J.Thi-Qar Sci. No. (3) Vol.1 Janu. /2009

ISSN 1991- 8690 الترقيم الدولي ١٩٩١ - ٨٦٩٠

Website: http://jsci.utq.edu.iq Email:utjsci@utq.edu.iq

Synthesis and Biological Activity study of some New Isonicotinic Acid Hydrazide (isoniazid) Derivatives

Kareem Salem Abass

Science College - Missan University – Amara – Iraq

Abstract

The work was included the synthesis of some new allyl , vinyl , tosyl and methyl derivatives for isonicotinic acid hydrazide(isoniazid). The products were characterized by elemental analysis besides the IR and ^{1}H-NMR spectroscopic identifications .

The work was also included the study of the biological activity of the products , starting material (isoniazid) and some antibiotics ,. This study shows that the biological activity of products is more than that of the isoniazid and the antibiotics.

Introduction:-

Isonicotinic hydrazide is a crystalline compound ($C_6H_7N_3O$, m.p 170-173 C) . It was consider as pyridine derivative and used in a new method for fluoresence detection in the high-performance liquid chromatography of ketosteroids [1]. Isonicotinic acid hydrazide (isoniazid) was used as drug to treat tuberculosis. Isoniazid is the most effective antituberculosis drug currently available. The drug inhibits or kills the tubercle bacilli that cause the disease. It is usually given together with some other antituberculosis drug such as streptomycin or aminosalicylic acid to prevent emergence of drug resistant organisms . Isoniazid is a prodrug and must be activated by bacterial catalase . The active form inhibits the sythesis of mycolic acid in the mycobacterial cell wall [2.3] .

Certain functionally Pyridines are potent inhibitors of the human immundodefficiency virus type 1 (HIV-1) reverse transcriptase, for example ateviridine has been selected for further clinical evolution anti-HIV agent [4] , the dihydropyridines e.g adalate are still the most widely used calcium channel blockers [5] . Another substituted pyridines , like amirone [5] and milrione [7]' were used for treatment of congestive heart failure . The reviews which related to our subject are [8- 16] . The aim of this study was a synthesis of some new isoniazid derivatives and compair their biological activity with the isoniazid and some famous antibiotic . This modification may help us to obtain derivatives with high biological activity properties and to develope new compounds of chemotherapeutic interest.

Experimental :-

Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected. Elemental analysis were carried out at the micro analytical center , University of Cairo , Giza- Egypt. IR spectra were recorded as KBr disc using a Pye-Unicam SP3-300 IR spectrophotometer . ^1H –NMR specter were recorded for only trimethyl isonicotonic hydrazide and tosyl isonictotonic hydrizide derivatives on a LOC ETHZ NMR GEMENTI 200 MHz at Lab. Fur Organic Chemistry , Zurich University , Swiss. The determination of the antibacterial activity properties were achieved at Lab. Of microbiology, AL-Sadar Hospital, Amara and Labs. Of Biology Dept. of Science College , Basrah University .

Preparation of allyl isonicotinic hydrazide [17] :-

Mixture of isonicotinic hydrazide (0.41 g, 3 mmo1) and potassium carbonate (0.60 g, 4 mmol) was dissolved in 30 ml of acetone. The mixture was stirred with heating for 25 minute at 40 ^{o}C . After addition of excess of allyl bromide (5- 6 ml) to the reaction mixture , reflux was continued for 5 hours and thin layer chromatography (T.L.C) test showed the presence of one spot and no starting material (isoniazid) was detected . The solvent was evaporated , and the residue was treated with mixture of benzene (40 ml) and water (25 ml) . The organic extract was dried with anhydrous sodium sulfate , filtered and evaporated to dryness . The crude derivative is recrystallized from methanol to obtain colorless crystals .(m.p=202-206 ^{o}C dec.).

Preparation of divinyl isonicotinic hydrazide [18] modifier :-

Isonicotinic hydrazide (0.55 g , 4 mmol) and potassium carbonate (0.60 g , 4

mmol) were dissolved in DMF (25ml) . The mixture was stirred for 15 minute at 50 °C , then excess of vinyl acetate (9 – 11 ml) was added . The reactants were stirred and refluxed for 4 hour and T.L.C test reveals no further reaction . The solvent was evaporated under vacuo . The residue was extracted with ether (50 ml) and water (20 ml) . The organic extract was dried with anhydrous sodium sulfate ,filtered and evaporated to dryness . The separated vinyl derivative was recrystallized from ethanol.(m.p=178-182 ^{o}C dec.) .

Preparation of tosyl isonicotinic hydrazide [17] :-

Isonicotinic hydrazide (0.27 g,2 mmol.) and recrystallized p.toluenesulfonyl chloride (0.38 g,2mmol.) were dissolved in dry pyridine (20 ml) . The reaction mixture was stirred for 4 hour at room temperature and the T.L.C showed no further reaction . The mixture was extracted from benzene (60 ml) and water (30 ml) . Both layers were washed with sodium bicarbonate solution until the medium became neutral . The organic extract was dried with anhydrous sodium sulfate , filtered and evaporated to dryness . The crude derivative was recrystallized from acetone to obtain white needle crystals .(m.p=213-216 ^{o}C) .

Preparation of trimethyl isonicotinic hydrazide [19] modifier :-

Isonicotinic hydrazide (0.55 g , 4 mmol) was mixed with (0.16 g , 3 mmol) of KOH and dissolved in 30 ml of toluene . The mixture was stirred for 25 minute at 70 °C , then excess of methyl iodide (12 ml) was added to the mixture . The reactants were stirred and refluxed for 7 hour and T.LC reveals no further reaction . The solvent was evaporated under vacuo . The residue was extracted with ether (60 ml) and water (30 ml) . The organic extract was dried with anhydrous Na_2SO_4 , filtered and evaporated to dryness . The crude derivative was recrystallized from ethanol to obtain colorless Crystals.(m.p=217-220 ^{o}C)

Determination of the biological activity properties :-

The products and stating material used as inhibitors of the activities of the standard micro-organisms (*Streptococcus pneumonia* NCTC 6303 Gr(+) and *Escherichia coli* NCTC 5933 Gr (-) to determined their inhibition diameters following a reported procedure [19,20] .

The minimum inhibitory concentrations (MIC) of the products and some famous antibiotics (tetracycline , cephalexine and ampiciline) against variety of medically important micro-organisms (*S.aureas , Paeroginosa, E.coli* and *K.pneumonia*) were determined according to a reported procedure [21].

The cytotoxicity of the products and tetracycline , cephalexine and ampiciline also are determined by following reported procedure [22].

Results and Discussion :-

The results of some physical measurements and elemental analysis are summarized in Table 1 .which revealed that the solid products have high melting points and showed that the difference between the found values and calculated values of carbon , hydrogen and nitrogen elements are situated within the range which confirmed the suggested structures of the products . The spectral data of IR and ^{1}H – NMR of the products are gathered in Table 2a and 2b respectively .

Table 2a showed that the IR spectra of allyl and divinyl derivatives have seven bands which ascribed to the stretching

vibrations of -NH , unsaturated C-H, saturated C-H , C=O , C=N, C=C and N-C groups , while the IR spectrum of the trimethyl derivative is similar to the spectra of the allyl and divinyl derivatives except the –NH band was disappeared because the three protons of the -NH and NH_2 groups are substituted by vinyl group . Also the spectrum of tosyl derivative is similar to the spectra of allyl and divinyl derivatives , in addition to the appearance of the stretching vibrations of S=O and para substituted ring bands of tosyl group.

^{1}H-NMR spectral data of tosyl and trimethyl derivatives are included in table 2b , and their spectra ae shown in Fig 1 and Fig 2 .

^{1}H-NMR spectrum of tosyl isoniazid is identified by the singlet signal of the –CH_3 of tosyl group at 2.02 ppm , and the multiplet signal of aromatic protons within the range 7.75 – 7.95 ppm . We noticed that the signal of the two protons of –NH_2 group is disappeared (exchanged with D_2O),. The ^{1}H-NMR spectrum of trimethyl isoniazid (DMSO solvent,3.8 ppm) is chraracterized by two singlet signals at 2.29 ppm and 2.52 ppm which ascribed to the –$N(CH_3)_2$ and –NCH_3 respectively . Also the spectrum showed multiplet signal within the range 7.13 – 7.86 ppm which attributed to the aromatic protons . The results of biological activity properties are summarized in Table 3 . Table 3a in general shows that the inhibition zone diameters of the products against standard *Escherichia coli* Gr (-) and *Streptococcus pneumonia* Gr (+) are more than of the inhibition zone diameters of starting material (isonicotinic acid hydrazide) especially tosyl derivative appeared more biological activity than of the other products (allyl , divinyl and trimethyl derivatives) against the same standard micro-organisms , because it was noted that the solubility of the tosyl derivative in water is increased than of solubility of other products in the same solvent . This factor encouraged us for the study of the biological activity properties of the products besides that the material which dissolve in water and used as chemotherapeutic agent exhibited more potential antibacterial than of the other materials which have less solubility in water . Table 3b included the results of the minimum inhibitory concentrations (MIC) of the products and some famous antibiotics . This Table appeared that the MIC of the prepared derivatives especially tosyl derivative are more than of the tetracycline , cephalexine and ampiciline antibiotics . For example , tosyl derivative exhibit of MIC of 1.00 ug/ml against *P.aeroginosa* . Whereas the MIC of tetracycline , cephalexine and ampiciline are (28.15 and 9.50) ug /ml respectively against the same micro-organism .

From Table 3c , we concluded that the cytotoxicity of the products are less than that of the ampicilline and tetracycline antibiotics . The cytotoxicity concentrations of the allyl , divinyl , tosyl and trimethyl derivatives are (650 , 600 , 750 , 600) ppm respectively. Whereas the cytotoxicity concentrations of the ampicilline and tetracycline are (300 and 550) ppm respectively .

Table 1 : Physical Properties and elemental analysis of the Products

Product	R_f value	Yield %	Molecular formula	Molecular weight g/mole	Elemental analysis					
					C %		H %		N %	
					Cal.	Fou.	Cal.	Fou.	Cal.	Fou.
Allyl isoniazid	0.78 6 : 4 Toluene:EtOH	67	$C_9H_{11}N_3O$	177	60.52	61.01	6.31	6.21	23.31	23.72
Divinyl isoniazid	0.66 3 : 7 Toluene:MeOH	71	$C_{10}H_{11}N_3O$	189	63.88	63.49	6.04	5.82	22.58	22.22
Tosyl Isoniazid	0.72 2 : 8 Benzene:EtOAC	83	$C_{13}H_{14}N_3O_3S$	292	52.90	53.42	5.08	4.79	13.90	14.38
Trimethyl isoniazid	0.69 5 : 5 EtOAC:MeOH	58	$C_9H_{13}N_3O$	179	60.80	60.33	6.90	7.26	23.90	23.46

Table 2 : Spectroscopic data of the products
2a : IR Spectra data of the products recorded as KBr discs (Cm^{-1})

The Product	NH Str.	=C-H Str.	Sat. C-H Str.	C=O Str.	C=N Str.	C=C Str.	N-C Str.	S-O Str. (s)	Para. Subst ring
Allyl isoniazid	3450(w)	3066(m)	2923(w)	1635 (s)	1696(w)	1595(m)	1120(m)	-	-
Divinyl isoniazid	3336(br.)	3115 (s)	2990(w) 2930(m)	1650(s) 1640(m)	1655(m)	1585(w)	1310(m)	-	-
Tosyl isoniazid	3515 (s)	3170(m) 3080(s)	2965 (m)	1670 (s)	1665 (s)	1625(m)	1010(m)	1180	810-840
Trimethyl isoniazid	-	3075(m)	2980(m) 2965(w)	1665 (s)	1626 (s)	1545(w)	1246 (s)	-	-

Str. = stretching Sat.=saturated br.=broad
S= strong m= medium w= weak Subst= Substituted

2b : ^{1}H-NMR Spectral data

The Product	Chemical Shift
Tosyl isoniazid	P-CH_3 (2.02 s) Aromatic Protons : (7.75 – 7.95 m)
Trimethyl isoniazid	- $N(CH_3)_2$ (2.29 s) - $N(CH_3)_2$ (2.52 s) Aromatic Protons : (7.13 – 7.86 m).

PPm = Part Per million. S = Singlet m = multiplet

Table 3 : Antimicrobial activity results
3a : Inhibition diameters (mm) of starting material (isoniazid) and its derivatives

Compound	Micro- organisms					
	Streptococcus pneumonia NCTC 6303 Gr (+)			Escherichia coli: NCTC 5933 Gr (-)		
	Concentratation Mg/ml			Concentratation Mg/ml		
	100	150	200	100	150	200
Isoniazid	8.80	12.30	16.20	7.30	13.20	18.40
Allyl isoniazid	14.30	17.50	20.30	15.10	17.60	21.30
Divinyl isoniazid	13.80	16.80	19.90	14.30	16.40	20.70
Tosyl isoniazid	18.20	20.10	23.00	19.30	21.40	22.10
Trimethyl isoniazid	12.90	15.60	18.00	13.80	15.00	19.80

3b: Minimum inhibitory concentration (MIC) of the Products and some famous antibiotics

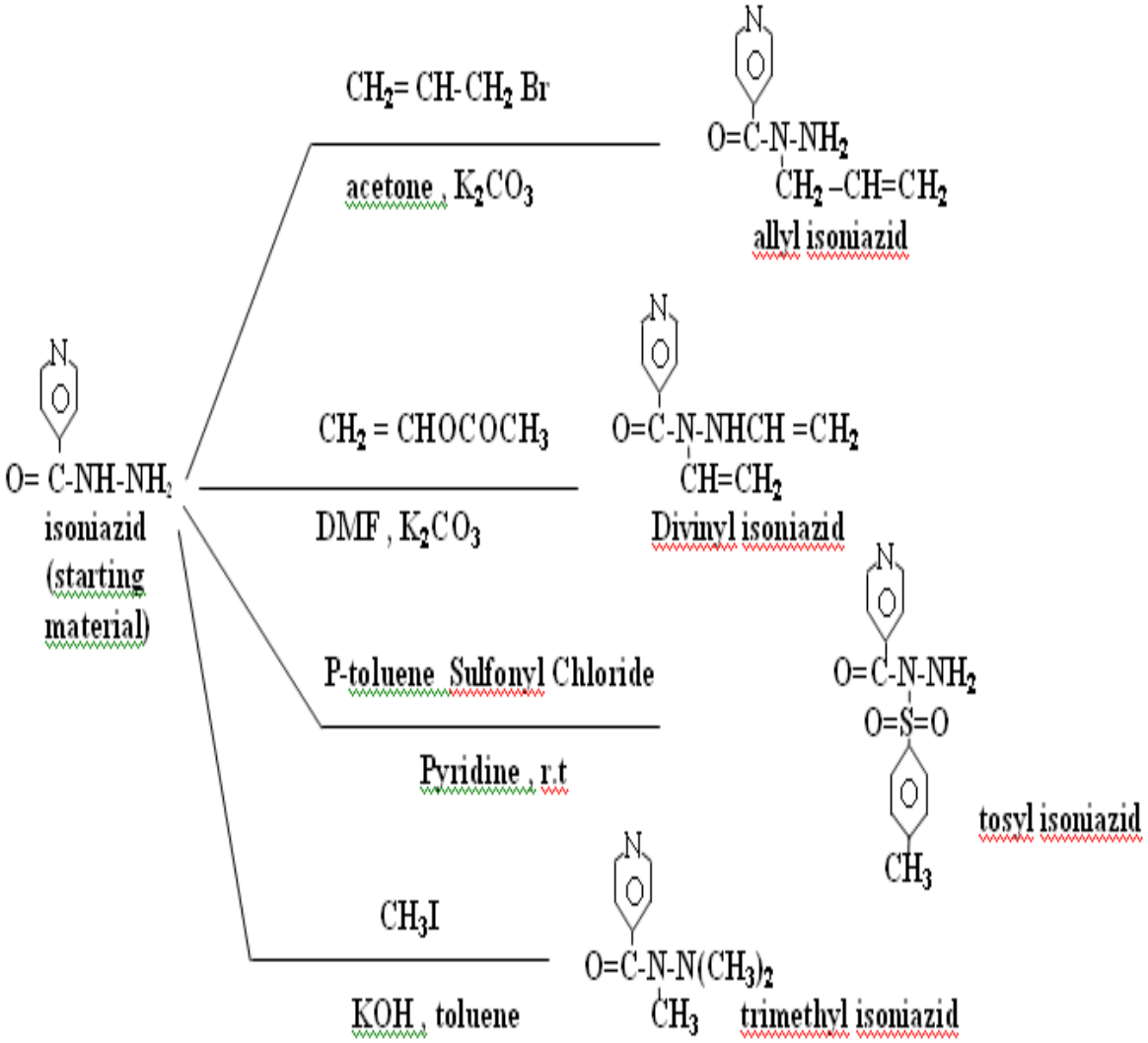
Micro-organisms	MIC Mg /ml						
	Allyl	Divinyl	Tosyl	Trimethyl	T C.	Cep	Amp.
S.aureas NCTC 6571	0.09	0.20	0.07	0.10	0.80	0.40	0.60
P.aeroginosa NCTC 6750	0.80	4.00	1.00	5.00	28	15	9.50
E . coli NCTC 5933	0.50	0.90	0.20	0.09	0.90	1.70	1.20
K.Pneumonia NCTC 6303	0.30	2.00	0.40	3.00	29	6.80	5.30

3c: Cytotoxicity Concentrations of the Products, Ampicilline and Tetracycline

Concentrations (PPm)	R.B.C toxicity after 1hr.					
	Allyl isoniazid	Divinyl isoniazid	Tosyl isoniazid	Trimethyl isoniazid	Ampicilline	Tetracycline
100	NT	NT	NT	NT	NT	NT
150	NT	NT	NT	NT	NT	NT
200	NT	NT	NT	NT	NT	NT
250	NT	NT	NT	NT	NT	NT
300	NT	NT	NT	NT	NT	NT
350	NT	NT	NT	NT	NT	NT
400	NT	NT	NT	NT	T	NT
450	NT	NT	NT	NT	T	NT
500	NT	NT	NT	NT	T	NT
550	NT	NT	NT	NT	T	T
600	NT	T	NT	T	T	T
650	T	T	NT	T	T	T
700	T	T	NT	T	T	T
750	T	T	T	T	T	T
800	T	T	T	T	T	T

Note: DMSO is used as reference (Conc. (PPM) = -) and the R.B.C toxicity after 1hr is NT.

T = Toxic NT = Non Toxic R.B.C = Red Blood Cells hr. = hour.



Scheme1 : Synthesis of the Products

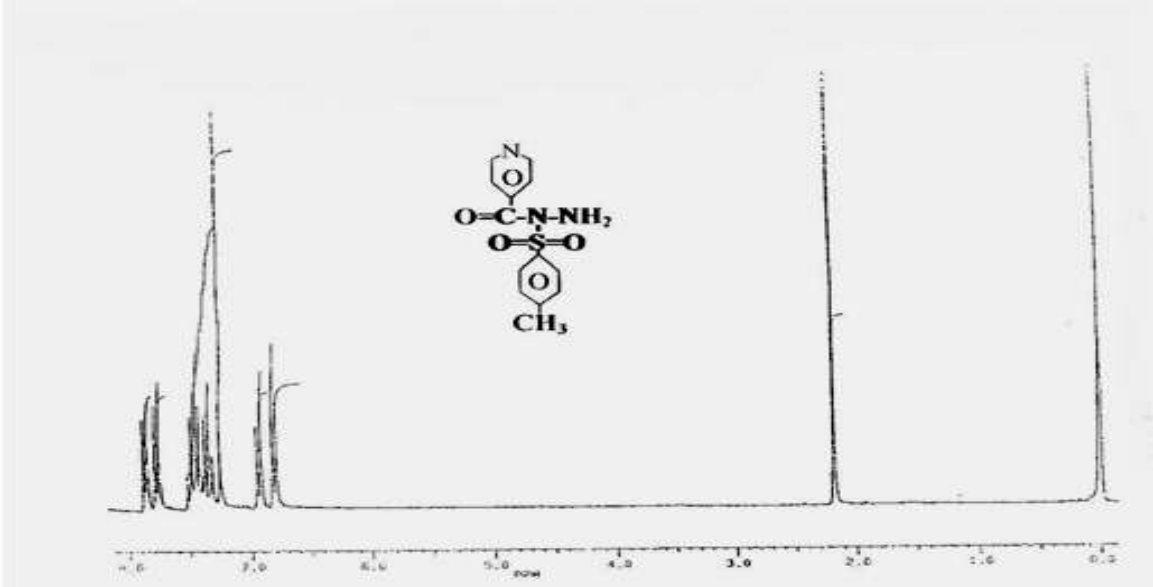


Fig 1 : ^{1}H – NMR of tosyl isoniazid

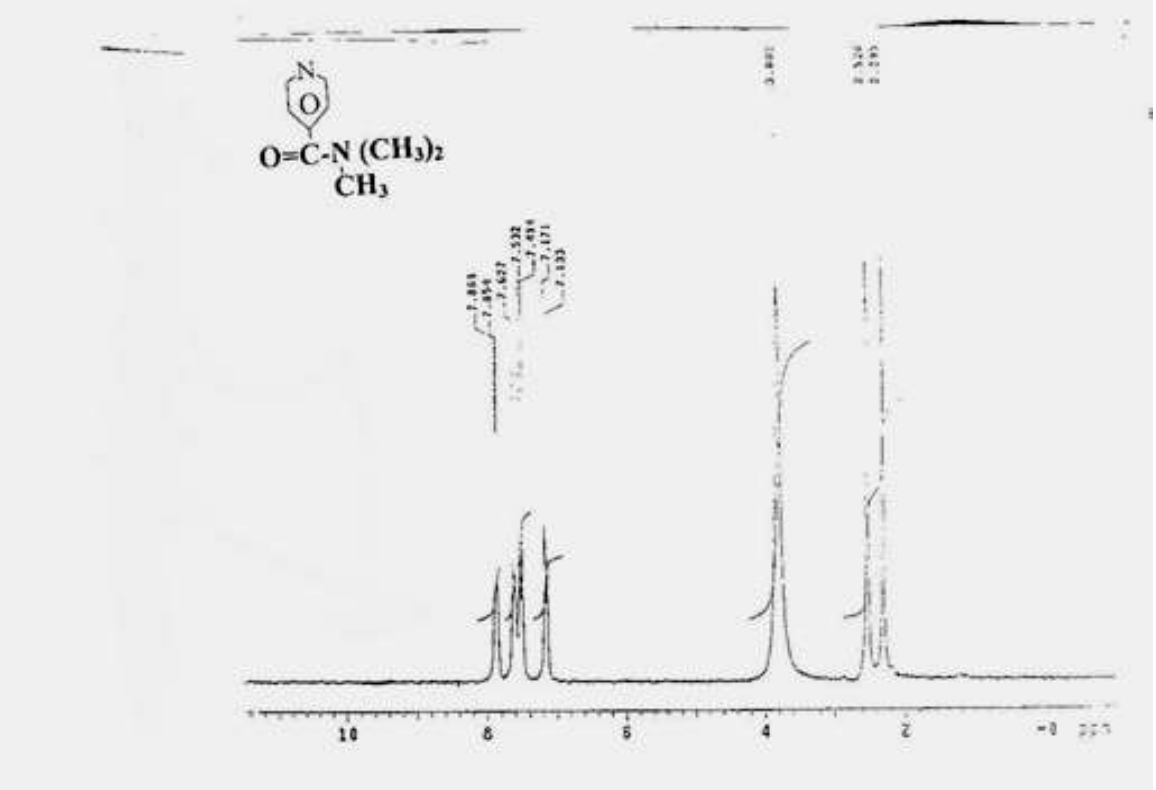


Fig 2 : ^{1}H – NMR of trimethyl isoniazid

References :-

1- Fluka Catalogue , Chemicals and Biochemicals , 1982 / 83.
2- Core Curriculum on Tuberculosis (2000) Division of Tuberculosis Elimination , Centers for Diseases Control and Prevention.
3- Isoniazid Overdose , Recognition and Management American Family Physican 1998 Feb 15.
4- R.Torschutz and A.Karger , *J.Heterocyclic Chem,* 1997 **, 34** , 1147.
5- R.M.Robertson and D.Robertson , The Pharmacological Basis of Therapeutic , Goodman and Gillman's , P 759 , 9 th ed. , A . G . Gilman , Consulting edd. , MC Graw-Hill Health Professions Divisions , New York (1996) .
6- A.E. Farah and A.A.Alousi , *Life Sci.* , 1978 ,**22**,543 .
7- J.Svetlik , N.Pronayova and V.Hanas , *J. Heterocyclic , Chem* . , 2000 , **37** , 395.
8- P . Dhamija , S.Malhota and P.Panhdi , *Pharmacology* , 2006 **,77,**2,1 .
9- M. Steffek , G.L.Newton , Y. Av-Gay and R. C. Fahey , Biochemistoy , 2003 , 42 , 12067.
10- M.P.Patel and Blanchard , *J.S* , *Biochemistry* , 1999 , **38** , 11827 .
11- G.L.Newton and R.C.Fahey , *Arch . Microbial.* , 2002 , **178** , 388 .
12- D.A. Rozwarski , G.A.Grant ,D.H.Barton,and W.R. Jacobs, *J.C., Science* , 1998 , **279** , 98.
13- R.N. Vogte, D.J.Steenkamp,R. Zheng and J.S.Blanchard, *J Biochem*, 2003 , 374 , 657.
14- N. Bergamini and G. Fowst , *Arzneim-Forsch* , 1965 , **15** , 951 .
15- Nicholas G.M. , Newton G.L. , Fahey R.C. and Bewely C.A. , *Org. Lett.* , 2002 **,3** , 1543.
16- G.M. Nicholas,L.L. Eckman , G.L. Newton,R.C. Fahey,S. Ray and C.A. Bewley , *Bioorg . Med . Chem.* , 2003 ,**11**,601.
17- Kareem Salem Abass , Ph.D Thesis ,2001, Basrah University , Iraq.
18- M.Al- Safi , M.Sc. Thesis , 1999 , Basrah University , Iraq.
19- D.J. Brown and J.D. Mason , *Jchem . Soc.* , 1955 , 211.
20- F.E.Hahn , Antibiotic , Vol.5 , Mechanism of action of antibacterial agent. Springler-Velgar , Berlin , 1979, 67.,
21- A .Bauer , W.Kinby and M. Yurk , Antibiotic Susceptibility testing by a standardization Single disk , *Amer . J Clim-Pathol.* , 1966 , **45,** 493.
22- S.M Finegold and E.J.Baron , Diagnostic Microbiology , (Bailey and Scottis the C.V. Mosby Company U.S.A.) , 1986 ,7th ,175.
23- M.G. Nair ,A.R. Putnam ,S.K Mishra ,M.H Mulks ,W.H. Taft ,J.E. Kaller and J.R. Miller , Faeriefungi, a new broad spectrum antibiotic from *Stretptomyces griseus* Var. autotrophicus , J . *Natural Products* , 1989 , **52 (4)** , 797 .

تخليق ودراسة الفعالية البايةلوجية لبعض المشتقات الجديدة لحامض ايزونيكوتينيك هايدرزايد (ايزونايزايد)

كريم سالم عباس
كلية العلوم – جامعة ميسان – العمارة – العراق

الـمستخلص

يتضمن البحث تخليق بعض المشتقات الجديدة (اليل ، قاينايل ، توسايل ومثيل لهايدرازيد حامض الايزونيكوتينيك). شخصت النواتجبواسطة التحليل العنصري ومطيافية الاشعه تحت الحمراء ومطيافية الرنين النووي المغناطيسي للبروتون . كذلك تضمن العمل تحديد الفعالية البايولوجيه للنواتج والمادة الاولية ومقارنتها بالمضادات الحيويه الشائعه . ان هذة الدراسة تظهر بان الفعالية البايولوجيه للنواتج اكثر من تلك التي تظهرها المادة الاولية والمضادات الحيويه التي استخدمت في هذة الدراسة.