SYNTHESIS OF NEW NICOTINIC ACID DERIVATIVES AND STUDYING THEIR EFFECTS ON CHO HINESTEES 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. Thiosemicarbazone is cyclized by using H₂SO₄ 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 CS₂ and KOH in absolute ethanol, then thiol group is converted into hydroxyl group by treating with H₂O₂ 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 A₂) cause inhibition to the enzyme activity. Kinetic parameters were studied and the results show that compounds (A₁, A₃, A₄ and A₇) cause competitive inhibition and compounds (A₅, A₆ and A₈) cause mixed type of inhibition.

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Introduction

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

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

5-acyl-1,2,4-thiadiazoles 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 been 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].
Experimental

Uncorrected melting points were determined on Gallen-Kamp apparatus. Infra-Red (IR) spectra were recorded on a Pye-Unicom SP3-100 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 \( (A_2) \) (0.01 mole, 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-pyridyl) 1,3,4-thiadiazol \( (A_5) \)**

A compound \( (A_3) \) (0.01 mole, 1.96 gm) was dissolved in cold conc. \( \text{H}_2\text{SO}_4 \) 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 \( \text{NaOH} \) 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 \( (1 \times 10^{-2}, 5 \times 10^{-3}, 1 \times 10^{-3}, 5 \times 10^{-4}, 1 \times 10^{-4}) \) 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:

\[
\text{The activity in the presence of inhibitor} - \text{The activity in the absence of inhibitor} \times 100
\]

3) A constant concentration of inhibitor \( (5 \times 10^{-4} \text{ M}) \) 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) \( K_i \)  
   b) Apparent \( V_{\text{max}} \) (\( V_{\text{mapp}} \)).  
   c) Apparent \( K_m \) (\( K_{\text{mapp}} \)).  
   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 A2, 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 increase 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 (A1, A3, A4 and A7) acte as competitive inhibitors, while compounds (A5, A6 and A8) cause mixed type of inhibition, figures (4 and 5). The kinetic properties of these inhibitors (K_{\text{mapp}}, V_{\text{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 A1 – A4

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>R</th>
<th>m.p. C⁰</th>
<th>Yield %</th>
<th>Punificat Solvent</th>
<th>Molecular formula</th>
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<tbody>
<tr>
<td>A₁</td>
<td>C-Cl</td>
<td>137-139</td>
<td>90</td>
<td>Benzene</td>
<td>C₆H₄NOCl</td>
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<tr>
<td>A₂</td>
<td>C-NH-NH₂</td>
<td>Oily</td>
<td>67</td>
<td>Benzene</td>
<td>C₆H₇N₃O</td>
</tr>
<tr>
<td>A₃</td>
<td>C-NH-NH-C-NH₂</td>
<td>130-132</td>
<td>80</td>
<td>Ethanol</td>
<td>C₇H₈N₄OS</td>
</tr>
<tr>
<td>A₄</td>
<td>C-NH-NH-C-NH-Ph</td>
<td>168-170</td>
<td>65</td>
<td>Ethanol</td>
<td>C₁₃H₇N₃OS</td>
</tr>
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</table>

Table (2): Physical properties of compounds A₅ – A₈
<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>U.V Λmax (nm)</th>
<th>Characteristic bands of IR spectra cm⁻¹</th>
<th>KBr disc</th>
<th>Others</th>
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<tbody>
<tr>
<td>A₁</td>
<td>332</td>
<td>υ(C=O) 1740, υ(C=Cl) 3050</td>
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<td></td>
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<tr>
<td>A₂</td>
<td>307</td>
<td>υ(C=O) 1630, υ(NH₂) 3060</td>
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<tr>
<td>A₃</td>
<td>264</td>
<td>υ(C=O) 1660, υ(C=S) 3080</td>
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<td></td>
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<tr>
<td>A₄</td>
<td>283</td>
<td>υ(C=O) 1640, υ(NH₂) 3030</td>
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Table (4): Spectra data of compounds (A₅-A₈)

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>U.V Λmax (nm)</th>
<th>Characteristic bands of IR spectra cm⁻¹</th>
<th>KBr disc</th>
<th>Others</th>
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<tbody>
<tr>
<td>A₅</td>
<td>310</td>
<td>υ(C=N) 1650, υ(NH₂) 3010</td>
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</tr>
<tr>
<td>A₆</td>
<td>329</td>
<td>υ(C=N) 1630, υ(SH) 3010</td>
<td></td>
<td></td>
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<tr>
<td>A₇</td>
<td>303</td>
<td>υ(C=N) 1650, υ(C=O) 3080</td>
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</tr>
<tr>
<td>A₈</td>
<td>384</td>
<td>υ(C=N) 1630, υ(SH) 3370</td>
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</tbody>
</table>
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 A₁, A₃, A₄, A₅, A₆, A₇ and A₈ on human serum ChE activity.

**Figure** (4): Lineweaver- Burk plot of ChE in the presence and absence of compounds A₁ and A₃.
**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.

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