Kinetic Study of the Effect of Some Novel Lipid Lowering Compounds on Activities of Creatine Kinase and 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase In Mice Induced Hyperlipidemia

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Abstract

Hyperlipidemia is one of the most important factors leading to atherosclerosis and heart disease, therefore, this study conducted to examine the effect of two newly synthesized compounds[3-(5-(ethylthio)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-2-(3-nitrophenyl)benzo[1-3-e] thiazin-4-one (I) and 5(4-dimethyl amino) benzylidene amino)-1,3,4-thiadiazole-2-thiol(II)] on the activities of creatine kinase(CK) and 3-hydroxy-3-methylglutaryl- CoA reductase (HMGR) in male Wister mice . Also to determine the type of inhibition of these compounds on the above enzymes .The study was carried out on sixty male Wister mice aged seven to eight weeks their weight ranged(180-200 g) . The mice were grouped as: group(1): control group (12 mice).Group(2):consisted of 48 mice in which the mice were daily administered cholesterol (25mg/kg/day) in coconut oil 6% and creamy cheese for 28 days. Lipid profile was measured for 12 of mice chosen randomely from G2 to assure hyperlipidemia. Then group2 is subdivided into three groups as:group (2.A): (12 mice) positive control group in which the mice were daily administered simvastatin (40mg /day) as standard drug for hyperlipidemia for 20 days.Group(2.B):(12 mice) in which the mice were daily treated with (10^{-4})M of compound (I)via drinking water for 20 days. Group(2.C):(12 mice) in which the mice were daily treated with (10^{-5})M of compound II via drinking water for 20 days.Lipid profile(Tch, TG, HDL-c, LDL-c and VLDL-c) were determined in all groups.The activities of CK and HMGR were determined in all groups. Lineweaver-Burk plot was used for determination of V_{max}, K_m and type of inhibition for treated and untreated groups with compounds I and II. The results showed significant elevation in levels of Tch, TG, LDL and VLDL, while there are significant reduction in HDL-c levels in G2 comparing to control group(G1), after administration of fat rich diet. Simvastatin, compound I with concentration (10^{-4}M) and compound I The results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated after administration of simvastatin, compound I and II in G2A, G2B and G2C respectively. The results showed that the activities of CK reduced for group G2B and G2C while it is increased for G2A . The results also showed that the activities of HMGR were reduced in the three groups. The effect of compound I on CK activity was found to be noncompetitive inhibitor with V_{max} values values(1000and 166.6) U/L respectively for the uninhibited and inhibited reactions and K_m value (0.6)mmol/L for compound I and with V_{max} values (1000 and 250)U/L and K_m value(0.84)mmol/L respectively for the uninhibited and inhibited reactions for compound II. Compounds I and II were found to be noncompetitive inhibitors on HMGR with V_{max} values (0.83 and 0.16)U and K_m value (0.34)mmol/L respectively for the uninhibited and inhibited reactions for compound I and V_{max} values (0.83 and 0.35) U and K_m value(0.28)mmol/L respectively for the uninhibited and inhibited reactions for compound II. In conclusion the
new compounds(I and II) showed different inhibitory effect on CK and HMGR activities that could be used in treatment of hyperlipidemia and related disease in future.

Key Words: lipid lowering compounds, CK and HMGR

Introduction

Dyslipidaemia is an increase in the lipids, and lipoproteins. Cholesterol and the triglycerides are the two lipids in the blood. Elevation of one or both of these is seen in dyslipidaemia. The study of hyperlipidaemia has recently gained considerable importance, mainly because of the involvement of lipids in cardiovascular disease[1].

Atherosclerosis is a condition in which arteries are blocked to a greater or lesser extent by the deposition of cholesterol plaques, which can lead to heart attacks. The major lipids are generally cholesterol and its esters, in which the hydroxyl group is esterifies to a fatty acid; triglycerols are also found in these aggregates. LDL-c and HDL-c also play a major role in increase the risk for heart disease [2]. As excess free cholesterol apoptosis. Over long periods of time, arteries become progressively occluded as plaques consisting of extracellular matrix material, scar tissue formed from smooth muscle tissue, and foam cell remnants gradually become larger. Occasionally a plaque breaks loose from the site of its formation and is carried through the blood to a narrowed region of an artery in the brain or the heart, causing a stroke or a heart attack[3].

Creatine kinase is a biologically important enzyme expressed by different tissues and cell types that catalyze the reversible conversion of creatine at the expense of adenosine triphosphate to phosphocreatine[4]. Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, the autoimmune myositis and in acute renal failure[5].

3-Hydroxy-3-methylglutaryl-CoA reductase is a transmembrane glycoprotein located on the endoplasmic reticulum. This enzyme catalyzes the four-electron reduction of HMG-CoA to coenzyme A , and mevalonate, which is the rate-limiting step in sterol biosynthesis [6] .

Schiff bases of thiadiazole is important class of the most widely used organic compounds which gained importance in pharmaceutical fields due to abroad spectrum of biological activities like anti-inflammatory[7], and antihyperlipidaemic[8] .

Thiazine are another useful units in the fields of medicinal and exhibit a variety of biological activities such as antidyslipidemic[9], antibacterial [10], antifungal[11], and anti-inflammatory[12].

The aim of the present study is to evaluate the effect of some derivatives of thiadiazole and thiazine in vivo on CK and HMGR activities. Also to determine the type of inhibition of these compounds which may be used as lipid lowering agents in future.

Material and Methods

The structure of compounds I and II which were used in this study as lipid lowering agent are shown in figure(1). These compounds were prepared in previous study [13].

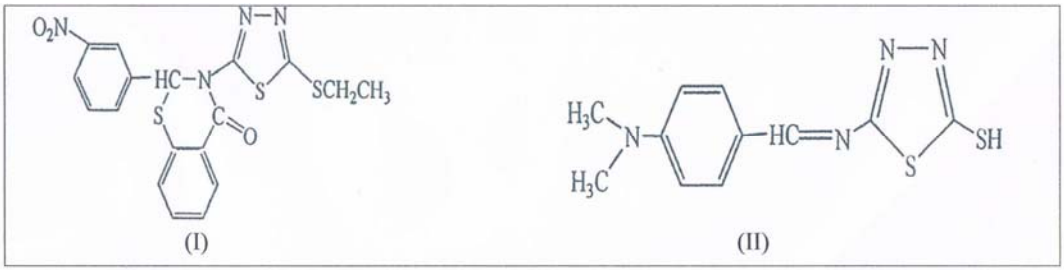

Figure No.(1)

The study were carried out with sixty male Wister mice aged seven to eight weeks (180-200 g) ,obtained from animal house , of college of Medicine, Baghdad University. The mice were grouped as follows:

Group(1): control group (12 mice)

Group(2**):**consists of 48 mice in which the mice were daily administered cholesterol (25mg/kg/day)[14] in coconut oil 6% and creamy cheese for 28 days. Lipid profile were measured for 12 mice chosen randomly from G2 to assure hyperlipidemia. Group2 were subdivided into three groups as follows:

Group (2.A): (12 mice) positive control group in which the mice were daily administered simvastatin (40mg/day) as standard drug for hyperlipidemia for 20 days.

Group(2.B):(12 mice) in which the mice were daily treated with (10^{-4})M of compound (I)via drinking water for 20 days.

Group(2.C):(12 mice) in which the mice were daily treated with (10^{-5})M of compound II for 20 days.

The chosen concentration was based on *in vitro* previous study which gave the best inhibition from the other concentrations[15].

The Tch, TG, HDL-c, LDL-c and VLDL levels were determined in all groups (G1, G2, G2A, G2B and G2C) according to the standard procedures of the biochemistry laboratory of the hospital.

The results were expressed as mean+ SD.T-test was used for comparison between the two studied groups. P-vales <0.05 was considered statistically significant .

The CK activity was determined in all studied groups by using a ready kit from Randox-UK by monitoring the concentration of creatine kinase. The absorbance was recorded at 340nm [16].

The HMGR activity was measured in all studied groups spectrophotometrically which HMG-CoA and NADPH performed from Sigma Alderage. The HMG-CoA dependent on oxidation of NADPH. Absorbance reduction at 340nm was measured in 6min interval[17].

Kinetic Study

The K_m and V_{max} for CK and HMGR were performed using different concentrations of substrate. Fixed concentrations of compounds I and II were used by utilizing the same concentrations of substrate without using both compounds were performed for the determination of the type of inhibition by Lineaver –Burk plot.

Results and Discussion

Simvastatin, compounds I and II were used in treatment of mice with dyslipidemia. The G2A was treated with simvastatin, G2B was treated with (10^{-4}) M of compound I and G2C was treated with (10^{-5}) M of compound II for twenty days.

Table(1) showed the levels of lipid profile in G1, G2and G2A, G 2B, G2C .The results showed significant elevation in levels of Tch, TG, LDL and VLDL, while there are significant reduction in HDL levels after administration of fat rich diet in G2 comparing to control group(G1). P-values <0.05 were considered statistically significant . The results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c were elevated after administration of simvastatin, compound I and II n G2A,G2B and G2C respectively.

Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms. Therefore, higher intake of dietary cholesterol increases serum lipid profile by down regulation of LDL-c receptor synthesis thus decrease uptake of LDL-c via these receptors[18].

Some researches demonstrated that the actions may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased

expression of hepatic LDL receptors and their protection etc. leading to an increased removal of LDL-c from the blood and its increased degradation and catabolism of cholesterol from the body[19].

Clinical trials have demonstrated that intensive reduction of plasma LDL-c levels could reverse atherosclerosis and decrease the incidence of CVDs. Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders in cholesterol metabolism, atherogenesis as well as possible treatments for the reduction of circulatory cholesterol levels[20].

Table (2) showed the effect of compound I with concentration (10^{-4}M) and compound II with concentration (10^{-5}M) on CK and HMGR activities for all experimental groups. The results showed that the activities of CK reduced for group G2B and G2C while it is increased for G2A which is in agreement with the literature that statin elevated the levels of CK[14].The results also showed that the activities of HMGR were reduced in the three groups.

The results revealed that compound I showed more potent antihyperlipidaemic effect than compound II. Also, compounds I and II exhibit more potent antihyperlipidaemic effect than simvastatin.

-Lineweaver-Burk Plot for Sera of CK and HMGR for Untreated and Treated Mice:

Figures (1) and (2) showed the type of inhibition using Lineweaver-Burk plot for compounds I and II on CK activity for G2 respectively. Compound I and II showed a noncompetitive inhibitor on CK activity with V_{max} values(1000and 166.6) U/L for uninhibited and inhibited enzyme respectively and K_m value (0.6) mmol/L for compound I and with V_{max} vales (1000 and 250) U/L for uninhibited and inhibited enzyme respectively and K_m value (0.84) mmol/L for compound II .

Figures(3) and (4) showed Lineweaver-Burk plot for compound I and II effect on HMGR activity for G2, respectively.Compound I and II showed a noncompetitive inhibitor on HMGR activity with V_{max} values (0.83 and 0.16)U for uninhibited and inhibited enzyme respectively, and K_m value (0.34) mmol/L for compound I and with V_{max} values(0.83 and 0.35) U for uninhibited and inhibited enzyme respectively, and K_m value (0.28) mmol/L for compound II .

Inhibition of HMGR blocks cholesterol biosynthesis, which in turn stimulates the synthesis of LDL receptors, which localize to the surface of the cell and bind and internalize circulating lipoproteins, thereby lowering the plasma LDL cholesterol concentration[20].

A series of keto-enamine Schiff bases, derived from 8-hydroxyquinoline was synthesized and subjected for in vitro antioxidant and antihyperlipidemic agents, The results demonstrated that Schiff bases compounds are found to be the most potent antihyperlipidemic agent[21].

A novel series of Schiff bases of 2-phenyl-3-(amino substituted arylidene) quinazoline-4-(3H)-ones, were synthesized and evaluated for antihyperlipidemic activity[22]. The nitrogen atom of Schiff bases may be involved in the formation of a hydrogen bond with the active centers of cells constituents and interferes in normal cell processes[23].

A new thiazine derivatives which was prepared by recent studies showed squalene synthase inhibitory/hypolipidemic activities which suggested that these compounds strongly inhibited in vitro microsomal lipid and LDL peroxidation[24].

A more recent research synthesized novel model of thiazine derivatives that significantly exhibited antidyslipidemic and antioxidant properties[25].

Recent studies revealed that organic compounds may be bound to the active site by molecular interactions such as hydrogen bound, hydrophobic, and cation-π interactions and interacting residues[26-28].

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Table No. (1): The Levels of Lipid profile in Control Group and Experimental Groups.

Parameters / Groups	G1 n=12	G2 n=12	G2A n=12	G2B n=12	G2C n=12
Tch (mg/dl)	155±2.4	600±20.5	340±0.97	172±0.91	250±0.86
TG (mg/dl)	100.3±5.1	325.01±15.6	299±9.3	205±10.3	280±6.4
HDL (mg/dl)	43±6.9	25.6±4.2	40.1±6.1	50.3±4.01	45±7.3
LDL (mg/dl)	62.1±3.4	510±9.3	240±2.7	81±2.4	149±3.6
VLDL (mg/dl)	30.1±0.19	65.3±0.25	59.8±0.43	41.6±0.74	56.4±0.86

P-values <0.05 was considered statistically significant

Table No. (2): Showed the Activity of CK and HMGR Activities in G1and G2 Before and After Addition of Simvastatin, Compound I and Compound II.

Parameters / Groups	CK activity (U/L)	HMG-CoA reductase activity(U)
G1	113.4	0.034
G2	989	0.51
G2A	1000	0.24
G2B	163.4	0.020
G2C	176.6	0.024

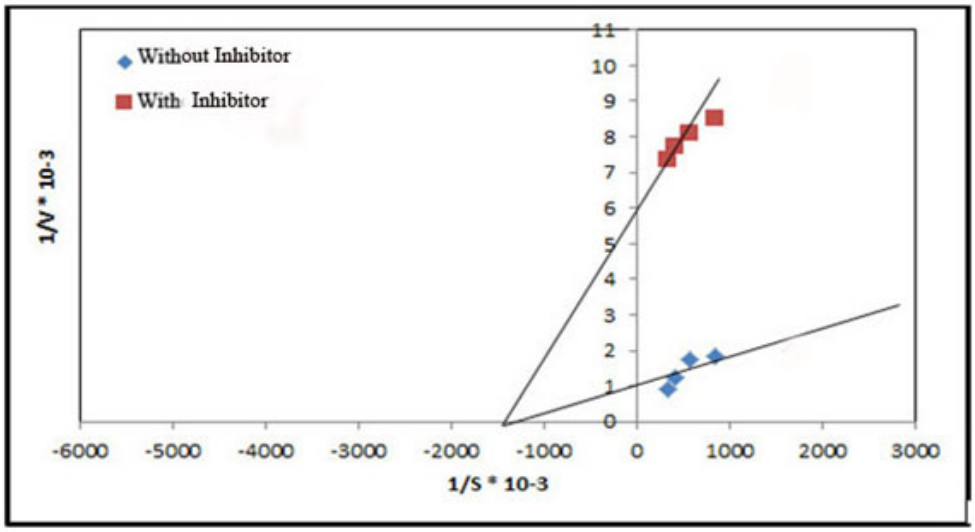

Figure No. (1): Lineweaver-Burk Plot for the effect of Compound I on CK Activity

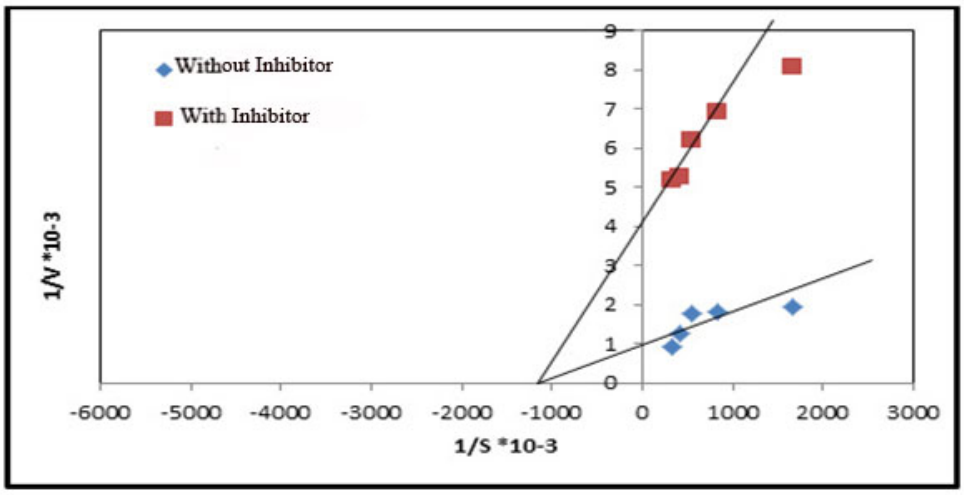

Figure No. (2): Line weaver-Burk Plot for effect of Compound II on CK Activity

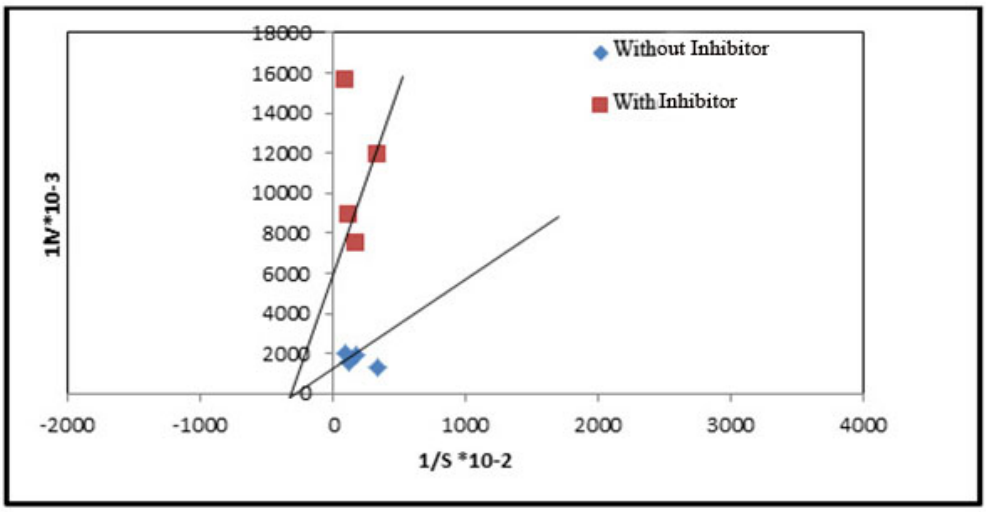

Figure No. (3): Line weaver-Burk Plot for Compound I Effect on HMGR

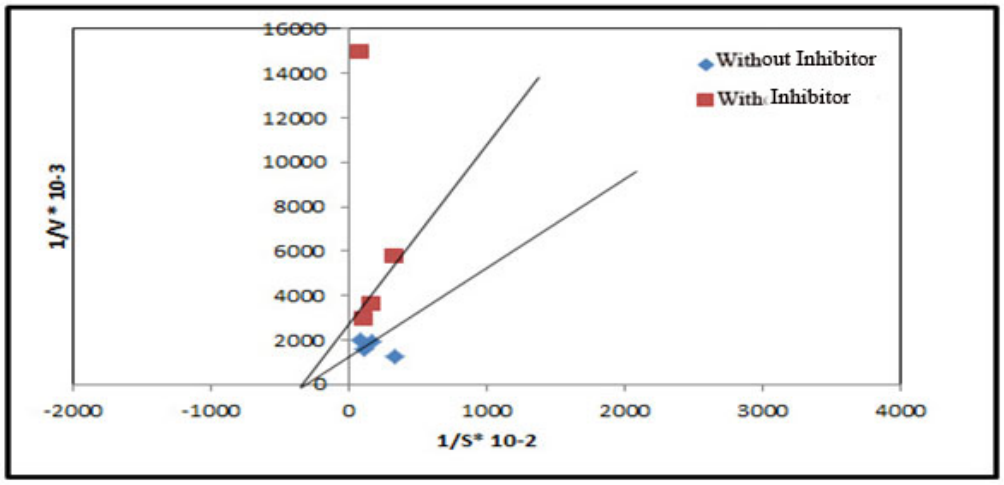

Figure No. (4) Line weaver-Burk Plot for Compound II Effect on HMGR

دراسة حركية لتأثير بعض المركبات المخفضة للدهون في فعالية كل من الإنزيمين الكرياتين كاينيز و3-هيدروكسي-3-مثيل كلوتاريل كو-إنزيم أي ريدكتيز في ألفئران المختبرية التي تم حث ارتفاع الدهون فيها

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استلم البحث في:7ايلول2014،قبل البحث في :21كانون الاول 2014

الخلاصة

الهدف من هده الدراسة هو تقدير تأثير بعض مشتقات الثياديازول والثيازين جديدة التحضير في فعالية كل من الإنزيمين الكرياتين كاينيز و3-هيدروكسي-3-مثيل كلوتاريل كو-إنزيم أي ريدكتيز فضلا" عن قياس صورة الدهون في ألفئران المختبرية التي تم حث ارتفاع الدهون لديها بواسطة تغذيتهم بالاغدية الغنية بالكولستيرول . تم اختبار تأثير المركب (I) بتركيز(10^{-4}) مولاري والمركب (II) *بتركيز* (10^{-5}) مولاري داخل الخلية الحية .

أجريت الدراسة على ستين فأرا" من الذكور الذين تتراوح اعمارهم بين سبعة الى ثمانية اسابيع (180-200 غم) التي تم الحصول عليها من البيت الحيواني في كليه الطب ,جامعة بغداد .تم تقسيم الفئران المختبرية كما يلي:

المجموعة الاولى: مجموعة السيطرة(12 فأرا") والمجموعة الثانية: تتكون من (48 فأرة) كانت تعطى يوميا" الكولسترول (25ملغم/كلغم/يوم), و زيت جوز الهند% 6 وجبنا" كامل الدسم لمدة (28)يوما" . تم قياس صورة الدهون لاثني عشر فارا" اختيروا عشوائيا" من المجموعة الثانية للتأكد من ارتفاع الدهون فيها. ثم قسمت بقية المجموعة الثانية الى ثلاث مجاميع على النحو الآتي:

المجموعة 2A: (12 فأرة) كمجموعة سيطرة موجبة في الفئران التي تعطى يوميا سمفاستاتين (*40ملغم/يوم*) كدواء قياسي لارتفاع الدهون لمدة (20 يوميا"). المجموعة 2B: (12 فأرة) إذ عولجت هذه الفئران يوميا" بالمركب الاول بتركيز (10^{-4}) مولاري عن طريق الشرب لمدة (20 يوميا").المجموعة 2C: (12فأرة) وعولجت هذه الفئران يوميا" بالمركب الثاني بتركيز (10^{-5}) مولاري عن طريق الشرب لمدة (20 يوميا")

تم قياس صورة الدهون ، وكذلك تم قياس فعالية كل من الانزيمين الكرياتين كاينيز و 3-هيدروكسي-3-مثيل كلوتاريل كو-إنزيم اي لكل المجاميع المدروسة . كما تم قياس قيمة الفعالية القصوى وثابت ميكاليز ونوع التثبيط للمجموعات المعالجة وغير المعالجة بالمركبين (I وII) .

أظهرت النتائج أن المركبين (I وII) أظهرا تأثيرا" تثبيطيا" لاتنافسيا" في الانزيم كرياتين كانيز بفعالية قصوى (1000 و 166.6) *وحدة/لتر* للانزيم المثبط وغير المثبط على التوالي و ثابت ميكاليز بقيمة (0.6) ملي مول /لتر للمركب (I) بفعالية قصوى (1000 و 250) وحدة /لتر للانزيم المثبط وغير المثبط على التوالي، وثابت ميكاليز بقيمة (0.84) *ملي مول /لتر* للمركب (II) .كما بينت النتائج بأن المركبين (I وII)اظهرا تثبيطاً لاتنافسياً على فعالية انزيم 3-هيدروكسي-3-مثيل كلوتاريل كو-انزيم اي بفعالية قصوى (0.16 و0.83) *وحدة* للانزيم المثبط وغير المثبط على التوالي وثابت ميكاليز بقيمة (0.34) ملي مول /لتر للمركب (I) بفعالية قصوى (0.35 و0.83) *وحدة* للانزيم المثبط وغير المثبط على التوالي ، وثابت ميكاليز بقيمة (0.28) ملي مول /لتر للمركب (II) .

استنتج من هذه الدراسة بأن المركبين (I وII) مهمان في تطور عوامل جديدة كمضاد لارتفاع الدهون التي ابدت تأثير تثبيطي على الكرياتين كانيز، في حين ان الستاتينات تسبب ارتفاع هذا الانزيم . كما ان المركبات اظهرت تأثيرا" تثبيطيا" في 3-هيدروكسي-3-مثيل كلوتاريل كو-انزيم اي ريدكتيزاكثر من السمفاستاتين وهو الانزيم المنظم لتخليق الكولسترول .

الكلمات المفتاحية:المركبات المخفضة للدهون,الكرياتين كارنز و-3 -مثيل كلوتاريل كو-انزيم.