Inhibitory Effect of Synthesized Acridone Derivatives Containing 1, 3, 4 –Thiadiazole on Cholesterol Enzymatic Activity

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Abstract

Synthesis of Diphenylamine-2, 4`-dicarboxylic acid {1}, 9(10H)-Acridone-2-carboxylic acid {2}, 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone {3}. Synthesized compounds were identified using spectral (FTIR, UV) .The results were discussed and found in agreement with the suggested structures.

Ten samples were collected from normal and patients with atherosclerosis and then addition of 50µl of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone to serum of patients with atherosclerosis in Al-Kadimia teaching hospital, diagnosis was established depending on clinical technique.

The study showed inhibition effect of the synthesized compounds on cholesterol level of patients using addition of 50µl of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone to patients that means the above compound work as inhibitor of cholesterol level.

Keywords: 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone, Cholesterol Enzymatic activity

List of abbreviations

FTIR : Fourier transforms infrared
UV : Ultraviolet
DMSO: Dimethyl sulfoxide
M.p. : Melting point
nm : Nanometer
λ : Wavelength
CHE : Cholesterol esterase
CHE : Cholesterol oxidase
POD : Peroxidase
W.R : Working reagent
S.D : standard deviation
TC : Total Cholesterol
OD : optical density

Introduction

There are numerous biologically active fused heterocyclic rings. Among these acridone is one such scaffold known to associate with several biological activities [1].Also acridone used in an antibody-based assay to detect catalysis for lipases and glycosidase [2]

1, 3, 4-thiadiazole derivatives have recently received significant importance because of their diverse biological properties [3], Cholesterol absorption inhibitor [4], enzyme inhibition activities [5, 6], hypoglycemic [7], antihypertensive [8] and antifungal [9].

Cholesterol and other fats can't dissolve in blood, only by special carriers called lipoproteins [10] LDL-c provides cells with their cholesterol requirements, excess amounts of it in man can lead to atherosclerosis [11].

Materials and Methods

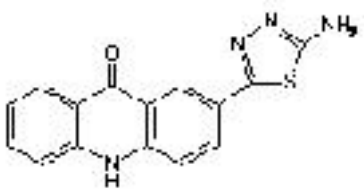
Instruments and equipments

Instruments	company
spectrophotometer	cencil
centrifuge	griffin
Incubator	Gallen Kamp
F.T.IR-8300 (Infrared spectra)	shimadzo
UV-Vis. 160A	shimadzo

Chemicals

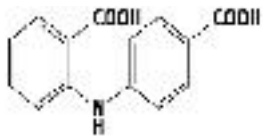
Compounds	Company
cholesterol kit	biomerieux
2-Chlorobenzoic acid (98%)	BDH
4-Aminobenzoic acid (99%)	BDH
Copper oxide powder (98%)	Merck
Amyl alcohol (99%)	BDH
Potassium carbonate (99%)	BDH
Hydrochloric acid (37%)	BDH
Sodium hydroxide (97%)	Fluka
Activated charcoal (high purity)	BDH
Sulfuric acid (95%)	BDH
Methanol (99+%)	BDH
Ethanol (absolute)	BDH
Phosphorus oxychloride (99 %)	Fluka
Thiosemicarbazide (99%)	Fluka
Potassium hydroxide (85%)	BDH
Dimethyl sulfoxide, DMSO (99.9%)	BDH

Preparation of 2-[(5-Amino)-1, 3, 4-thiadiazol-2-yl]-9(10H)-acridone {3}



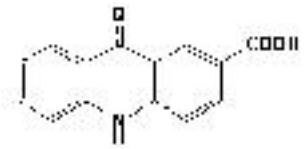
Include

A- Preparation of Diphenylamine-2, 4'-dicarboxylic acid {1}*:*



To a mixture of 2-chlorobenzoic acid (12.5g, 0.08mol), 4-aminobenzoic acid (10.97g, 0.08 mol) and copper oxide powder (0.2g) in (60 ml) of amyl alcohol, dry potassium carbonate (12g) was slowly added and the contents were allowed to reflux for (6h) at about (100^{o}C). The amyl alcohol was removed by evaporation and the mixture poured into (250 ml) of hot water, cool and acidified with concentrated hydrochloric acid. The greenish black precipitate which formed was filtered, washed with cold water and collected. The crude acid was dissolved in aqueous sodium hydroxide solution, boiled in the presence of activated charcoal and filtered. Acidification of the filterate with concentrated of hydrochloric acid white precipitate of compound {1} was obtained which was washed with water and recrystallized from ethanol [12]

B-Preparation of 9(10H)-Acridone-2-carboxylic acid {2}:



Compound {1} (5g, 0.04 mol) was placed in a round bottom flask and (50 ml) of concentrated sulfuric acid, was added then shaken well and heated on water bath at (100^{o}C) for (3hrs). Appearance of yellow color indicated the completion of the reaction. Then, it was poured into (250 ml) of hot water. The yellow precipitate which formed was filtered, washed with water and collected. The sample 9(10H)-acridone-2-carboxylic acid {2} was recrystallized from methanol [12].

C-Preparation of 2-[(5-Amino)-1, 3, 4-thiadiazol-2-yl]-9(10H)-acridone {3}:

A mixture of compound {2} (1g, 0.004 mol), thiosemicarbazide (0.364g, 0.004 mol) and phosphorus oxychloride (5 ml) was refluxed gently overnight. After cooling, water was added (50 ml). The mixture was refluxed for (3 hrs) and filtered. The solution was neutralized with potassium hydroxide. The precipitate was filtered and washed with distilled water and recrystallized from ethanol to give compound {3}

Lab. Test

Determination of total cholesterol in serum

Cholesterol is present in the originates a colored complex, according to the following reaction [13]

$$\text{Cholesterol-ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4 -Colestenona} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

Scheme(1)

Reagents

R1 buffer	Pipes ph 6.9	90 mmol/L
	Phenol	26 mmol/L
R2 Enzymes	Cholesterol esterase(CHE)	300 U/L
	Cholesterol oxidase(CHOD)	300 U/L
	Peroxidase(POD)	1250 U/L
	4-aminophenazone(4-Ap)	0.4 mmol/L
Cholesterol Cal	Cholesterol standard 200mg/dl	

Working reagent: dissolve the contents of one vial R2 Enzymes in one bottle of R1 buffer cap and mix gently to dissolve content.

Procedure

1-Adjust the instrument to zero with distilled water
2-Pipette into acuvette:

Reagent	Blank	Standard	Serum
Working reagent ml	1	1	1
Standard µl		10	
Sample µl			10

3-The mixture was incubated at 37 C^o for 5 min.
4- Absorbance of the incubated mixture was achieved at 505 nm.

Inhibitory study:
Used micropipette for added 50µl of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone to serum of patients in step (3) and repeat the same steps.

Calculation

$$\text{Cholesterol conc. mmol/L} = \frac{\text{OD. Sample}}{\text{OD. Standard}} \times (200) \text{ conc. standard} = \text{mg/dl}$$

Conversion factor: mg/dl × 0.0258 =mmol/l

Results

F.T.IR spectrum and ultra-violet (UV) spectrophotometry technique with melting point (M.p) is used to characterize the synthesized compounds found on Table-1.

Table (2), **Figure (1)** shows elevation in cholesterol level in patients compared with control, but cholesterol level decrease in patients using addition of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone.

Discussion

These λ max could be assigned to (π-π*), (n-π*) [(acridone, thiadiazole, C=N (azomethine)] transitions. The spectra data found to be quite similar to other acridone derivatives reported in literature [14, 15]

The melting point of compounds {1} was (208-210^{o}C) using F.T.IR spectra analysis a disappearance of absorption bands due to NH_2 stretching of amino group in p-amino benzoic acid and the appearance of a band at 3315.4 cm^{-1} for (N-H),occurs ..

The presence of a carboxyl group (-COOH) is recognized by the presence of O-H stretching absorption band at (2500-3500 cm^{-1}), as well as the C=O absorption at 1685 cm^{-1}.

Evidence of the presence of aromatic ring was confirmed by the presence of a stretching band at (1596.9 cm^{-1}) for C=C aromatic and also two sharp bands at (754.1 cm^{-1}) and at (842.8 cm^{-1}) that were assigned to the out of plane bending of o- and p-disubstituted benzene ring, respectively.

Compound {2} found it has very high melting point (>330^{o}C) and F.T.IR spectra analysis , which displays a broad (O-H) stretching absorption band at (3267.2 cm^{-1}) as well as the carboxylic acid (C=O) absorption band at (1685.7 cm^{-1}) , and ketone (C=O), aromatic , (C=C) and amine (N-H) absorption band at (1631.7 cm^{-1}), (1587.3 cm^{-1}) and (3423.4 cm^{-1}),respectively.

The structural assignments to the product {3} based on their M.p. and F.T.IR spectral data. Melting points are (>340C^{o}). F.T.IR spectrum of compound {3} exhibited significant two bands about (~3300, 3168.8 cm^{-1}) which could be attributed to asymmetric and symmetric stretching of NH_2 group. Beside this, a band at (1620 cm^{-1}) due to C=N stretching of thiadiazole ring is also observed.

The UV-Visible spectra that reflects the absorbance (λ nm) of compounds {1, 2, and 3} were resulted in different values of each compound, all these on (Table-1).

Amount that don't effect on enzyme activity was litle therefore used (50µl) of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone.

When added 50µl of 2-[5-amino-1, 3, 4-thiadiazole-2-yl]-9(10H)-acridone to serum of patients present decrease on cholesterol level compared with before addition of patients because it was converted androgens to estrogens, that Estrogen decreases level of cholesterol [16]

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Table (1): Physical properties and spectral data of synthesized compounds

Compd. No	M. p.	Mol Formula	Yield%	FTIR (cm-1)	UV-Visible (λnm)
1	208-210	$C_{14}H_{11}NO_4$	62	1. N-H stretch., 3315.4 2. O-H stretch., near 2500-3300 3. C=O stretch., of acid 1685 4. C=C stretch., 1596.9 5. Out-of-plane C-H bend., (p-disub. benzene) 842.8 6. Out-of-plane C-H bend., (o-disub. benzene) 754.1	399, 387, 330, 293, 272, 243
2	>330	$C_{14}H_9NO_3$	72	1. N-H stretch., 3423.4 2. O-H stretch., 3267.2 3. Aromatic C-H stretch.,3103 4. C=O stretch., of acid 1685.7 5. C=O stretch., of ketone 1631.7 6. C=C stretch., 1587.3	538,396, 378, 325, 286, 262, 239
3	> 340	$C_{15}H_{10}N_4OS$	48	1. N-H stretch. 1° amine coupled doublet, Asym near 3250 sym.,3168 2. Aromatic C-H stretch. about 3000 3. C=N stretch. Overlapped with C=O stretch. of ketone, 1625.9 4. Ring C=C stretch., 1587.3	488, 396, 377, 324, 286, 259, 213

Table (2): Descriptive static with their comparison of Total Cholesterol (TC)

Parameter	Statistics	Normal	Before addition	After addition
Total Cholesterol	Mean	164.5	289.6	258.6
	S.D	3.366	3.717	3.502

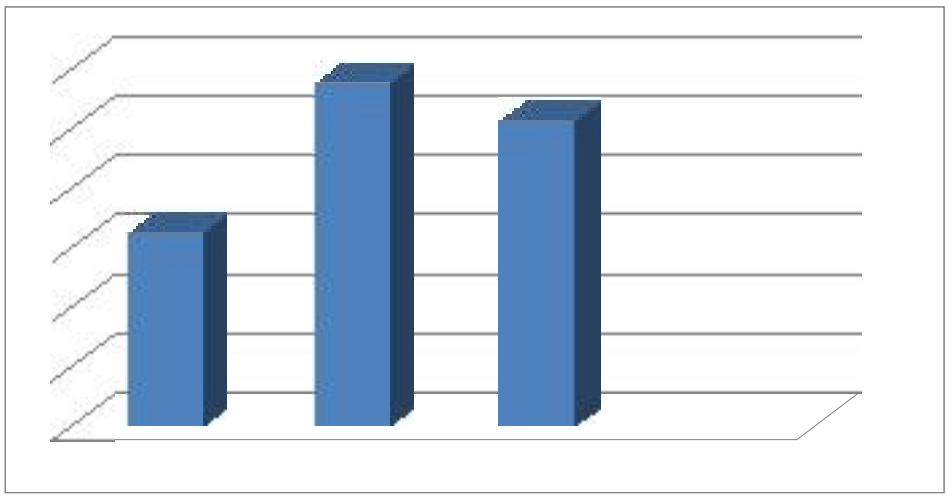

Fig. (1): Total Cholesterol (TC) parameter distribution

التاثير التثبيطي لمشتقات الاكريدون المحضرة الحاوية على 4,3,1- ثاياداياازول على الفعالية الانزيمية للكولسترول

اسراء برهان رؤوف
كلية الصيدلة ، الجامعة المستنصرية

استلم البحث في : 15 آذار 2011
قبل البحث في : 10 آيار 2011

الخلاصة

حضر ثنائي فنيل أمين-2، 4`- ثنائي حامض كاربوكسيلي و 9(H10)-أكريدون-2- حامض كاربوكسيلي و2- [5-أمينو- 4,3,1-ثاياداياازول-2-إيل]-9(H10)-أكريدون . المركبات المحضرة شخصت باستعمال أطياف (F.T.IR، UV) وكانت النتائج المستحصلة متوافقة مع التراكيب المقترحة .

جمعت عشر عينات من الاصحاء والمرضى المصابين بتصلب الشرايين وبعدها اضيف 50 مايكروليتر من 2- [5-أمينو- 4,3,1-ثاياداياازول-2-إيل]-9(H10)-أكريدون في مستشفى الكاظمية التعليمي وشخص المرض بالاعتماد على الفحوصات السريرية .

وبينت الدراسة التاثير التثبيطي للمركبات المحضرة في مستوى الكولستيرول للمرضى باضافة 50 مايكروليتر من 2- [5-أمينو- 4,3,1-ثاياداياازول-2-إيل]-9(H10)-أكريدون مما يدل على ان المركب اعلاه يعمل مثبطا للكولسترول .

الكلمات المفتاحية: 2- [5-أمينو- 4,3,1-ثاياداياازول-2-إيل]-9(H10)-أكريدون ، الفعاليه الانزيمية للكولسترول