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Synthesis, characterization and Molecular docking simulation of novel Atorvastatin Aspirin ester, and study activation effect on lactate dehydrogenase enzyme in atherosclerosis patient

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ABSTRACT

This study involved synthesis of Atorvastatin Aspirin ester prodrug by reacting of aspirin drug with thionyl chloride $SOCl_2$ to produce aspirinyl chloride that mixed with Atorvastatin drug which dissolved with acetone to produce corresponding ester. The structure of new compound was estabilished by spectral techniques that includes FT-IR and ^{1}H-NMR. The biochemical part encompassed collecting of blood samples from atherosclerosis patients then many diluted concentrations of new prepared prodrug and study its effect on the lactate dehydrogenase activity where the outcomes indicated the new ester have activation effect on lactate dehydrogenase enzyme. Molecular docking study was a theoretical investigation to study the interaction of new synthesized ester with Lactate dehydrogenase enzyme (PDB:ID=**4i8x)**, the molecular docking study clarified the synthesized compound showed good docking score: -8.9 kcal\mol with RMSD value 2.819, 5.803 A^o, and binding patterns

Keywords: Atorvastatin, Aspirin, Molecular docking, lactate dehydrogenase, Atherosclerosis

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تحضير وتشخيص ومحاكاة الارساء الجزيئي لأستر جديد للاتورفاستاتين والاسبرين ودراسة التاثير التنشيطي له على فعالية انزيم اللاكتيت ديهايدروجنيز في مرضى تصلب الشرايين

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قسم الكيمياء، كلية العلوم، جامعة تكريت، تكريت، العراق

الملخص

تضمن هذا البحث تحضير دواء مصاحب استري للاسبرين والاتورفاستاتين من خلال مفاعلة دواء الاسبرين مع كلوريد الثايونيل للحصول على كلوريد الاسبرينيل الذي مزج مع عقار الاتورفاستاتين المذاب في الاسيتون للحصول على الاستر المقابل شخص المركب المحضر باستحدام التقنيات الطيفية : FT-IR, 1H-NMR ، الجزء الحياتي من هذا العمل تضمن جمع عينات دم من المرضى المصابين بتصلب الشرايين واخذت عدة تخافيف من محلول الاستر المحضر ودرس تاثيرها على فعالية انزيم اللاكتيت ديهايدروجنيز حيث اظهرت النتائج ان الاستر الجديد يمتلك تاثير تنشيطي جيد للانزيم . كذلك تضمن البحث دراسة الارساء الجزيئي لتوضيح بين الاستر الجديد وانزيم لاكتيت ديهايدروجنيز نظريا حيث اظهرت نتائج تحليل الارساء الجزيئي قيم جيدة لكل من درجة الارساء (-8.9 kcal\mol) وRMSD (2.819, 5.803 A°) وRMSD ووجود نماط ارتباط جيدة

INTRODUCTION

Atorvastatin is considered one of the most important statin drugs that work to lower cholesterol levels in vitro [(1)]. It is used to prevent the risk of cardiovascular disease and to treat abnormal lipid levels [(2)]. It is considered the first line of treatment for these diseases that taken orally [(3)]. Atorvastatin is a competitive inhibitor of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA)[(4)]. It is a synthetic compound, as the HMG-CoA reductase enzyme (3-hydroxy 3-methylglutaryl coenzyme A) catalyzes its reduction into mevalonate [(5)]. This step determines the rate of hepatic cholesterol biosynthesis. This inhibition reduces the level of cholesterol in liver cells and reduces harmful Low Density Lipoprotein LDL cholesterol., triglycerides in the bloodstream, and there is a slight increase in the levels of High Density Lipoprotein HDL cholesterol [(6)].

Aspirin or acetyl salicylic acid. It is an organic compound with the chemical formula $C_9H_8O_4$ [(7)]. It is considered one of the most famous, most produced and best-selling drugs in the world [(8)]. The German pharmacists, at the Bayer chemical factory, called, This name is based on salicylic acid [(9)]. Aspirin has been used to treat fever and rheumatic pain over the past century, and to this day it remains a treatment distinct from its alternatives [(10)]. Aspirin is used to avoid the formation of clots that cause heart attacks and strokes [(11)]. Aspirin is used to treat Kawasaki disease, pericarditis, and rheumatic fever[(12)].

Some therapeutic drugs, in general, possess undesirable properties that constitute an obstacle to the clinical applications of these drugs. Therefore, derivatives of these drugs are used that are degradable within the body into the original drug and are currently known as prodrugs, which have achieved the optimal clinical application of the drug and have shown high flexibility and efficiency. They are described as Concomitant drugs are drugs that contain a specialized non-toxic conjugate group used to modify or eliminate undesirable properties of the drug[(13)].

The International Union of Applied Chemistry system defines a concomitant drug as a compound that undergoes a life change before its

pharmaceutical effect appears [14]. The purpose of preparing the prodrug is to increase absorption and distribution, increase the solubility of drugs that are poorly soluble in water, and increase the stability of the drug [15].

Lactate dehydrogenase enzyme (E.C.1.1.1.27) works to catalyze the reverse reaction by oxidizing lactate and converting it to pyruvate (reducing Nicotinamide adenine dinucleotide NAD to NADH). The molecular weight of the enzyme is 134 kilodaltons [16].

It is considered one of the oxido-reductase enzymes, is considered one of the enzyme analogues [17]. It exists in five forms that can be separated by electrophoresis technology. Each form contains four polypeptide chains found in skeletal muscle (M) and in Heart(H) [18].

The molecular docking technique has emerged as a prominent tool in computer-assisted drug design in recent years, since it may significantly increase efficiency and lower research costs while predicting binding affinity and analyzing interactive mode [19, 20].

MATERIALS AND METHODS

All chemicals were obtained from Sigma Aldrich. Melting points were recorded by using the Gallen-Kamp MFB-600 melting point apparatus. The FT-IR spectra were recorded on an FT-IR-8400S-Shimadzu spectrophotometer. NMR spectra were recorded on VARIAN-INOVA 500 MHZ spectrophotometer (Germany), deuterated solvent (DMSO-d6) was used, and tetramethylsilane (TMS) was used as an internal standard.

Organic part:

Preparation of aspirin Atorvastatin ester

(2.85g, 0.015mol) of aspirin was reacted with 2 ml of thionyl chloride $SOCl_2$ with stirring of mixture at room temperature for two hourses until of hydrogen chloride and sulfur dioxide ceased, other steps involved adding of aspirinyl chloride (3.97g, 0.02 mol) into Atrovastatine (5.56g, 0.01 mol) that dissolved with 15 ml of acetone drop by drop continuously in presence 1 ml of pyridine to get rid of HCl the reaction was left for 6hrs, the precipitate formed. The acetone was removed by heating the mixture at 58 ^oC, then the precipitated formed dried, washed by n-hexane the final product have brown color and characterized by TLC (R_f=0.36) [21].

Biochemical part

Blood was drawn from the vein using (5 ml medical syringe) and placed in new sterile tubes free of additives and the blood was left for (10) minutes after which the blood serum was separated from the clotted part using a centrifuge at a speed of (4000 rpm) for (10) minutes, then the pure blood serum was drawn and placed in small tubes (Epindroff) and stored in the refrigerator at a temperature of (-20°C). Part of the serum was used for examination purposes (Lactate dehydrogenase) using the enzyme linked immunosorbent assay ELAIZA.

preparation of diluted concentrations of Atorvastatin Aspirin ester

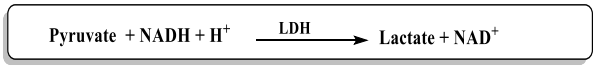
This part included preparation of many diluted concentrations of Atorvastatin Aspirin ester by serial dilution to obtain the following concentrations :(500,5,5,0.5, 0.05, 0.005, 0.0005) mg/mL.

estimation of Lactate dehydrogenase (LDH) activity

The activity of LDH activity was estimated as follow**:**

a-principle:

The LDH activity of was estimated in serum of atherosclerosis patients by ELISA kits that provided by Giesse Diagnostics company, the principle of this protocol rely on the converting of pyruvate into lactate through time units at 340nm, where NADH transform into NAD+, the decreasing of NADH units indicate to LDH activity that recorded by change of absorbance at 340nm activity was estimated according to the procedure that reported by [22], as follow:



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Molecular docking protocol:

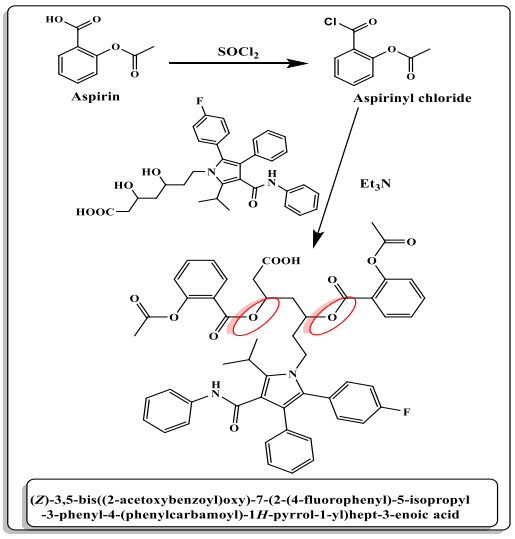
This study includes molecular modeling study; this term indicates designing the candidate lead compound by computer software. Molecular docking studies were investigated via employing Auto Dock vina programs. The **Lactate dehydrogenase** enzyme (**PDB:ID=4i8x**), was abstracted from RCSB, PDB (Protein Data Bank) site. The ligand was drawing by chem office and prepared by minimizing energy through MMFF94 tool in chem 3D. In regarding enzyme, water molecules were removed and polar hydrogens were added as well as Kollman charge this process were performed by Auto Dock tool 1.5.6 program. The 2D,3D binding mods of ligand were visualized by Discovery Studio 2020 Client [23] .

RESULTS AND DISCUSSION

This research included two parts as follow:

organic part

In this section the aspirin drug was converted into aspirinyl chloride by thionyl chloride $SOCl_2$, the aspirinyl chloride added into Atrovastatine to afford ultimate product according to the following scheme:



The prepared organic compound was characterized by observing the changes in physical characteristics. The resulting solid was brown to yellow in color and had a distinctive odor that indicated the formation of an ester product with a melting point of (138-140)°C. The product was diagnosed using spectroscopic methods FT-IR, 1H-NMR proton nuclear magnetic resonance spectroscopy:

FT-IR spectrum of aspirin atorvastatin ester

The functional groups of new prodrug was characterized by FT-IR as follow: It was observed that a band appeared in the range (3500-2800) cm-1 attributed to the stretching frequency of the (OH) carboxyl group. Likewise, the appearance of a band in the range (3562) cm-1 attributed to the (NH-) amide group, and the presence of a band in the range (2873) cm-1 was observed. indicates to symmetric stretching vibration of CH_2. The appearance of a stretch band at 2960 cm-1 indicates the asymmetric stretching vibration of the aliphatic methylene group (CH_2), and the appearance of a band at frequency (3056) cm-1 is evidence of the (C-H) group in aromatic system. t was also observed that there was a strong broadband at frequency (1731) cm^{-1}, evidence of the presence of ester (C=O) groups in atorvastatin moiety, in addition to the presence of the band with frequency (1664) cm-1, evidence of the presence of (C=O) groups of atorvastatin acid. The presence of a sharp and strong band at frequencies 1598 and 1529 cm-1 indicates aromaticity (C=C), the presence of a band at frequency (1313) cm^{-1} is evidence of the (C-N) group, in addition to the appearance of a sharp band at frequency (1076) cm^{-1} for the presence of (C-F), and the two bands at the frequency (1224-1242) cm-1, evidence of the presence of CH_3 groups for aspirin associated with atorvastatin. As clarified in fig. (1).

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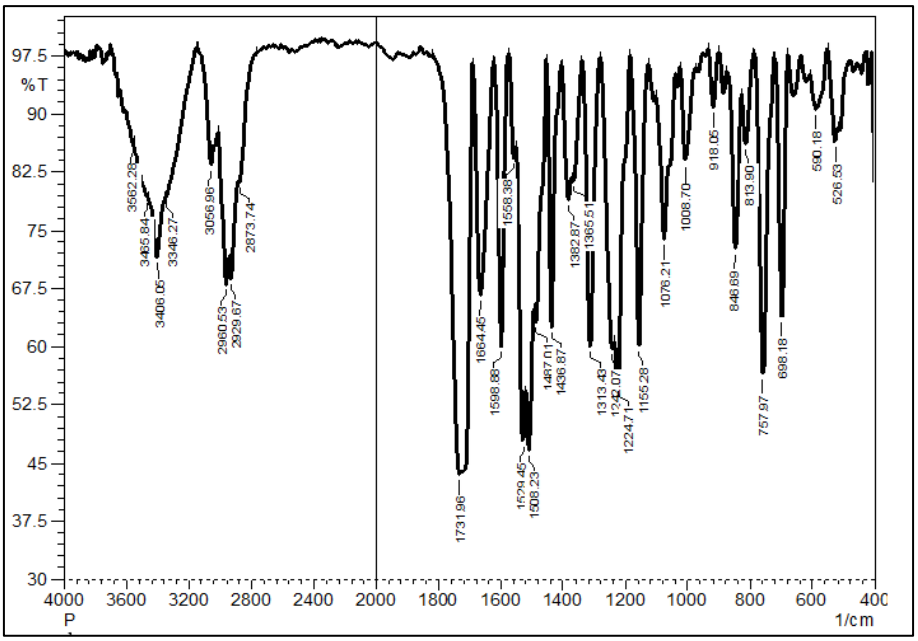

Fig. 1: FT-IR spectrum image for aspirin atrovastatin ester

^{1}H-NMR spectrum of aspirin atrovastatin ester

The prepared ester was identified using 1H-NMR spectroscopy, and the analysis results showed the following:

The appearance of a doublet signal at the value of the chemical shift of 1.18-1.29 ppm is due to the isopropyl moiety in the clip of the drug Atrovastatin. The appearance of a doublet signal at the value of the chemical shift of 1.38-1.39 ppm is due to the protons of the methine group, and the indicator towards it is the value of the chemical shift in the figure. A singlet signal appears at the value of the chemical shift of 1.38-1.39 ppm It is attributed to the protons of the methine group and the pointer towards it is the value of the chemical shift in the figure. The appearance of a single signal at 2.25 ppm is due to the protons of the methine group in the isopropyl portion. The appearance of a single signal at 2.29 ppm is due to the protons of the acetyl group in the aspirin portion of the drug derivative. The appearance of a multiple signal at the range 2.38-2.39 ppm, refers to the protons of the methylene group and the indicator is shown in the figure. The triplet splitting signal at 3.29 ppm refers to the methylene group attached to the nitrogen atom of the pyrrole ring in the atrovastatin part of the ester derivative. The multiple signal at the chemical shift value of 2.37 ppm belongs to the group of two examples indicated in the figure below, while the aromatic protons appeared in the range 6.88-7.81 ppm, and finally the proton of the NH group appeared at the chemical shift value of 9.85 ppm. As clarified in fig. (2) and fig. (3).

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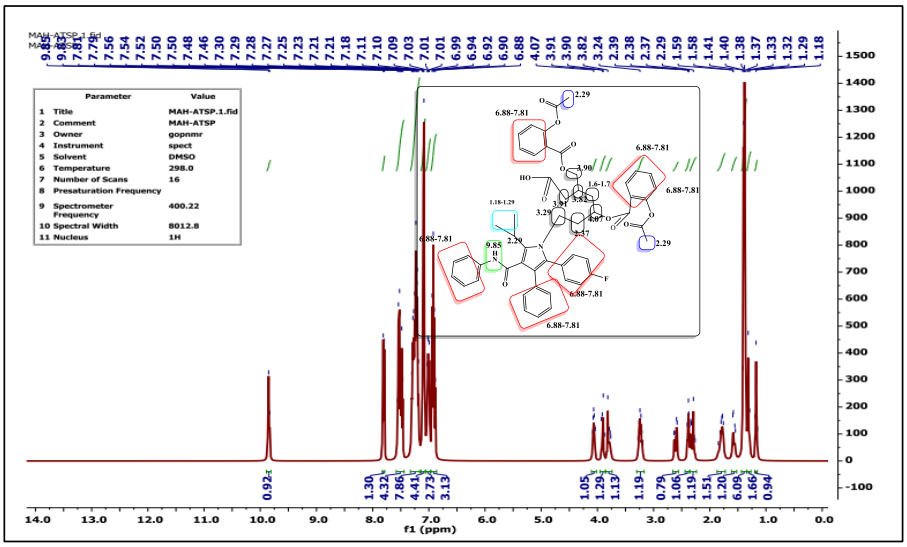

Fig. 2: 1H-NMR spectrum image for aspirin atrovastatin ester

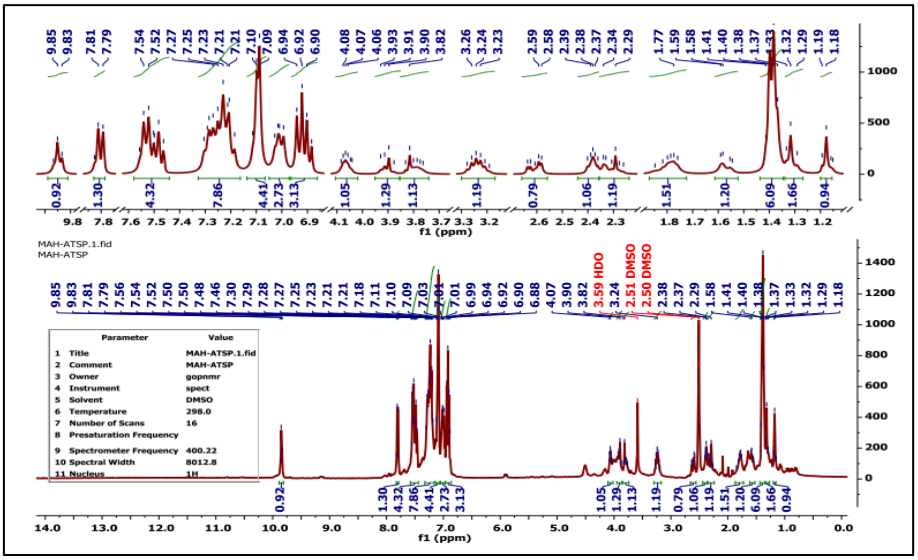

Fig. 3: 1H-NMR spectrum image for aspirin atrovastatin ester

study effect of Atorvastatin Aspirin ester on the activity of LDH enzyme

The study of of Atorvastatin Aspirin ester on activity of LDH was gave the following outcomes:

Table 1: the effect of synthesized ester on the activity of LDH enzyme

Normal Value=225-450U/L			
ENZYME	**PONI**		
COMPOUND NAME	**Concentration of comp. (mg/mL)**	**Activity**	**%of Activation**
Atorvastatin Aspirin ester	500	339.34	259
	50	310.54	237
	5	278.91	213
	0.5	248.04	189
	0.05	189.47	144
	0.005	167.32	127
	0.0005	151.58	115

From the table above observed that the increasing the concentration of the prepared compound leads to increasing the effectiveness of the enzyme. Therefore, this pharmaceutical derivative behaves as an activator for the lactate dehydrogenase enzyme, as it binds to the positive catalytic site of the enzyme, which leads to a conformational change in the shape of the enzyme or act as coenzyme then leads to increase the flexibility of the binding site of the enzyme, which leads to an increase in the Enzymatic reaction speed

Molecular docking study

Molecular docking results that resulted in this computational simulation indicated that the compound **Atrovastatin aspirin ester** exhibited a good values that encompass of docking score, RMSD, hydrogen bonds, where interact with binding site of protein at docking score -8.9 kcal\mol with RMSD value 2.819, 5.803 A^o, 2D and 3D screened visualized images exhibited presence five hydrogen bonds(donor) : (ARG:C:72) with carbonyl group (ASP:D:42) with carboxyl group of atorvastatin part, (LYS:C:42), (LYS:A:42) with carbonyl and acyloxy respectively at aspirin part (LYS:C: 264) with carbonyl group of acetyl group at aspirin section), as well as other interactions that compares: pi-sigma, pi-anion, pi-cation, fluorine interactions , as illustrated in the following:

Table 2: Molecular docking result of the synthesized compound with binding site of of lactate dehydrogenase enzyme (PDB:ID: 4i8x)

Comp No.	Affinity (Kcal/mol)	RMSD l.b. (A^o)	RMSD u.b. (A^o)	Distance (A^o)	Main residue	H-bond interaction
Atrovastatin aspirin ester	-8.9	2.819	5.803	2.35867	A:LYS264:HZ1 - :UNK1:O	Conventional Hydrogen Bond
				2.13151	C:LYS41:HZ2 - :UNK1:O	Conventional Hydrogen Bond
				2.27079	C:LYS41:HZ3 - :UNK1:O	Conventional Hydrogen Bond
				2.45322	C:LYS41:HZ3 - :UNK1:O	Conventional Hydrogen Bond
				2.57697	C:ARG72:HE - :UNK1:O	Conventional Hydrogen Bond
				2.64175	:UNK1:H - D:ASP42:OD2	Conventional Hydrogen Bond
				2.5956	:UNK1:H - :UNK1:O	Conventional Hydrogen Bond

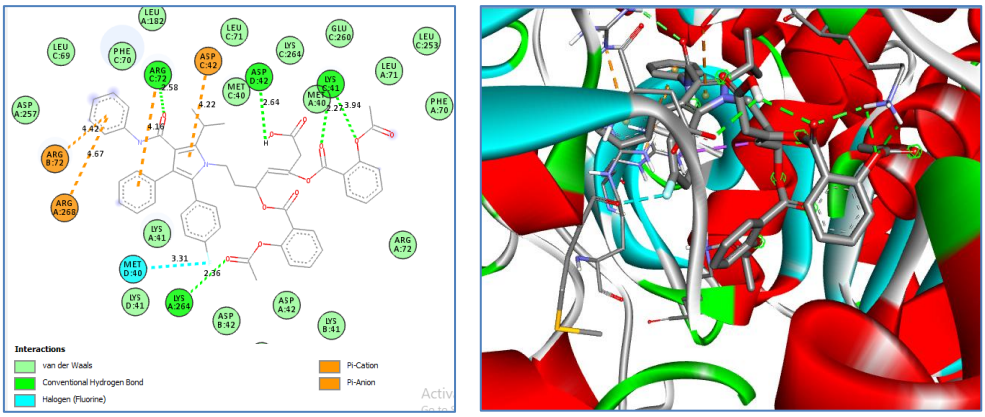

Fig. 4: The interaction between Atrovastatin aspirin ester with binding site of lactate dehydrogenase enzyme in 3D and 2D dimensions.

CONCLUSION

The [synthesized compound was prepared with high yield, the biochemical investigation of synthesized ester showed an excellent activity to promote of lactate dehydrogenase enzyme, the molecular docking study clarified the synthesized compound showed good docking score with best RMSD values and binding patterns.

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Author contribution: Author contributed in the study.

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