Synthesis and Investigating Hypoglycemic and Hypolipidemic Activities of Some Glibenclamide Analogues in Rats

Abbas Ahmadi\textsuperscript{a}, Mohsen Khalili\textsuperscript{b}, Khadijeh Khatami\textsuperscript{a}, Majid Farsadrooh\textsuperscript{a} and Babak Nahri-Niknafs\textsuperscript{a}

\textsuperscript{a}Department of Chemistry, Faculty of Science, Karaj Branch, Islamic Azad University, Karaj, Iran; \textsuperscript{b}Neurophysiology Research Center, Shahed University, Tehran, Iran

Abstract: Glibenclamide (5-chloro-N-(cyclohexyl)-1-[4-(cyclohexylcarbamoyl)] sulfamoyl]-2-methoxybenzamide, Glyburide, \textit{E}) is a well-known and potent second-generation of sulfonylurea oral hypoglycemic drug which is most widely used in type 2 diabetes recently. It acts upon pancreatic \(\beta\)-cells by stimulating insulin secretion in glucose and lipid-lowering activities. So far, many derivatives of \textit{E} have been synthesized by adding new structural moieties to its structure while preserving its binding affinity to the receptor before their anti-hyperglycemic and anti hyperlipidemic activities being evaluated. In this study, new analogues of \textit{E} after changing lipophilic side chain (5-chloro-2-methoxy benzamide) with 4-bromo-3, 5-dimethoxy benzamide and 2, 4-dichloro benzamide were synthesized. Also, their glucose and lipid-lowering activities were evaluated and compared to \textit{E} and Tolbutamide (a famous first-generation of sulfonylurea oral hypoglycemic drug) by the known procedures. Findings showed that chloride substitution on lipophilic side chain of Glibenclamide could possibly increase the affinity of drug for receptor/or its half life time that resulted in more lasting anti-hyperglycemic and anti lipidemic activities in diabetic rats. However, bromide substitution with additional methoxy groups in benzamide ring could slightly improve the anti-hyperglycemic potency of the new drug compared to the root drug (\textit{E}).

Keywords: Glibenclamide, Hypoglycemic and lipid-lowering effects, Sulfonylurea, Type 2 diabetes.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetes [1]. It is a metabolic disorder associated with three basic pathophysiological abnormalities: impaired insulin secretion, excessive hepatic glucose production, and insulin resistance in skeletal muscle, liver, and adipose tissue [2]. Insulin plays a key role in the pathogenesis of type 2 diabetes and is often associated with a variety of other disorders like obesity, dyslipidemia (hyper triglycerideremia), low levels of high-density lipoprotein (HDL) cholesterol, and hypertension [3, 4]. Investigations on hypolipidemic activity of anti-diabetic drugs are also available in literature [5-8]. Over the last forty years of oral therapy for type 2 diabetes mellitus has focused on sulfonylureas and biguanides [9]. Sulfonylureas (SU) are the most widely used as anti-diabetic agents which improve glucose levels by stimulating insulin secretion in the pancreatic \(\beta\)-cells [10-13]. There are two generations in the class of (SU) anti-diabetic drugs (first and second generations) where the latter is more potent. Glibenclamide (5-chloro-N-[4-\(\text{(cyclohexylcarbamoyl)}\) sulfamoyl]-2-methoxybenzamide, Glyburide, \textit{E}) is a well-known and potent second-generation type 2 of anti-diabetic drug [14]. So far, many derivatives of \textit{E} were synthesized and their anti-hyperglycemic and anti hyperlipidemic activities were evaluated [15-19]. According to SAR (Structure-Activity-Relationships) studies, the search for novel Glibenclamide derivatives is originated to influence the \textit{in vivo} behaviors by adding new structural moieties to its structure while preserving its binding affinity to the receptor (Scheme 1). [18] Therefore in this study, new derivatives of Glibenclamide, with changing 5-chloro-2-methoxy benzamide (B moiety in Scheme 1) by 4-bromo-3, 5-dimethoxy benzamide (additional methoxy with halogen groups, \textit{E}), and 2,4-dichloro benzamide (additional halogen groups, \textit{E'}) [20]) were all synthesized and their glucose and lipid-lowering activities evaluated and compared to \textit{E} and Tolbutamide (a famous first-generation of sulfonylurea oral hypoglycemic drug) by known procedures [21-23].

2. MATERIALS AND METHOD

2.1. General

2,4-dichloro benzoic acid, 4-bromo-3,5-dimethoxy benzoic acid, thionyl chloride, chlorosulfonic acid, ethyl chloroformate, cyclohexyl isocyanate, 2-phenethyl amine, dimethyl formamide (DMF), acetone and all other chemicals were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich chemical Co. Tolbutamide was synthesized according to our previous published article [24]. Melting points (uncorrected) were determined with a digital Electrothermal melting point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). \(^1\)H and \(^{13}\)C-
NMR spectra were recorded with a Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded with a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, U.S.A.) spectrometer. Mass spectra were recorded with an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Column chromatographic separations were performed over Acros silica gel (No.7631-86-9 particle size 35-70 micrometer, Geel, Belgium). Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. Sixty adult male Wistar rats, weighing 190-220 g, (Razi Institute, Iran) were used in the pharmacological testing.

2.2. Preparations (Scheme 2)

4-Bromo-3, 5-Dimethoxy-benzoyl chloride (A’)

SOCl$_2$ (8.65 ml) and a drop of DMF were added to 4-bromo-3, 5-dimethoxy-benzoic acid (0.015 mol). The mixture was refluxed for 72 hours and the excess of thionyl chloride was evaporated under vacuum to obtain the desired compound [25].

4-Bromo-3, 5-dimethoxy-N-(2-phenethyl) benzamide (B’)

A’ (0.014 mol) in chloroform was added dropwise to the chloroform solution (20 ml) of 2-phenylethylamine (0.014 mol) at 0°C. The reaction mixture was stirred for 24 hours. After completing the reaction by TLC monitoring, it was washed with 2 M HCl. The organic layer was dried with anhydrous sodium sulfate. Chloroform was removed under reduced pressure to give the pale viscous brown compound.

4-[(4-bromo-3, 5-dimethoxy benzamido) ethyl] benzene sulfonyl chloride (C’)

Chlorosulphonic acid (0.07 mol) was added dropwise to a cooled and stirred chloroform solution (30 ml) solution of B’ (0.009 mol). Stirring was continued for additional 24 hours in room temperature before it was poured into ice-water. The organic layer was separated and the aqueous layer was extracted with chloroform. The combined organic layers were dried over magnesium sulphate and the organic solvent was evaporated to yield the yellowish viscous compound.

4-[(4-bromo-3, 5-dimethoxy benzamido) ethyl] benzene sulphonamide (D’)

50 ml 25% ammonium hydroxide solution was added to C’ (0.007 mol) and the mixture was stirred for 2 hours in room temperature and additional 24 hours at 70 °C. Then it was extracted with water and chloroform to yield the yellowish brown crude.

N-[4-{(4-bromo-3, 5-dimethoxy benzamido) ethyl} phenylsulfonyl]-N’-cyclohexyl urea (E’)

A dried acetone solution (50 ml) of E’ (0.001 mol) and potassium carbonate (0.3 g) was refluxed for 1 hour. Cyclohexylisocyanate (0.001 mol) was added to the reaction mixtures and refluxed for additional 16 hours. Then, it was extracted with 10% HCl and the yellowish brown compound was obtained.

All the above procedures were repeated for synthesizing E’’ and its intermediates (A’’-D’’), according to the published methods [20, 26].

2.3. Pharmacological Methods

2.3.1. Animals

Initially, 60 adult male Wistar rats, weighing 190-220 g, (Razi Institute, Iran), with blood glucose under 150 mg/dl (which was measured by glucometer from a blood drop from the cutting tail) as non-diabetic animals were randomly selected and housed three to four per cage in a temperature-controlled colony room under a 12 hour light/dark cycle. Animals were given free access to water and standard laboratory rat chow (Pars Company, Tehran, Iran). All the experiments were conducted between 11a.m. and 4p.m. under the normal room light and 25°C. This study was carried out in line with the policies provided in the Guide for
the Care and Use of Laboratory Animals (NIH) and those in the Research Council at Shahed University of Medical Sciences (Tehran, Iran).

For inducing diabetes, a single dose of streptozotocin (STZ) (Sigma, U.K.) in 60 mg/kg [24, 25] (immediately before usage dissolved in 0.9% saline) was injected intraperitoneally to all selected animals (with serum glucose content of higher than 250 mg dl\(^{-1}\) were selected as diabetics for the following measurements [21-23]. LDL and a low-density lipoprotein (VLDL) cholesterol levels were calculated using following formula:

\[
\text{VLDL} = \frac{\text{Triglyceride}}{5} \\
\text{LDL} = \frac{\text{Total cholesterol} - \text{HDL cholesterol} - \text{VLDL}}{}
\]

3.2.3. Statistical Analysis

Measurement data were tabulated as means ± S.E.M. Comparisons were carried out using one way analysis of variances (ANOVA) followed by post-hoc Tukey test and p-value<0.05 as the level of significance.

3. RESULTS

3.1. Chemistry

In this study, some derivatives of Glibenclamide and its intermediates were synthesized and their glucose and lipid-lowering activities evaluated and compared to E and tolbutamide by the known procedures [21-23]. It is well known that the lipophilic side chain (5-chloro-2-methoxy benzamide) of E is critical for its better activity and its markedly increased selectivity of sulphonylurea receptors (SUR) [19]. Moreover, halogen and methoxy groups on phenyl ring in this moiety can enhance binding affinity and insulin secretion by the drug [18]. Accordingly, new E derivatives with more methoxy and halogen groups as well as their intermediates for increasing the binding affinity of SUR and insulin secretion from pancreatic islets were synthesized and evaluated to examine their glucose and lipid-lowering activities in rats. The known procedures for synthesizing the compounds A’ and A”-E” with the appropriate modifications were also used [20, 26, 27]. Spectroscopic data (IR, \(^1\)H and \(^13\)C NMR, Mass, CHN) confirmed the structure of the compounds B’-E’. The purity of the compounds was determined by TLC using ethyl acetate-hexane as the eluent.

3.1.1. Analytical data

3.1.1.1.4-Bromo-3, 5-dimethoxy-N-(2-phenethyl) benzamide (B’)

IR (KBr) cm\(^{-1}\): 3316, 2940, 1741, 1628, 1579, 1544, 1453, 1463, 1406, 1331, 1241, 1127, 1034, 699; \(^1\)H-NMR: 3.33-3.88 (2H, m), 3.77-3.87 (2H, m), 3.86-3.95 (6H, m), 6.87-7.2 (2H, m), 7.26-7.8 (4H, m), 7.98-8.02 (1H, m); \(^13\)C-NMR: 35.5, 41.25, 56.5, 103.1, 106.9, 126.6, 128.8, 135, 138.7, 156, 167.8; MS: m/z (Regulatory Intensity): 363 (14), 365 (15), 215 (8), 243 (98), 245 (100), 259 (49), 261 (50), 91 (27), 106 (9);
Anal. Calcd. for C\textsubscript{17}H\textsubscript{18}BrNO\textsubscript{3}: C, 56.06%; H, 4.98%; N, 3.85%. Found: C, 56.16%; H, 5.05%; N, 3.84%.

3.1.1.2. 4-[(4-bromo-3,5-dimethoxy benzamido) ethyl] benzene sulfonyl chloride (C’)

IR (KBr) cm\textsuperscript{-1}: 3305, 2944, 1687, 1582, 1461, 1408, 1331, 1229, 1122, 1069, 1007, 850; \textsuperscript{1}H-NMR: 2.96-3.14 (2H, m), 3.50-3.84 (2H, m), 3.93-3.97 (6H, m), 6.83-7.33 (7H, m), 7.9-8.1 (1H, m); \textsuperscript{13}C-NMR: 38.9, 40.9, 56.7, 103.1, 105.5, 127.4, 130, 140.1, 143.8, 157.1, 165.9, 169.8; MS: m/z (Regulatory Intensity): 463 (3), 461 (2), 217 (40), 215 (33), 243 (73), 245 (62), 260 (94), 262 (100), 288 (82), 290 (86), 177 (11), 175 (9), 77 (48); Anal. Calcd. for C\textsubscript{17}H\textsubscript{17}BrClNO\textsubscript{5}: C, 44.12%; H, 3.70%; N, 3.03%. Found: C, 44.18%; H, 3.75%; N, 3.08%.

3.1.1.3. 4-[(4-bromo-3,5-dimethoxy benzamido) ethyl] benzene sulfonamide (D’)

IR (KBr) cm\textsuperscript{-1}: 3308, 2940, 1624, 1579, 1460, 1407, 1335, 1237, 1123, 1035, 594; \textsuperscript{1}H-NMR: 2.91-3.76 (4H, m), 3.85-3.88 (6H, m), 7.1-7.74 (6H, m), 8.6-8.8 (1H, m); \textsuperscript{13}C-NMR: 38.9, 47.9, 56.9, 104.1, 126.1, 126.7, 129.6, 135.4, 143.3, 144.1, 156.8, 166.8; MS: m/z (Regulatory Intensity): 444 (7), 442 (6), 243 (100), 245 (99), 259 (14), 261 (15), 361 (6), 363 (7), 200 (10), 202 (9), 77 (10); Anal. Calcd. for C\textsubscript{17}H\textsubscript{19}BrN\textsubscript{2}O\textsubscript{5}S: C, 46.06%; H, 4.32%; N, 6.32%. Found: C, 46.11%; H, 4.35%; N, 6.28%.

3.1.1.4. N-[4-2-(4-bromo-3,5-dimethoxy benzamido) ethyl] phenylsulfonyl-N’-cyclohexyl urea (E’)

IR (KBr) cm\textsuperscript{-1}: 3327, 2930, 2850, 1627, 1579, 1406, 1335, 1238, 1123, 640.5; \textsuperscript{1}H-NMR: 1.14-1.58 (10H, m), 2.47-2.84 (2H, m), 2.87-3.41 (3H, m), 3.84 (6H, m), 7.1-7.8 (6H, m), 8-8.1 (1H, m); \textsuperscript{13}C-NMR: 24.3, 25.7, 35.2, 38.9, 40.9, 47.9, 56.9, 103.2, 104.2, 126.7, 128.5, 129, 135.5, 140.2, 156.7, 157.1, 165.8; MS: m/z (Regulatory Intensity): 566 (11), 568 (5), 470 (6), 126 (56), 141 (16), 243 (7), 245 (6), 259 (4), 261 (3), 287 (7), 362 (94), 205 (11), 77 (31), 169 (100); Anal. Calcd. for C\textsubscript{24}H\textsubscript{30}BrN\textsubscript{3}O\textsubscript{6}S: C, 50.71%; H, 5.32%; N, 7.39%. Found: C, 50.76%; H, 5.35%; N, 7.34%.

3.2. Pharmacology

3.2.1. General Consideration

Mortality (number of death), morbidity (defined as any abnormal condition or behavior due to a disorder), irritability (a condition of aggressiveness or increased response on handling) and other related abnormal states were observed in experimental animals. However, the motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant differences between treated rats.

3.2.2. Blood Serum Glucose

There was no blood serum level glucose difference between the control and the treatment groups 4 days after STZ injection (Fig. 1). However on days 9 to 16 after STZ application, a significant reduction in glucose level was found between treatment and control animals. On day 9, 48.46, 68.20, 72.43 and 74.40 % decrement were found in serum glucose level in Br-derivative (E’), Cl-derivative (E”), tolbutamide and Glibenclamide (E) animal groups respectively in comparison with the controlled ones. However, the related values on day 16 after STZ injection reached 55.56, 70.28, 43.62 and 52.28 %. In addition, as shown in Figure 1, both Br and Cl-derivatives of E were able to yield more decremental effects on serum glucose level than other treatment groups.

![Fig. (1). The effects of Br-derivative (E’), Cl-derivative (E”), tolbutamide and Glibenclamide (E) on blood serum glucose on days 4, 9 and 16 after STZ injection. Bars show the mean ± SEM serum glucose (n = 12) in each group. * and $ P< 0.05 and show the difference compared to control and other treatment groups, respectively.](image-url)
groups on phenyl ring of this side of the molecule (E'' in comparison to E' and E). Regarding to halogen and methoxy groups responsibility for high affinity and selectivity joining to SUR, respectively, it might be concluded that adding two chloride atoms in R₁ and R₂ of benzamide ring yields a long half-life with higher anti-hyperglycemic activity drug (E''). Also, probably the high efficiency of E'' in reducing serum LDL level which results in a high HDL/LDL ratio as a good lipid profile is the outcome of high anti-hyperglycemic activity in this drug. Although the other liver, cytoskeleton, fat tissue and cellular LDL uptake direct mechanisms must be accounted.

5. CONCLUSION

The results by the present study showed that the additional number of chloride or methoxy substitution in lipophilic side chain of Glibenclamide could improve and reinforce the potency in these drugs (E' and E'') better and more markedly for decreasing the serum glucose and LDL level and increasing their lasting time. However, these effects for additional number of chloride substitutions were more than methoxy ones.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was done as a research project at Karaj Branch, Islamic Azad University. The authors would like to express their gratitude to both. They thank Fariba Ansari for her assistance with the pharmacological tests. They appreciate Dr. Natasha Pourdana, the certified editor in Journal of Language Education in Asia (LEiA), for proofreading the initial draft of this article. Finally, they would like to appreciate Mr. Mojtaba Chaichi for latter proofreading and language editing.

REFERENCES


[27] Rickenbacher, H.R. 1.5-bis-(2,4'-dihalo-benzamido)-4'-hydroxy(4,8-dihydroxy) anthraquinones. US patent, 3,439,003, 15, April 1969.


Received: March 20, 2013 Revised: December 16, 2013 Accepted: December 27, 2013