ,8.8(s, 1Ha, Ar-H in nicotinic acid nuclei), 8.5(s,1H, -NH), 8.7(d, 1He, Ar-H in nicotinic acid nuclei), 6.9(t, 1H, Ar-H in 2-methoxy phenyl ring),7.0(t,2H,Ar-H in benzene ring of phthalimide), 7.8(m, 3H, Ar-H in 2-methoxy phenyl ring). **^{13}C-NMR**:167,168, 172, 173(amide, cyclic amide and carboxylic), 38(CH_2, aliphatic), 55 (CH3, methoxy), 66(C-H in seven membered ring). The signals of Aromatic carbon atoms appeared in the range of (111-151ppm).

{3-Furan-2-yl-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-acetic acid **(15)**: (IR(KBr cm^{-1}): (νC=O amide 1659),(νC=O cyclic amide 1719), (νC=O carboxylic 1771), (νN-H 3205), (ν=C-H Ar 3037). **MS, negative ion nanoelectrospray technique m/z (Irel, %):** The molecular ion peak for compound 15 is observed at m/z 419.100, [M-H]-. The observed data were quite identical to the theoretical data.

^{1}H-NMR :(Chloroform-d_6,ppm)δ at 4.5(s, 2H, CH_2), 11.3(s, 1H, carboxylic OH), 9.3(s, 1H, -NH), 8.7(s, 1Ha, Ar-H in nicotinic acid), 8.9(d, 1He, Ar-H in nicotinic acid), 5.3(s, 1H in seven membered ring), 8.8(d,2H, Ar-H in benzene ring of phthalimide), 7.8(t, 2H, Ar-H in benzene ring of phthalimide), 7.9(t, 1Hd, Ar-H in nicotinic acid nuclei), 7.5(d, 1Hc, Ar-H in nicotinic acid nuclei), 6.5-7.0(m, 3H, Ar in furan ring). **^{13}C-NMR**: 166,169,174,175(amide, cyclic amide and carboxylic), 38(CH_2, aliphatic), The signals of Aromatic carbon atoms were appeared in the range of (107-150ppm).

{3-(4-Hydroxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3] diazepin-2-yl}-acetic acid**(16)**: (IR(KBrcm^{-1}): (νC=O amide 1659),(νC=O cyclic amide 1715), (νC=O carboxylic 1772), (νN-H 3213), (ν=C-H Ar 3041). **^{1}H-NMR:**(DMSO-d_6,ppm)δ at 4.3(s, 2H aliphatic), 12.3(s, 1H carboxylic OH), 11.1(s, 1H phenolic OH), 4.7(s, 1H in seven membered ring), 8.6(s, 1Ha, Ar-H in nicotinic acid), 8.7(s,1H, NH), The aromatic protons appeared in the range of (6.8-8.3ppm) . **^{13}C-NMR**: 161,167,169,170(amide, cyclic amide and carboxylic), 31(CH2 aliphatic), 67(C-H in seven membered ring). The signals of Aromatic carbon atoms appeared in the range of (116-157ppm).

General procedure of synthesis {3-(Aryl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-Benzoic acid (17-20): A mixture of an equimolar ratio of compound 9 and compounds (1-3) respectively was transferred into a morter and ground perfectly then transferred to a conical flask and subjected to microwave irradiation for 2-3 minutes. The mixture was cooled and recrystallized from the appropriate solvent.

4-{3-(4-Methoxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-benzoic acid**(17).IR(KBr,cm^{-1})**: (νC=O amide 1658),(νC=O cyclic amide 1695), (νC=O carboxylic 1765), (νN-H 3245), (ν=C-H Ar 3055). **MS (EI, 70 eV), m/z (Irel, %):**a molecular ion peak (M.+) at m/z =522.508, 15%, base peak at m/z=305.03 100%. **^{1}H-NMR:** (DMSO-d_6,ppm)δ at 3.6(s, 3H aliphatic), 11.5(s, 1H carboxylic OH), 8.5(s, 1Ha , Ar-H in nicotinic acid nuclei), 8.6(s, 1H, NH), 4.8(s, 1H in seven membered ring), 6.9-8.2(m,16H,Ar). **^{13}C-NMR**: 161.5, 161.8, 167.1, 167.2ppm (amide, cyclic amide and carboxylic), 156(C-O Ar of methoxy group). 55(CH_3 of methoxy) 72(C-H in seven membered ring). The signals of Aromatic carbon atoms were appeared in the range of (115-151ppm).

4-{3-(2-Methoxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo [e] [1,3]diazepin-2-yl}-benzoic acid**(18).IR(KBr,cm^{-1})**: (νC=O amide 1653),(νC=O cyclic amide 1701), (νC=O carboxylic 1745), (νN-H 3239), (ν=C-H Ar 3051). **^{1}H-NMR:**(DMSO-d_6,ppm)δ at 3.8(s, 3H aliphatic), 11.9(s, 1H carboxylic OH), 8.7(s, 1Ha , Ar-H, in nicotinic acid nuclei), 8.4(s, 1H, NH), 4.7(s, 1H in seven membered ring), 7.0-8.1(m,16H,Ar). **^{13}C-NMR**: 161.5,161.8,167.1,167.2 (amide, cyclic amide and carboxylic), 150(C-O Ar of methoxy group). 55(CH_3 of methoxy), 72 (C-H in seven membered ring). The signals of Aromatic carbon atoms were appeared in the range of (114-150ppm).

4-{3-(2-Hydroxy-phenyl)-1,5-dioxo-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-benzoic acid**(19).IR(KBr,cm^{-1})**: (νC=O amide 1666),(νC=O cyclic amide 1689), (νC=O carboxylic 1737), (νN-H 3199), (ν=C-H Ar 3052). **^{1}H-NMR:**(DMSO-d_6,ppm)δ at 11.6(s, 1H carboxylic OH), 11.0(s, 1H phenolic OH) 8.2(s, 1Ha , Ar-H in nicotinic acid nuclei), 8.5(s, 1H, NH), 4.5(s, 1H in seven membered ring), 6.8-8.1(m,16H,Ar). **^{13}C-NMR**: 164,166,169,171(amide, cyclic amide and carboxylic), 149(C-O Ar of phenol group). 55(CH_3 of methoxy), 68(C-H in seven membered ring). The signals of Aromatic carbon atoms were appeared in the range of (114-152ppm).

4-{1,5-Dioxo-3-phenyl-4-[(pyridine-3-carbonyl)-amino]-1,3,4,5-tetrahydro-benzo[e][1,3]diazepin-2-yl}-benzoic acid**(20).IR(KBr,cm^{-1})**: (νC=O amide 1661),(νC=O cyclic amide 1705), (νC=O carboxylic 1743), (νN-H 3203), (ν=C-H Ar 3045). **^{1}H-NMR (fig8):** (DMSO-d_6,ppm)δ at 12.1(s, 1H carboxylic OH), 8.4(s, 1Ha , Ar-H in nicotinic acid), 8.7(s, 1H, NH), 4.7(s, 1H in seven membered ring), 7.6-8.8(m,17H,Ar). **^{13}C-NMR(fig9)**: 166.7, 166.8, 169, 175 (amide, cyclic amide and carboxylic), 69(C-H in seven membered ring). The signals of Aromatic carbon atoms appeared in the range of (121-150ppm).

Anti bacterial and anti fungal study:

Antibacterial activity of the synthesized compounds has been tested using Gram-negative strains such as

Escherichia coli, Salmonella enteric, Pseudomonas aeruginosa and Gram-positive strains such as *Staphylococcus epidermidis, Bacillus subtilis* and *Micrococcus luteus*. Additionally, antifungal activity has been tested using different Candida strains such as *Candida albicans and Candida glabrata* . Using cup-plate agar diffusion method, the zones of inhibition were measured in cm as a parameter of antimicrobial activity[17].

Table2: Anti microbial activity of compounds 10-19

Compound / Organism	10 concentration			11 concentration			12 concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0.3	--	--	0.4	--	--	0.1	--	--
Salmonella enterica	0.4	--	--	0.2	--	--	0.4	--	--
Pseudomonas aeruginosa	0.2	--	--	0.3	--	--	0.2	--	--
Staphylococcus epidermidis	0.6	0.3	--	0.6	0.5	0.2	0.6	0.5	0.4
Bacillus subtilis	0.5	0.2	--	0.7	0.3	--	0.6	0.4	--
Micrococcus luteus	1.3	1.1	0.6	2.1	2.0	1.0	2.4	2.1	1.4

Compound / Organism	13 concentration			14 concentration			15 Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0.4	--	--	0.5	--	--	0.3	--	--
Salmonella enterica	0.3	--	--	0.4	--	--	0.1	--	--
Pseudomonas aeruginosa	0.3	--	--	0.3	--	--	0.4	--	--
Staphylococcus epidermidis	0.8	0.4	0.2	0.7	0.4	--	0.5	0.3	--
Bacillus subtilis	0.7	0.4	--	0.7	0.5	0.1	0.7	0.4	0.1
Micrococcus luteus	2.9	2.3	1.8	2.8	2.5	2.0	1.4	1.2	0.5

Compound / Organism	16 concentration			17 concentration			18 Concentration		
	60,000	30,000	15,000	60,000	30,000	15,00	60,000	30,000	15,000
Escherichia coli	0.3	--	--	0.3	--	--	0.2	--	--
Salmonella enterica	0.4	--	--	0.3	--	--	0.4	--	--
Pseudomonas aeruginosa	0.3	--	--	0.2	--	--	0.3	--	--
Staphylococcus epidermidis	0.6	0.5	--	0.7	0.6	0.4	0.6	0.6	0.1
Bacillus subtilis	0.6	0.3	--	0.6	0.2	--	0.7	0.5	--
Micrococcus luteus	2.5	1.7	1.3	0.4	0.2	--	3.1	2.8	1.8

Compound / Organism	19 concentration			Ampicillin concentration			Geniticin Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Escherichia coli	0.3	--	--	1.2	0.9	0.5	1.8	1.3	1.1
Salmonella enterica	0.2	--	--	1.6	1.4	0.8	1.0	0.7	0.3
Pseudomonas aeruginosa	0.3	--	--	1.2	0.8	0.5	1.8	1.3	1.0
Staphylococcus epidermidis	0.8	0.4	--	3.8	3.5	2.8	1.9	1.5	1.2
Bacillus subtilis	0.8	0.8	0.3	2.4	2.0	1.6	1.1	0.7	0.2
Micrococcus luteus	2.7	2.4	1.7	3.6	3.3	2.5	1.5	1.2	0.5

Table 3: Anti-fungal activity of compounds 10-19 measured in cm.

Compound / Organism	10			11			12		
	concentration			concentration			Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.4	0.1	--	0.3	--	--	0.9	0.6	0.2
Candida glabrata	0.3	--	--	0.7	0.3	--	0.7	0.4	--

Compound / Organism	13			14			15		
	concentration			concentration			Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.8	0.3	--	0.9	0.5	--	0.6	0.2	--
Candida glabrata	0.6	0.2	--	0.6	0.2	--	0.6	0.2	--

Compound / Organism	16			17			18		
	Conc.			concentration			Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.4	--	--	0.6	0.1	--	0.7	0.3	--
Candida glabrata	0.8	0.5	--	0.9	0.5	0.1	0.8	0.6	0.2

Compound / Organism	19			Ampicillin			Geniticin		
	Concentration			concentration			Concentration		
	60,000	30,000	15,000	60,000	30,000	15,000	60,000	30,000	15,000
Candida albicans	0.7	0.4	--	0.8	0.4	--	1.0	0.6	0.2
Candida glabrata	0.9	0.5	--	0.7	0.5	--	0.8	0.3	--

Results and discussion

The structures of Compounds 1-7 were proved by the physical properties and FT-IR spectral data which showed the significant and charachteristic bands in the range of (1601-1619)cm^{-1} which belong to νC=N of hydrazide-hydrazone. In addition, the bands of νC=O amide were appeared in the range of (1649-1671)cm^{-1} and another significant bands in the range of (3189-3260)cm^{-1} belong to νNH confirming the formation the hydrazones . The structure of Compounds 8-9 were proved by the comparison of the physical properties with literatures and by FT-IR which showed the bands of νC=O cyclic amide at 1724 and 1698 respectively. While the bands of νC=O carboxylic have appeared at 1739 and 1724 respectively confirming the formation of the phthalimide. The structure of Compounds 10-20 were proved by FT-IR which showed three significant bands , the first one in the range of (1651-1677)cm^{-1} belongs to νC=O amide, the second one in the range of (1689-1717)cm^{-1} belongs to νC=O of cyclic amide and the third band was in the range of (1737-1778)cm^{-1} belongs to carboxylic νC=O group, confirming that these compounds contain the amide group, cyclic amide and carboxylic group.

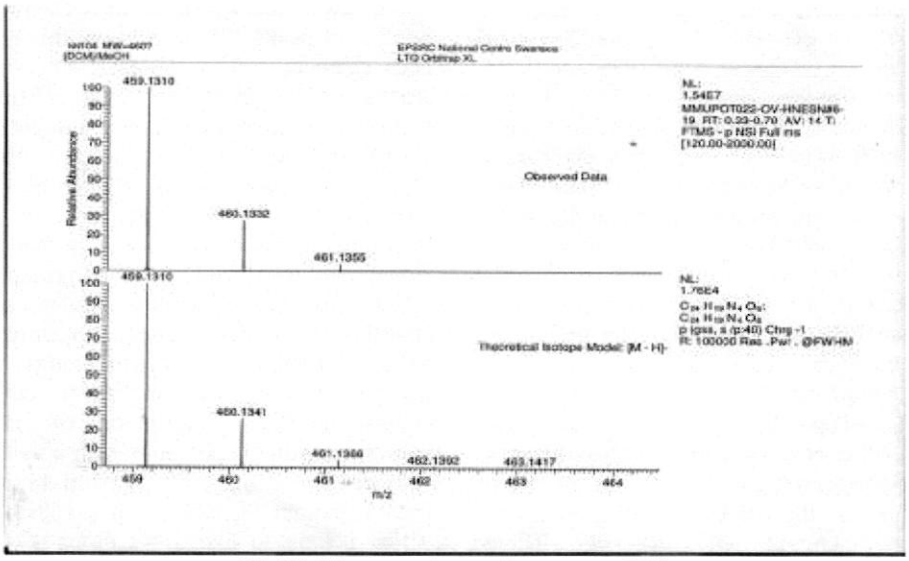
^{1}H-NMR of compound 10 has shown significant signals , s at 3.82ppm belongs to methoxy group protons, s at 4.4ppm belongs to the –CH2- protons , s at 3.88ppm belongs to the C-H proton in the seven membered ring and the most significant was s at 9.9ppm which belongs to OH carboxylic. These observations suggest the incorporation of nicotinic acid, glycene phthalimide and p-methoxy benzaldehyde in one molecule. In addition,the structure of compound 10 was proved by mass spectrum, fig(1) which showed the molecular ion peak [M-H]- at m/z 459.13, 100%which is the base peak. ^{13}C-NMR spectrum of compound 12 showed four significant signals in the range of at 161, 169, 170 and 172 which belong to four carbonyl groups in the compound. Another significant signals was at 69ppm belongs to C-H in the seven membered ring. Compound 13 was proved by mass spectrum fig(2) which showed a molecular ion peak (M.+) at m/z =475, 4.6%, base peak at m/z=160.0 100% , total number of peaks 105.In addition, this compound has been characterized by ^{1}H-NMR and ^{13}C-NMR. The structure of compound 14 was confirmed by mass spectrum, fig (3) which showed a molecular ion peak (M.+) at m/z =460.10, 13.4%, base peak at m/z=268.1, 100% , total number of peaks 59. In addition, this compound was proofed by ^{1}H-NMR spectrum, fig(4) which showed a significant singlet signal at 4.9ppm belongs to C-H proton in the seven membered ring. Another singlet signals have appeared at 3.8ppm belongs to three protons of methoxy group, at 4.4ppm belongs to –CH2 in glycene and at 11.2 belongs to carboxylic OH confirming the incorporation of nicotinic acid hydrazone with the phthalimide in a seven membered ring fig4. ^{13}C-NMR of compound 14,fig(5) showed four important signals at 167, 168, 172 and 173 which belong to four C=O group .On comparison with DEPT,fig(6) these four signals were disappeared

because they are not attached to hydrogen atoms. Also, the signal of $-CH_2$ was appeared at 38ppm and on comparison with DEPT appeared downward. The most significant signal in ^{13}C-NMR was at 66ppm which belongs to C-H in seven membered ring and didn't disappeare in DEPT because it is attached to hydrogen atom. Compound 15 was proved by mass spectrum fig(7) which showed a molecular ion peak[M-H]- at m/z =419.100, 15%, base peak at m/z=204.03 100%. ^{1}H-NMR spectrum of this compound showed a significant singlet signals at 11.3ppm belongs to carboxylic OH, at 5.3ppm belongs to C-H proton in seven membered ring, at 4.3ppm belongs to CH_2 protons and at 8.6ppm belongs to aromatic proton in nicotinic acid ring. These singlet signals confirm the incorporation of nicotinic hydrazide- hydrazone and phthalimide via a seven membered ring. ^{1}H-NMR of compound 16 showed three significant singlet signals at 4.7ppm belongs to C-H proton in seven membered ring, at 12.3ppm belongs to carboxylic OH and at 11.1ppm belongs to phenolic OH confirming the incorporation of nicotinic hydrazide-hydrazone with phthalimide via seven-membered ring. ^{13}C-NMR of compound 17 showed a significant signal at 72ppm belongs to C-H carbon atom in the seven membered ring and four signals at 161.5, 161.8, 167.1, 167.2ppm belong to four carbonyl group in the structure. ^{1}H-NMR spectrum of compound 18 showed three significant singlet signals at 3.8ppm belongs to CH_3 protons, at 4.7ppm belongs to C-H proton in the seven membered ring and at 11.9ppm belongs to carboxylic OH confirming the incorporation of nicotinic hydrazide-hydrazone and phthalimide. ^{1}H-NMR spectrum of compound 20, fig(8) showed a significant singlet signal at 12.1ppm belongs to carboxylic OH, singlet signal at 4.7ppm belongs to C-H proton in the seven membered ring and s at 8.4ppm belongs to aromatic proton in the nicotinic acid ring. ^{13}C-NMR of of this compound, fig(9) showed four significant signals at 166.7, 166.8, 169,175ppm belong to four C=O in this structure and on comparison with DEPT, fig(10) these four signals were disappeared because they are not attached to hydrogen atoms. Another significant signal was at 69 belongs to C-H carbon atom in the seven membered ring and this signal still appear in DEPT confirming its attachment to hydrogen atom.

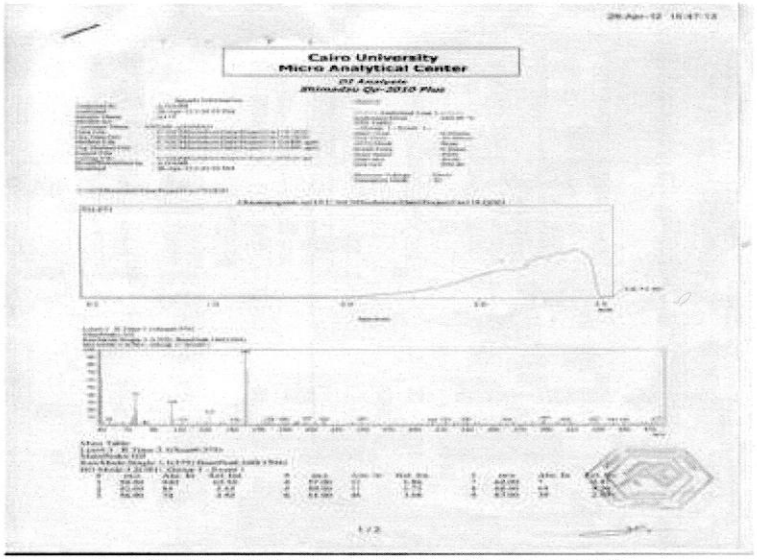
Biological activity

A different ranges of biological activities against all tested strains have been obtained compared to the standard drugs; Ampicillin and Geniticin with all synthesized compounds. As for Gram-negative bacteria, antibacterial activities of all compounds were lower than that of Ampicillin and Geniticin (Table2). On the other hand, the higher activity was clear against Gram-positive bacteria when compared to Geniticin, but similar to lower activity when compared to Ampicillin. The antifungal activities were obviously similar to what achieved compared with standard drugs Ampicillin and Geniticin (Table3). All the synthesized compounds at high concentration; 60000ppm; exhibited antibacterial and antifungal activities against all tested strains with inhibition zones ranged from 3.1cm for compound 18 against *Micrococcus luteus* down to 0.1cm for some compounds mainly against Gram-negative bacteria. Very limited activity against Gram-negative bacteria was observed with all compounds. Among such organisms, *Micrococcus luteus* exhibited the highest inhibitory effect with inhibition zones as high as 3.1cm for compound 18 at 60,000ppm down to 0.5cm for compound 15 at 15,000ppm compared to standard drugs 3.6cm for Ampicillin at 60,000ppm down to 0.5 for Geniticin at 15,000.

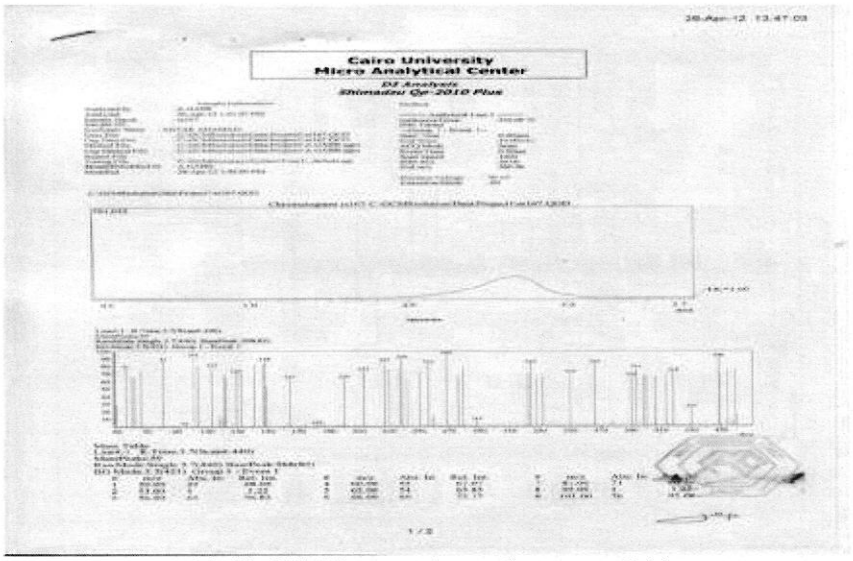
Compound 17 exhibited limited antibacterial activity against *Micrococcus luteus* with inhibition zones of 0.4cm for 60,000ppm and no inhibition zone at 15,000ppm.



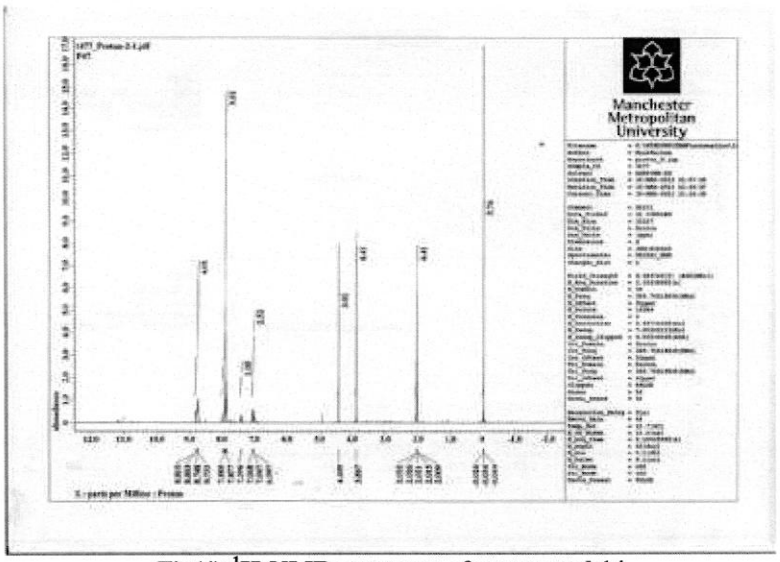
Fig(1):Mass spectrum of compound 10



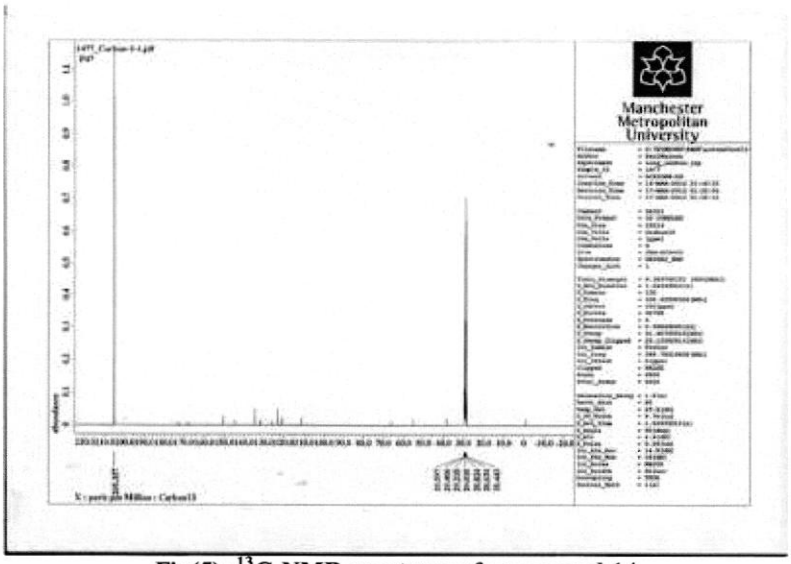
Fig(2):GC-Mass spectrum of compound 13



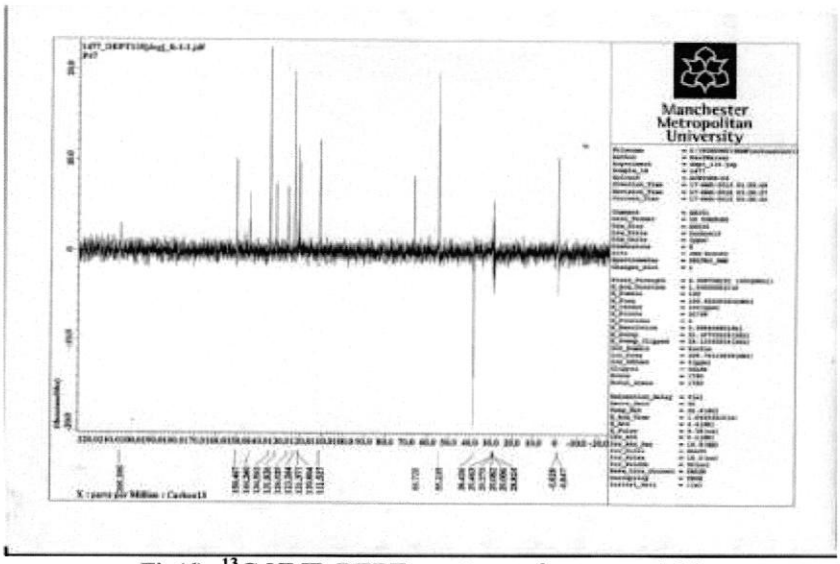
Fig(3):GC-Mass spectrum of compound 14



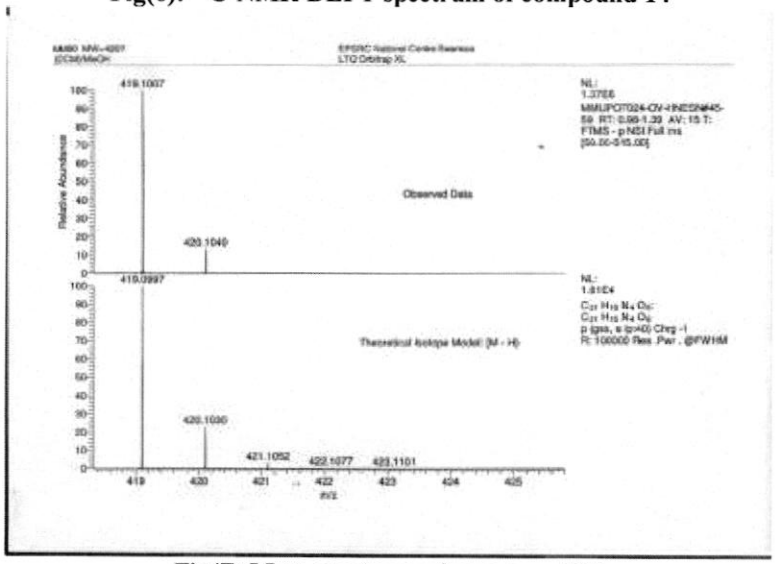
Fig(4):^{1}H-NMR spectrum of compound 14



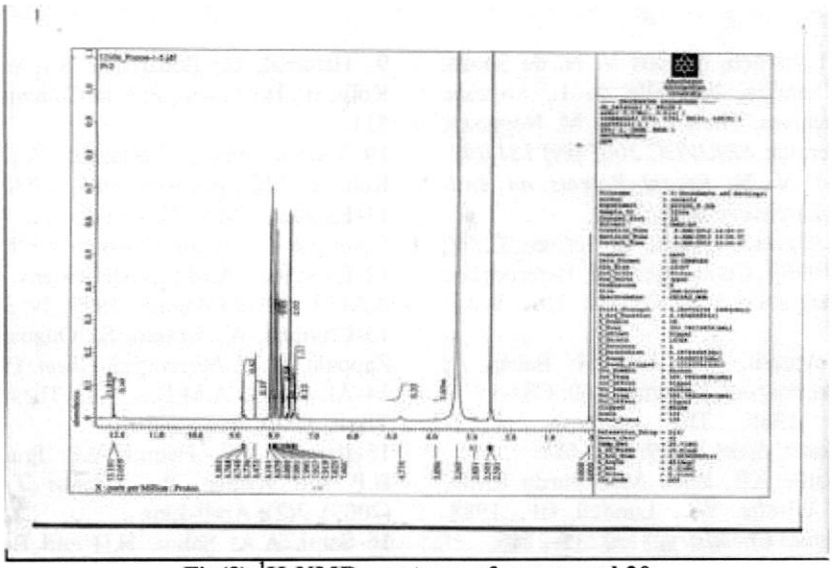
Fig(5): ^{13}C-NMR spectrum of compound 14



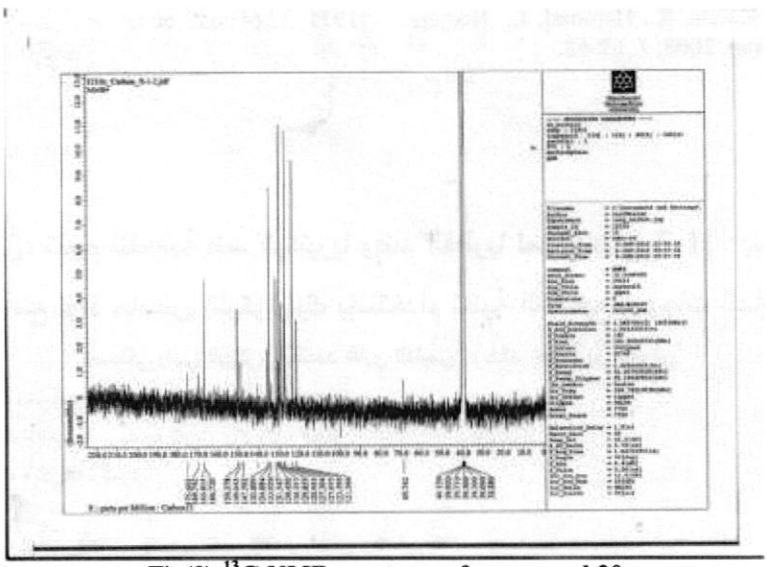
Fig(6): ^{13}C-NMR-DEPT spectrum of compound 14



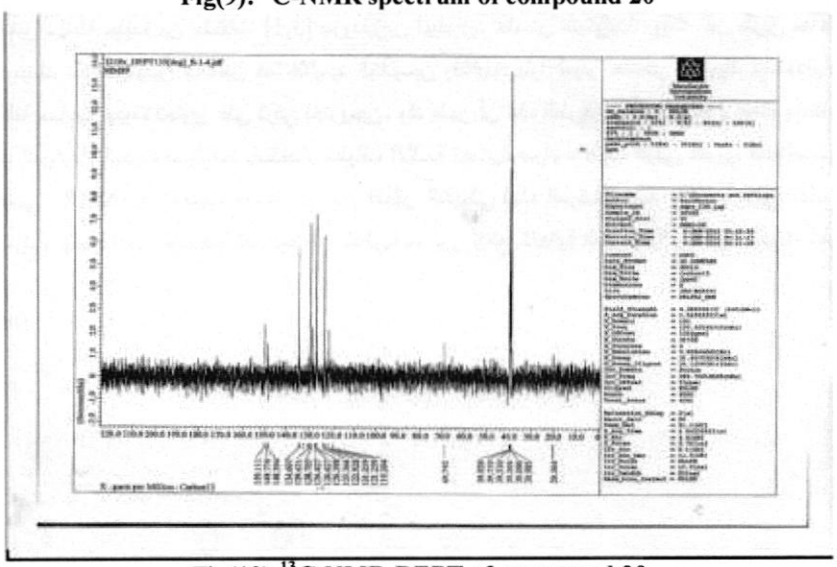
Fig(7):Mass spectrum of compound 15



Fig(8):^{1}H-NMR spectrum of compound 20



Fig(9):^{13}C-NMR spectrum of compound 20



Fig(10):^{13}C-NMR-DEPT of compound 20

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تحضير، تشخيص، تقييم للفعالية ضد البكتيريا وضد الفطريا لمشتقات [1،3] -بنزودايزبين جديدة مندمجة مع نواة حامض النيكوتينيك بإستخدام تقنية التشعيع بموجات المايكروويف

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[3] كلية الزراعة ، جامعة تكريت ، تكريت ، العراق

الملخص

تم في هذا البحث تحضير سلسلة جديدة من مشتقات [3,1]-بنزودايزبين لهيدرازيد حامض النيكوتينيك وذلك عن طريق مفاعلة هيدرازونات مختلفة لهيدرازيد حامض النيكوتينيك مع فثاليدين مختلفين هما فثاليميد الكلايسين وفثاليميد بارا-أمينو حامض البنزويك بإستخدام تقنية التشعيع بموجات المايكروويف لإعطاء حلقة سباعية جديدة تحتوي على ذرتي نايتروجين. وقد ظهر أن هذه الطريقة سهلة وكفوءة لإعطاء مشتقات [3،1]-بنزودايزبين بحصيلة عالية. تراكيب المركبات الجديدة تم إثباتها بإستخدام مطياف الأشعة تحت الحمراء ، طيف الرنين النووي المغناطيسي ^{1}H-NMR وطيف الرنين النووي المغناطيسي ^{13}C-NMR وطيف الكتلة. تم تقييم التأثير التثبيطي لهذه المركبات ضد البكتيريا خارج الأنسجة بإستخدام سلالات موجبة وسالبة لصبغة كرام بالإضافة إلى تقييم فعاليتها المضادة للفطريات. أبرز نتائج الفعالية البايولوجية والفطرية ظهرت للمركب 18 .