Chapter

Antidiabetogenic Features of Benzimidazoles

Alexander A. Spasov, Pavel M. Vassiliev, Vera A. Anisimova and Olga N. Zhukovskaya

Abstract

Literature data on the insulinogenic effect of 2-aminobenzimidazole prompted us to investigate its novel derivatives, particularly those containing an additional fused cycle in C1,2-α position, including imidazole, dihydroimidazole, or tetrahydropyrimidine ring. Consensus analysis of the hypoglycemic effect of these compounds performed with IT Microcosm and PASS system revealed that activity is mostly characteristic for N^9-2,3-dihydroimidazo[1,2-a]benzimidazole derivatives. Substructural analysis of hypoglycemic activity identified substituents that determine the greatest pharmacological effect. According to the in silico assessment of the ADME properties, RU-254 was nominated as a lead compound due to the most optimal calculated and experimental activity and pharmacokinetic parameters. Preclinical studies have shown that identified compound has a pronounced insulinogenic effect and hypoglycemic effect, both in intact animals and in animals with experimental diabetes mellitus. RU-254 also reduces the level of glycated hemoglobin upon chronic administration, slightly decreases the activity of DPP-4, and increases the average number of Langerhans islets in the pancreas. Pharmaceutical drug formulation of RU-254 was developed and investigated for pharmacokinetic, pharmacodynamic, and toxicological properties. The dosage form of the drug under the name limiglidol (compound RU-254, diabenol) was evaluated in the full cycle of clinical studies that confirmed the safety, tolerability, and prominent antidiabetic properties of the drug.

Keywords: in silico, IT Microcosm, consensus prediction, antidiabetic effect, aminobenzimidazoles, cyclic benzimidazoles, pharmacodynamics, pharmacokinetics, toxicology, diabenol

1. Introduction

The history of drug discovery for the treatment of diabetes mellitus was and still is strongly determined by achievements in the field of fundamental medicine. Initially, the role of the pancreas and islets of Langerhans in the development of this pathology was proved; later, the structure of insulin, insulin receptor, and glucose transporters was deciphered; the role of the liver glycogenolysis and gluconeogenic enzymes, contributing to increased glucose output and hyperglycemia, was established; molecular mechanisms for the development of insulin resistance, the importance of the incretin system and Na^+/glucose transporters in the kidneys, and intestinal α-glucosidase were revealed, which led to the introduction of novel antidiabetic drugs into clinic [1–4].

The basis of insulin resistance at the cellular level primarily resides in the disruption of insulin signaling pathway at the level of the insulin receptor and insulin receptor substrate (IRS) proteins. The underlying mechanism of this phenomenon is impaired phosphorylation of serine amino acid residues, catalyzed by a number of intracellular protein kinases. The muscles, liver, and adipose tissue are the primary target organs of concern for the development of insulin resistance [5]. It was established that the severity of insulin resistance correlates, first of all, with intracellular lipid accumulation [6]. It is intracellular lipids that hamper signal transmission from the insulin receptor and cause a decrease in insulin-dependent glucose uptake. The pivotal role of AMP-dependent protein kinase (AMPK), which is an energy "sensor" of the cell, is also established, since AMPK through TORCI, the first mTOR-based protein complex, serves as a metabolic switch between catabolic and anabolic processes of the cell. Metformin is a biguanide derivative, which is the first-line drug for the treatment of type 2 diabetes. In 2001, it was shown that the molecular mechanism of its action is at least in part mediated by AMPK [7]. It is believed that indirect activation of AMPK by metformin-induced Ser172 phosphorylation determines its pleiotropic effects [8].

At the same time, it is important to note that course of type 2 diabetes mellitus characterized by several consecutive phases. It begins with primary insulin resistance and compensatory hyperinsulinemia with the subsequent development of β-cell dysfunction, thus creating the need for administration of insulin secretagogues or insulin formulations at the late stages of the disease [9–11].

Given that the previous works described the insulinogenic effect of the anthelminthic drug mebendazole [12], which can be considered as a new scaffold (2-aminobenzimidazole or cyclic guanidine) that exhibits an insulinogenic effect, we performed an experimental study of the novel cyclic guanidine derivatives, designed by introduction of additional fused cycle (imidazole, dihydroimidazole, and tetrahydropyrimidine).

2. Results

The synthesis of novel 2-aminobenzimidazole (AmBI) [13, 14] derivatives and fused benzimidazole derivatives was carried out, including N^9-imidazo[1,2-*a*]benzimidazoles (N^9-ImBI) [15–17], N^1-imidazo[1,2-*a*]benzimidazoles (N^1-ImBI) [18, 19], N^9-2,3-dihydroimidazo[1,2-*a*]benzimidazoles (N^9-DhImBI) [20, 21], N^1-2,3-dihydroimidazo[1,2-*a*]benzimidazoles (N^1-DhImBI) [18, 19], and 2,3,4,10-tetrahydropyrimido[1,2-*a*]benzimidazoles (PrmBI) [22–24].

In order to identify the most promising antidiabetic substances using IT Microcosm [25, 26] and PASS computer systems [27, 28], a step-by-step detailed in silico analysis of the hypoglycemic properties of the new compounds was carried out. Programs DruLiTo [29] and QikProp [30] were employed to assess key ADME properties and characteristics.

Hypoglycemic effect of the newly obtained derivatives was initially studied in rats upon intraperitoneal administration at a dose of 50 mg/kg. Blood sampling was carried out 4 hours after treatment with test compounds. Blood glucose concentration was determined with the glucose oxidase method using a commercial Glucose FKD kit [31]. The ratio of glucose concentrations in the blood plasma of the experimental and control group animals served as an indicator of hypoglycemic activity [32].

It was found that among condensed benzimidazole derivatives, a number of substances exceeded hypoglycemic activity of metformin, which served as a reference drug.

2.1 In silico study

IT Microcosm [25, 26] and PASS [27, 28] computer systems were employed to determine the most promising chemical class of compounds. A training set of known hypoglycemic drugs and a library of tested benzimidazole derivatives were subjected to a consensus prediction of the level of hypoglycemic activity. The average informativity coefficient K_{Pr} was calculated and used as a metric for comparison of AmBI, N^9-ImBI, N^1-ImBI, N^9-DhImBI, N^1-DhImBI, and PrmBI derivatives. K_{Pr} value ranges from 0 for inactive compounds to 5 for highly active compounds.

According to value of K_{Pr}, the potential of benzimidazole derivatives classes as sources of substances with hypoglycemic activity decreases in the following order: N^9-DhImBI (K_{Pr} = 4.50) > PrmBI (K_{Pr} = 4.25) > AmBI (K_{Pr} = 2.50) > N^1-ImBI (K_{Pr} = 2.00) > N^1-DhImBI (K_{Pr} = 1.25) > N^9-ImBI (K_{Pr} = 0.25) [33].

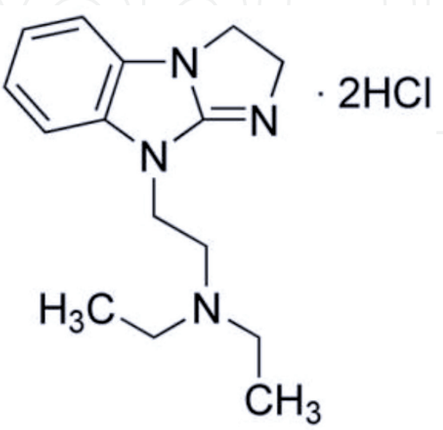
Thus, it was shown that N^9-2,3-dihydroimidazo[1,2-*a*]benzimidazole and 2,3,4,10-tetrahydropyrimido[1,2-*a*]benzimidazole derivatives have the most promising blood glucose lowering activity. That is, these tricyclic structures containing embedded guanidine group turned out to be more active than 2-aminobenzimidazole derivatives.

Subsequently, employing substructural analysis [34] and analysis via median [35] and supremal [36] estimates, the class of N^9-2,3-dihydroimidazo[1,2-*a*]benzimidazoles was selected as the most promising for the development of hypoglycemic compounds (**Figures 1** and **2**). It was shown that this scaffold is more preferable than 2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazole.

Substructural analysis [34] of the level of hypoglycemic activity among the N^9-2,3-dihydroimidazo[1,2-*a*]benzimidazole derivatives allowed us to reveal a chemical feature (substituent) that largely determines high hypoglycemic activity—diethylaminoethyl substituent at the N^9 atom of the N^9-DhImBI scaffold.

According to the frequency analysis of physicochemical parameters [37] of experimentally studied derivatives of N^9-DhImBI scaffold, a significant feature of high hypoglycemic activity was revealed—a charge on the internal imidazole cycle of the condensed system, namely, $Q(Imid1)_{cs} \geq -0.109$, which is a characteristic for compound RU-254 (diabenol).

Taken together, the results of a complex consensus in silico analysis of the hypoglycemic activity of six classes of benzimidazole derivatives revealed 9-diethylaminoethyl-2,3-dihydroimidazo[1,2-*a*]benzimidazole dihydrochloride (RU-254, diabenol) as the most promising highly active compound.



To assess the feasibility of a further study of the pharmacological properties of compound RU-254, we calculated parameters of drug-likeliness and ADME properties (absorption, distribution, metabolism, excretion) for RU-254 and reference antidiabetic drugs metformin and glibenclamide.

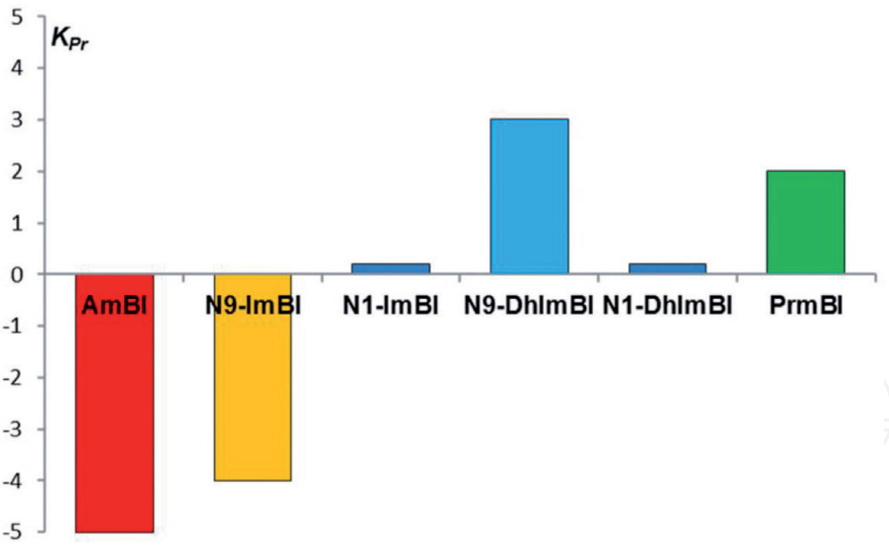


Figure 1.
Informativity coefficients describing the influence of basic benzimidazole structure on high hypoglycemic activity level (according to the substructural analysis).

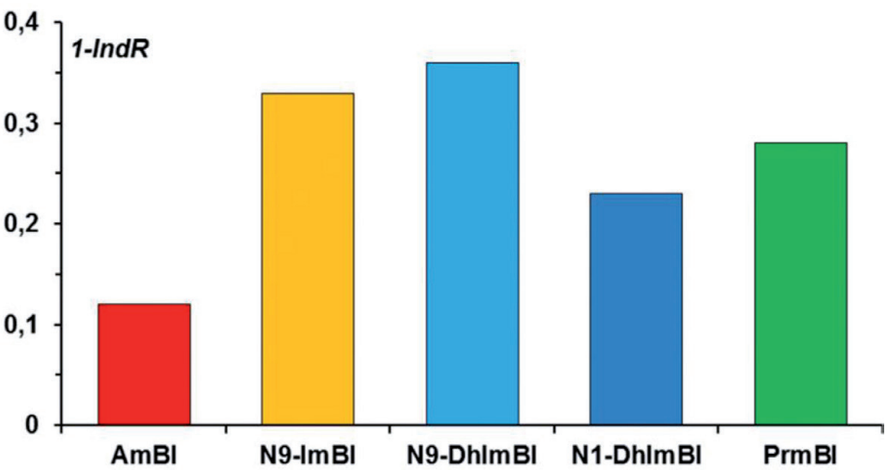


Figure 2.
Supremal evaluations of the effect of basic benzimidazole structure on high hypoglycemic activity level.

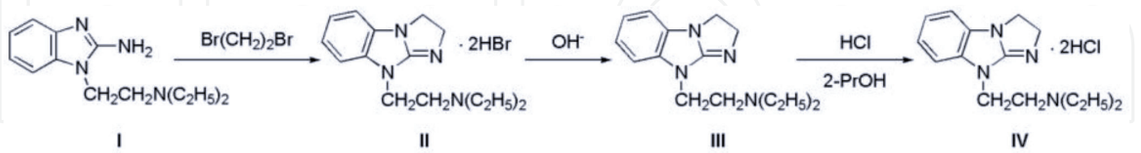
Using the DruLiTo program [29], it was found that diabenol satisfies the boundary conditions of all eight drug-likeliness filters, while metformin and glibenclamide correspond only for two of them.

Water solubility, serum albumin binding parameters, cellular permeability, and absorbability through the gastrointestinal tract for the three aforementioned substances were calculated with QikProp program [30]. A comparative analysis of the obtained characteristics showed that water solubility and the degree of binding to serum albumin of diabenol are higher than that of glibenclamide and lower than that of metformin. Indicators of bioavailability and absorbability through the gastrointestinal tract in diabenol are higher than that of glibenclamide and metformin. Thus, in terms of the total pharmacokinetic characteristics calculated in the QikProp program, diabenol is superior to metformin and glibenclamide. It should be noted that the calculated values of pharmacokinetic parameters of all three compounds are in the ranges that are recognized as appropriate for drug molecules.

Summarizing the results of the evaluation of ADME properties obtained using two computational approaches, it can be argued that diabenol in regard of its calculated drug-like and pharmacokinetic characteristics is not inferior to metformin and glibenclamide and is a very promising substance for performing advanced preclinical studies.

2.2 Synthesis

Synthesis of 9-diethylaminoethyl-2,3-dihydroimidazo[1,2-*a*]benzimidazole dihydrochloride (RU-254, diabenol) is readily realized through condensation of 2-amino-1-diethylaminoethylbenzimidazole with an excess of dibromoethane and subsequent transformation of the resulting of 9-diethylaminoethyl-2,3-dihydroimidazo[1,2-*a*]benzimidazole dihydrobromide to the base and the desired dihydrochloride [21].



Example. A stirred suspension of 69.6 g (0.3 mol) of 2-amino-1-diethylaminoethylbenzimidazole (I) in 104 ml (1.2 mol) of dibromoethane is gently heated in a glycerin bath. At 60–70°C, the initial amine dissolves completely, and at 100–105°C, an exothermic cyclization reaction occurs (the bath is set aside at the beginning), accompanied by strong boiling up of the reaction mass while temperature rises to 140°C and a heavy colorless precipitate begins to form. After 5–7 minutes, the reaction virtually ends, and, in order to complete it, the mixture is heated for an additional 20 minutes at 140–145°C. After that, 80 ml of DMF are added to the thick mass with vigorous stirring, and the mixture is heated for another 10–15 minutes. Cooling to 20–25°C, filtering the precipitate, and washing with DMF (3 × 20 ml) and acetone (3 × 25 ml) afford 106 g of dihydrobromide (II) in 84% yield. The latter is dissolved in 230 ml of water and boiled for 10 minutes with 3–5 g of activated carbon. Carbon is filtered off, and the filtrate after cooling is brought to pH 10 with 40% sodium hydroxide solution. The light yellow oil (III) which separates on the surface is extracted with toluene. The toluene extracts are washed with water and dried with anhydrous potassium carbonate. The desiccant is filtered off and washed with toluene. Combined toluene fractions are acidified by gradual addition of a saturated solution of hydrogen chloride in 2-propanol to pH 1. The heavy colorless precipitate of dihydrochloride (IV) is filtered off after 4–5 hours at 20–25°C, washed with acetone, and dried at 100–110°C for 2–3 hours to a constant weight. Yield is 75 g (90.9%) from dihydrobromide (III) and 75% from the initial amine (II). The crude product can be recrystallized from 2-propanol to pharmacopeia grade purity.

2.3 Preclinical studies

Diabenol had a pronounced hypoglycemic effect and antihyperglycemic activity in carbohydrate tolerance tests performed on white outbred rats and rabbits. The compound studied showed a marked decrease of glycemia in animals with impaired glucose tolerance (in rats with severe streptozotocin diabetes and insulin resistance syndrome), in rats with alloxan diabetes, and in rabbits with acute insulin deficiency, induced with administration of anti-insulin guinea pig serum. In experiments on pancreatomic dogs, diabenol did not reduce blood glucose but enhanced the hypoglycemic effect of exogenously administered insulin [38–40], thus confirming insulin-mediated mechanism of action.

Detailed study of antidiabetic action revealed not only pancreatotropic but also extrapancreatic components of diabenol action. Its pancreatotropic effect is determined by the enhancement of phase 1 insulin secretion, especially in glucose-stimulated conditions (**Figures 3** and **4**).

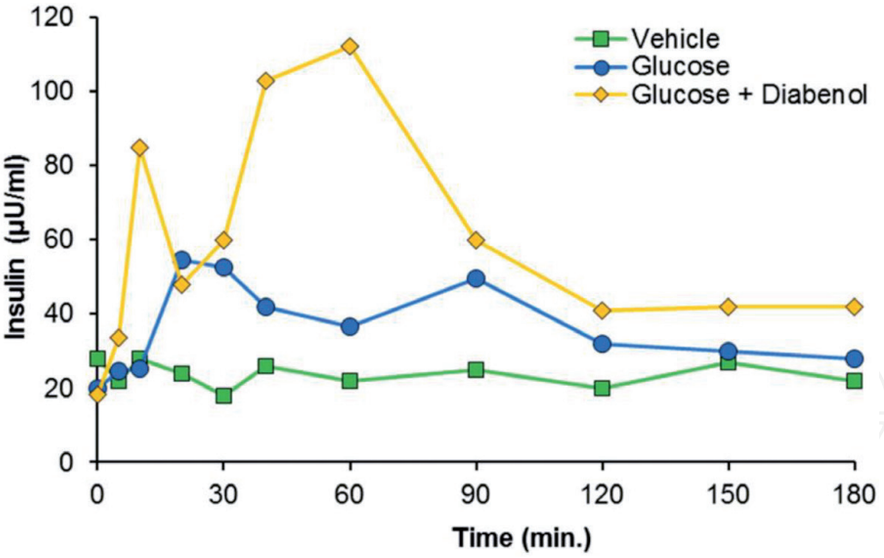


Figure 3.
Effect of diabenol (10 mg/kg, intravenously) in blood insulin levels during glucose tolerance test (1 g/kg) in cats.

Diabenol increases the insulin-dependent glucose uptake in muscles of rat diaphragm. Under conditions of alloxan-induced diabetes in rats, diabenol restored liver glycogen content and glycolysis rate and inhibited glycogenolysis in insulin-dependent organs and tissues (liver, striated muscles) while having no significant effect on these parameters in kidneys, which are insulin-independent organs [38].

It could be assumed that increased insulinotropic effect of diabenol is associated with a possible incretinomimetic effect. Studies [41] showed the ability of diabenol to inhibit the incretin-degrading DPP-4 enzyme, leading to a modulation of the insulin response. In our studies, diabenol also inhibited DPP-4, but in substantially higher concentrations (IC_{50} 1.35–2.05 mM), which cannot be achieved in the animal's body. Along with that, a 28-day administration of diabenol to rats with streptozotocin-induced diabetes was found to slightly and statistically insignificantly decrease the plasma activity of DPP-4 [42], which could be attributed to the action of its metabolites.

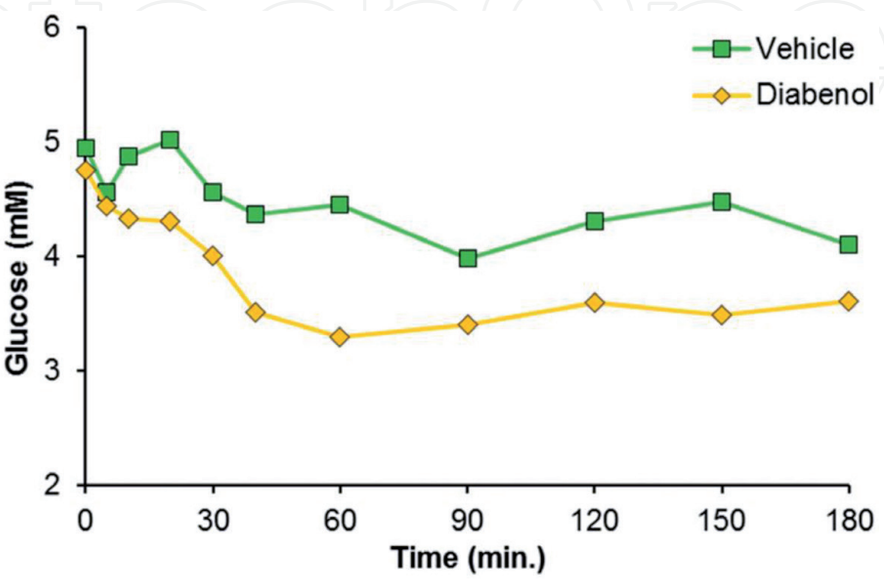


Figure 4.
Effect of diabenol (10 mg/kg, intravenously) on the basal portal vein blood glucose levels in cats [38].

Long-term administration of diabenol to streptozotocin-nicotinamide-induced diabetic rats allowed us to obtain interesting and valuable results. Oral administration of diabenol in a dose of 25 mg/kg for 4 weeks reduced blood glucose levels and volume of consumed liquid by more than 2 times, and level of glycated hemoglobin by 2.2%, and increased the content of C-peptide [43]. Moreover, diabenol administration resulted in a significant increase in the average number of islets of Langerhans in the splenic region of the pancreas and a significant increase in the area of the pancreatic β-cells (**Table 1**) [44].

The studied compound did not affect the apoptosis index (fraction of caspase-3 positive cells) and the proliferation index (PCNA-positive and Ki-67-positive cells) of the endocrinocytes of Langerhans islets. That is, diabenol had a cytoprotective effect on the cells. These data confirm the possibility of increasing the synthetic activity of β-cells under the influence of diabenol [44].

Given the complex nature of type 2 diabetes mellitus and aiming to increase the effectiveness of antidiabetic therapy in clinical practice, combination drugs (fixed combinations) are actively used to simultaneously target several key pathogenesis factors of the underlying disease or its complications [7, 9]. The optimal ratios of diabenol with metformin (1:4) and glibenclamide (5:1) were determined in experiments on rats with streptozotocin-nicotinamide-induced diabetes. Administration of these fixed combinations proved to be effective in terms of key metabolic markers, including blood glucose level, dynamics of glycated hemoglobin reduction, C-peptide level, and recovery of pancreatic β-cells, and has a positive impact on carbohydrate metabolism—liver glycogen content and glycogenolysis [45].

A very important aspect of diabetes pathogenesis is the activation of lipid peroxidation, which facilitates development of β-cell dysfunction and peripheral insulin resistance [11]. In order to address this issue in clinical practice, combination therapy regimens for diabetes have begun to include an antioxidant, for example, lipoic acid [46]. At the first stage of our study, the direct effect of some antidiabetic agents on free radical processes was studied in vitro. It was established that diabenol is a scavenger of superoxide anion, hydroxyl, and peroxyl radicals; rosiglitazone is active only against the superoxide anion, gliclazide has an antiradical effect in experiments with DPPH, and metformin and glibenclamide were unable to interfere with these processes. At the same time, the established direct antioxidant properties of some studied drugs are difficult to be expected in vivo, since they require relatively high concentrations to exert antiradical activity in vitro [47, 48].

Experimental groups	Islet area (μm^2)	Volume fraction of islets (%)	Relative number of β-cells (%)	Volume fraction of β-cells (%)	Nuclear area of β-cells (μm^2)
Intact control	15,448.2 ± 9819.4	11.0 ± 1.2	63.8 ± 7.2	74.2 ± 5.6	26.4 ± 3.7
Streptozotocin-nicotinamide-induced diabetes	12,801.5 ± 11,252.3	5.1 ± 2.3*	47.1 ± 3.5*	55.3 ± 6.1*	30.5 ± 6.2
Streptozotocin-nicotinamide induced diabetes +25 mg/kg diabenol	9559.6 ± 11,513.8	7.5 ± 1.5	54.3 ± 9.5	63.1 ± 4.6	25.4 ± 6.4

**Statistically significant compared with the intact control group.*

Table 1.
Morphometric parameters of pancreatic islets in splenic region of the pancreas of streptozotocin-nicotinamide-induced diabetic rats after administration of diabenol for 21 days (M ± m) [44].

A further study [48] determined the optimal ratios for a combination of diabenol and lipoic acid (2.8: 1 and 5.6: 1). Its activity was studied in a streptozotocin-nicotinamide-induced diabetic rat model. It was established that this combination possesses a more pronounced antidiabetic effect than monotherapy with diabenol. The more important finding of this study is a significantly reduced content of lipid peroxidation products in the liver, pancreas, and kidneys. In the pancreas under streptozotocin intoxication conditions, β-cells were significantly preserved by the combined treatment with diabenol and lipoic acid.

It is known that diabetes is associated with the increased thrombogenic potential of the blood and impaired rheology properties [49]. This effect is attributed not only to hyperosmolarity of the blood due to hyperglycemia but also to an increased aggregation of platelets and red blood cells. Among the antidiabetic agents used in clinical practice, only gliclazide has a direct inhibitory effect on platelets [8]. For other drugs, a similar effect is observed only with prolonged therapy. Given the fact that diabetes increases the frequency of thrombosis events, we studied the effect of diabenol on aggregation properties of platelets and red blood cells and its influence on microcirculation in experimental diabetes.

It was established that diabenol, both in vitro and in the conditions of the whole organism, has an antiplatelet activity. Probably, the effect on functional activity of platelets is determined by the influence of diabenol on balance of prostacyclin and thromboxane A2 systems. Diabenol showed an antithrombogenic effect on the model of thrombosis of the carotid induced by electric current and in systemic adrenaline-collagen thrombosis, exceeding the activity of gliclazide [50–52].

Diabenol reduced the aggregability and increased the erythrocyte deformability in normal conditions and, more profoundly, in experimental diabetes. Using fluorescent probes, it has been shown that diabenol was able to increase electronegativity and reduces the microviscosity of the red blood cells membrane, which results in the increase in their deformability [53–56].

Amelioration of thrombogenic potential and blood viscosity gives diabenol ability to enhance the survival of skin graft (a model of the diabetic foot) in both intact and alloxan-induced diabetic animals [57].

Toxicological study of the diabenol pharmaceutical substance and the dosage form (tablets containing 0.2 g of the active ingredient) involved acute and chronic toxicity, examination of cumulative properties, immunotoxicity, effects on carcinogenesis, and transplacental action. The therapeutic dose of the drug had no adverse effects. Subtoxic doses of diabenol lead to a sharp decrease in pancreatic β-cell secretion along with platelet hemorrhages.

Upon long-term administration of diabenol to low-cancer NMRI mice, transgenic HER-2/neu mice, and LIO rats with drinking water, no toxic or carcinogenic effect was observed. An interesting fact has been demonstrated, that is, in NMRI mice, diabenol delayed the development of age-related disorders of the extra function and increased the life span of animals. The drug inhibited the occurrence of spontaneous tumors, reduced the incidence of malignant lymphomas, and inhibited the onset and development of colon cancer induced with 1,2-dimethylhydrazine in rats. The authors of this study conclude that diabenol has an anticarcinogenic and geroprotective effect. Hence, both diabenol and metformin contain a guanidine group in their structure and exert experimentally proven antitumor effect and geroprotective activity [58–60].

The data on the pharmacokinetic study of diabenol established the values of the drug half-life and average retention time, which suggest that the substance undergoes significantly rapid elimination. The drug penetrates well into organs and tissues, especially in those with a high degree of vascularization. An important role in the processes of elimination of a compound is played by processes of its metabolism [61].

2.4 Clinical studies

Clinical studies of diabenol were conducted on 180 patients with type 2 diabetes. The drug was administered orally in solid dosage form (0.2 g tablets) 2 times a day. The study design was a randomized controlled comparative study of efficacy, tolerability, and safety. Glidiab (gliclazide) served as a reference drug. The course of diabenol administration ameliorated both fasting and postprandial hyperglycemia, reduced glycated hemoglobin level by 1.1% at the end of the third month, and increased postprandial insulin levels. Diabenol reduced platelet aggregation, increased erythrocyte deformability, and reduced their aggregability, thus normalizing coagulation hemostasis [62, 63].

Clinical and laboratory parameters of patients allowed to conclude that diabenol administered in a dose of 0.4 g per day for 3 months had no adverse or toxic effects [63].

3. Conclusions

Based on the studies performed, it can be stated that aminobenzimidazole is a universal privileged substructure that can be used as a source for development of novel antidiabetic agents. Introduction of structural modifications by means of transition to tricyclic structures led us to the identification of N^9-2,3-dihydroimidazo[1,2-*a*] benzimidazole as an optimal scaffold, and in this chemical class of compounds, agents with high glucose lowering activity were identified.

As a result, RU-254, or diabenol, was developed as the most active compound. Both in the experimental and clinical settings, it restores insulin secretion and ameliorates peripheral tissue glucose uptake. Another important effect of diabenol that has been established experimentally and confirmed in clinical studies is a reduction of thrombogenic potential and viscosity of blood. It has been demonstrated that diabenol, much like the biguanide derivative metformin, exerts an anticarcinogenic and geroprotective effect in rodents.

Thus, a new cyclic aminobenzimidazole derivative, diabenol, containing a guanidine group, combines pharmacological effects characteristic for both biguanide derivatives (reduction of hyperglycemia and liver glycogenolysis, improved glucose tolerance, anticarcinogenic and geroprotective effects) and for sulfonylurea derivative gliclazide (restoring insulin secretion, antiplatelet, and antiradical activity). It is quite possible that all of the identified effects are not a manifestation of the multi-target action but are pleiotropic effects of diabenol.

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