Research Article

Synthesis, characterization, and *in vitro* oxidative properties study of the new Schiff base 2-((4-nitrobenzylidene) amino) acetic acid

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ABSTRACT

Background: There are many evidences that the exposure of red blood cells (RBCs) to toxic chemical materials able to form free radicals causes formation of methemoglobinemia. One of these toxic chemical materials is nitrite, which leads to an autocatalytic reaction of hemoglobin converted to methemoglobin (Met-Hb). Methemoglobinemia is a pathological condition studied mostly under *in vitro* conditions, in which the ferrous iron of hemoglobin (Hb) converts to ferric iron if the oxy-Hb of RBC mixed with a sodium nitrite in supraphysiological concentrations. This report describes the synthesis of a novel Schiff base compound glycene-4-nitrobenzylidene-Ishraq (GBI), study its characterization, and test the oxidative properties of this new compound on the RBCs. **Materials and Methods:** Preparation of the new compound by the condensation reaction of glycine together with 4-nitrobenzaldehyde prepared new Schiff base compound, with methanol and potassium hydroxide. The spectral data include H-NMR, infrared, and C.N.H characterized a prepared compound. Then, the GBI screened to assess its ability to a mechanism of oxidation or anti-oxidation as *in vitro* study on human hemolyzed blood through the reaction of the synthesized compound (GBI) with the Hb of the erythrocyte with the present of sodium nitrite as a control, which is a known oxidative material. **Results:** It appears from the results an excessive oxidative effect in a dose dependent increases in the concentrations of GBI on the human blood cells through conversion of oxy-Hb into Met-Hb. **Conclusions:** The chemical reaction of 4-nitrobenzaldehyde and glycine led to the formation of the new compound (2-(4-nitrobenzylidene) amino) acetic acid which was found to be a strong oxidizing compound that caused the conversion of oxyhemoglobin to methemoglobin when the compound was tested on erythrocytes in invitro study.

KEY WORDS: *In vitro*, Methemoglobin, Novel compound, Oxidative properties

INTRODUCTION

The oxidants caused damage erythrocytes by different endogenous and exogenous agents[1] used as a model for the oxidative damage of red blood cell (RBC) during their life in the bloodstream.[2] There are many studies dealt with the RBC oxidation and formation of methemoglobinemia.[3-5] Methemoglobinemia is a condition characterized by the formation of abnormal hemoglobin (Hb) (methemoglobin [Met-Hb]), in which the iron moiety of heme suffered from oxidation forming the ferric iron (Fe^{3+}) instead of the ferrous iron (Fe^{2+}).[1,6] This condition may attribute to a genetic defect in erythrocyte metabolism or Hb structure, or it may gain following exposure to various oxidant drugs or toxins such as exposure to aniline, local anaesthetic agents, or nitrite.[6] Nitrite makes the hemoglobin unable to carry oxygen and that lead to varying degrees of cyanosis[6,7] which regard the initial sign of methemoglobinemia.[8] Nitrite cause occurance of 10%-20% concentration of Met-Hb, while the normal physiological concentration of Met-Hb no exceeded 1%-2%.[8] Oxidation of Hb is a reversible reaction, two mechanisms reduced Met-Hb back to normal Hb. The first one either by cytochrome-b5 reductase also called reduced nicotinamide adenine dinucleotide-dependent Met-Hb reductase,[9,10] which requires flavin adenine dinucleotide as a cofactor, this reaction accounts for 95–99% of the daily reduction and it is corrective rather than protective this enzyme accounts for the remaining reaction.[6,10] Sodium nitrite is an inorganic compound regard as a useful precursor of many organic compounds such

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as pesticides, pharmaceuticals, and dyes, and it has a positive and negative effects on the health,[11] from the harmful effects of high levels of sodium nitrite are an increased incidence of brain tumors, leukemia,[11] intrauterine growth retardation, cardiac, and central nervous system defects.[12,13] In the blood, nitrite reacts with oxy-Hb in an oxidation reaction to produce Met-Hb[14] as mention above. In other sides, Schiff bases are compounds contain (-HC=N-) group formed from condensation products of aromatic aldehydes or ketones with primary amines. The formation process of Schiff base takes place in special conditions such as under acid or base catalysis or with heat. Schiff base regards one of the very important classes of organic compounds because have a wide application in many biological aspects, decarboxylation reactions, proteins, enzymatic aldolization, and visual pigments.[15] Hence, in this search, we study the *in vitro* effect of several concentrations of synthesized Schiff base on the hemolyzed blood in a present of sodium nitrite and observed the degrees of Hb oxidation. The objective of the present work was to synthesis a novel Schiff base compound glycene-4-nitrobenzylidene-ishraq (GBI), study its characterization, and to determine the effect of oxidative effects of the new compound.

MATERIALS AND METHODS

Chemistry Study

Starting materials and solvents purchased from Aldrich and Fluka and used without purification. Fourier-transform infrared spectroscopy (FT-IR) measurements recorded on Shimadzu.^{1}H-NMR spectra obtained with 250.13 MHz in CHLOROFORM-D solution with the tetramethylsilane as an internal standard.

Preparation Method of Amino Acids Schiff Bases

We prepared the Schiff base derived from 4-nitrobenzaldehyde and glycine employing a method similar to one given in the reference.[10] Add 4-nitrobenzaldehyde (0.144 g, 0.953 mmol) in methanol (10 mL) and glycine (1.0 mmol) in methanol (12 mL) containing potassium hydroxide (0.054 g, 1.0 mmol), then stirred the resulted solution magnetically for a complete 8 h producing brownish-red solution. We reduced the next stage of the preparation volume of the obtained solution in vacuum utilizing a rotary evaporator and washed with absolute ethanol and anhydrous diethyl ether added for wash the product at 20–25°C temperature and dried. The physical properties FT-IR,^{1}H- NMR, and C.N. H analysis are listed in Tables 1 and 2.

Table 1: Physical properties of synthesis compound

Molecular formula	Molecular weight	m.p.°C	Yield %	Solvent
$C_9H_8N_2O_4$	222	245–247	80	Ethanol

Physiological Study

Blood sample collection and hemolysate preparation

Samples of blood collected from cubital vein of healthy men in an ethylenediaminetetraacetic acid anticoagulated tubes. Separated blood sample components centrifuged at 2500 rpm for 10 min and removed all blood components except RBC which washed with phosphate buffer saline (PBS, pH 7.4) 3 times, then the RBC lysed using phosphate buffer (pH 7.4) at ratio 1:20 (blood:PBS), respectively. At the end, the lysate centrifuged at 3000 rpm for 10 min to elevate the stroma.[16,17]

Preparation of the New Schiff Base Solutions

Several new Schiff base concentrations prepared by dissolving 100 mol in 1000 ml of distilled water as a stock solution (100 µM) and from the stock solution, prepared serial dilutions included concentrations of 10 µM, 20 µM, 30 µM, 40 µM, 50 µM, 60 µM, 70 µM, 80 µM, and 90 µM of new compound solutions.

Determination of Met-Hb Spectrophotometrically

By use of NaNO2 to produce methemoglobin (Met-Hb), the reaction initiated with additional of 1 ml freshly prepared hemolysate blood, 1 ml of different concentrations of new shiff base complex (10 µM-100 µM) for each time concomitantly with 0.5 ml $NaNO_2$ (final concentration 6.0 mM) as cited in Table 3, finally, recorded the form of Met-Hb spectrophotometrically at 631 nm for 60 minutes using computerized UV-visible spectrophotometer.[18]

RESULTS AND DISCUSSION

Synthesis

Condensation of 4-nitrobenzaldehyde and glycine in ethanol give the Schiff base according to the following reaction:

Table 2: Spectral data of synthesis compound

Fourier-transform infrared spectroscopy spectra data, v, cm^{-1}	^{1}H-NMR spectra data δ, ppm	C.N.H analysis (%)	
		Calculate	Found
v. 3377 (OH), *v.* 1435(C=C), *v.* 1516 (CH=N-), *v.* 1622(C=O), *v.* 3209(CH aliphatic)	(4.6,2H, s,-N-CH_2),(7.5-8,5H, m, aromatic ring), (8.15,1H, s, CH=N-), (10.8,1H, s, OH)	C: 52.4, H: 3.90 N: 20.34	C: 51.60, H: 310 N: 19.50

Table 3: Addition of $NaNO_2$ to hemolysate containing 10–100 µM of new compound, control without new compound

Solution	Blank	Control	Test
Hemolysate	1 mL	1 mL	1 mL
Distilled water	1.5 mL	1 mL	-
Glycene-4-nitrobenzylidene-ishraq	-	-	1 mL
Sodium nitrite	-	0.5 mL	0.5 mL

Table 4: Antioxidant *in vitro* activity of the new compound at 10 µM–50 µM in comparison with $NaNO_2$

Time	$NaNO_2$ 6.0 mM	GBI 10 µM	GBI 20 µM	GBI 30 µM	GBI 40 µM	GBI 50 µM
1	0.017	0.006	0.002	0.008	0.011	0.007
2	0.400	0.044	0.052	0.028	0.019	0.020
3	0.488	0.122	0.339	0.073	0.049	0.057
4	0.790	0.464	0.992	0.225	0.107	0.156
5	0.802	1.047	1.084	0.902	0.393	0.463
6	0.802	1.112	1.090	1.097	1.059	1.052
7	0.802	1.115	1.091	1.098	1.119	1.112
8	0.802	1.117	1.091	1.098	1.119	1.112
9	0.802	1.117	1.091	1.098	1.119	1.112
10	0.802	1.117	1.091	1.098	1.119	1.112

GBI: Glycene-4-nitrobenzylidene-ishraq

Table 5: Antioxidant *in vitro* activity of the new compound at 60 µM–100 µM in comparison with $NaNO_2$

Time	$NaNO_2$ 6.0 mM	GBI 60 µM	GBI 70 µM	GBI 80 µM	GBI 90 µM	GBI 100 µM
1	0.017	0.003	0.008	0.012	0.024	0.017
2	0.400	0.030	0.054	0.053	0.089	0.208
3	0.488	0.321	0.221	0.128	0.621	0.796
4	0.790	0.972	0.602	0.226	0.089	1.038
5	0.802	1.069	1.008	0.832	0.926	1.049
6	0.802	1.079	1.027	1.057	1.082	1.150
7	0.802	1.080	1.030	1.120	1.128	1.152
8	0.802	1.080	1.030	1.121	1.128	1.152
9	0.802	1.080	1.030	1.121	1.128	1.152
10	0.802	1.080	1.030	1.121	1.128	1.152

GBI: Glycene-4-nitrobenzylidene-ishraq

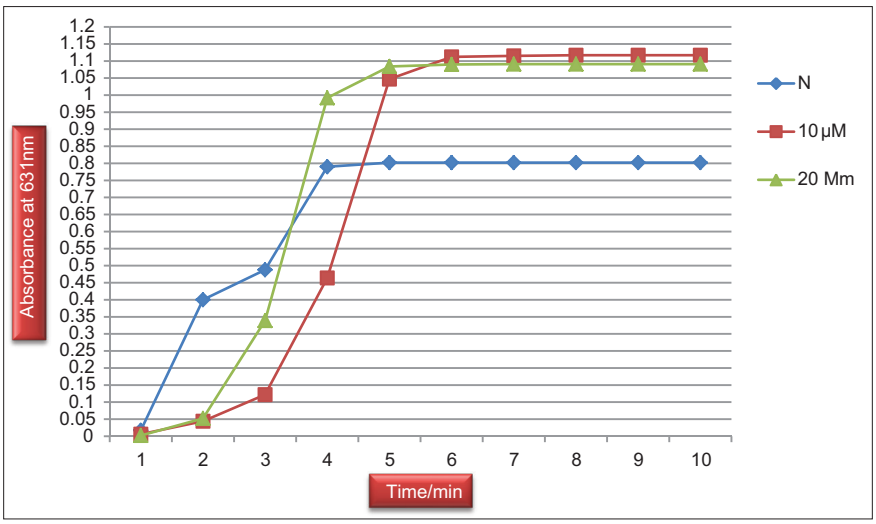

Figure 1: Effect of glycene-4-nitrobenzylidene-ishraq (10 µM and 20 µM) concentrations on the time course of nitrite-induced oxidation of hemoglobin in hemolysate solution

We list the physical, FT-IR, and electronic spectral data of this compound in Tables 1 and 2, which is soluble in methanol, ethanol, and other polar solvents. We attribute the strong absorption bands near 1435 cm^{-1} to the ring C=C stretching band. We observe the azomethine stretching band near 1516 cm^{-1}. We assign also the band near 1622 cm^{-1} to the carbonyl stretching band, while the band near 3377 cm^{-1} is an attribute to the hydroxyl group of acid for Schiff bases compound.

^{1}H-NMR spectrum of Schiff base compound GBI signals at 8.15 and 10.8 ppm, attributed to CH=N and

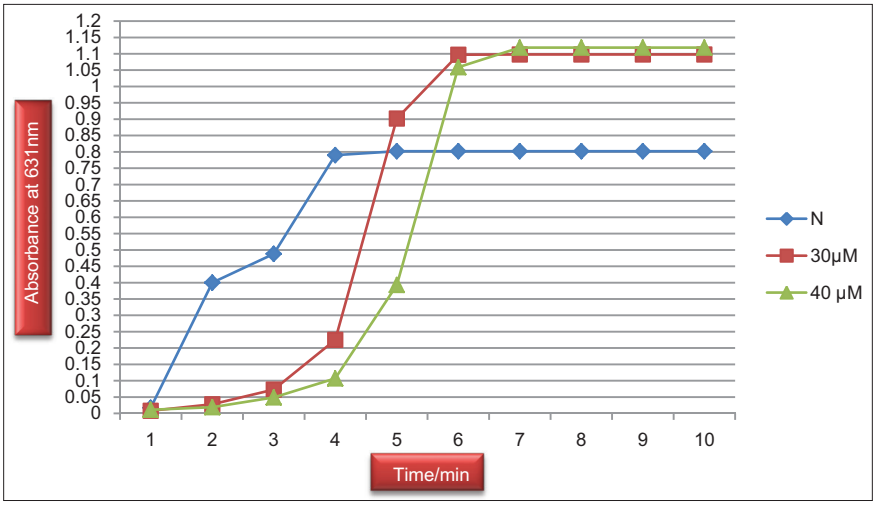

Figure 2: Effect of glycene-4-nitrobenzylidene-ishraq (30 µM and 40 µM) concentrations on the time course of nitrite-induced oxidation of hemoglobin in hemolysate solution

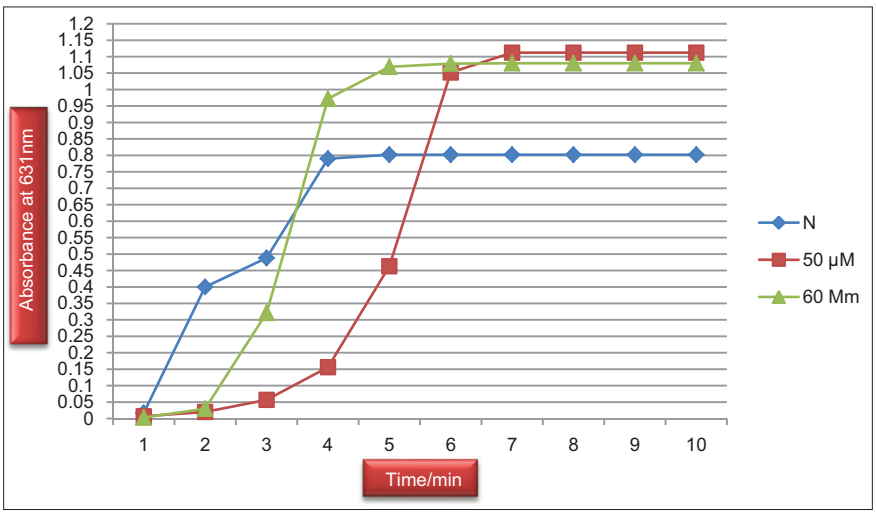

Figure 3: Effect of glycene-4-nitrobenzylidene-ishraq (50 µM and 60 µM) concentrations on the time course of nitrite-induced oxidation of hemoglobin in hemolysate solution

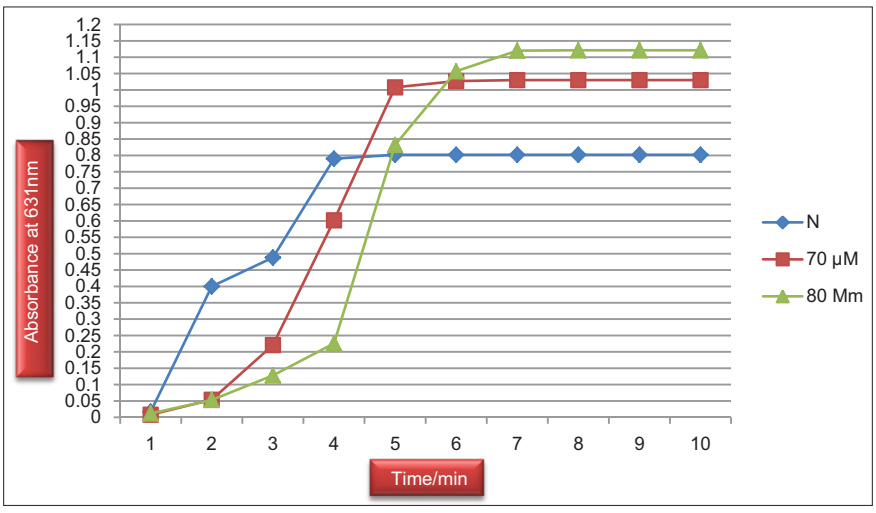

Figure 4: Effect of glycene-4-nitrobenzylidene-ishraq (70 µM and 80 µM) concentrations on the time course of nitrite-induced oxidation of hemoglobin in hemolysate solution

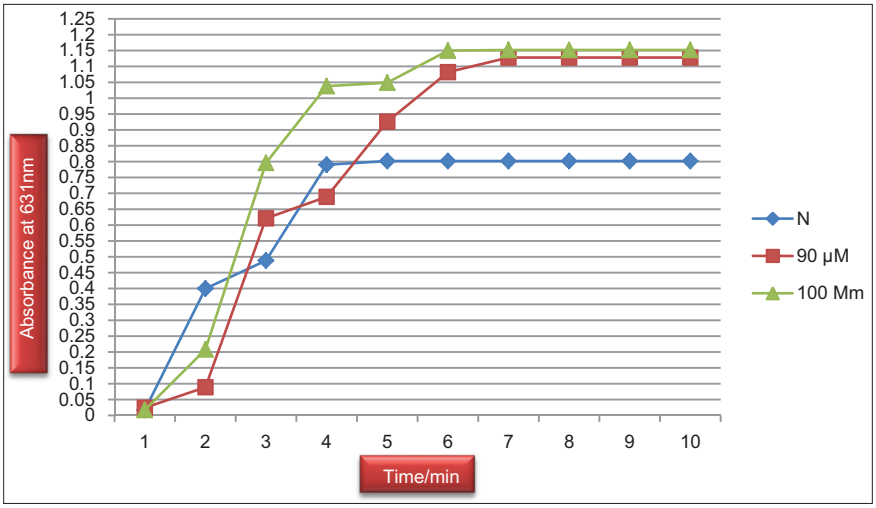

Figure 5: Effect of glycene-4-nitrobenzylidene-ishraq (90 µM and 100 µM) concentrations on the time course of nitrite-induced oxidation of hemoglobin in hemolysate solution

COOH protons, respectively. We assign the multisignal within the 7.5–8 ppm range to the aromatic protons of the benzene ring. The two signals at 4,6 ppm are assigned to the –N-CH2.

In vitro Study

Exposure of hemoglobin in the hemolysate solution to sodium nitrite revealed a rapid alteration of Oxy-Hb to Met-Hb (during only 5 min) as showed in the reaction of the control sample. The results revealed that the GBI compound in a dose dependent manner has further oxidative effect in comparison with sodium nitrite despite it take a slightly longer duration for complete conversion from oxy-Hb to met-Hb (7-8 min) as appear in the followed [Tables 4, 5] and figures. Its occur from the results that the kinetic profile of the reaction of the GBI compound was appeared as two phases of the oxidation process, an initial slow phase (lag phase) and a rapid autocatalytic phase which leaded the reaction to finalization so that S-shaped curve was obtained [Figures 1-5]. Level of Met-Hb formation measured by the light absorbance at 631 nm.

It shows from the results that the most concentrations of the new compound GBI complete the Hb oxidation and production Met-Hb at time 7 min only (concentrations 20 µM, 30 µM, 40 µM, 50 µM, 60 µM, 70 µM, 90 µM, and 100 µM) and the reminder concentrations (10 µM and 80 µM) delayed till 8 min. In addition to the time, the results revealed that the absorbance of the GBI rose progressively with raising of its concentration as take place in 20 µM, 30 µM, 40 µM, 60 µM, 80 µM, 90 µM, and 100 µM, except in 10 µM was higher than 20 µM and 30 µM and concentration of 70 µM was the lowest one among the all other concentrations.

CONCLUSION

Hb is an easy target when attacking free radicals of RBCs because it is an interactive molecule that can lose electrons and acquire them relatively easily and interact with a variety of biochemicals such as redox drugs and xenobiotics.[19] One of these oxidize agents is nitrite poisoning,[6] so, according to the present findings, the free radicals liberation due to the exposure of sodium nitrite on erythrocyte lysate leads to oxidative damage and production of Met-Hb,[10] and in case of adding of the new compound GBI, the oxidation increased that may be due to the effect of presence of glycine which used as starting material for the synthesis of the new compound GBI, and according to the Dhavskar and Srinivasan, when sodium nitrite added into a solution has glycine results in nitrous acid (HNO_2) formation which regards one of the strong oxidizing agents[20] and it is established that an oxidative agent leading to converting of Hb to Met-Hb,[6,7,21,22] so the activity of HNO_2 and nitrite collectively at the same time increased Met-Hb production[2,5] because both of them act as potent oxidative agents,[14,15] moreover, the effect of HNO_2 form of nitrite is more potent as an oxidizing agent than nitrite itself.[4]

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