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Kinetic Study of the Effect Some Novel Lipid Lowering Compounds on Creatine Kinase and 3-Hydroxy-3-Methy-Glutaryl-CoA Reductase Activities

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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 (HMG-CoA redutase) in serum of hyperlipidemic patients *in vitro* study. Also to determine the type of inhibition of these compounds on the above enzymes which may be used as lipid lowering agents in future. The results revealed that compound I showed the best inhibition effect at 10^{-4} M concentration, while compound II showed the best inhibition effect at 10^{-5}M concentration for both enzymes. The effect of compound I on CK activity was found to be noncompetitive inhibitor with V_{max} values (1000 and 344.82)U/L respectively for the uninhibited and inhibited reactions and K_m value (10)mmol/L while compound II was found to be competitive inhibitor with V_{max} value (588.23)U/L and K_m values (5.51 and 4)mmol/L respectively for the uninhibited and inhibited reactions. Compounds I and II were found to be competitive inhibitors on HMG-CoA reductase with V_{max} value (0.020)U/L and K_m values(0.339 and 0.125)mmol/L respectively for the uninhibited and inhibited reactions for compound I and V_{max} value (0.021) U/L and K_m values(1.111 and 0.256)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 HMG-CoA reductase activities that could be used in treatment of hyperlipidemia and related disease in future.
Key words: lipid lowering compounds, CK and HMG-CoA reductase.

Introduction:

Elevation in the blood lipids termed hyperlipidemia. The increase in cholesterol, TG, LDL-c and reduction HDL-c increase atherosclerotic plaque formation resulting in risk of heart attack[1-3]. Investigations into vascular consequences of chronic hypercholesterolemia, the mechanisms through which these consequences occur, and the potentially beneficial effects of ameliorative therapies have received considerable attention during the last decade[4,5].

Creatine Kinase (CK), which catalysis the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP), expressed by various tissues and cell types. 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 [6].

3-Hydroxy-3-Methyl Glutaryl-CoA Reductase (HMG-CoA reductase) is the rate limiting step in the biosynthesis of cholesterol and isopernoides [6,7]. Statin, and its derivatives are HMG-CoA rreductase inhibitors, which used for decades in lipid lowering [8,9]. But the high prevalence of their adverse effects such as myopathy myotoxicity [10], liver damages enhances anticoagulant effect [11] and potential drug-drug interactions has been reported [12].

Schiff bases and thiadiazole the important classes of the most widely used organic compounds which gained importance in pharmaceutical fields due to abroad spectrum of biological activities like anti-inflammatory, antimicrobial, and antidyslipidemic effects[13,14].

Thiazine are six member heterocyclic that contain in their structure a nitrogen and a sulfar atom. It is a very useful unit in the fields of medicinal and pharmaceutical chemistry and have been reported to exhibit a variety of biological activities such as antioxidant [15], antidyslipidemic and anti-inflammatory[16].

The aim of the present study is to evaluate the effect of compounds(I and II) on CK and HMG-CoA reductase activities in hyperlipidemia patients. Also to determine the type of inhibition of these compounds which may be used as lipid lowering agents in future.

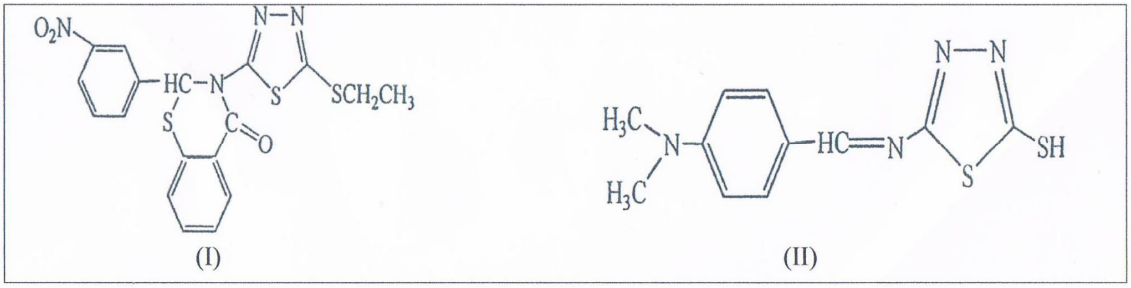
Material and Methods:

Sixty individuals with age ranged between (40-60) years were enrolled in this study. They were divided into groups; first group (G1) consisted of 30 healthy individuals as a control group with body mass index (BMI) 25.67. The second group (G2) consisted of 30 patients with hyperlipidemia and BMI 26.48. The patients attended the Ibn-Alnaphaes hospital during November 2013 to February 2014 who they were diagnosed by physician as hyperlipidemia. Patients with high blood viscosity, diabetes mellitus, and renal failure as well as those who are under treatment with statins were excluded.

Ten milliliters of fasting blood were collected from all subjects. The serum obtained used in determination of lipid profile[total cholesterol (Tch), triglyceride (TG), high density lipoprotein(HDL), very low density lipoprotein(VLDL)],fasting blood glucose(FBG),alanine transaminase(ALT),Aspartate transaminase(AST) and C-reactive protein(CRP) according to the standard procedures of the biochemistry laboratory of the hospital.

Data are presented as mean± SD. The differences between two groups were analyzed by student's t-test. P-value of <0.05 and 0.001 considered significant and highly significant, respectively.

The structure of compounds I and II that used in this study as lipid lowering agent are wshowen in figure(1). These compounds were prepared in previous study[17].



Figure(1): The structure of compounds I and II

Determination of Biological Activity of Compounds (I and II) as Lipid Lowering Agents:

-Determination of CK and HMG-CoA Reductase Activities:

The CK activity was determined by using a ready kit from Randox-UK by monitoring the concentration of creatine kinase, hexokinase and glucose-6-phosphate dehydrogenase. The absorbance was recorded at 340nm[18].

HMG-CoA reductase activity was measured spectrophotometrically which HMG-CoA reductase, 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[19].

Fourdifferent dilutions (10^{-2}, 10^{-3} , 10^{-4} and 10^{-5})M for both compounds were prepared from a stock solution (10^{-1}) M in distilled water with addition of few drops of ethanol.

Activities of CK and HMG-CoA reductase were determined in G1 and G2 before and after addition of different concentrations of compound I and compound II.

Simvastatin, which considered as standard drug for lipid lowering, was used for comparison with the potency of compounds I and II on HMG-CoA reductase activity in treatment of hyperlipidemia.

Determination of K_m and V_{max} for CK and HMG-CoA Reductase:

The K_m and V_{max} for CK and HMG-CoA Reductase were performed using different concentrations of substrate.

Determination of Percentage inhibition:

The percentage of inhibition was determined by using different concentrations of compounds I and II while the concentration of substate [S] was fixed.

The activity of the enzymes without addation of any compound was considered to be100% and all the results were relative to it. The concentration of compounds that gives the highest percentage of inhibition was used throughout the study to obtain the type of inhibition.

Determination of the type of the inhibition:

Fixed concentrations of compounds I and II were used by utilizing the same concentrations of substrate without using both compounds were performed. The effect of solvent, which used as diluents, was determined by adding a quantity equivalent

to the sample and all steps completed as in the method of determination of CK and HMG-CoA reductase activities. Its inhibition effect was determined and subtract from the results before determining the inhibition or activation of the compounds.

Results and Discussion:

The levels of charastastic parameters in patients and control groups are summarizes in table (1). The results which exptressed as (mean± SD), showed highly significant elevation in Tch, TG, LDL, VLDL, ALT, AST and CRP while there are significant reduction in HDL levels when comparing between G1 and G2. The results, also showed no significant elevation in FBG level when comparing between G1 and G2.

Table(2 and 3) represent the activities of CK and HMG-CoA reductase, respectively,in G1 and G2 before and after addition of compounds I and II in different concentrations (10^{-2} , 10^{-3} ,10^{-4} and 10^{-5})M.

The results revealed that compound I showed inhibitory effect on CK activity at (10^{-3} ,10^{-4} and10^{-5}) M, while it showed activator effect for enzyme at concentrations (10^{-2})M. Compound II showed inhibitory effect on CK activity at (10^{-2} and 10^{-5}) M, while it showed activator effect for enzyme at concentrations (10^{-3} and 10^{-4})M. The results also revealed that compounds I and II showed inhibitory effect on HMG-CoA reductase in all concentrations.

The results demonstrated that compound I showed the best inhibition effect at 10^{-4} M concentration, while compound II showed the best inhibition effect at 10^{-5}M concentration for both enzymes which were chosen for kinetic study.

Figure(1 and 2) showed the type of inhibition for compounds I on CK and HMG-CoA reductase activities using Lineweaver-Burk plot. The results revealed that compound I was found to be a noncompetitive inhibitor for the CK with the V_{max} values(1000 and 344.82) U/L respectively for the uninhibited and inhibited reactionsand K_m value (10) mmol/L, while compound I was found to be competitive inhibitor on HMG-CoA reductase with V_{max} value (0.020) U/L and K_m values (0.339 and 0.125) mmol/L respectively for the uninhibited and inhibited reactions.

Figure(3 and 4) showed the type of inhibition for compounds II on CK and HMG-CoA reductase activities using Lineweaver-Burk plot. The results revealed that compound II was found to be a competitive inhibitor for both enzymes with the V_{max} value (588.23) U/L and K_m values (5.51 and 4)M respectively for the uninhibited and inhibited reactions for CK and V_{max} value (0.021) U/L and K_m
Values (1.111 and 0.256) mmol/L respectively for the uninhibited and inhibited reactions for HMG-CoA reductase.

The activity of simvastatin on HMG-CoA reductase activity was shown in table(4).The results revealed that compounds I and II found to be more potent as lipid lowering agents than simvastatin.

Statins are the major drug to treat hyperlipidemia that are even prescribed to the healthy population, to prevent heart disease development. However, the statin use is an open discussion because of their side effects such as myostis, define as the presence of muscle symptoms, including aches, soreness, or weakness, and an increase in serum CK[20]. Taking into consideration that millions of people are treated with statins, a lot of them complain of side effects especially in high dose treatment and this suggests that,despite the success of statins, efforts in the development of novel hypolipidemic compounds should be needed [21].

A dozen of the synthetic compounds mimic the inhibition of purified HMG-CoA redutase activity caused by pravastatin, fluvastatin and sodium salt of lovastatin, simvastatin in the cell free assay, suggesting direct interaction with the rate limiting enzyme of cholesterol biosynthesis[22].

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[23,24].This could be due to the affinity of such compounds to compete on binding to the active sites of the enzyme.

In conclusion the novel synthetic compounds (I and II) seem to be of interest in development of a new antihyperlipidemic agents that exhibit inhibition effect on CK while statins cause increasing in this enzyme. Also these compounds exhibit inhibition effect on HMG-CoA reductase activity more than simvastatin.

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Table(1):Descriptive parameters in the studied groups (G1, G2)

Groups / Parameters	G1 n=30	G2 n=30	P*
BMI (kg/m)	25.67±4.55	26.48±5.22	NS
Tch (mg/dl)	121.7±13.84	458.05±71.75	<0.001
TG (mg/dI)	97.73±5.07	199.16±53.07	<0.05
HDL-c (mg/dI)	42.43±4.07	28.37±5.73	<0.001
LDL-c (mg/dI)	60.36±14.15	390.68±54.58	<0.001
VLDL-c (mg/dI)	19.67±1.19	39.96±19.56	<0.05
FBG (mg/dI)	95.8±17.22	96.2±9.40	NS
ALT (U/L)	14.39±3.04	63±5.83	<0.001
AST (U/L)	13.89±2.77	85.3±7.66	<0.001
CRP (mg/dI)	0.40±0.16	2.99±0.11	<0.05

NS, No Significant

Table(2): The CK activity in U/L in G1, and G2 before(B) and after(A) addition of different concentrations of compound I and compound II

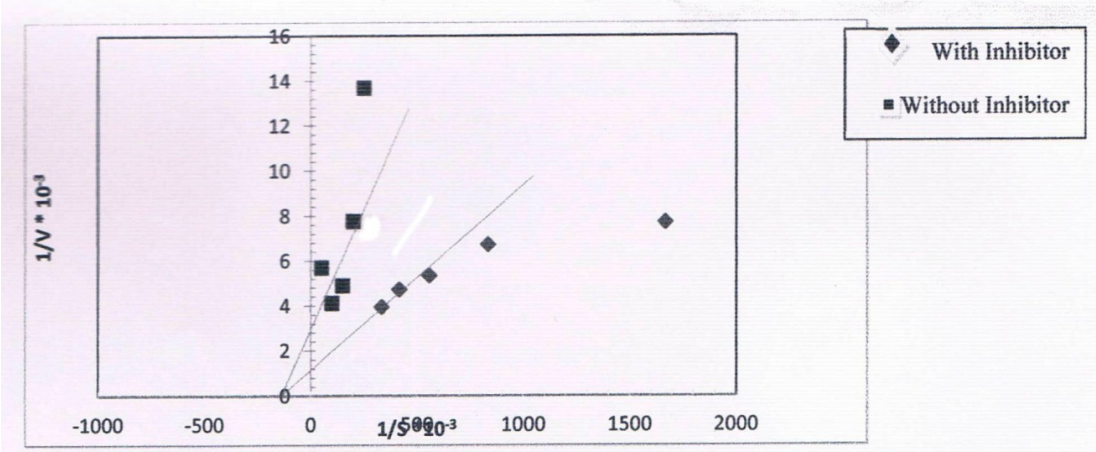
Groups	HMG-CoA red. Activity(U)	Compounds	HMG-CoA red activity (U)in G2(A)			
			10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M
G1	85.34	Compound I	340.06	208.41	175.39	229.04
G2(B)	252.5	Compound II	215.28	356.98	316.95	189.01

Table(3): HMG-CoA reductase activity in U/L in G1, and G2 before(B) and after(A) addition of different concentrations of compound I and compound II

Groups	HMG-CoA red. Activity(U)	Compounds	HMG-CoA red activity (U)in G2(A)			
			10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M
G1	0.009	Compound I	0.020	0.020	0.012	0.020
G2(B)	2.00	Compound II	0.021	0.019	0.023	0.017

Table(4): HMG-CoA reductase activity (U) in G1, and G2 before(B) and after(A) additionof simvastatin

Groups	HMG-CoA reductase activity
G1	0.009
G2(B)	0.200
G2(A) (Simvastatin)	0.024



Figure(1): Lineweaver- Burk plot for compound I effect on CK activity of Hyperlipidemia patients

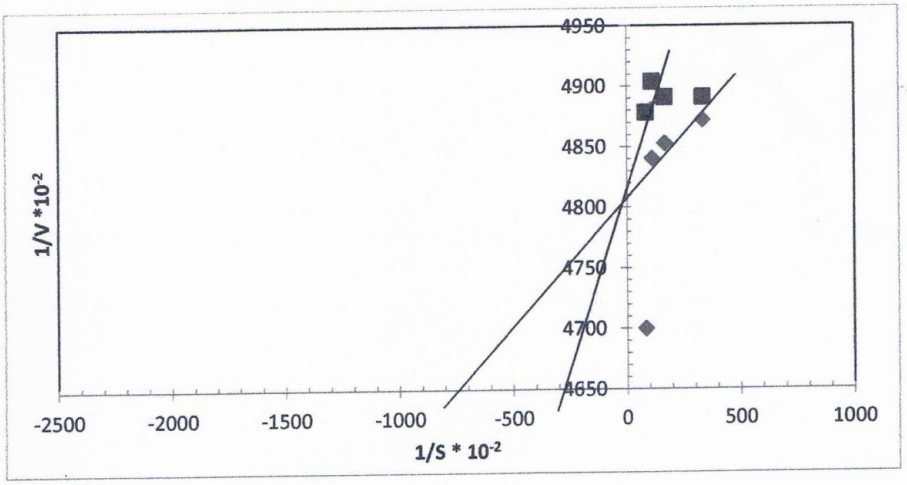


Figure (2): Lineweaver- Burk plot for compound I effect on HMG-CoA reductase activity of Hyperlipidemia patients

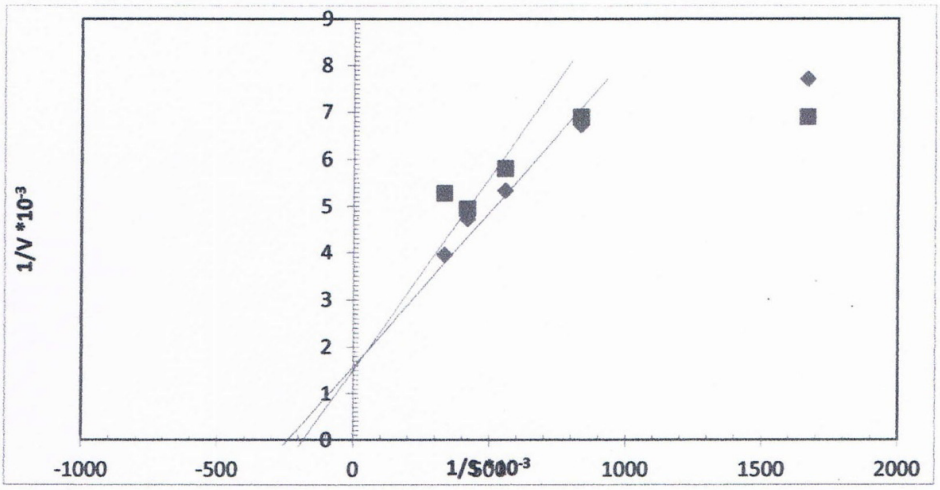
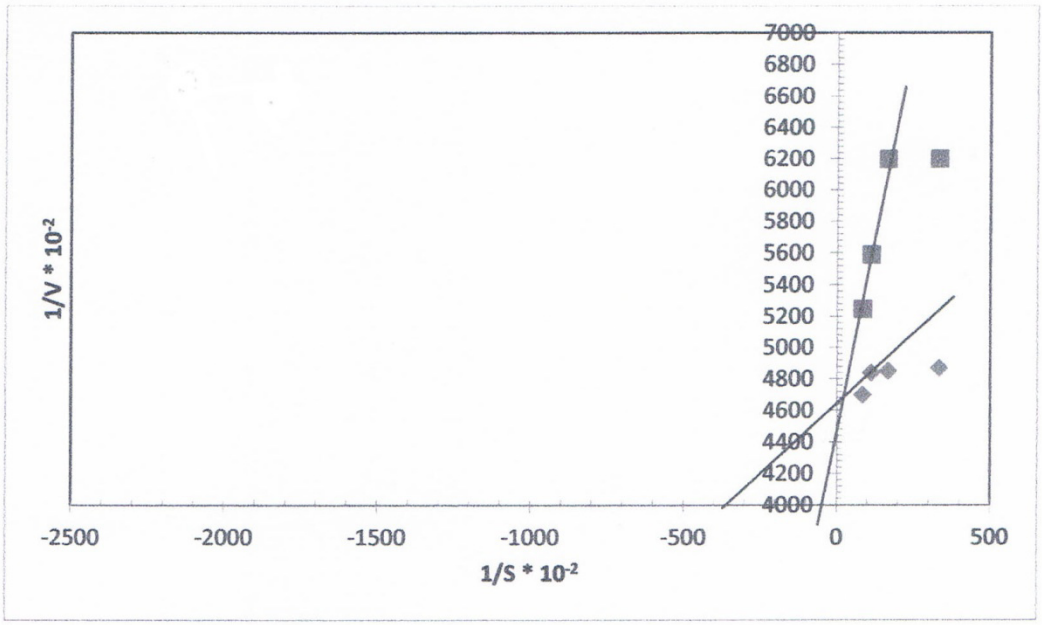


Figure (3): Lineweaver- Burk plot for compound II effect on CK activity of Hyperlipidemia patients



Figure(4). Lineweaver- Burk plot for compound II effect on HMG-CoA reductase activity of Hyperlipidemia patients

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