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# Synthesis and Charactrization of New Schiff Base Derived from PVA and Erythroascorbic Acid Derivative and Study Its Effect on the Activity of ACh Enzyme (In Vitro)

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**Abstract**
Schiff base derived from PVA and Erythroascorbic acid derivative (pentulosono-ɣ-lactone-2, 3-enedianisoate) was synthesized and characterized by Thin Layer Chromatography (TLC) and FTIR spectra, aldehyde was also characterized by (U.V-Vis), $^{1}$HNMR, $^{13}$CNMR and mass spectra.
The inhibitory effect of prepared polymer on the activity of human serum Cholinesrerase has been studied in vitro. The polymer showed a remarkable activity at low concentration ($4.5*10^{-3}$ – $4.5*10^{-8}$ M).
**Keywords:** Schiff base, PVA, Acetylcholinesterase.

## Introduction

Schiff bases are the important compound owing to their wide range of biological activities and industrial application.They have been found to posses the pharmacological activities such as antimalarial, anticancer, antibacterial, antifungal, antitubercular, antiinflammetery, antimicrobial and antiviral. They also serve as a back bone for the synthesis of various heterocyclic compounds (ZS Yang, Zhang, Cao & Wang 2003), (Villar, Encio, Migliaccio & Martinez-Merino 2004), (Venugopal & Jayashree 2008), (Pandey, Lakshmi & Pandey 2003), (Bhat, Imran, Khan & Siddiqui 2005), (Wadher, Puranik, Karande & Yeole 2009), (Karthikeyan, Prasad, SubrahmanyaK Bhat & Holla 2006).
Schiff bases-bimolecular condensation products of primary amines with aldehydes-represent valuable intermediates in organic synthesis and at the same time, compounds with various applications (Ugras, Basaran, Kilic & Cakir 2006). They are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to presence of imine (-N=CH-) which imports in elucidating the mechanism of transformation and rasemination reaction in biological system (Rajavel, Senthil & Anitha 2008). These compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation (Mohamed, Omar & Hindy 2006).
Thousands of compounds have been synthesized and tested as a cholinesterase inhibitors.They belong to different types of organic and organometalic classes such as alkaloids, physostigmine, organophosphrous (Ballantyn & Marrs 1992).
Acetylcholinesterase (EC3.1.1.7) (ACh), also known as RBC Cholinesterase, or acetyl choline acetylhydrolase (Silver & 1974), (Ligand, Perrier & Noureddine 2008), it was found in many types of conducting tissue: nerve and muscle, central and peripheral tissue, motor and sensory fibers, and cholinergic and non cholinergic fibers. It was also found in smooth cardial muscle, blood, and gland (Francis, Azzolos & Foldes 1978), (Massoulie, Pezzementi, Bon, Kerjic & Vallette 1993). The physiology function of AchE in cholinergic transmission has been well established. At the post synaptic site of excitable tissue. The enzyme termining the synpating transmission through the inactivation of Ach function by hydrolyzing it to choline & acetic acid (Quinn 1987).

## Experimental

### Preparations of Poly Schiff base

Melting points were determined by electrothermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on 8400s Shimadzu FT infrared spectrophotometer. $^{1}$HNMR spectrum was recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. $^{13}$CNMR spectrum was recorded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECl 7200 England. Mass spectrum was recorded on IEOLJMS-7high resolution instrument. Thin layer chromatography (TLC) were performed on aluminum plates coated with layer of silica

gel, supplied by Merck. The spots were detected by iodine vapor. All chemical were obtained from Fluka or BDH.

**Synthesis of 5,6-*O*-isopropylidene-L-ascorbic acid (2)**

Dry hydrogen chloride was rapidly bubbled with stirring for 20 minutes into a (250ml) flask containing (10g, 57mmol) of powdered L-ascorbic acid (1) and (100ml) of dry acetone.

After addition of (80ml) n-hexane, stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with (154ml) of acetone-hexane mixture (4:7) (v/v), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (2) (95.35%) as a white crystalline residue (Salomon 1963), m.p (206-208˚C). $R_f$ (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, $cm^{-1}$): 3240 (O-H), 2993 (C-$H_{ali.}$), 2908 (C-$H_{ace.}$), 1751 (C=$O_{lac.}$), 1662 (C=C), 1431 (-CH-$_{asym}$), 1388 (-CH-$_{sym}$), 1141-1026 (C-O), 767 δ(O-H) (O.O.P.) (Dudley & Fleming 1995).

**Synthesis of 2,3-*O*-dianisoyl-5,6-*O*-isopropylidene-L-ascorbic acid (3)**

To a cold solution of (2) (10g , 46mmol) in pyridine (50ml), anisoyl chloride was added as dropwise (17.5ml , 129mmol) with stirring .The resulting mixture was stirred for 2 hours , then kept in dark place at room temperature for 22 hours.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. The oil layer was extracted with chloroform (150 ml), washed with water, dilute hydrochloric acid (5%) (2 × 100ml.), saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate, Chloroform was evaporated to produce a brown syrup and purified from chloroform: petroleum ether (60-80˚C) (1:5) (v/v) to give (3) (15g, 76.5%) as a pale yellow solid (Mukhlis, AL-Rawi, Tomma & Al-Dujaili 2012), m.p (102-104˚C). $R_f$ (0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, $cm^{-1}$): 3028 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2939 (C-$H_{ace.}$), 2843 (OC-$H_{ali.}$), 1749 (C=$O_{lac.}$), 1683 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-H) (O.O.P.).

**Synthesis of 2, 3-*O*-dianisoyl-L-ascorbic acid (4)**

Compound (3) (10g , 23.6mmol) was dissolved in mixture (65%) acetic acid (30ml) and absolute methanol (10ml) and stirred for 48 hours at room temperature. The TLC showed that the reaction was complete (benzene: methanol, 6:4).

To the resulting solution a benzene (40ml) was added and evaporated (repeat this process four times) (Mukhlis, AL-Rawi, Tomma & Al-Dujaili 2012).The residue recrystallized from chloroform and then diethyl ether to yield (4) (7g, 77.7%) as a white crystals, m.p (130-132˚C), $R_f$ (0.42). FTIR (KBr, $cm^{-1}$): 3444 (O-H), 3008 (C-$H_{ar.}$), 2972 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 1741 (C=$O_{lac.}$), 1681 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1606 (C=$C_{ar.}$), 1319-1112 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.) (Mukhlis, AL-Rawi, Tomma & Al-Dujaili 2012).

**Synthesis of pentulosono-γ-lactone-2,3-enedianisoate (5)**

To the stirred solution of sodium periodate (5.6g, 26mmol) in distilled water (60ml) at (0˚C), a solution of (4) (10g, 26mmol) in absolute ethanol (60ml) was added dropwise. After stirring for 15 minutes, ethylene glycol (0.5ml) was added as dropwise, stirring was continued at room temperature for 1 hour (Mukhlis, AL-Rawi, Tomma & Al-Dujaili 2012).

The mixture was filtered and to the filtrate water (40ml) was added then the product was extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate, then filtered and the solvent was evaporated and the residue recrystallized from benzene to yield the pure product of compound (5) (4g, 45%) as a white crystals, m.p (156-158˚C). $R_f$ (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, $cm^{-1}$): 3040 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), (2671, 2559) (C-$H_{ald.}$), 1782 (C=$O_{lac.}$), 1749 (C=$O_{ald.}$), 1685 (C=$O_{est.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.). $^{1}$HNMR (DMSO δ ppm): 12.5 (s, 1H, $CHO_{.}$), 7.00-7.97 (dd, 8H, aromatic), 3.86 (s, 1H, $H_4$), 3.82 (s, 6H, $2OCH_3$) (Dudley & Fleming 1995). $^{13}$CNMR (DMSO δ ppm): 167.50 (C=$O_{lac.}$), 163.32 (C=$O_{est.}$), 131.86 (C-4), 131.83 (C-3), 131.81 (C-2), (123.44, 114.31, 114.28, 114.26) ($C_{ar.}$), 55.90 ($OCH_3$). The signal of aldehydic carbonyl was disappeared due to it showed out of the scale (Carey 2006). MS, (positive ion) m/z (relative intensity): 413 [M+1, (100)], UV ($\lambda_{max}$, nm, $CHCl_3$): 300.

**Synthesis of poly (vinyl-4-aminobenzoate) (PVAB) (6)**

4-Aminobenzoic acid (0.5g, 0.003mol) was dissolved in $SOCl_2$ (2ml, mol) and refluxed for 5hrs. The excess of $SOCl_2$ was evaporated under vacuum to obtained quantitative yield of 4-Aminobenzoyl chloride. The 4-Aminobenzoyl chloride was co-evaporated with diethyl ether to remove traces of $SOCl_2$. The resulting 4-Aminobenzoyl chloride was dissolved in DMSO (5ml) and to this solution of polyvinyl alcohol (PVA) (0.5g), in

DMSO (10ml), KOH (0.5g, 0.008mol) was added. The reaction mixture was stirred for 48 hrs at room temperature. After completion of the reaction, the mixture was poured into ice-water and chloroform was added to extract the materials were not reacted. The residue was evaporated to yield the ester (PVAB) (6) (0.89g), as a pale brown solid. FTIR (KBr, $cm^{-1}$): (3392, 3365) ($NH_2$), 3000 (C-$H_{ar.}$), 2926 (-$CH_2$-), 2856 (-CH-), 1688 (C=$O_{est.}$), 1604 (C=$C_{ar.}$), 1419 (-CH-$_{asym.}$), 1315 (-CH-$_{sym.}$), 1261-1111 (C-$O_{est.}$), 900-600 δ(C-$H_{9ar.}$) (O.O.P.) (Dudley & Fleming 1995).

**Synthesis of poly (vinylbenzoate-imine-pentulose-γ-lactone-2,3-enedianisoate) (7)**

A mixture of poly (vinyl-4-aminobenzoate) (6) (0.14g), aldehyde (5) (0.14g), dry benzene (5ml) and 2 drops of glacial acetic acid was refluxed for 24 hrs, then collected the pale brown solid by filtration and washed with dry benzene to give compound (7) (0.3 g). FTIR (KBr, $cm^{-1}$): 3030 (C-$H_{ar.}$), 2983-2879 (C-$H_{ali}$), 2843 (O-C-$H_{ali}$), 1770 (C=$O_{lac}$), 1699-1664 (C=$O_{est.}$), 1612 (C=N), 1577 (C=$C_{ar.}$), 1425 (-CH-$_{asym}$), 1311 (-CH-$_{sym}$), 1271-1107 (C-$O_{est}$), 927-615 δ(C-$H_{ar.}$) (O.O.P.) (Dudley & Fleming 1995).

**Enzyme assay**

This compound was dissolved in dimethyl sulphoxide (DMSO) and stock solutions were made for this compounds. Different volumes from stocks were added to the assay mixture and the enzyme activity determined according to a slightly modified WHO procedure (WHO 1978).

A volume of 2.250 ml of phosphate buffer pH= 7.2 , 50µl of DTNB solution and 10µl serum was served as the assay mixture. In a 3ml cuvette, 2ml of this mixture was taken and 34µl of the substrate (acetylthiocholineiodide) was added and the absorbance was measured at 430nm.

DMSO was used as a vehicle solution (control) and showed no inhibitory effect on the activity of the enzyme (Jaffer, Mahmod & Al-Azzawi 1988).

**Results & Discussion**

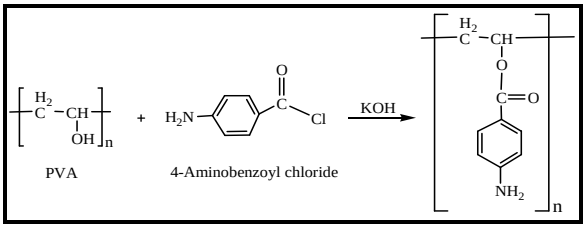
**Spectroscopic studies**

In the present work the synthesis of new Schiff bases derivative was achieved from pentulosono-γ-lactone-2,3-enedianisoate (5). The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L-ascorbic acid with acetal formation leading to compound (2) using dry acetone in acidic media, following Salomon[1] method. This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of anisoyl chloride in dry pyridine.

The FTIR spectra for compound (2) and (3) were confirmed the formation of compound (3) by disappearance of the bands for (O-H) of compound (2) and exhibited the band at (1683) $cm^{-1}$ for (C=O) of the ester in compound (3) spectrum.

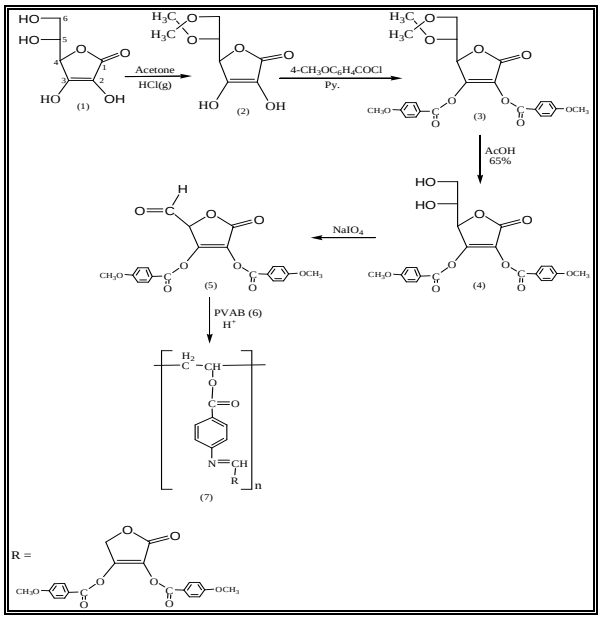
In order to prepare aldehyde (5), the acetal moiety was cleaved under acidic condition (Gazivoda et al. 2006) (65% acetic acid) for compound (3) to give (4) and oxidation of the product with sodium periodate to result (5), which gave a positive Tolen's test by formation a silver mirror (Vogel 1989). The FTIR spectra for compound (4) and (5) were confirmed the formation of compound (5) by disappearance of the bands for (O-H) of compound (4) and exhibited the band at (1749) $cm^{-1}$ for (C=O) in compound (5) spectrum. The structure of (5) was confirmed by $^{1}$HNMR which exhibited a signal at δ (12.5) ppm for (C**H**O) and was characterized by $^{13}$CNMR and (U.V-Vis) spectrum which showed one peak at (300) nm assigned to ($\pi \longrightarrow \pi^*$) and ($n \longrightarrow \pi^*$) transitions. Finally, the mass spectrum showed a highest mass signal at [M+1] =413 with signal intensity 100%.

Compound (6) was prepared from treatment of polyvinyl alcohol with 4-aminobenzoyl chloride in presence of potassium hydroxide and DMSO as solvent with stirring for 48 hours, scheme (1), which was converted to compound (7) by reaction with aldehyde (5) in presence of glacial acetic acid and dry benzene, scheme (2). The FTIR spectra for compound (6) and (7) exhibited the band at (3392, 3365) $cm^{-1}$ due to ($NH_2$) for compound (6) spectrum and disappearance this band in compound (7) spectrum and display the band at (1612) $cm^{-1}$ due to (C=N) for compound (7) spectrum.




**Scheme (1):** The scheme of prepared polyester.




**Scheme (2):** The scheme of prepared polyschiff base.

**Enzymic Study**

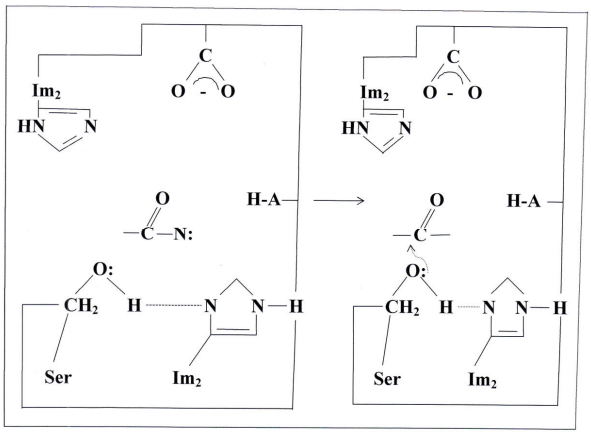
The effect of Schiff base on serum cholinesterase activity invitro were carried. This compound shows an encouraging inhibitory action as compound to aknown inhibitors such as Dibucaine and NaF (Stepankova & Komers 2008). The percentages of inhibition of this compound are shown in table (1):

**Table (1):** The effect of different concentrations of (poly (vinylbenzoate-imine-pentulose-γ-lactone-2, 3-enedianisoate) on (AchE) in serum.

| Inhibitor Conc. (μ) | Enzyme activity, μmol/ml/3min | Inhibition % | Recovery % |
|---|---|---|---|
| Nil | 6.75 ± 0.38 | 0.00 | 100 |
| $4.5x10^{-3}$ | 0.90 ± 0.54 | 86.66 | 13.34 |
| $4.5x10^{-4}$ | 1.12 ± 0.24 | 83.41 | 16.59 |
| $4.5x10^{-5}$ | 1.21 ± 0.17 | 82.07 | 17.93 |
| $4.5x10^{-6}$ | 1.39 ± 0.22 | 79.41 | 20.59 |
| $4.5x10^{-7}$ | 1.95 ± 0.54 | 71.11 | 28.89 |
| $4.5x10^{-8}$ | 2.91 ± o.42 | 56.89 | 43.11 |

The inhibition effect of this compound may be attributed to the similar structure as the structure of Choline ester that's because of containing several (C=O) groups, and then (OH) group of serine (Ser-$CH_2$-OH) will attack (C=O) group of the compound instead of attacking the (C=O) group of the enzyme, which will cause inhibitory effect of enzyme mechanism, as shown in the suggested chart:

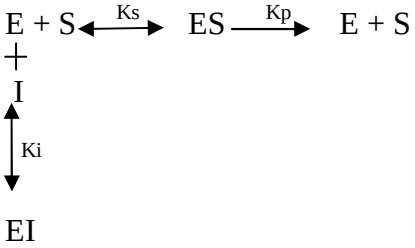
 




**Suggested Chart**

**Kinetic of Inhibition**

Type of inhibition was determined by Lineweaver-Burk plot, Figure (1) which indicate that the inhibition proceeds as noncompetitive inhibition due to change in $V_{max}$ value while Km remained constant. The noncompetitive inhibition could be represented as follows (Al-Mudaffer 1985), (Cook & Celand 2007):




$V_{mapp.}$ Was calculated from the point of intercept with Y axis, where $K_i$ was calculated from $V_{mapp.}$ (Segal 1975) According to eq. (2)

$$\frac{1}{V} = \frac{Km}{Vmax}\left[1 + \frac{I}{Ki}\right]\frac{1}{[S]} + \frac{1}{Vmax}\left[1 + \frac{I}{Ki}\right] \ldots\ldots\ldots (1)$$

$$\frac{1}{Vmapp} = \frac{1}{Vmax.\ Ki}[I] + \frac{1}{Vmax}$$

$$\frac{1}{Vmapp} = \frac{\left[1 + \frac{1}{Ki}\right]}{Vmax} \ldots\ldots\ldots (2)$$

**Table (2):** Shows Kinetic properties of (AChE) using Lineweaver-Burk plot.

| No. of Comp. | Conc. of Inhib | Vmapp (µmol/ml/3min) | Ki (M) |
|---|---|---|---|
| 1 | $4.5x10^{-8}$ | 0.4444 | $2.47x10^{-8}$ |

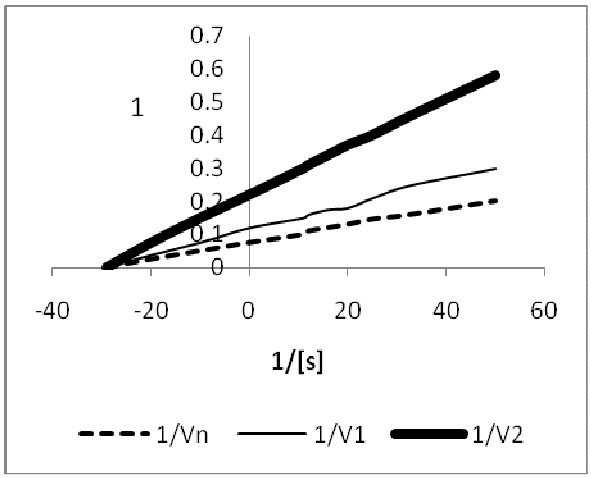



**Fig.(1) Lineweaver Burk plot determination $K_1$ and $V_{mapp.}$ Values of inhibition (AChE); ( - - - )normal, ( ——— ) 4.5x10$^{-8}$ M,( ▬▬ )4.5x10$^{-3}$ M.**

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