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SYNTHESIS, CHARACTERIZATION AND ANTI-HYPERGLYCEMIC EVALUATION OF NEW METFORMIN DERIVATIVES

Yasir K. Mahdi*

Department of Pharmacy, Al-Israa University College, Baghdad, Iraq.

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***Corresponding Author**
Dr. Yasir K. Mahdi
Department of Pharmacy,
Al-Israa University
College, Baghdad, Iraq.

ABSTRACT

Metformin is a cornerstone in the treatment of type 2 diabetes particularly in obese patients. Its main actions are the suppression of gluconeogenesis and the improvement of glucose uptake and insulin sensitivity. A series of metformin derivatives (III a-b) have been synthesized by reaction of metformin, chloroacetyl chloride and an appropriate phenol. The chemical structure of the intermediates and the final compounds were confirmed by measuring their melting points, FT-IR spectroscopy and HNMR spectroscopy of the final compounds. All the new compounds were evaluated for their anti-diabetic activity using streptozotocin induced diabetic rats model. Fasting blood glucose level was estimated on hours 0,2,5,6 and 24 from rat tail vein using glucometer. The newly synthesized compounds showed significant anti-hyperglycemic activity compared to metformin.

KEYWORDS: metformin, anti-hyperglycemic activity, phenols, streptozotocin.

INTRODUCTION

Metformin, a principle biguanide is a widely used antihyperglycemic agent for treatment of non-insulin-dependent diabetes mellitus with significant effectiveness in decreasing the risk of disease development.[1] The discovery of metformin began with the synthesis of galegine-like compounds derived from *Gallega officinalis* which is a summer-flowering perennial herb with white, blue or purple flowers found in most temperate regions that is traditionally employed in Europe as a drug for diabetes treatment for centuries.[2]

Metformin reduces serum glucose level by several different mechanisms, notably through nonpancreatic mechanisms without increasing insulin secretion. It increases the effects of insulin; hence, it is termed “insulin sensitizer”.[3] Metformin also suppresses the endogenous

glucose production by the liver, which is mainly due to a reduction in the rate of gluconeogenesis and a small effect on glycogenolysis. Moreover, metformin activates the enzyme adenosine monophosphate kinase (AMPK) resulting in the inhibition of key enzymes involved in gluconeogenesis and glycogen synthesis in the liver while stimulating insulin signaling and glucose transport in muscles.[4] Besides lowering the blood glucose level, metformin may have additional health benefits, including weight reduction, lowering plasma lipid levels, and prevention of some vascular complications.[5] Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS/RNS) which by the transfer of their free unpaired electron causes the oxidation of cellular machinery.[6] In order to encounter the deleterious effects of such species, body has got endogenous antioxidant systems or it obtains exogenous antioxidants from diet that neutralizes such species and keeps the homeostasis of body. Any imbalance between the RS and antioxidants leads to produce a condition known as "oxidative stress" that results in the development of pathological condition among which one is diabetes.[7] large number of naturally occurring compounds have been identified as antioxidants e.g. (figure 1) thymol (a) and guaiacol (b) which are viewed as promising therapeutic agents for treating free radical mediated diseases.[8]

This study aims to investigate the effect of metformin derivatives of phenol on the blood sugar level of streptozotocin induced diabetic rat model.

MATERIALS AND METHODS

2.1 Chemicals and Instrumentation

All reagents and anhydrous solvents were of analar type and generally used as received from the commercial suppliers (BDH, England, Merck, Germany, Fluka AG, Switzerland and Sigma-Aldrich, Germany). Metformin was supplied from pioneer company, USA. Melting points were determined by using electro-thermal melting point apparatus, Stuart England. Thin layer chromatography was run on silica gel (60) F254, Merck (Germany), for checking the purity of the products and monitoring the progress of the reaction. The identification of compounds was done using IR spectra recorded on FT-IR spectrophotometer SHIMADZU 8400s, Japan in University of Al-Mustansiriyah at college of science. CHN elemental microanalysis was done using Perkin- Elmer model, Italy in AL-Byat University, Jordan. ^{1}HNMR bands were documented on 300 MHZ spectrometer in Al-Byat University, Jordan.

The antidiabetic activity was evaluated using streptozotocin induced diabetic rat model in Al-Israa university college, Iraq.

2.2 Synthesis

2.2.1 Method of preparation of 2- Chloro-N- [2-(dimethylamino)-2-iminoethanimidoyl]acetamide (II)

0.01 Mole of metformin (I) was dissolved in methanol 50 mL, then TEA (0.01 mole) was added. The reaction mixture was stirred in ice bath and chloroacetylchloride (0.01 moles in 10 mL benzene) was added drop wise with continuous stirring over a period of 1 hr. at -10^0C, followed by reflux for 3 hrs. The mixture was cooled by addition of cold to give a solid product.[9]

The progress of reaction was checked by TLC using methanol: ammonium sulfate 10%: water (1:20:1) solvent system.[10]

(II) is a white powder which was produced with percent of yield equal to 68%, melting point at 272^0C, Rf value: 0.54. FT-IR spectra of II show peaks at 3000-3320 for NH stretching of amide overlapped with CH stretching vibration, 1680 for C=O of amide and a broad band at 1548 for C=N of imine.

2.2.2 Method of preparation of N-[2- (dimethylamino)-2-iminoethanimidoyl]-2-phenoxyacetamide (III a, b)

A reaction assembly was arranged on Magnetic Stirrer. The Phenol (0.01mole) dissolved in sufficient quantity of acetone and anhydrous K2CO3 (0.01 mole) were placed in Iodine flask and were Refluxed for 1hr. After 1hr the chloro compound (2-chloro-N-[2-(dimethylamino)-2-iminoethanimodoyl] acetamide) (0.01 mole) dissolved in dry acetone was added to the above reaction mixture along with Pinch of KI(200mg). The reaction mixture was stirred by magnetic stirrer and was refluxed for 14-18hrs.[11]

The progress of reaction was checked by TLC using methanol: ammonium sulfate 10%: water (1:20:1) solvent system.[10]

(III a, b) compounds are faint yellow powders which were produced with percent of yield equal to 53% and 60% respectively. melting point at 260^0C and 275^0C, Rf value: 0.34 and 0.44 respectively. FT-IR spectra of IIIa show broad peak extended from 3100- 3454 for OH

band overlapped with NH stretching of secondary amine,2850 for CH_3 stretching of thymol, 1666 for C=O of amide, 1600 for C=C aromatic and 1579, 1543 (C=N of imine).

FT-IR spectra of III b show broad band extended from 2800-3600 for OH overlapped with NH stretching of secondary amine and CH stretching vibration, a broad band at 1650 for C=O of amide overlapped with C=C aromatic and 1543,1498 (C=N of imine).

^{1}HNMR spectra of III a show signals at 0.93 δ doublet for the six protons of CH_3 thymol, 1.95 δ septet for CH proton of thymol, 2.50 δ singlet for three protons of thymol, 3.32 δ singlet for the six proton of N-CH_3, 4.63 δ singlet for two protons of CH_2, 5.5 δ singlet for phenolic proton, 7.10- 7.83 δ multiplet for aromatic protons and 8.0 δ singlet for amine and imine protons.

^{1}HNMR spectra of III b show signals at 3.05 δ singlet for the six protons of N-CH_3, 4.25 δ singlet for the two protons of CH_2, 4.54 δ singlet for OCH_3 of guaicol, 5.51 δ for phenolic proton, 6.83-7.10 δ multiplet for aromatic protons, 7.69 δ singlet for three protons of amine and imine functional groups.

2.3 Evaluation of antidiabetic activity

In vivo antidiabetic activity of the chemically synthesized compounds (III a, b) were evaluated using streptozotocin induced diabetes in rats.[12] Their evaluation for antidiabetic activity based on the percent decrease in blood glucose level in comparison to metformin standard treatment.[13]

2.3.1 Method of assessment

Albino rats of either sex weighing (200 ± 10 g) were supplied by National Center for Drug Control and Research, Iraq and were housed in Al-Israa university college/ department of pharmacy under standerized condition. Animals were fed commercial chaw and had free access to water and libitum. Diabetes was induced by intraperitoneal injection of sterptozotocin (60mg per Kg) and rats were divided into 4 groups (each group consists of 6 rats) as follow: Group 1: six diabetic rats served as positive control and treated with the vehicle (water). Group 2: six diabetic rats treated with metformin as reference substance in a dose of 150mg/kg dissolved in distilled water (orally via gavage needle).[14] Group 3-4: six diabetic rats treated orally via gavage needle with the tested (III a) and (III b) respectively. Each compound was dissolved in distilled water in doses that determined below:

150 mg/kg/165.62 = Dose/ M.Wt. of the tested compounds.

Table 1: compounds with their molecular weight

Compounds	Molecular weight	Dose mg/kg
metformin	165.92	150
III a	334.32	302.8
III b	308.23	279.16

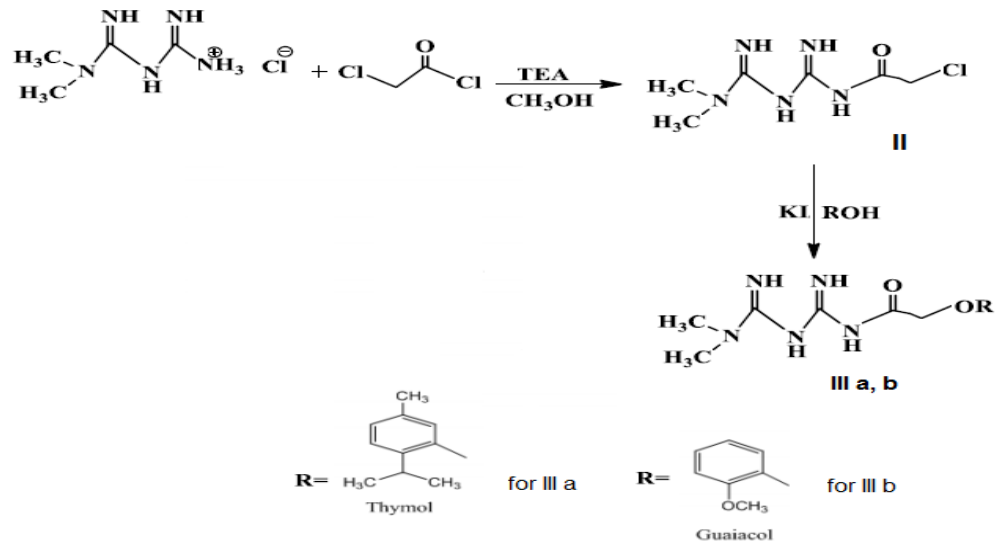
2.3.2 Experimental design

Rats were adapted for 7 days then diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/ kg), dissolved in distilled water and prepared for immediate use within 5 min. After 48 hours, rats were starved overnight and fasting blood glucose level was checked via glucometer (ACCU-CHECK). Rats with hyperglycemia (fasting blood glucose >100 mg/dl) considered diabetic. Metformin treatment and compounds (III a, b) were administered orally via gavage needle and blood glucose level for each rat was measured via glucometer at seven time intervals (0, 0.5, 1, 2, 4, 5 and 24 hr.) after drug administration.

2.3.3 Statistical analysis

The data are expressed as the mean ± SEM and analyzed for statistical significance using student t-test for comparison between mean values. Comparisons between different groups were made using ANOVA (one-way analysis using GraphPad prism 7 software) Probability (P) value of less than 0.05 was considered significant.

RESULT AND DISCUSSION



Scheme 1; Synthesis of intermediates and final compounds

The tested compounds showed significant decrease in blood glucose level after 5 hrs. of the treatment (*: $p<0.05$, **: $p< 0.001$). Compound (III a) produced higher percent of decrease in blood glucose level 63% compared to metformin (42%) at 5hrs while compound (III b) showed slow release property in which it appeared to decrease blood glucose level in a percent equal to 50% after 24hrs.

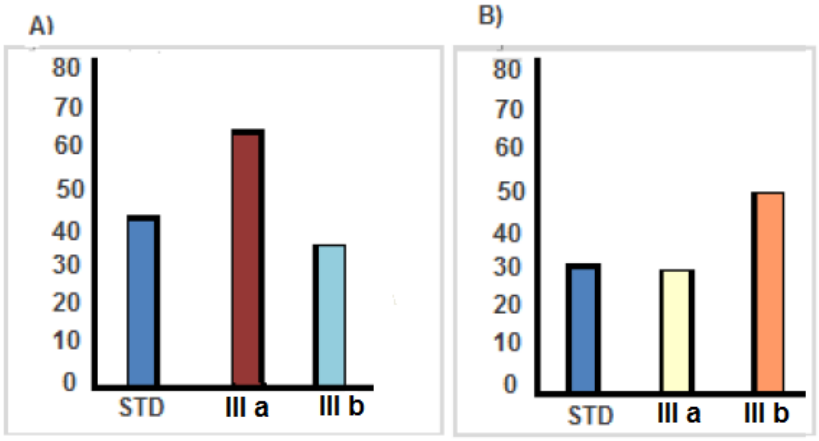


Figure 1: percent decrease in blood glucose level for metformin (STD) and the tested compounds (III a, III b) after A) 5hrs and B) 24hrs. Results are expressed as mean ± SEM (n= 6). ANOVA one-way analysis was used for statistical analysis.

CONCLUSION

In vivo antidiabetic activity revealed that incorporation of thymol and guiacol antioxidants into metformin enhanced it's antihyperglycemic activity which might be attributed to increase metformin bioavailability since metformin is a hydrophilic drug with net positive charge at physiological pH. Thymol and guaicol increase metformin lipophilicity which leads to increase bioavailability and as a result increase metformin absorption.

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