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Synthesis and Charactrization of some Hetrocyclic Compounds from Ninhydrin and Study of its Effect on Amylase Enzyme in Serum

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Abstract

Ninhydrin is a chemical compound that is commonly used in analytical chemistry. In this research, ninhydrin was utilized to prepare some heterocyclic derivatives. It was reacted with hydrazine to make compound (1), which was the starting material to synthesize other heterocyclic derivatives in this research. These compounds were characterized by (FT-IR) and (H-NMR) techniques. The biological effect of the prepared compounds were tested against the activity of amylase enzyme in serum. These compounds resulted in deactivating the amylase enzyme in a percentage rate of (11.43, 36.49) %, which proves their effectiveness against the enzyme.

Introduction:

Hetero cyclic compound and their derivatives have an important and fundamental role in many areas, particularly in biological and industrial filed [(1)]. Imidazole, an organic compound that has formula ($C_3H_4N_2$), is a hetero cyclic compound. Imidazole ring is a part of Histidine's structure. It is also part of the theofylene molecule found in tea leaves and coffee [(2)]. Imidazole is involved in the manufacture of certain anti-cancer drugs [(3)]. Benzimidiazole, which is a benzene attached to pentahetero cyclic ring, was synthesized for the first time by Ho brecker in 1872 by reducates compound "2-nitro-4-methyl acetanilide [(4)] , Benzimidiazole derivatives are great important in medical chemistry . They are used as antimicrobial, anti-inflammatory, anti-Hivi2, anti-oxidants [(5)]. These compounds have been widely used as anti-biotic such as penicillin [(6)]. Also, they are

being used as enzyme inhibitors [7]. Ninhydrin which is a common name of (2,2-dihydroxy -1-indine -1,3-dayon), is used in protein and peptide chains' analysis. It has the ability to detect peptide bonds, particularly, amino groups (primary or secondary amines) [8]. It was discovered in 1910 by (siegferied rohmann) [9]. In 1954 (vdine) and ((van hofsten)) proposed the use of ninhydrine as a fingerprint detector [10]. Amyleas enzyme is one of the enzymes that the pancreas secrete. It's an enzyme responsible for digesting carbohydrates [11]. Amylase enzyme is found in the salivary glands, where carbohydrates are digested from the mouth and in the small intestine [12]. The enzyme is effected by any disorder that occurs in the pancreas, either by increasing or decreasing its concentration [13]. Enzyme inhibitors are chemical compounds that reduce the rate of enzymatic reaction or stop its function by effecting one or more of the following factors [14]. 1-Active site in enzyme 2-Apo enzyme 3- Co enzyme 4-Prosthatic group. Enzyme inhibitors can be classified into three classes [15]. 1-Competitive inhibitors 2-Non–Competitive inhibitors 3-Un-Competitive inhibitors.

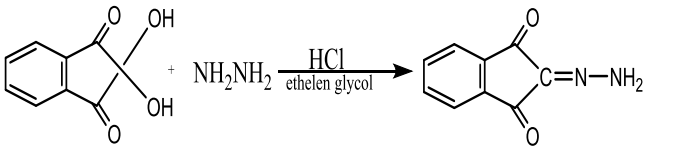
Materials and methods:

Melting points were determined on FALC instrument (s.r.I) 50/60 HZ (Italy).FT-IR spectra of compounds were recorded on ((Shimadzu fourier transform infrared Spectrophoto meter FT-IR 8400 s (KBr) scale (4000 – 400)cm^{-1})). HNMR spectra (solvent DMSO-d6) were recorded on broker ultra shield 400 MHz spectro meter with TMS on internal slanders in Al-Bayt university – Jordan.

1- Chromatography class thin TLC

This test is done for all the prepared compounds . It was performed at different periods of preparation .The checked using U.V Lights , sheets silkjel and moble phase (ethanol absolute).

1-1:Synthesis:2-hydrazineylidene-1H-indene-1,3(2H) – dione

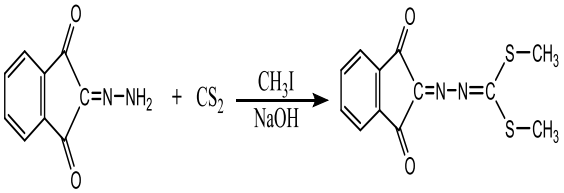


phthalic acid — 2-hydrazineylidene-1*H*-indene-1,3(2*H*)-dione

Two solution were prepared separately, solution(1) : (1 ml) of HCl was added in droplets to (1 ml) of hydrazine with stirring .

Solution(2): (0.01 ml) of ninhydrine was dissolved in ethylene glycol . Solution (1) was added to solution (2) dropwise. The mixture was then refluxed for 6 hours. The solution was left to set at room tempreture for 24 hours. Followed by filtration and re–crystallized with absolute ethanol to yield a pale yellow crystals .M.P (124 – 126) °C yield 72 %.Anal. calcd. for $C9H6N_2O_2$ (174): C, 62.066; H, 3.448; N; 16.091 ; found %: C, 62.041; H, 3.391; N, 16.089.

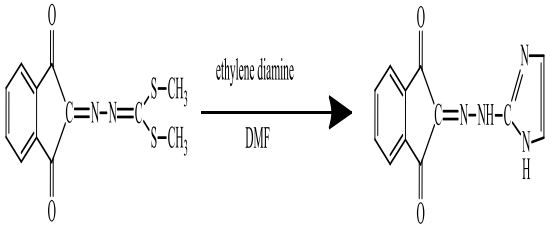
1-2:Synthesis:dimethyl(1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene)carbonyl hdrazono dithioate(II)



dimethyl (1,3-dioxo-1,3-dihydro-2*H*-inden-2-ylidene)carbonohydrazonodithioate

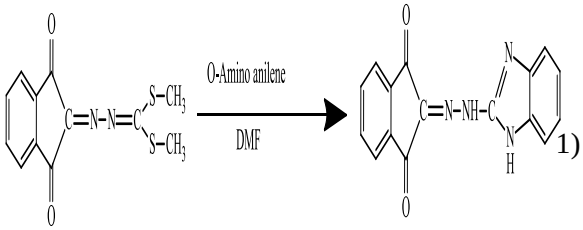
Added (4 ml) of NaOH (10 ml) for the solution of (0.025 ml) of compound dissolved in (10 ml) (0.05) ml of CS_2 , (0.025 ml) CH3I , then stirred this mix for (4 hours). After that, pour the mixture over the ice and collect precipitation by filtration and wash it.Several times with distilled water. Re-crystallized with absolute ethanol .M.P (280 – 282)°C. yield 67% colour orange. Anal. Calc.%. for $C_{12}H_{10}N2O_2S_2$ (278): C, 51.798; H, 3.59; N, 10.071, found %: C,51.802; H, 3.61; N,10.054.

1.3 Synthesis: 2-(2-(1H-imidazol-2-yl)hydrazineylidene)-1H-indene-1,3(2H)-dione(III)



This compound was prepared by dissolving (0.0025 mol.) of 2-hydrazineylidene-1H-indene-1,3(2H)- ione in (10 mL) DMF , then it was followed by addition of (0.002 mol.) ethylene diamine in (15 mL) DMF. The mixture was stirred for an hour at room temperature, then, the mixture was refluxed for 8 hours. Afterward, the mixture was poured into a crushed ice cold water to yield a solid. The product was filtered and re-crystallized from absolute ethanol to yield yellow crystals. M.P(205-208)°C . Yield 63% . Anal. Calc.%. for: $C_{12}H_8N_4O_2$ (240). C, 59.504; H, 3.305, N, 23.140; found %: C, 59.813; H, 3.33; N, 23.146.

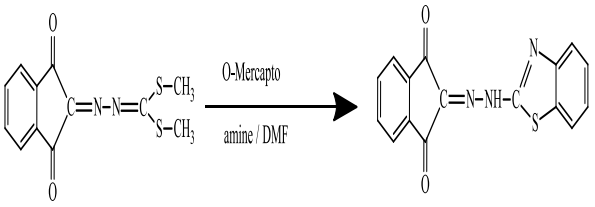
1.4:synthesis:2-(2-(1H-benzo[d]imidazol-2-yl)hydrazineylidene)-1H-indene-1,3(2H)-dione(IV)



1)

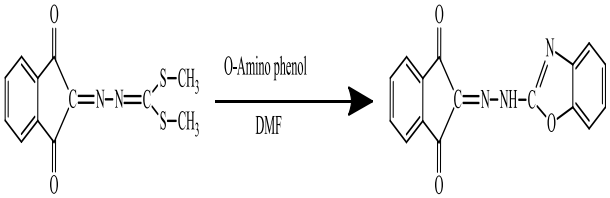
A similar method of work was used to synthesize compound III .M.p (242-244) °C, yield 52%, color white yellowish. Anal. Calc.% for: $C_{16}H_{10}N_4O_2$ (290). C, 66.206; H, 3.448, N, 19.310; found %: C, 66.213; H, 3.49; N, 19.306.

1.5 :synthesis (VI):



A similar method of work was used to synthesized compound III .M.p (165-167) °C , yield 51% , color white. Anal. Calc.% for: $C_{16}H_9N_3O_2S$ (290). C, 66.206; H, 3.448, N, 19.310; found %: C, 66.213; H, 3.49; N, 19.306.

1.5 :synthesis (VII):



A similar method of work was used to synthesized compound III .M.p (189-192) °C , yield 64% , colorless. Anal. Calc.% for: $C_{16}H_9N_3O_3$ (290). C, 65.753; H, 3.082, N, 14.383; found %: C, 65.749; H, 3.08; N, 14.366.

The biological study;- Sample Preparation:

1) Serum sample
After taking a blood sample, blood was centrifuged (6000) rpm to (10) min. then serum was transferred into disposable plane tube, and stored in deep freeze.

2) Preparation of solutions according to the following steps :-

A) Solution(I)

starch base solution :- (3.33) gm of (Na_2HPO_4) and (2.2) gm of a (C_6H_5COOH) benzoic acid , were dissolved in (150 mL) of distilled water , and the mixture was heated.

B) Solution(II)
In a beaker , (0.2 gm) of starch was dissolved in (10 mL) of cooled water , then solution 1 and solution 2 were mixed together. The mixture was boiled for (1 min.) and then was left to cool to room temperature. The mixture was transferred into a (500 mL) volumetric flask and the volume was completed to (500ml) with distilled water.

3) Stock iodine solution (0.01N)

A) Solution 1 : (3.25)gm of pure I_2 was dissolved in a solution of (8) gm KI in (25)ml of distilled water , the volume was completed to (250 mL)

B) Solution 2: (1.25gm) of KI was dissolved in a little amount if distilled water, and followed by the addition of (25ml) of solution A , the volume was completed to 1L with distilled water , the solution was stored at (4°C) .

4) Calculate the enzyme activity.

To calculate the enzyme activity, follow the step in Table (1).

After preparation (T and C) measured immediately the absorbance at (660) nm ($1*10^{-3}$)M,then Add the prepared compound with addition of the serum and read absorption.

Calculation:

A-The control tube (C), the amount of starch.

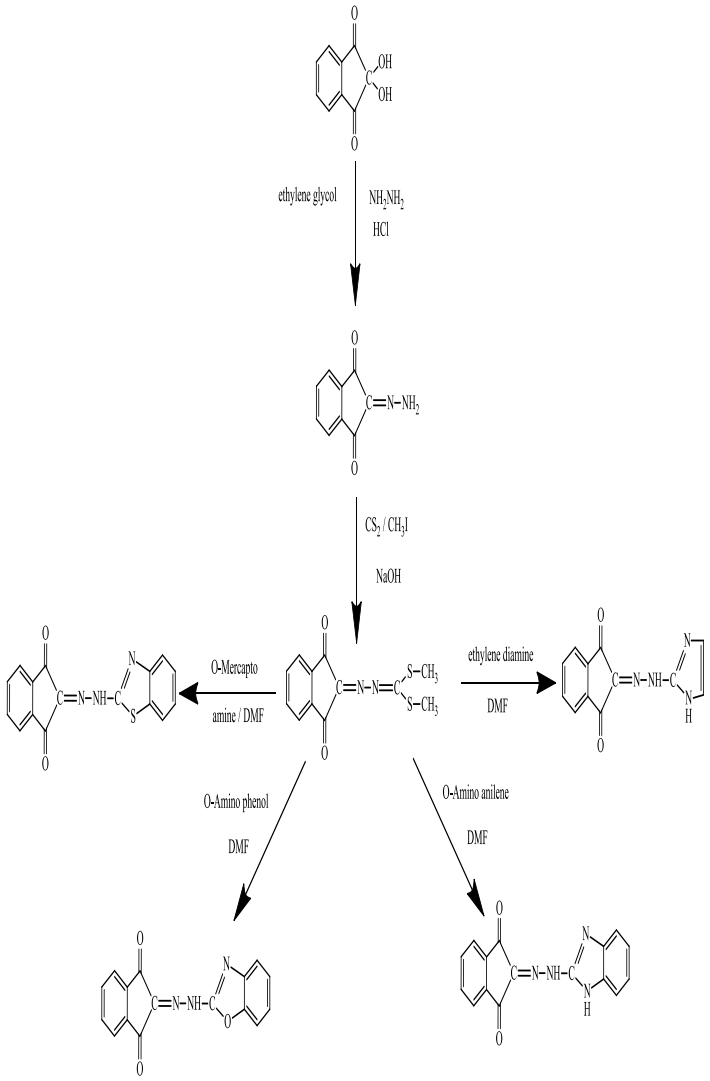
$$= \frac{C-T}{C} * 0.4 \text{ mg} \qquad = \frac{C-T}{C} * \frac{0.4}{S} \text{ s.u}$$

B-Test (amylase activity) in s.u/di.

$$= \frac{C-T}{C} * \frac{0.4}{S} * \frac{100}{0.01} = \frac{C-T}{C} * 800$$

Results and Discussion:

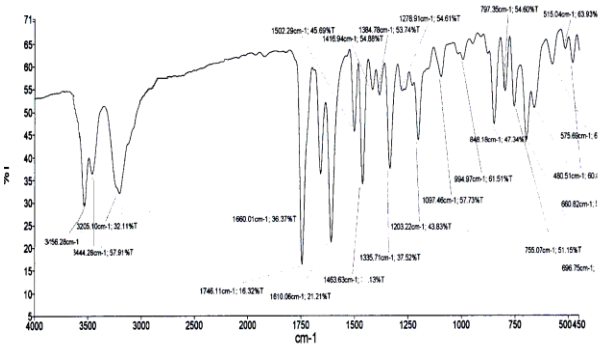
Routes of the syntheses can be summarized in the following scheme (1):



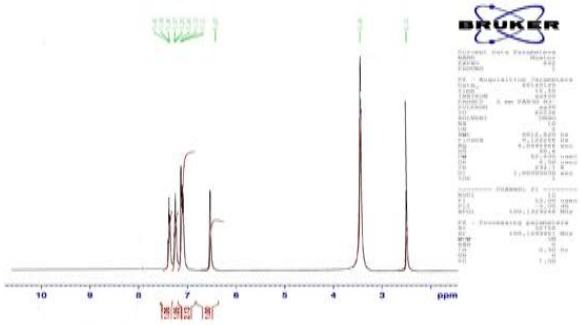
Scheme (1).

The present investigation deals with the synthesis of heterocyclic compounds as starting material required to carry up a biological effect. The one pot synthesis:Synthesis of compound(1) was prepared by reacting ninhydrin with hydrazine monohydrate. This compound was confirmed by analytical and spectral techniques. The FT-IR spectrum of compound (1) showed absorption bands at (3500-3457) cm^{-1} and (1698) cm^{-1} which was associated with ν (NH_2) and carbonyl group frequency, respectively. Disappearance band at (3300-3250) cm^{-1} was due to ν (OH) group removal. The spectrum also shows that carbonyl groups frequencies have not changed, which is an

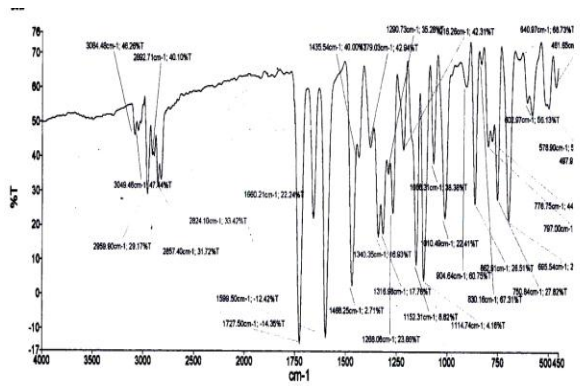
evidence that (NH_2) group of hydrazine did not interact with the carbonyl group, but rather it interacted with the (OH) groups of the ninhydrine.



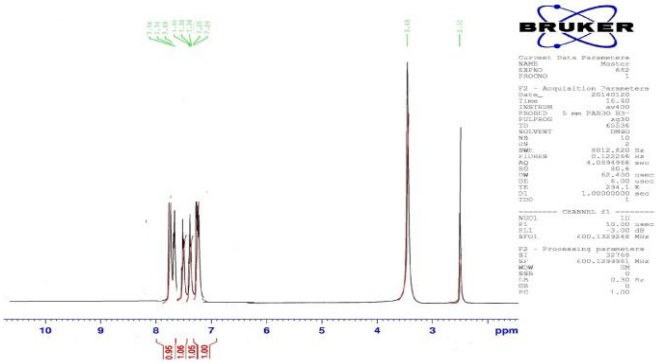
H-NMR spectrum of compound 1 showed the following characteristic signals at (δ,ppm,DMSO,d^6) 6.36ppm (S,2H,NH) ,(7.7 to 7.97)ppm (m,H,Ar)



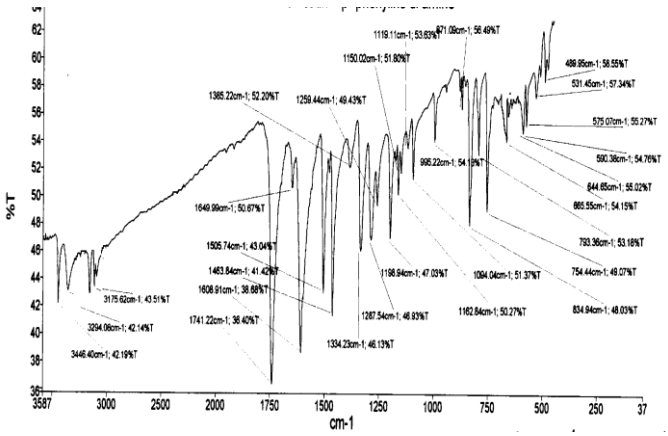
Treating compound (1) with SC2 in CH_3I/NaOH, afforded compound (2). The latter compound was characterized by (FT-IR, ^{1}H-NMR) spectroscopy. The IR spectrum of compound (2) showed disappearance of symmetrical and non-symmetrical stretch of (NH_2) group. Appearance absorption bands at (2929-2923) cm^{-1} and (1134) cm^{-1} was attributed to ν(C-H)alph and ν(C-S-C), respectively.



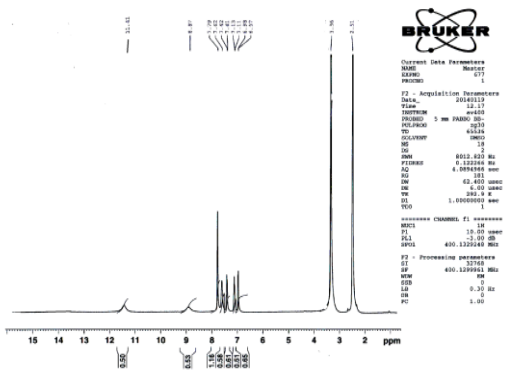
The H-NMR spectra of compound (II), showed following signals at (δ, ppm, DMSO-d^6):3.08 ppm(s, 6H, 2CH3), (7.45-7.47) ppm (m,H,aromatic).

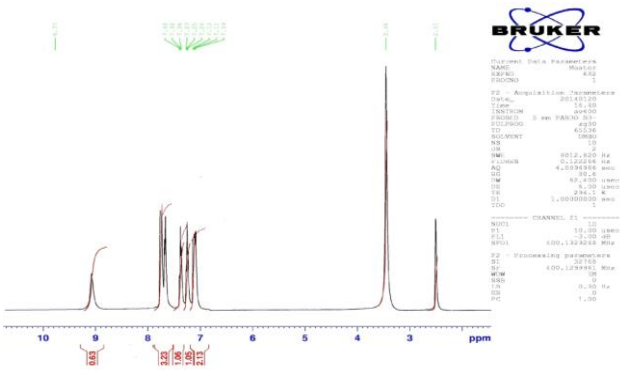


On the treatment of compound (II) ,after confirmed by analytical and spectral techniques, withethelen diamine, o-amino aniline ,o_marcapto aniline and o-amino phenpl respectively, (III,IV,VI,VII), Beside the correct values in elemental analyses, the IR and 1H-NMR spectra of , (III,IV,VI,VII), showed agreeable data with the proposed structures (c. f. exp.). The FT-IR spectra of compound (III) showed absorption peaks at (3454) $cm^{-1}$and (3427) cm^{-1} due to ν (NH) hdrazin and ν (NH) imidazole. And disappearance Absorption bands at (2929-2923) cm-1 and (1134) cm-1 was attributed to ν(C-H)alph and ν(C-S-C), respectively.

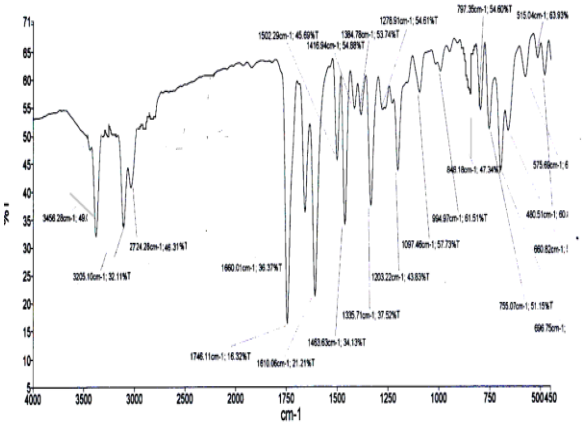


The H-NMR spectra of compound (III),showed following signals at (δ,ppm,DMSO-d6):10.91-978(s,H,NH), 8.45-7.47 (m,H,aromatic)





Compound **VI** showed the following characteristic spectral features, IR (ν/cm^{-1}): 3345-3250 (NH). And (NH) ring and 1660(C=N) . H-NMR (DMSO): δ/pp=-11.34-9.15(s, H,NH and NH ring) and 8.35-7.06(m,H,aromatic). Compound **VII** gave significant peak in IR (ν/cm^{-1}): 3353 (NH) and 1648 (C=N) and in ^{1}H-NMR (DMSO): δ/ppm = 10.84 (s, H,NH) and 8.05-6.89(m,H,aromatic). FT-IR spectra of compound(VII) showed absorption peaks at (3454) cm^{-1} due to ν(NH) hdrazin ,and showed absorption peaks at (1620) cm^{-1} due to (C=N) grop. And disappearance Absorption bands at (2929-2923) cm^{-1} and (1134) cm^{-1} was attributed to ν(C-H)alph and ν(C-S-C), respectively. Spectrum (H-NMR) of compound (VII): Showing signals at δ7.2 ppm (d,H,NHamidazole ring),δ(8.04-7.41)ppm (m,H,aromatic).These signals in H-NMR are proof of compound.



2): The biological effect of the prepared compounds against enzyme activity

The biological effect of the prepared compounds were studied in vitro. It was observed that these compounds inhibited the enzyme activity. The cause of the inhibition may be due to our understanding of enzyme action .which is the association of compounds with active site of the enzyme. I expect that the effect of the prepared compounds will different effect on enzyme's activity within the living cell in side body, prepared compound suffer from metabolic and physiological processes, these processes change the chemical properties of these compounds. Table (2) shows the amounted of amylase inhibitory and percentage of inhibition by prepared compounds.

Conclusions:

In this research, we reported the synthesis and characterization of four different compounds from Ninhydrin. These compounds have been confirmed by IR and HNMR spectroscopy. These compounds showed biological activity against amylze enzyme .The reaction between the functional groups of the compounds with the enzyme lead to deactivating it in a rate of (32.43, 32.71, 33.02, 33, and 49). Also, it has been found that there is a relationship between the number of aromatic rings in the prepared compounds and the deactivation rate, in which the deactivating rate increases as the number of the aromatic ring increases.

Table (1): Calculate the enzyme activity.

Seq.	Solution	Test,T	Control (C)
1	starch substrate * Stored the starch substrate in water bath for (3min) at 37°C,	1 ml	1 ml
2	Serum sample *Stored the serum sample in water bath for (15min) at 37°C	0.1 ml	-
3	Stock I_2 solution *mixed it	0.4 ml	0.4 ml
4	D.W	8.5 ml	8.5 ml

Table (2): The amounted of amylase inhibitory and percentage of inhibition by prepared compounds.

No. compound (conc.[M] [$1*10^{-3}$]	Activity (s.u/di)	Inhibition (%)
Control	365.04±166.35	0
III	259.06±164.35	32.43
IV	258.02±165.41	32,71
VI	253.02±153.52	33.02
VII	258.08±160.08	33.49

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