Uracil Substitution on a Hippuric Acid Containing 1,3,4-thiadiazole Scaffold: The Exploration of the Anti-Hyperglycaemic Potential

*Debarshi Kar Mahapatra, Kanhaiya M. Dadure, Animeshchandra G. M. Haldar

1Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur 440037, Maharashtra, India
2Department of Chemistry, J. B. College of Science, Wardha 442001, Maharashtra, India
3Department of Applied Chemistry, Priyadarshini Bhagwati College of Engineering, Nagpur 440009, Maharashtra, India

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Abstract:
The human civilization has witnessed diabetes mellitus as a curse which has already affected 400 million individuals and is expected to affect 600 million people by the end of 2030. Compromised pharmacokinetics, reduced pharmacological efficacy, etc. of the modern-day drugs has motivated researchers across the world to look for better alternatives. 1,3,4-thiadiazoles have been rising as a prominent scaffold in reducing the blood glucose level through various mechanisms. While moving towards the glorified path of drug design, a novel molecule with anti-diabetic interest was developed with an intention of having a better pharmacological profile than the existing drugs by substituting a uracil moiety at 5th position of a hippuric acid containing 1,3,4-thiadiazole scaffold and screened using streptozotocin-induced hyperglycemic method in Swiss albino rats. The uracil-containing 1,3,4-thiadiazole expressed an impressive hypoglycemic activity with a 28.89% reduction in the blood glucose level at 6 hrs. The compound also exhibited comparable pharmacological activity with that of the standard drug glibenclamide (39.12%) at 6 hrs. The compound may be believed to successfully reduce the glucose level by either an expression of PPAR-γ or inhibition of α-glucosidase. The research has opened new prospects in the rational designing of the next generation anti-hyperglycemic drug molecules with pronounced pharmacodynamics and pharmacokinetic effects.

Keywords: Antidiabetic, Antihyperglycemic, Hypoglycemic, Hippuric acid, Thiadiazole, Uracil.

Introduction

The human civilization has witnessed diabetes mellitus as a curse which has already affected 400 million individuals and is expected to affect 600 million people by the end of 2030 [1]. Although, five classes of therapeutic agents; dipeptidyl peptidase-4 (DPP-4) inhibitors, protein tyrosine phosphatase 1B (PTP1B) inhibitors, α-glucosidase inhibitors, aldose reductase (ALR) inhibitors, and peroxisome proliferator activated receptor-γ (PPAR-γ) activators [2] have been into applications for treating hyperglycemia, but several complications, like compromised pharmacokinetics, reduced pharmacological efficacy, etc. has motivated researchers across the world to look for better alternatives [3]. Drug discovery is a continuous process which aims at developing the best inhibitors having intense pharmacodynamics and pharmacokinetics attributes [4]. Thiadiazole is a vital
The structure of uracil gets liberated by the uracil moiety at 5th position of a previously reported compound from our group [10] with 6-(chloromethyl)pyrimidine-2,4(1H,3H)-dione (2) in presence of triethylamine under aprotic solvent dichloromethane under room temperature to obtain N-((5-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (3). The chemical reaction was a simple step where amine gets converted into amide where HCl (hydrochloride) gets liberated by the abstraction of a proton from compound (1) and chloride from compound (2). The neutrality of the media was maintained by triethylamine, the basic component, which is also a good nucleophile. The

Materials and Methods

Chemical and Instrumentation: The analytical grade reagents and solvents used in this study were exclusively obtained from Sigma-Aldrich, Germany through a local vendor. The progress of the chemical reaction was monitored by Merck silica gel G-coated thin layer chromatography plates. The structure of the final product was confirmed initially by the FT-IR spectrum, recorded in IRAffinity-1 instrument by utilizing the KBr discs. The mass spectrum was recorded on JEOL-JMS-DX 303 instrument. The 1H-NMR (400 MHz) spectrum was obtained on a Bruker spectropor NMR DPX-300 instrument, and calibrated using internal standard tetramethylsilane. The elemental analysis report was obtained on a Perkin-Elmer 240C analyzer instrument. The Glucose strips (One TouchTM) were purchased from a local pharmacy.

Animals: After receiving approval from the Department Ethical Committee and CPCSEA (1389/a/10/CPCSEA), the hypoglycemic potential of the uracil-containing compound was screened on Swiss male albino rat of weight in the range 150-250g, having age 5-7 weeks, provided free access to water, fed with standard rodent pellets, kept in polypropylene cage under hygienic conditions in the animal house under temperature 24–25°C, humidity 50–60%, and 12 hr of light and darkness.

Synthesis of target compounds: The present chemical synthesis involved reaction of N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide (1), with 6-(chloromethyl)pyrimidine-2,4(1H,3H)-dione (2) in presence of triethylamine under aprotic solvent dichloromethane under room temperature to obtain N-((5-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)methyl)amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (3). The chemical reaction was a simple step where amine gets converted into amide where HCl (hydrochloride) gets liberated by the abstraction of a proton from compound (1) and chloride from compound (2). The neutrality of the media was maintained by triethylamine, the basic component, which is also a good nucleophile. The chemical synthesis outline is described in the Scheme 1.

Scheme 1. Synthetic outline for uracil-containing 1,3,4-thiadiazole

Synthetic protocol for N-((5-((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) methyl) amino)-1,3,4-thiadiazol-2-yl) methyl benzamide (3)

0.01 M of N-((5-amino-1,3,4-thiadiazol-2-yl) methyl) benzamide (1) was dissolved in aqua-free methanol at a three-neck flask equipped with a stirrer. The solution was thoroughly stirred at high RPM for 20 minutes and triethylamine was dropwise added to the above content. Afterward, 6-(chloromethyl) pyrimidine-2,4(1H,3H)-dione (2) (0.01 M) in the form of a methanolic solution was added and the reaction mixture was stirred vigorously for the duration of 30 min. The final content was poured over crushed ice-water to filter off the solid product (3). The obtained product was thoroughly washed with cold water, air dried, and recrystallized suitably. 43% yield; FTIR (KBr) ν (cm⁻¹): 3155 (-NH, stretching), 3068 (C-H, aromatic), 1733 (C=O, stretching), 1681 (C=N, five-membered), 1635 (C=C, aromatic), 1546 (-NH, bending), 1472 (-CH2, bending), 1221 (C-N, stretching); 1H NMR (δ, ppm, CDCl3): 10.22 (19,
uracil amide, 1H), 8.14 (9, Amide, 1H), 7.5-8.2 (Aromatic, 5H), 6.18 (17, Amide, 1H), 4.53 (10, Methylene, 2H), 4.19 (14, Amide, 1H), 3.99 (15, Methylene, 2H). MS: M+ 358. Anal. Calcd. for C15H14N6O3S: C, 50.27; H, 3.94; N, 23.45. Found: C, 49.68; H, 3.31; N, 23.09

Anti-diabetic screening: The hypoglycemic potential of the newly synthesized compound was screened by using the protocol given by Mahapatra et al., 2017. Initially, the rats having the blood glucose level of 60-75 mg/dl were chosen and fasted overnight. For inducing hyperglycemia, streptozotocin (60 mg/kg) solution was carefully prepared in the citrate buffer of pH 4.5 and administered to the selected rats via the i.p. route. The blood glucose of the fasted male albino rats was estimated by utilizing the commercial glucose strips after the lapse of 48 hrs. The rats demonstrating a high blood glucose levels, i.e. 200–300 mg/dl, were chosen. The blood glucose levels were re-estimated on the 4th day to endorse a steady state of hyperglycemia in the experimental animals. The selected animals were divided into 3 groups, each comprising 6 rats. The control group (first) received 1% Gum acacia (carrier), the standard group (second) received glibenclamide, and the experimental group (third) received the uracil-containing compound at a dose of 100 mg/kg b.w. Here, an initial sugar load of 5 g/kg was administered orally and after 30 min interval, the test compound was fed similarly. The blood glucose level was monitored at time intervals of 0 hr, 1 hr, 3 hrs, and 6 hrs using the glucometer. The potential of the test molecule was determined according to the AUC method and expressed in percentage (%) [11].

Result and Discussion
Chemistry: The spectroscopic data supported the formation of the hybrid thidiazole compound. The synthesis of the uracil-containing compound was confirmed by the vanishing of the –NH2 peak at FT-IR spectra of (1) at 3264 cm-1. The presence of the two amide peaks at 10.22 ppm (at position 19) and 6.18 ppm (at position 17) of the uracil, additionally substantiate the fabrication of novel compound. Moreover, the amide stretching and amide bending in the FT-IR spectrum at 3155 cm-1 and 1546 cm-1 supported the formation of the desired pharmacophore. The carboxamide NH (at position 9) and five-membered heterocycle amide (at position 14) were noticed predominantly at 8.14 ppm and 4.19 ppm, respectively. The conversion of amine to amide was also suggested by the proton peak at 3.99 ppm, depicting the –CH2- linkage. Furthermore, the methylene component was observed chiefly at 1472 cm-1 in the FT-IR spectrum. The aromatic protons were located on the spectrum in the range of 7.5-8.2 ppm. The C-H and C=C of the aromatic portion were seen primarily at 3068 cm-1 and 1635 cm-1, respectively. These two above results authenticated the presence of an aromatic fragment. The C=N and C-N components of the five-membered heterocycle were corroborated by the FT-IR spectral peaks at 1681 cm-1 and 1221 cm-1, respectively. On analyzing the mass spectra, it was noticed that the base peak corresponded closely with the theoretically calculated molecular mass (358) of the compound (3), also with the appearance of several fragment peaks. Additionally, the % of the elements (C, H, and N) of the compound (3) matched in closed agreement with the theoretical calculated % in the CHN analyzer.

Hypoglycemic potential: The uracil-containing 1,3,4-thiazole expressed an impressive hypoglycemic activity with a 28.89% reduction in the blood glucose level at 6 hrs. The compound also exhibited comparable pharmacological activity with that of the standard drug glibenclamide (39.12%) at 6 hrs. The compound may be believed to successfully reduce the glucose level by either an expression of PPAR-γ or inhibition of α-glucosidase.

Conclusion
The present study revealed the hypoglycemic perspective of a newly fabricated uracil-containing 1,3,4-thiazole with an impressive and comparable activity with the standard drug glibenclamide. The novel fabricated molecule demonstrated a 28.89% reduction in the blood glucose level at 6 hrs either an expression of PPAR-γ or inhibition of α-glucosidase. The research has opened new prospects in the rational designing of the next generation anti-hyperglycemic drug molecules with pronounced pharmacodynamics and pharmacokinetic effects.

References
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