SYNTHESIS OF NEW NICOTINIC ACID DERIVATIVES AND STUDYINY THEIR EFFECTS ON CHO HINESTEEAS ENZYWR ACTIVITY[+]

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Abstract

The nicotinic acid is refluxed with thionyl chloride to give acid chloride, which is converted to acid hydrazide by treatment with hydrazine hydrate. Thiosemicarbazone and 4-phenyl-thiosemicarbazone are synthesized by treatment of acid chloride with thiosemicarbazide and acid hydrazide with phenyl isothiocyanate, respectively.

The cyclization of thiosemecarbazone is achieved by using H_2SO_4 and NaOH to give 1,3,4-thiadiazole derivative and 1,3,4-triazole derivative respectively.

The acid hydrazide is also converted into 5-marcapto 1,3,4-Oxadiazole by refluxing with CS_2 and KOH in absolute ethanol, then thiol group is converted into hydroxyl group by treating with H_2O_2 and formic acid.

IR and U.V spectra were used to characterize the prepared compounds.

Effect of the synthesized compounds on human serum ChE activity was studied, the results show that all compounds (except compound A_2) cause inhibition to the enzyme activity. Kinetic parameters were studied and the results show that compounds (A_1, A_3, A_4 and A_7) cause competitive inhibition and compounds (A_5, A_6 and A_8) cause mixed type of inhibition.

المستخلص

يتناول هذا البحث تحويل مجموعة الكاربوكسيل في حامض النيكوتينيك الى مشتق الهيدرازايد عن طريق تفاعلها مع $SOCl_2$ ثم تفاعل كلوريد الحامض الناتج مع الهيدرازين المائي. بعد ذلك تم الحصول على مشتق الثايوسيميكاربازايد و 4 – فنيل – ثايوسميكاربازايد بتفاعل مشتق كلوريد الحامض مع الثايوسميكاربازايد و بتفاعل مشتق الهيدرازيد مع فنيل ايزو ثايوسيانيت على التوالي ثم اجراء الغلق الحلقي لمشتق الثايوسميكاربازايد للحصول على ثايودايازول والترايازول عن طريق استخدام $NaOH$, H_2SO_4 على التوالي اما مشتق الاوكساديازول فقد تم تحضيره عن طريق تفاعل مشتق الهيدرازيد مع CS_2 و KOH في الايثانول المطلق .. وبعدها تم تحويل مجموعة الثايول في مشتق الاوكساديازول الى مجموعة OH بمعاملتها مع بيروكسيد الهيدروجين وحامض الفورميك. تمت دراسة تأثير المركبات المحضرة في فعالية أنزيم الكولين أستريز في مصل الدم حيث ظهر أن جميع المركبات (باستثناء المركب A_2) لها القابلية على تثبيط فعالية الأنزيم . أخيرا تمت دراسة الخواص الحركية لهذه المركبات مع

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الأنزيم حيث وجد أن المركبات (A_1 و A_3 و A_4 و A_7) تسبب تثبيط تنافسي للأنزيم بينما المركبات (A_5 و A_6 و A_8) تسبب تثبيط من النوع المختلط.

Introduction

Acid hydrazide and acid thiosemicarbazid are starting materials for the preparation of a large numbers of heterocyclic compounds and their derivatives such us thiadiazole, triazole and oxadiazole.

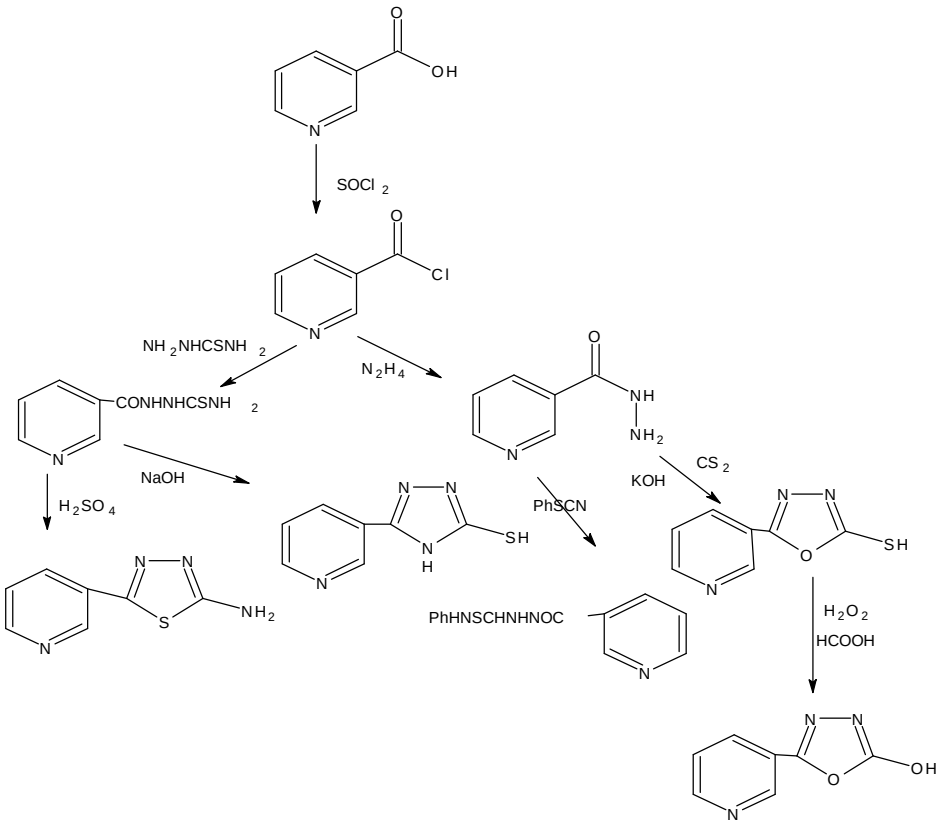
1,3,4-thiadiazole derivatives are associated with diverse biological activities probably due to toxophoric –N = C-S group.[2]

5-acyl-1,2,4-thiadazoles have been prepared by cyclo addition of nitrile sulfide to acylcyanides[3].

Substituted 1,3,4-triazole moieties can be found in a vast number of compounds displaying biological activity. For example 3-thio-1,3,4-triazoles have been shown as antiasthmatic and anti inflammatory[4] .

Katritzky and coiworkers[5] have prepared some of 2-amino-5-Aryl-1,3,4-Oxadiazole derivatives from imine and benzene carbohydrazide, which have be found to be antidiabetic, antiarthritic and anti inflammatory.

Cholinesterase, which is an enzyme, breaks acetylcholine (neurotransmitter in the nervous system) down into choline and acetate[6]. It also breaks down succinyl choline, a neuromuscular blocking agent which is widely used for producing brief paralysis during surgery. Cholinesterase is required for normal nervous function. Impulses process from one nerve to another, or from a nerve cell to an effecter organ or gland (e.g. a muscle or gland), by a process of chemical transmission. After a series of events, the chemical (namely, acetylcholine) is destroyed by cholinesterase. This allows the muscle membrane or nerve to return to its resting state, ready for another nerve impulse if need be[7] .



Scheme (1)

Experimental

Uncorrected melting points were determined on Gallen-Kamp apparatus.
Infra-Red (IR) spectra were recorded on a Pye-Unicom SP_3-100 spectrophotometer.

Ultra Violet (UV) spectra were taken with Cintra-5-Gbes scientific equipment.

Preparation of nicotinoyl chloride (A_1)

A mixture of nicotinic acid (0.0/mole, 1.23 gm) and thionyl chloride (10 ml) was refluxed gently for 2 hours. After cooling the excess of thionyl chloride was removed under vacuum. The product was yellow crystal.

Preparation of nicotinoyl hydrazide (A_2)

To a stirring mixture of compound (A_1) (0.01 mole, 1.415 gm) in dry benzene (15ml), a mixture of hydrazine (99%) (0.01 mole, 0.325 gm) and benzene (10ml) was added dropwise. After that, the mixture was refluxed for 1 hour.
After cooling, the excess of benzene was removed under vacuum. The product was collected as oily compound.

Preparation of nicotinoyl thiosemicarbazide (A_3)

To a stirring mixture of compound (A_1) (0.01 mole, 1.415 gm) in dry benzene (25 ml), thiosemicarbazide was added.

After that, the mixture was refluxed for 4 hours. After cooling, the product was filtered and recrystallized from an appropriate solvent.

Preparation of nicotinoyl-4-phenyl thiosemicarbazide (A_4)

A methanolic solution of (A_2) (0.01mole, 1.37 gm) and phenyl isothiocyanate (0.01 mole, 1.34 gm) was refluxed for 4 hours. The contents were poured onto crushed ice, filtered and the product was recrystallized from an appropriate solvent.

Preparation of 2-amino-5-(3-pynidyl) 1,3,4-thiadiazol (A_5)

A compound (A_3) (0.01 mole, 1.96gm) was dissolved in cold conc. H_2SO_4 and contents were kept at room temperature for 2 hours, stirred occasionally and then poured onto crushed-ice, filtered and the product was recrystallized from an appropriate solvent.

Preparation of 2-thiol-5-(3-pyridiyl) 1,3,4-triazol (A_8)

A compound (A_3) (0.01 mole, 1.96 gm) was refluxed in NaOH solution (20 ml,4%) for 7 hours, cooled, poured into excess of water, stirred and filtered. On acidification the obtained filtrate was solid, which was recrystallized from an appropriate solvent.

Procedure to measurement enzyme activity:

1) ChE activity was assayed in human serum using the modified Ellman method[8] with acetylthiocholine iodide ASChI (0.06 M) as substrate in (0.2 M) Sodium phosphate buffer (pH= 7.3). Units of activity are (µmole of ASChI hydrolyzed / 3 min/ml) .The procedure went as follows:

(50 µl) of 5,5-Dithio bis-2-nitrobenzoic acid solution (DTNB 0.001M) were added to (2.25 ml) of sodium phosphate buffer solution (pH= 7.3, 0.2M), then (10µl) of serum were added, mixed well and (2 ml) of the mixture was transferred to a measuring cell (3 mm), then (34 µl) of ASChI was added, the change in absorbency was measured before and after adding the substrate at (430 nm) for (3 min).

2) A stock solution (0.1 M) concentration of each compound synthesized, was prepared and then the following concentrations ($1X10^{-2}$, $5X10^{-3}$, $1X10^{-3}$, $5X10^{-4}$, $1X10^{-4}$) M were prepared by diluting with dimethyl sulfoxide (DMSO) solvent using the stock solution. For the measurement of the activity the method described in section (1) was followed with the exception that the inhibitor was added to the buffer (0.25 ml of inhibitor mixed with 2 ml of buffer (pH=7.3, 0.2M)). The inhibition percentage was calculated by comparing the activity with and without the inhibitor and under the same conditions, according to the equation:

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}}\right) X\ 100$$

3) A constant concentration of inhibitor ($5X10^{-3}$ M) was used with different substrate concentrations (0.02, 0.04, 0.05, 0.06, 0.08) M to determine the type of inhibition. The enzyme activity was determined with and without the inhibitor. Using the Lineweaver-Burk[9] method by plotting 1/V vs. 1/[S] the following values were calculated:

a) Ki. b) Apparent Vmax (Vmapp). c) Apparent Km (Kmapp). d) Type of inhibition.

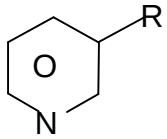
Results and Discussion

Scheme 1 summarizes all reactions in this work. Structure and physical properties of the synthesized compounds are given in tables (1 and 2). The IR and U.V. spectra data are given in tables (3 and 4).

The biological activities of these synthesized compounds on the activity of cholinesterase enzyme in human serum were studied *in vitro*. The results obtained show that all compounds (except compound A_2, which did not show any significant effect on the enzyme activity) cause inhibitory effects on the cholinesterase enzyme activity as in figures (1 and 2) which show the relationships between compounds concentrations versus percentage of inhibition. From these figures we observe that the percentage of inhibition increass with increasing the compound concentration and the inhibition percentage ranging between (51%-70%) at concentration up to (0.01M) as shown in figure (3).

Different concentrations of the substrate were used to study the type of inhibition, the results obtained from lineweaver-burk plots indicate that compounds (A_1, A_3, A_4 and A_7) acte as competitive inhibitors, while compounds (A_5, A_6 and A_8) cause mixed type of inhibition, figures (4 and 5). The kinetic properties of these inhibitors (K_{mapp}, V_{amp} and K_I) were also determined from Lineweaver-Burk plots as shown in figures (4 and 5) and table (5).

Table (1) Physical properties of compounds $A_1 - A_4$



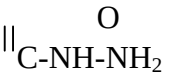
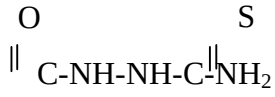
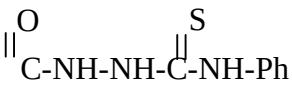
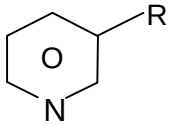
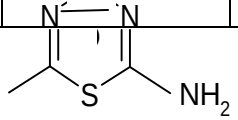
Comp. No.	R	m.p. C°	Yield %	Punificat Solvent	Molecular formula
A_1		137-139	90	Benzene	C_6H_4NOCl
A_2		Oily	67	Benzene	$C_6H_7N_3O$
A_3		130-132	80	Ethanol	$C_7H_8N_4OS$
A_4		168-170	65	Ethanol	$C_{13}H_7N_4OS$

Table (2): Physical properties of compounds $A_5 - A_8$



Comp. No.	R	m.p. C°	Yield %	Punificat Solvent	Molecular formula
					

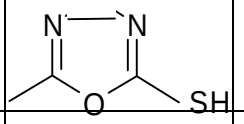
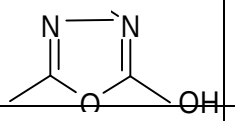
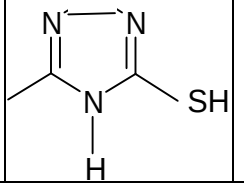
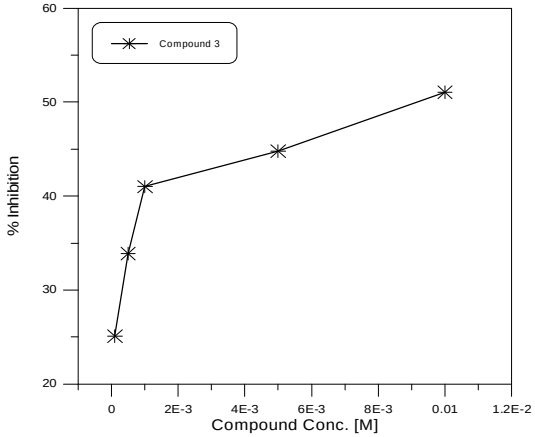
A_5		210-212	56	Ethanol	$C_7H_6N_3S$
A_6		158-160	69	Ethanol	$C_7H_5N_3OS$
A_7		200-202	42	Ethanol	$C_7H_5N_3O_2$
A_8		196-198	73	Ethanol	$C_7H_6N_4S$

Table (3): Spectra data of compounds (A_1-A_4)

Comp. No.	U.V Λmax(nm)	Characteristic bands of IR spectra cm^{-1} KBr disc			
		υ (C=O)	υ (C=C)	υ $(C-H)_{ar}$	Others
A_1	332 268	1740	1610 1500	3050	7800υ(C-Cl)
A_2	307 280	1630	1600 1500	3060	3200-3450υ(NH_2)
A_3	264 232 219	1660	1610 1500	3080	3210-3380(NH-NH_2) 1200 (C=S)
A_4	283 256 230	1640	1590 1480	3030	3250-330(NH-NH) 1210 (C=S)

Table (4) :Spectra data of compounds (A_5-A_8)

Comp. No.	U.V Λmax(nm)	Characteristic bands of IR spectra cm^{-1} KBr disc			
		υ (C=N)	υ (C=C)	υ $(C-H)_{ar}$	Others
A_5	310 261 243	1650	1590 1500	3050	3210-3300(NH_2st) (Sy and asy)
A_6	329 210	1630	1610 1510	3060	2430 υ(SH)
A_7	303 268	1650	1600 1520	3080	1780 υ(C=O)
A_8	384 329 253	1630	1600 1500	3010	2400 (SH) 3370 (NH st)



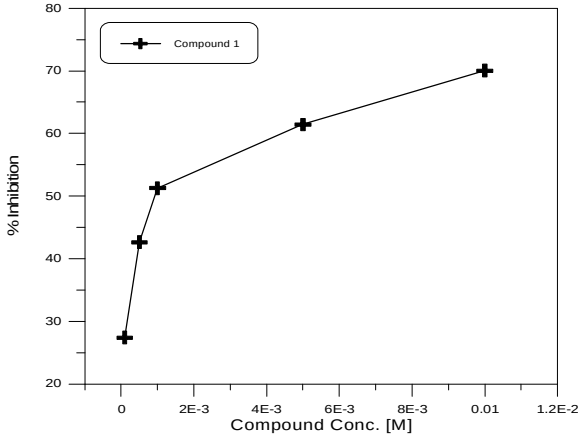


Fig. (1): Effect of different concentrations of compounds A_1 and A_3 on human serum ChE activity.

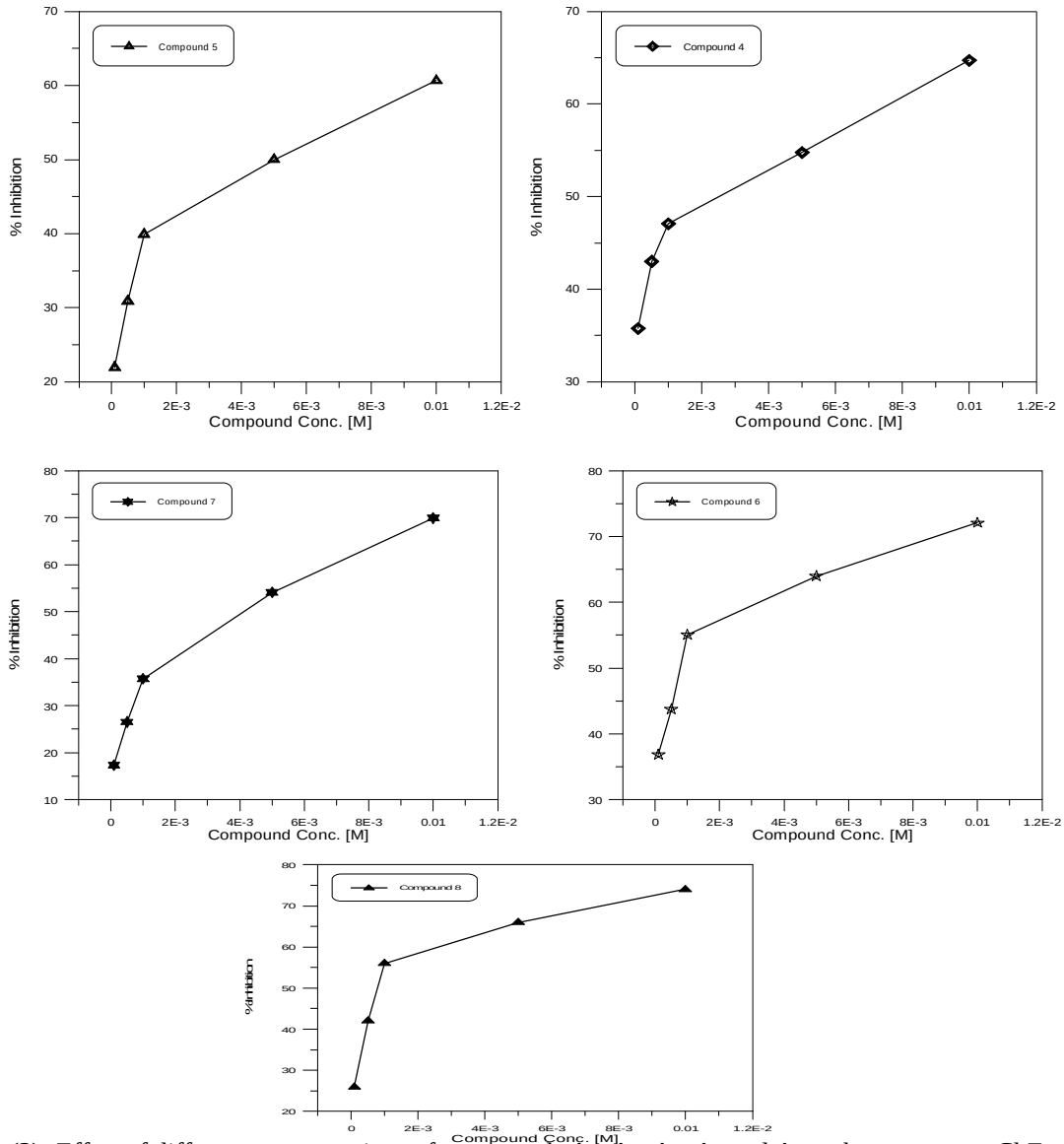


Fig. (2): Effect of different concentrations of compounds A_4, A_5 ,A_6, A_7 and A_8 on human serum ChE activity.

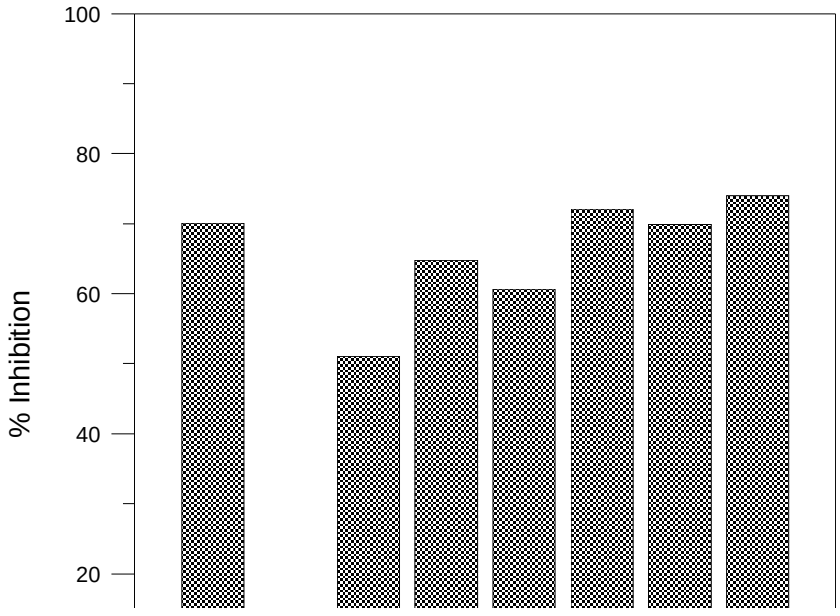


Fig. (3) The effect of (0.01 M) of compounds A_1, A_3, A_4, A_5, A_6, A_7 and A_8 on human serum ChE activity.

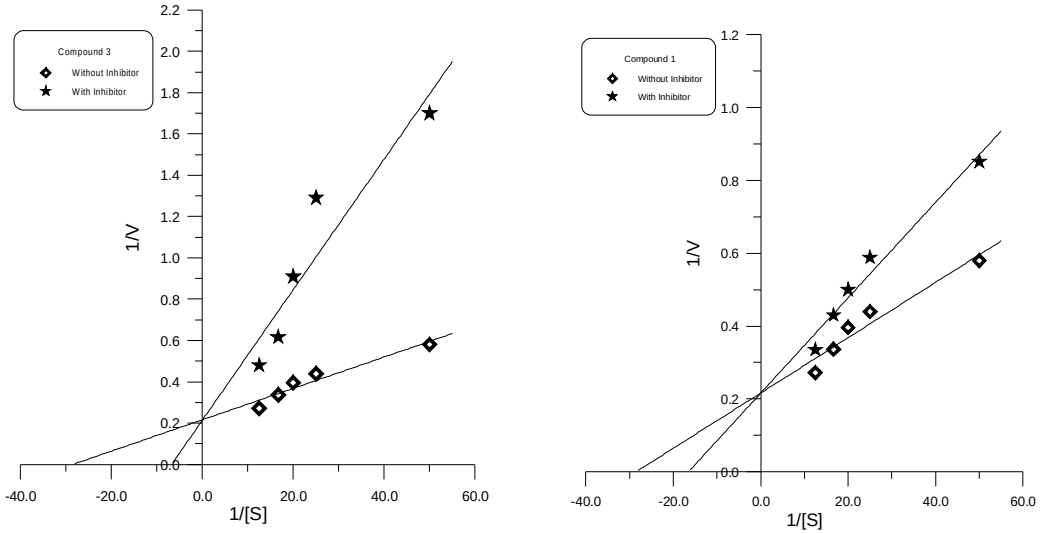
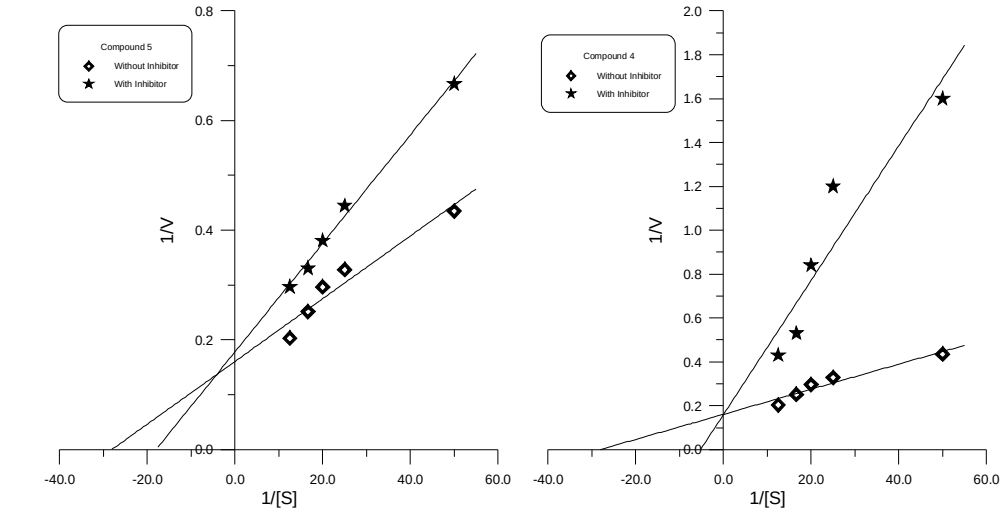


Figure (4) : Lineweaver- Burk plot of ChE in the presence and absence of compounds A_1 and A_3.



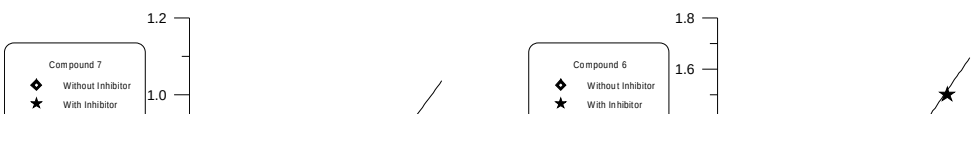


Fig. (5): Lineweaver- Burk plot of ChE in the presence and absence of compounds A_4, A_5, A_6, A_7 and A_8.

Table (5): Kinetic parameters and type of inhibition of ChE with compounds synthesized.

Comp. number ($5*10^{-4}$ M)	**Kmapp (M)**	**Vmapp (µmole/ml/min)**	**Ki (M)**	**Type of inhibition**
A_1	0.061		$7.1*10^{-4}$	Competitive
A_3	0.143		$1.6*10^{-4}$	Competitive
A_4	0.182		$1.2*10^{-4}$	Competitive
A_5	0.056	5.56	$1.5*10^{-4}$	Mixed
A_6	0.118	4.55	$5.2*10^{-4}$	Mixed
A_7	0.125		$1.95*10^{-4}$	Competitive
A_8	0.067	5.89	$7.3*10^{-4}$	Mixed

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