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Synthesis, Characterization, Acute Toxicity and Investigation of the new Pyrimidine Derivative as antihyperlipidemic activity on Male Mice.

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Abstract

A new pyrimidine derivative was prepared by reaction of Pyridimine-4,5-diaminewith 3-Phenyl propanal in good yield. This new Schiff base was characterized by elemental analysis, Infra-red and Proton Nuclear Magnetic Resonance (^{1}H- NMR) spectroscopy. The toxicity of the compound was also assayed via the determination of their LD50 value by using Dixon method. LD50 of the novel compound was 618.75 mg/Kg BW when administered Intraperitonially. In Atorvastatin has been used as a controlled drug for the treatment of HFD-induced hyperlipidemia as improvement drug, both atorvastatin and pyrimidine derivative prevented the increase in serum and hepatic levels of total cholesterol (TC), total glycerides (TGs), low density lipoprotein (LDL-C), and increased serum and hepatic levels of high-density lipoprotein (HDL-C) in HLD-fed rats. The results of the histopathological study of sections of the liver of the study groups confirmed their conformity with the physiological study.

Keywords: Schiff base, Acute toxicity, Histopathology

Introduction:

In recent years, researchers have been interested in preparing pyrimidine derivatives, due to the wide variety of biological activity of these derivatives and their use as medicines for serious and deadly diseases that are currently spreading in the world, such as HIV disease (AIDS) (1). The pyrimidine ring system has wide occurrence in nature as substituted and ring fused compounds and derivatives, including the nucleotides cytosine, thymine and uracil, thiamine (vitamin B1) and alloxan (2). The first pyrimidine derivative to be isolated was alloxan in 1818 by Brugnatelli, oxidizing uric acid with nitric acid (3). Methoprim, which is one of the pyrimidine

derivatives, is one of the effective medicines against many bacterial infections, including urinary tract infection and pneumonia. It is given with sulfamethoxazole (4). Rilpivirin has been proven effective for the treatment of AIDS (5) as well as the compound Etravirin, which is one of the pyrimidine derivatives that was prepared and presented among the global medicines for the treatment of AIDS (6). The pyrimidine compound known as Monastrol, which was prepared by Mayer and co-workers (7), is highly effective in inhibiting the protein kinesin Eg5, which is responsible for some cancerous tumors, and thus the pyrimidine derivative called Monastrol is an anti-tumor compound. Hyperlipidemia is a group of metabolic disorders characterized by an increased in the levels of lipids, which is a major Predisposing factor risk factor for cardiovascular and atherosclerosis. These lipids include cholesterol, cholesterol esters, triglycerides and phospholipids, increase levels of LDL are related to the development of atherosclerosis (8). A novel anti-hyperlipidemic compound named4-substituted-2-substitutedmethyltriazino[6,1-*b*] quinazolin-10-ones and 2,4-disubstituted-6,7-dimethoxy quinazoline derivatives were evaluated for antihyperlipidemic activity (9).

Materials and Methods

Instruments: The IR spectra were recorded in the range 4000-200 cm^{-1} on a Pye-Unicam SP3-300 spectrometer using KBr discs at College of Science, University of Basrah, Iraq. ^{1}H and ^{13}C-NMR spectra were measured on a Bruker at 300 and 600 MHz, with TMS as internal reference at Konstanz University, Germany, and Al-Elbiat University, Gordan. Melting point was measured by a Philip Harris melting point apparatus.

Acute toxicity (LD50)

All experiments were performed on 10-14 weeks old male and female mice- weighing 50-75 g at the time of treatment by using up-and-down method (9) . Male and female rats were injected intraperitonially with different doses of the pyrimidine derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD50 were determined after reading final result (response-dead (X) or non-response alive (O), then the following equation was applied LD50 = XF + K×d (1) where, LD50: Median lethal dose; XF: Last dose administered; K = Value from Table 1; D = Difference between dose levels (Table 1) (10).

Experimental design : Sixty male mice were divided randomly into six groups,
(Ten in each group) as following :Group 1(control) :Animals will be fed standard pellet for 3 weeks and considered as control. Group 2: Animals treated with 0.1ml DMSO for 3 weeks Group 3: Animals fed a high- fat diet for 3 weeks Group 4: Animals treated with new derivative 1.54 mg (1/10 of LD50)/kg/day for 3 weeks

Group 5: Animals with high -fat diet treated with new derivative 1.54 mg/kg/day Group 6: Animals treated with a high-fat diet received atorvastatin 2.1 mg/kg/day for 3 weeks. After anesthesia of the mice, blood

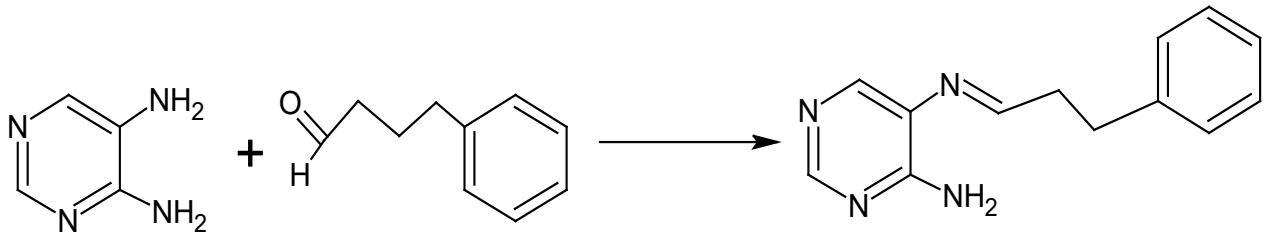
collected, blood samples were collected from the heart by using disposable syringes of 5cc capacity. And then samples analyzed.

Synthesis of the novelcompoundof 5-[(*E*)-(3-phenylpropylidene) amino] pyrimidin-4-amine :Pyridimine-4,5-diamine (10mmol, 1.10 g) in 15 mL ethanol was added to hot ethanolic solution of 3-Phenyl propanal (10mmol, 1.34 g), three drops of glacial acetic acid was added and resulting solution was refluxed for 4 h and then lift overnight in refrigerator. The solid product obtained was filtered and washed with ethanol and the final product was recrystallized by using chloroform: ethanol (8:2, *v*: *v*) to yield yellow crystals of 5-[(*E*)-(3-phenylpropylidene) amino] pyrimidin-4-amine.

It was obtained as yellow crystals from ethanol, yield 78%; m.p. 174-1176 ºC. IR (KBr): υ cm^{-1}, 3416-3 (NH), 3005 (CH-$_{arom.}$), 2945(CH-$_{aliph.}$), 1664(C=N), 1591(C=C);^{1}H NMR (DMSO-d_6): δ = 9.49 (s, 2H, NH_2), 7.58-6.94 (m, 7H, Ar-H), 7.58 (s, 1H, CH=N), 3.42(m,CH_2-imine), 3.18(t,CH_2-Ar);^{13}C NMR (DMSO-*d6*) δ :27.54(CH_2-Ar), 30.75(CH_2-imine), 99.93-137.07(C-Ar), 150.15-153.79(C-pyrim.); Anal. Calc. for $C_{13}H_{14}N_4$; (226.27): C, 68.94; H, 6.18; N, 24.74% Found: C, 69.25; H, 6.33; N, 24.98%.

Table (1):Dixon values. (Dixon 1980)

	Kre presented serial tests				
	O	OO	OOO	OOOO	
Xooo	0.157-	0.154-	0.154-	0.154-	oxxx
Xoox	0.878-	0.861-	0.860-	0.860-	oxxo
Xoxo	0.701	0.747	0.741	0.741	oxox
Xoxx	0.084	0.169	0.181	0.182	oxoo
Xxoo	0.305	0.372	0.380	0.381	ooxx
Xxox	0.305-	0.169	0.144-	0.142-	ooxo
Xxxo	1.288	1.500	1.544	1.549-	ooox
Xxxx	0.555	0.0897	0.985	1.000	OOOO
	X	Xx	xxx	Xxxx	



Scheme 1 : Preparation of 5-[(*E*)-(3-phenylpropylidene) amino [pyrimidin-4-amine

Results and Discussion

Chemistry

Condensation of pyrimidine derivative by reaction of Pyridimine-4,5-diamine with 3-Phenylpropanal in 1:1 ratio to yield new Schiff base derivative.
The IR spectrum confirms
the existence of the azomethine group (CH = N) extending about 1591 cm^1 with a sharp area.
^{1}H NMR spectrum of synthesized compound show chemical shift at δ 9.49 ppm due to amine group. The region at δ 7.56-6.94 ppm due to aromatic protons in aromatic and pyrimidine rings. The ^{1}H NMR spectrum show signal at δ 7.58 to azomethine proton (CH=N). The ^{1}H NMR spectrum show signals at δ 3.42 and 3.18ppm due to methylene groups CH_2.
^{13}C NMR of the structure detected signals at 27.54 and 30.75 ppm due to 2(CH_2).
A multiplet signals at δ at 99.93-137.07 ppm assigned to aromatic carbons and signals at 150.15-153.79 ppm due to carbons in pyrimidine ring.
Determination of the 50% of lethal dose (LD50) of the synthesized Schiff base in-vivo The LD50 of synthesized new compound was detected in the mice by using the "up-and-down" procedure described by Dixon (10) in the experiment we using 10 animals of white mice 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (450, 500, 550, 600 mg/kg.bw) in 0.1 mL dimethyl sulfoxide (DMSO) were administered and chosen with equal spacing (concentrations) between doses.
Mortality was recorded after 24 h that each one animal treated with one dose and after 24 h was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being (OOXX)and according for Dixon value was get and the LD50 was determined according to the formula employed by Dixon.
(LD50 = Xf + Kd; LD50 = 600 + 0.375 × 50 = 618.75 mg/kg.bw).

Biological activity

The result from the present study indicated that the animals feeding HFD caused many changes in lipid profiles like significant increase in the level of TC, TG. LDL and decreased in HDL in hyperlipidemia groups of animals compared with DMSO and control. The hyperlipidemia plus new

pyrimidine derivative and hyperlipidemia plus give 2.1 mg /kg of atorvastatin can ameliorative the effect of HLD but not reach the value of control. The results agreement with (11), they used rats fed with HFD for 5 weeks exhibited significantly increased serum TC and LDL-C levels. the lipid accumulation in the livers of rats fed an HLD, and a marked increase of liver TG levels was observed in the HFD group compared with the control group. There potential reasons for these findings HLD, caused increased serum TC level, induced TC accumulation in the liver.

The HFD-fed rats had higher serum of hepatic TC, TGs, and LDL-c and caused lower levels of HDL-C. All these data are in accordance with the many other similar studies done (12). previous studies have reported that serum TG increased after HLD and this may be due to increase in serum very low-density lipoprotein (VLDL) concentration, it acts as a carrier for TG in the blood (13). Also, the results were similar to that reported by (14), they suggested that elevation in TG due to partial difference of lipoprotein lipase associated with increase output of lipoprotein from liver due to consumption high cholesterol diet. The decreased in serum HDL level table 2 may be due to the action of hepatic lipase on this class of lipoprotein on HDL. These results were agreement with (Taylor and Fan (15), they reported that the hepatic lipase enzyme act on the HDL surface through its phospholipase activity led to altering the lipid environment to favor transfer of cholesterol to cell membranes, also the reduction of HDL level may be due to an increase catabolism, due to increase its clearance from plasma or decrease rate of their production (16).

In the present study, we have used atorvastatin as a control drug, demonstrated by the others drug of statin it is more effective hypolipidemic drug as compared with other statins including Fluvastatin lovastatin, and pravastatin (17). Both atorvastatin and pyrimidine derivative prevented the increase in serum and hepatic levels of TC, TGs, and LDL-C, and increased serum and hepatic levels of HDL-C in HLD-fed rats. Atorvastatin and other statins such as lovastatin are the best known 3-hydroxy-3- methylglutaryl coenzyme A (HMG CoA) reductase inhibitors lipid-lowering effects in both human and animals(18).In general, it is more accepted that increase in LDL-C is atherogenic which is caused stimulated the deposition of LDL-C in the arterial walls at higher levels and in the presence of oxidative stress stimuli, also the HDL-C protective cholesterol and prevent these events to reduces the oxidation of LDL-C(19),or maybe statin caused competitively blocking the active site of the first and key rate-limiting enzyme in the mevalonate pathway, HMG-CoA reductase. Inhibition of this site prevents substrate access, thereby blocking the conversion of HMG-CoA to mevalonic acid. Within the liver, this reduces hepatic cholesterol synthesis, leading to increased production of microsomal
HMG-CoA reductase and increased cell surface LDL receptor expression. This facilitates increased clearance of LDL-c from the bloodstream and a subsequent reduction in circulating LDL-c levels by 20% to 55%. In addition to reducing LDL-c

and cardiovascular morbidity and mortality (20). The results of pyrimidine derivative compound ameliorate in the TC, TG and LDL and increased HDL levels. That may be due to the effect of pyrimidine derivative on thyroid gland, causes hypothyroidism effect in some studies, the major anti thyroid drugs namely propylthiouracil (6-propyl-2-thioxo-3,4-dihydropyrimidin-2-(1H)-one; PTU), interfere with iodine into the tyrosine residues of thyroglobulin and therefore ceases the biosynthesis of thyroid hormones namely (T3) and (T4). Which plays a critical role in lipid metabolism by regulating genes which involved in the lipolysis and lipogenesis or may act as other pyrimidine derivatives that effect on the Ca+2 level in the blood such as thiazolopyrimidines which caused calcium channel blocker activities(21),thecalcium channel blockers prevent cholesteryl ester (CE) accumulated by increasing CE through increasing intracellular cyclic AMP with resultant decrease in CE accumulation and increase the lipolysis process, and that leads to increase the free lipids in the blood, this is a good effect by preventing accumulation of lipid in the tissue such as arteries.

Table (2): Effect of pyrimidine derivative and atorvastatin on male mice lipid profile (Mean ± SD) n= 10

Group	TC mg/dL	TG mg/dL	LDL mg/dL	HDL mg/dL
Control	133.02±3.23 c	96.57±4.61 b	18.03±1.19 c	45.14±4.57 b
DMSO	135.32±2.49 c	98.54±5.01 b	21.78±2.54 c	41.43±3.32 b
HFD	265.07±3.17 a	278.22±9.15 a	85.21±7.23 a	22.03±3.07 c
The novel compound	143.11±3.22 c	85.15±.4.11 b	25.31.0±3.71 c	59.11±4.81 a
HFD + novel compound	166.15 ±4.03 b	102.22±7.04 b	44.49±3.10 b	39.25±3.14 c
HFD + atorvastatin	156.35 ±4.33 b	98.04 ±5.41 b	42.01 ±3.75 b	41.24±4.14 b
LSD	11.19	14.11	4.23	5.21

The different small letters refer to significant differences at (p≤05).

Histopathology

The liver section of male mice showed normal structure and distribution of hepatocytes and central vein as shown in Figure 1. The cross sections of liver treated with DMSO for twenty-one days showed normal of the central vein and radiate liver

hepatocytes plates Figure 2. The examination of liver tissue which treated with diet rich cholesterol shown aggregation of Kupffer cells, appearance of lipid drops, hypertrophy of hepatocytes and dilated of sinusoids Figure (3).While the section of liver rats treated with new derivative plus 1/10 of LD50 of pyrimidine derivative for twenty one days shows accumulation of lipid drops, appearance of Kupffer cells and dilated of sinusoids Figure (4) Finally, the section of liver mice treated with HFD plus atorvastatin shows degeneration and necrosis of hepatocytes, pyknotic of the hepatocytes nuclei and aggregation of Kupffer cells,(Figure 5).

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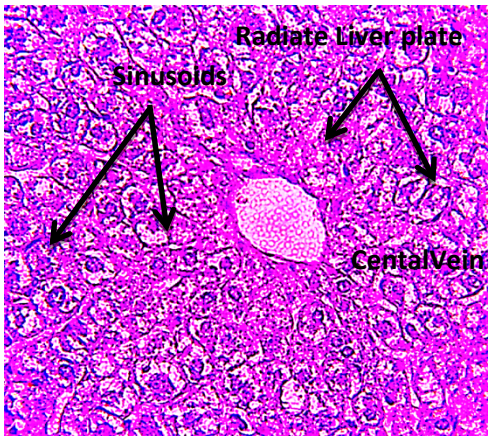

Figure (1). Liver tissue section (control) shown normal hepatocytes and central vein (H and E 400X).

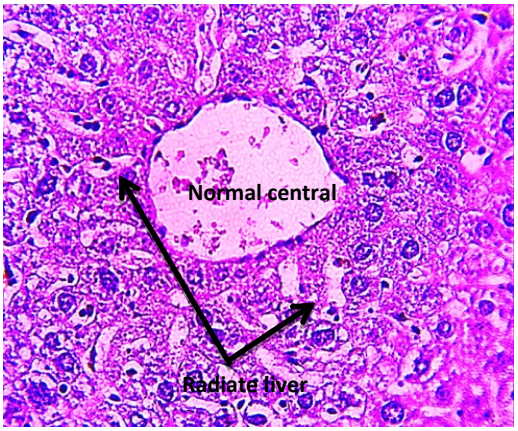

Figure (2). liver tissue section (group DMSO) shown normal of the central vein and radiateliver hepatocytes plates. (H andE 400X).

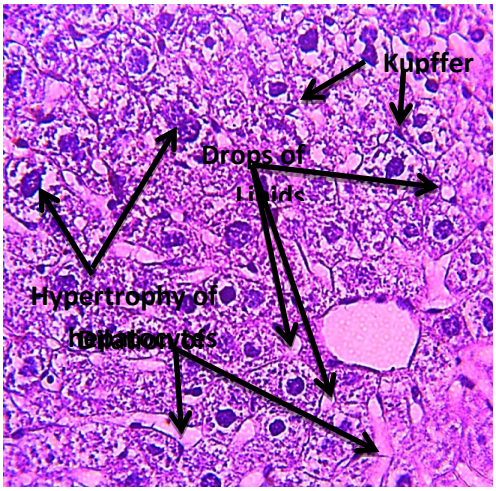

Figure (3). liver tissue section (hyperlipidemia group) shown aggregation of Kupffer cells, apparency of lipid drops, hypertrophy of hepatocytes and dilated of sinusoids. (H and E 400X).

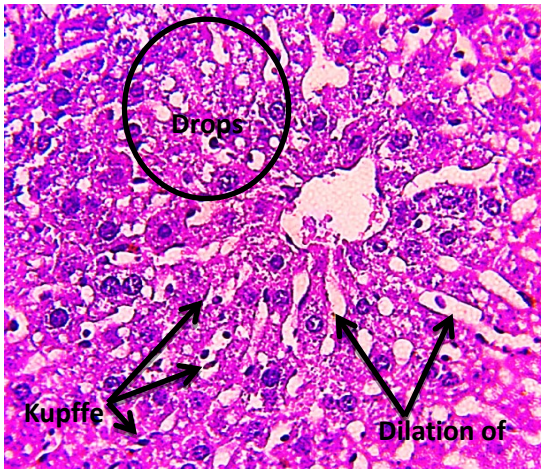

Figure (4). liver tissue section (hyperlipidemia plus 1/10 pyrimidine derivative group) shown accumulation of lipid drops, apparency of Kupffer cells and dilated of sinusoids. (Hand E 400X).

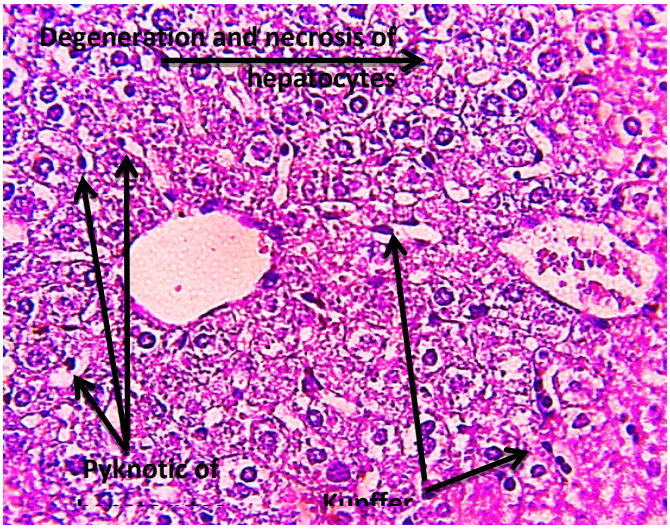

Figure (5). liver tissue section (hyperlipidemia plus atorvastatin group) showndegeneration and necrosis of hepatocytes, pyknotic of the hepatocyte's nuclei and aggregation of Kupffer cells. (H and E 400X).

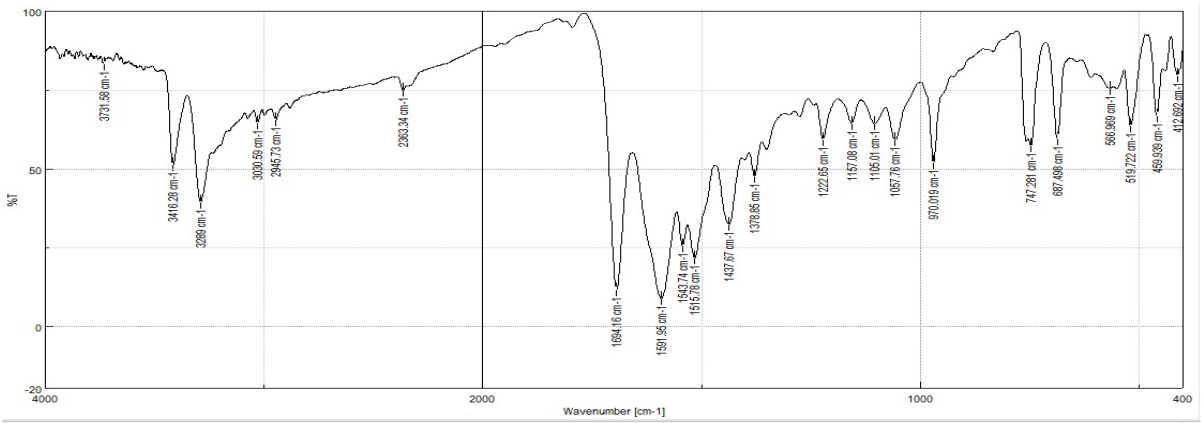

Figure 6: Infra-red spectrum of 5-[(*E*)-(3-phenylpropylidene) amino]pyrimidin-4-amine

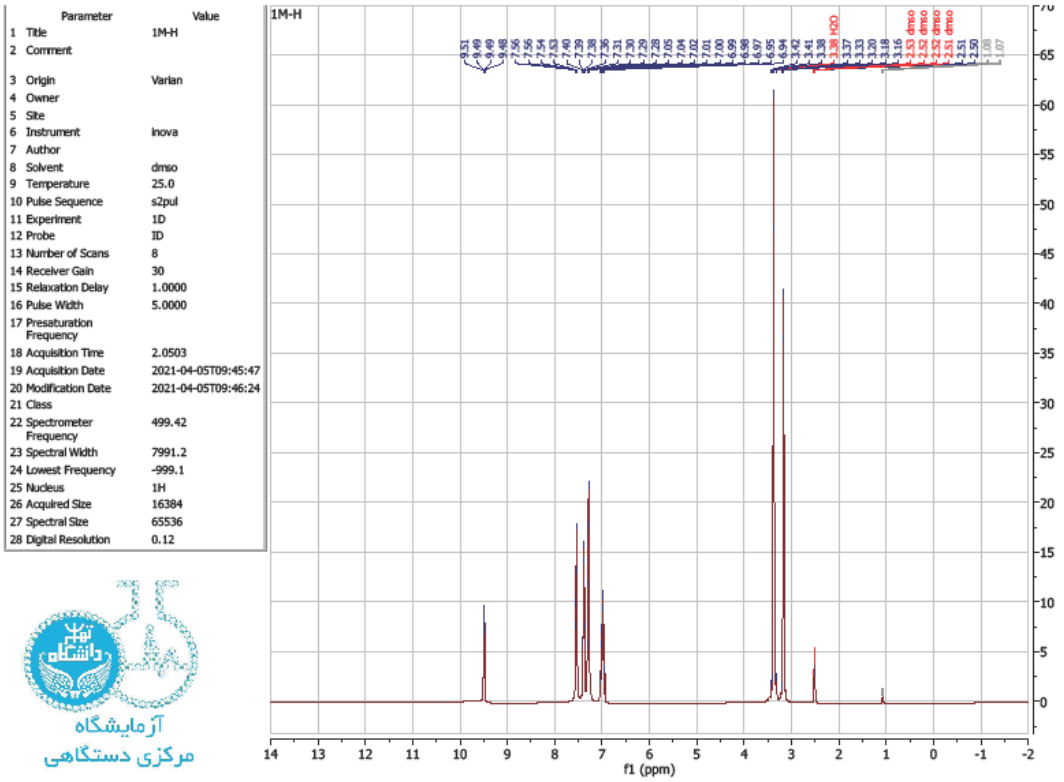

Fig. 7: ^{1}HNMR Spectrum of 5-[(*E*)-(3-phenylpropylidene) amino]pyrimidin-4-amine

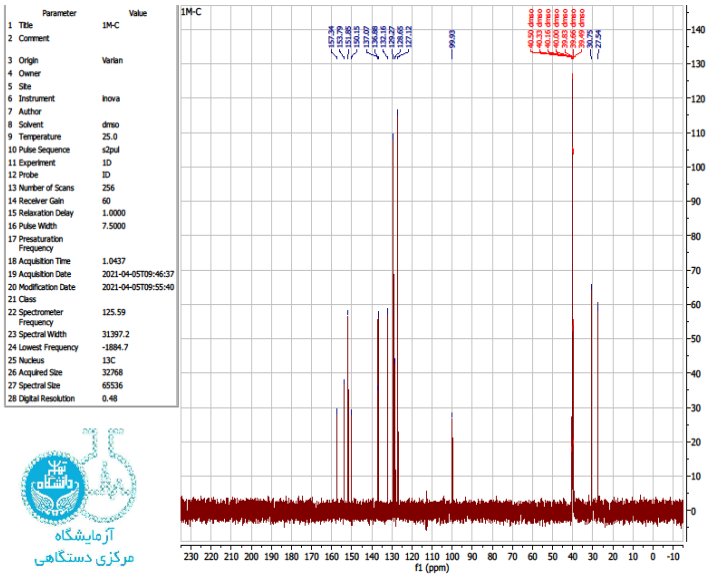

Figure 8: ^{13}CNMR Spectrum of 5-[(*E*)-(3-phenylpropylidene) amino]pyrimidin-4-amine

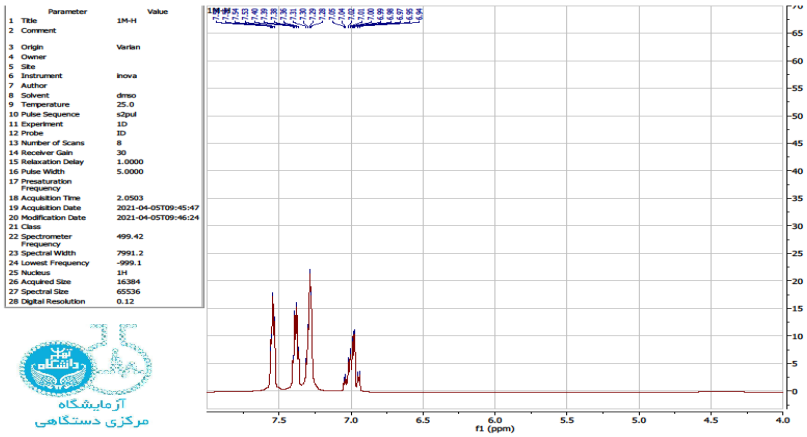

Figure 9: ^{1}HNMR Spectrum (Expansion) of 5-[(*E*)-(3-phenylpropylidene) amino]pyrimidin-4-amine

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تصنيع وتشخيص وتحديد جرعة النصف قاتلة لمشتق البريمدين كمضاد لفرط الشحوم في ذكور الفئران

الخلاصة

تم تحضير مشتق بيريميدين جديد عن طريق تفاعل 4-Pyridimine،3-Phenyl propanal with 5-diaminew. كناتج جيد. حيث تم تميز قاعدة شيف الجديدة هذه من خلال اجراء تحليل العنصري، والتحليل الطيفي للرنين المغناطيسي النووي بالأشعة تحت الحمراء والبروتون (H- NMR1). تم أيضًا تقييم سمية المركب عن طريق تحديد قيمة جرعة النصف قاتلة باستخدام طريقة ديكسون للمركب الجديد حيث كانت 618.75 مجم / كجم من وزن الجسم. اماعقار أتورفاستاتين، تم استخدامه كعلاج لفرط شحميات الدم الناجم عن استخدام غذاء عالي الدهون كدواء محسن، وكانت النتائج ان مشتق بيريميدين وعقار أتورفاستاتين منع زيادة مستويات المصل والكبد. الكوليسترول الكلي، وثلاثي الجليسريد، البروتين الدهني منخفض الكثافة، وزيادة مستويات بروتينات الكبد البروتين الدهني عالي الكثافة في غذاء الفئران عالي الدهون. أكدت نتائج دراسة التشريح النسيجي المرضي للكبد لمجموعات الدراسة مطابقتها للدراسة الفسيولوجية.

الكلمات المفتاحية : قاعدة شف, علم السمية الحاد, علم الانسجة المرضي.