Conference paper

Alexander A. Spasov*, Pavel M. Vassiliev, Karina V. Lenskaya, Vera A. Anisimova, Tatyana A. Kuzmenko, Anatolii S. Morkovnik, Vadim A. Kosolapov and Denis A. Babkov

Hypoglycemic potential of cyclic guanidine derivatives

Directed search, pharmacology, clinics

DOI 10.1515/pac-2016-1024

Abstract: Guanidine derivatives are widely used antidiabetic drugs. Metformin (biguanide) is the first-line therapy for the type 2 diabetes mellitus due to its multi-target and pleiotropic effects. Compounds that comprise guanidine moiety integrated in a heterocycle, i.e. cyclic guanidines, represent an increasing area of interest. We have synthesized and studied hypoglycemic effects of a range of cyclic guanidines, namely 2-aminobenzimidazoles and structurally related imidazo[1,2-*a*]-, pyrimido[1,2-*a*]-, pyrrolo[1,2-*a*]-, triazolo[1,5-*a*]benzimidazole tricyclic derivatives. We have determined the potential of these scaffolds using molecular modeling and QSAR analysis. Experimental studies have shown that N^9-(diethylamino)ethyl-2,3-dihydroimidazo[1,2-*a*]benzimidazole (RU-254, diabenol) exhibits potent antidiabetic effects along with a low toxicity. We have found that diabenol exerts long-term glucose-lowering effects in prediabetic and diabetic animals, stimulates the first phase of insulin secretion and reduces the rate of liver glycogenolysis. Additionally, diabenol has been shown to exhibit antiplatelet, geroprotective and antitumor activities in animals. Clinical trials confirm that diabenol produces a range of antidiabetogenic effects such as improved glycemia, reduced HbA_{1c}, stimulation of insulin secretion, decreased thrombogenic potential of blood and ameliorated hemorheology.

Keywords: computer-aided molecular design; diabetes; heterocyclic chemistry; medicinal chemistry; Mendeleev XX; structure-activity.

Introduction

Type 2 diabetes mellitus (T2DM) is a widely recognized medical and social concern, which is due to the high prevalence, growing incidence, chronic nature, high disability and mortality rates of patients due to development of late vascular complications (micro- and macroangiopathies), as well as a need for specialized care system [1]. No less alarming is the fact that T2DM is ranked third among the primary death causes after cardiovascular diseases and cancer [2–4]. According to statistics, every 6–7 diabetes patients out of 10 die from the macroangiopathic complications. Accordingly, the socio-economic impact associated with the development of severe disabling complications is growing [5].

Article note: A collection of invited papers based on presentations at the XX Mendeleev Congress on General and Applied Chemistry (Mendeleev XX), held in Ekaterinburg, Russia, September 25-30 2016.

***Corresponding author: Alexander A. Spasov,** Volgograd State Medical University, Pavshikh Bortsov Sq. 1, Volgograd 400131, Russia, e-mail: aspasov@mail.ru
Pavel M. Vassiliev, Karina V. Lenskaya, Vadim A. Kosolapov and Denis A. Babkov: Volgograd State Medical University, Pavshikh Bortsov Sq. 1, Volgograd 400131, Russia
Vera A. Anisimova, Tatyana A. Kuzmenko and Anatolii S. Morkovnik: Southern Federal University Institute of Physical and Organic Chemistry, Stachka Ave. 194/2, Rostov-on-Don 344090, Russia

The current armamentarium against T2DM includes the following groups of drugs with a high level of clinical evidence: insulin secretagogues (sulfonylureas glibenclamide, glipizide, gliclazide, gliquidone, glimepiride); postprandial insulin secretion regulators (repaglinide and nateglinide); biguanides (metformin); insulin sensitizers (rosiglitazone, pioglitazone); incretin mimetics (vildagliptin, sitagliptin, saxagliptin). Nevertheless, there is an evident urge for more potent pharmacotherapies with a novel mechanisms of action [6–9].

Cyclic guanidines as a source of antidiabetic agents

Presence of a guanidine moiety in well-known alkyl and aralkyl biguanides (metformin, buformin, phenformin) has made it an indispensable anchor for the development of new antidiabetic agents. One of the first extensive studies in the field was performed by Ishikawa, Koasasayama and coauthors. They described synthesis and biological evaluation of a broad chemical class which is defined as a "cyclic guanidines" (Fig. 1), particularly, monocyclic imidazolines (**1**) [10], bicyclic imidazo[1,2-*a*]pyrimidines (**2**) [11], and tricyclic imidazo[2,1-*b*]quinazolines (**3**) [12]. Structure-activity studies revealed that guanidine fragment is a pre-

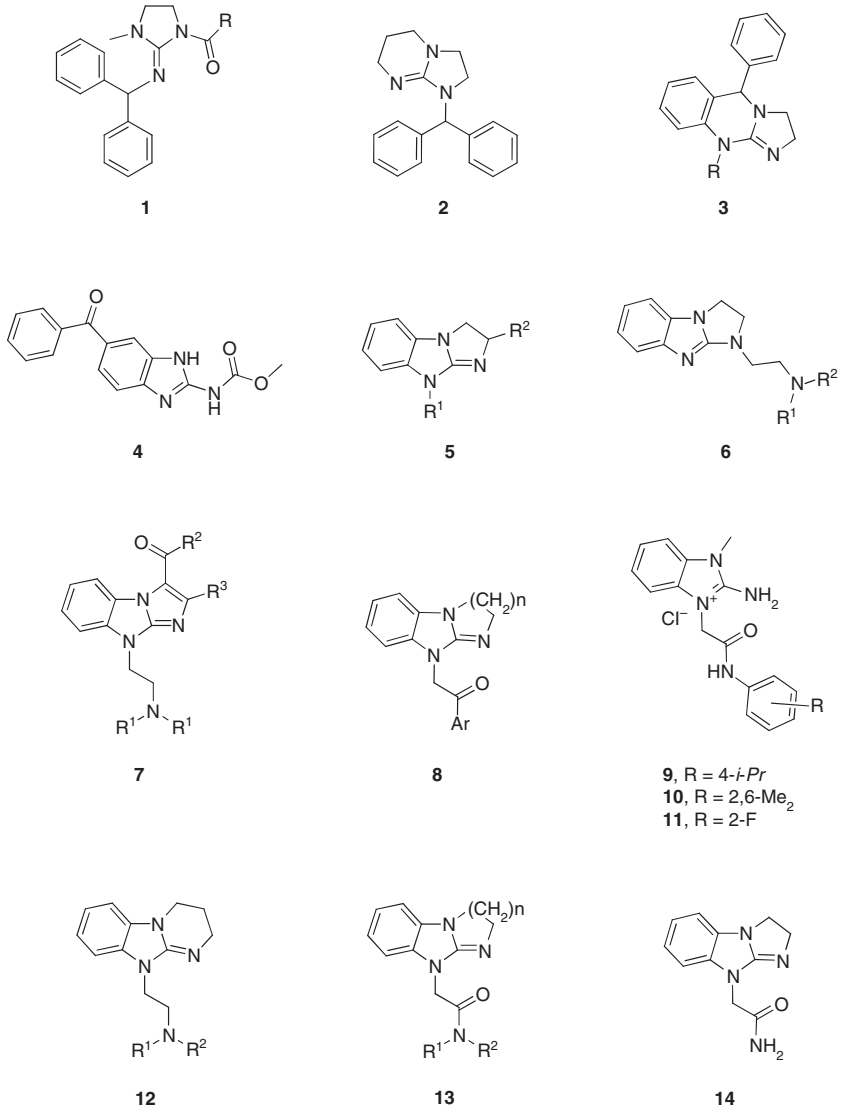


Fig. 1: Cyclic guanidines possessing hypoglycemic properties.

requisite for hypoglycemic activity, while bulky benzyl or benzhydryl substituents maximize insulinogenic properties. Also, a number of compounds were found to inhibit platelet aggregation.

Through serendipity, it was observed that antihelmintic drug mebendazole (**4**) improves glycemic control in diabetic patients through stimulation of glucose-induced insulin secretion [13, 14]. This finding influenced further research on a potential of 2-aminobenzimidazole scaffold [6], which is also considered as a cyclic guanidine.

Later, Anisimova et al. [15] reported the 2,3-dihydro-imidazo[1,2-*a*]benzimidazoles (**5**) as a hypoglycemic agents. Introduction of a methyl, ethyl or butyl as R substituent rendered the maximal hypoglycemic effect 4 h after administration. In case of a propyl or piperidinoethyl substituents the maximum effect was developed after 2 h. All 2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives **5** exceeded chlorpropamide (4-chloro-*N*-(propylcarbamoyl)benzenesulfonamide), but were less active than buformin (1-butylbiguanide).

As a development of this work, 1-aminoethyl-2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives (**6**) were synthesized and evaluated to explore the influence of the N^1 versus N^9 substitution [16]. It was established that dihydrochlorides **6** bearing pyrrolidine or piperidine moieties as NR^1R^2 caused statistically significant reduction of blood glucose levels exceeding that of the reference drugs chlorpropamide and buformin. Introduction of morpholine, cyclohexylamine or phenylamine residues is associated with a slightly lower activity comparable with chlorpropamide.

Later, several works were performed to confirm hypoglycemic potential of benzimidazoles. Among 14 synthesized 3-aroyl and 3-hetaroyl-imidazo[1,2-*a*]benzimidazoles (**7**) 6 compounds were found to lower blood glucose [17]. Noteworthy, derivatives comprising the N^9-(diethylamino)ethyl moiety are more potent than (piperidino)ethyl counterparts.

A series of aroylmethyl-substituted benzo[*d*]imidazo[1,2-*a*]imidazoles and related derivatives **8** were synthesized and found to be hypoglycemic as well. Structure-activity relationship study revealed that 4-hydroxyphenyl as R produces marked and sustainable hypoglycemia greatly exceeding the reference drug chlorpropamide [18].

A variety of heterocyclic compounds with a broad spectrum of pharmacological actions including hypoglycemic properties were obtained by introduction of an acetamide fragment [19]. It was shown that certain 3-(*N*,*N*-disubstituted)acetamido-N^1-substituted 2-amidobenzimidazolium chlorides produce marked and prolonged hypoglycemic effect. The highest hypoglycemic activity within 6-h period was associated with lipophilic NR^2R^3 moiety, e.g. alkyl substituted phenyl (4-isopropyl, 2,6-dimethyl), along with alkyl present in R^1 position. These substances (**9** and **10**) surpass the potency of the reference drugs glibenclamide and metformin in experimental animals. Replacement of the alkyl substituents in benzene ring with electronegative fluorine atom decreased hypoglycemic activity. Thus, compound **11** in terms of duration and level of a hypoglycemic effect did not exceed, but was only comparable to the glibenclamide *in vivo*.

The study of 10-(dialkylamino)ethyl-2,3,4,10-tetrahydro-pyrimido[1,2-*a*]benzimidazoles (**12**) confirmed their hypoglycemic action [20]. *N*-Acetamide derivatives of fused tricyclic benzimidazole systems comprising partially hydrogenated imidazole or pyrimidine ring also exhibit hypoglycemic activity. Out of 20 compounds tested nine compounds exceed the reference drug metformin in terms of plasma glucose lowering. Experiments established that substituted amides of 2,3-dihydro-imidazo and 2,3,4,10-tetrahydro-pyrimido[1,2-*a*]benzimidazolyl-*N*-acetic acids (**13**) exhibit different degrees of hypoglycemic activity [21]. Compounds bearing amine or piperidine as the NR^1R^2 substituent reduced blood glucose level stably for 6 h. The most active compound **14** significantly exceeded the activity of metformin and was comparable in strength to the hypoglycemic action of glibenclamide.

QSAR-guided lead selection

Substructural analysis of the hypoglycemic activity of cyclic guanidine derivatives

One of the problems to solve during drug discovery project is to identify the basic core among chemically diverse structures that is determinant of a particular activity [22]. Classification machine learning techniques have been used successfully to pursue this issue [23], in particular, employing the Bayesian substructural analysis approach [24].

In the course of our study, substructural analysis allowed to determine basic chemical structures responsible for the manifestation of high a hypoglycemic activity among discussed benzimidazole derivatives [25]. Totally, 109 substances of seven chemical classes were studied: 2-aminobenzimidazoles (2-AmBI, Fig. 2); N^9-imidazo[1,2-*a*]benzimidazoles (N^9-ImBI); N^1-imidazo[1,2-*a*]benzimidazoles (N^1-ImBI); N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazoles (N^9-DHImBI); N^1-imidazo[1,2-*a*]benzimidazoles (N^1-DHImBI); N^1,N^9-imidazo[1,2-*a*]benzimidazoles (N^1,N^9-DHImBI) and 2,3,4,10-tetrahydro-pyrimido[1,2-*a*]benzimidazoles (PrmBI). Ind_R value served as an indicator of hypoglycemic activity and was determined as an average lowering of blood glucose level of experimental animals 4 h after administration of the substance at a 50 mg/kg dose as compared to the control group.

In terms of Ind_R cluster analysis was performed with *k*-means method [26]. Twenty-two of the tested compounds belong to a class of a highly active compounds having $Ind_R < 0.82$. As a structural feature, responsible for the manifestation of a high hypoglycemic activity, we have considered the core heterocycles. Analysis of the data (Table 1) allowed us to conclude that N^9-DHImBI class is the most promising in terms of potency.

PrmBI and N^1-DHImBI derivatives can also be considered as a source of hypoglycemic agents, but the influence of this basic structure is not statistically significant enough. However, the introduction of substituents that define activity could result in a high hypoglycemic effect. According to the informativity coefficient 2-AmBI and N^9-ImBI classes were found to be definitely not promising. No final conclusions could be done for N^1-ImBI class since only three compounds were sampled, which is statistically insufficient.

Therefore, we concluded that N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazole class is the most likely to offer highly active hypoglycemic agents.

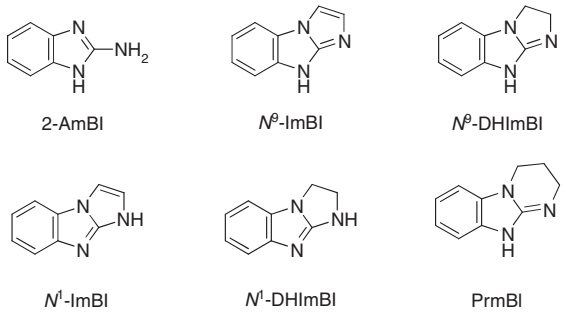


Fig. 2: QSAR-analysed cyclic guanidine classes.

Table 1: Substructural analysis of the influence of the heterocycle core on a high hypoglycemic activity.

Core structure	N^a	P_h^b	P_{nh}^c	Pr^d	K_{Pr}^e
2-AmBI	11	0.1125	0.8875	0.0008	−5
N^9-ImBI	22	0.3056	0.6944	0.0095	−4
N^1- ImBI	3	0.6068	0.3932	0.2824	0
N^9-DHImBI	35	0.5985	0.4015	0.0380	+3
N^1-DHImBI	8	0.5558	0.4442	0.3414	0
PrmBI	30	0.5848	0.4152	0.0851	+2

[a]Number of compounds.

[b]Frequency of occurrence of compounds with high hypoglycemic activity.

[c]Frequency of occurrence of compounds with non-high hypoglycemic activity.

[d]Significance of differences between P_h and P_{nh} according to binomial test.

[e]Informativity coefficient for differences between P_h and P_{nh}: 0 – $Pr > 0.2$; 1 – $0.1 < Pr \leq 0.2$; 2 – $0.05 < Pr \leq 0.1$; 3 – $0.01 < Pr \leq 0.05$; 4 – $0.001 < Pr \leq 0.01$; 5 – $0.001 < Pr$; positive values correspond to the positive influence and vice versa.

Analysis of the prospects of cyclic guanidine classes using median and supremal evaluations

A study was carried out to assign median [27] and supremal [28] evaluations in oreder to elucidate the impact of the heterocyclic core of a cyclic guanidine derivatives on the level of hypoglycemic activity. We analyzed 105 experimentally studied compounds of five chemical classes, which contained statistically sufficient number of derivatives. According to the median evaluations (Table 2) the following order of activity could be defined: N^1-DHImBI > PrmBI > N^9-imBI > N^9-DHImBI >> 2-AmBI. Median IndR value of 2-AmBI class corresponds with hyperglycemic activity. Other classes demonstrate rather subtle estimation differences. Thus, 2-aminobenzimidazoles were found to be the least potent, while the remaining chemical classes are quite similar to each other.

Liability of a pharmacological effect upon mutation of substituents is an important characteristic of a chemical class. In mathematical statistics, variance is used to assess variability of a random value, and can be defined with several kinds of supremal functions [29]. Additionally, supremal evaluations are of interest for drug design since they reflect the maximal activity exerted by derivatives of a particular chemical class.

In order to prioritize cyclic guanidine classes and thus maximize probability of identifying highly active antidiabetic agents we performed the supremal evaluations based on activity change upon introduction of different substituents. Results collected in Table 2 show that activity of N^9-DHImBI class is the most susceptible to the influence of substituents, i.e. derivatives of N^9-DHImBI are more likely to increase their potency in response to the substituents change. Less pronounced influence of substituents was observed for N^9-ImBI derivatives.

Considering the above mentioned analysis one can conclude that N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazoles are the most promising class of cyclic guanidines as a source of potential hypoglycemic agents due to both core heterocycle and potential of activity increase induced with substituents, which contribute to the potency.

Substructural analysis of the hypoglycemic activity of N^9-2,3-dihydro-imidazo[1,2-*a*] benzimidazole derivatives

We performed substructural analysis for the 56 experimentally studied N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives [25] aiming to establish the effect of substituents on a high level of hypoglycemic activity ($Ind_R < 0.82$). We considered substituents R^1, R^2, R^3 and type of acid HX as determinants of high activity (Fig. 3). Totally, 52 structural features were analyzed, and their impact on activity level was assessed with

Table 2: Median and supremal evaluations of hypoglycemic activity of the cyclic guanidine classes.

Core structure	Number of compounds	Ind_R median	Median cliff[a]	Maximal (1-Ind_R)[b]
N^1-DHImBI	7	0.87	–	0.23
PrmBI	30	0.88	0.01	0.28
N^9-ImBI	22	0.93	0.05	0.33
N^9-DHImBI	35	0.95	0.02	0.36
2-AmBI	11	1.10	0.15	0.12

[a]Difference between Ind_R values of the nearest classes.
[b]Relative blood glucose lowering effect in experimental animals, supremal evaluations.

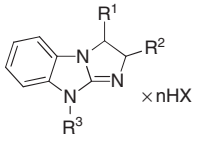


Fig. 3: N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives.

informativity coefficient K_{Pr}. As a result, 19 structural features were defined that contribute to a high hypoglycemic potency (Table 3).

Sixteen of them have a very slight, statistically insignificant impact on the high activity level. Two features have a little, statistically significant impact: phenoxymethyl in R^2 position affects adversely, and iodide as an acid residue affects positively. Diethylaminoethyl residue at R^3 was considered as a statistically significant determinant of a high level of hypoglycemic activity for N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazoles.

Frequency analysis of physicochemical parameters of N^9-2,3-dihydro-imidazo[1,2-*a*] benzimidazole derivatives

In order to identify additional QSAR that are specific for N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazoles with a high hypoglycemic activity ($Ind_R < 0.82$) we carried out the frequency analysis of a lipophilic, steric and electronic parameters of 56 tested compounds [30].

Lipophilicity was assessed as *logP* calculated using various fragment approximation methods for entire molecule and certain R substituents separately [31]. Molar refractivity *MR* served as a steric parameter, which values were calculated using classical additive scheme [32]. Atomic charges *Q* were calculated with AM1 approach after molecular geometry optimization with MM2 molecular mechanics [33]. A total of 40 physicochemical parameters were calculated.

The impact of each parameter was estimated by substructural analysis algorithm [25] and rated with the informativity coefficient K_{Pr}. Table 4 collects the most relevant physicochemical features of a high hypoglycemic activity ($K_{Pr} > +2$ threshold). Thus, 10 physicochemical parameters were found to reliably determine the presence or absence of a high hypoglycemic activity of the discussed compounds. The data obtained could direct further search for a novel highly active substances in N^9-DHImBI chemical class.

To sum up, the substructural, median and supremal analyzes of 109 experimentally tested cyclic guanidines rendered N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazole as a promising scaffold for a novel hypoglycemic agents due to the core heterocycle influence and potential to activity increase upon its decoration. Substructure analysis of 56 N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazoles revealed that diethylaminoethyl at N^9 favors hypoglycemic potency. According to a frequency analysis of the physicochemical parameters the charge on the inner imidazole ring $Q(Imid_1)_{CS} \geq -0.109$ is a significant sign of a high hypoglycemic activity.

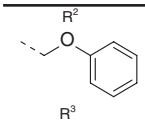
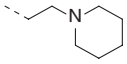
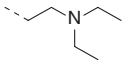
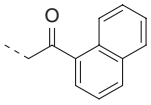
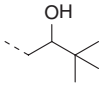
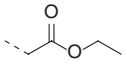
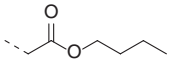
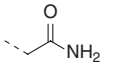
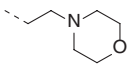
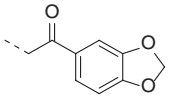
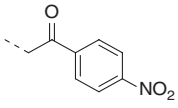
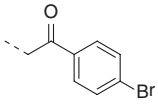
Thus, the results of computational analysis taken together led us to a conclusion that compound RU-254 (diabenol, 9-diethylaminoethyl-2,3-dihydro-imidazo[1,2-*a*]benzimidazole dihydrochloride, Fig. 4) represents all the attributes of a high hypoglycemic activity, and thereby is the most promising cyclic guanidine derivative that should be pursued to further evaluate its antidiabetogenic properties.

Experimental and clinical evaluation of RU-254 (diabenol)

The effect of diabenol on insulin secretion was subsequently studied *in vivo*. We have shown that diabenol increases plasma insulin concentration in lean cats more than four times as compared to the baseline at the 10th min of experiment reflecting recovery of the first phase of insulin secretion. This effect was maintained for 60 min, bringing insulin to an average of 80.7 mU/ml. At 60–90-min period insulin concentration decreased twice, and 30 min later it has dropped another 33.3%. On the 120th min the hormone concentration in the blood of cats returned to the original values [34].

Dose-dependent hypoglycemic effect of diabenol was determined in a single and repeated administration experiments. In rat studies ED_{30} of diabenol matches that of gliclazide and it exceeds glibenclamide 1.5 times. Rabbits treated with diabenol 2 h after administration produced more pronounced hypoglycemia than animals treated with gliclazide. After 4 h, diabenol exceled gliclazide by 10.1%. It has been found that duration of the diabenol hypoglycemic action is independent from experimental animal species and lasts 20–22 h, which is similar to the duration of sulfonylureas action.

Table 3: Substructural analysis of *N*⁹-2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives ($R^1 = H$).[a]

Structural feature	P_h	P_{nh}	Pr	K_{Pr}
	0.2407	0.7593	0.0932	−2
–Et	0.2910	0.7090	0.1828	−1
–Bn	0.7258	0.2742	0.1504	+1
–Me	0.6381	0.3619	0.1903	+1
	0.6381	0.3619	0.1903	+1
	0.7064	0.2936	0.0355	+3
	0.7258	0.2742	0.1504	+1
	0.7258	0.2742	0.1504	+1
	0.7258	0.2742	0.1504	+1
	0.7258	0.2742	0.1504	+1
	0.7258	0.2742	0.1504	+1
	0.2910	0.7090	0.1828	−1
	0.7258	0.2742	0.1504	+1
	0.7258	0.2742	0.1504	+1
 n HX	0.2910	0.7090	0.1828	−1
2 HNO_3	0.7258	0.2742	0.1504	+1
HCl	0.4171	0.5829	0.1110	−1
2 HBr	0.2910	0.7090	0.1828	−1
I^-	0.7142	0.2858	0.0899	+2

[a]Designations are the same as in Table 1.

It is important to note that diabenol has no effect on a blood glucose in dogs with removed pancreas, but combination of diabenol with exogenous insulin has more pronounced and long lasting effect than administration of insulin alone [34], suggesting that diabenol improves peripheral insulin sensitivity. Another

Table 4: Frequency analysis of the physicochemical parameters of N^9-2,3-dihydro-imidazo[1,2-*a*]benzimidazole derivatives.

Parameter[a]	P_h	P_{nh}	Pr	K_{Pr}
$0.029 \le Min(Q^+)_{CS} < 0.037$	0.7834	0.2166	0.0056	+4
$60.69 \le MR_0 < 75.04$	0.7373	0.2627	0.0146	+3
$13.16 \le MR_3 < 27.51$	0.7030	0.2970	0.0170	+3
$M(Q)_3 \ge 0.066$	0.7984	0.2016	0.0103	+3
$Min(Q^+)_{CS} \ge 0.037$	0.8267	0.1733	0.0191	+3
$Q(N_4)_{CS} < -0.232$	0.6964	0.3036	0.0326	+3
$0.111 \le M(Q)_2 < 0.156$	0.6119	0.3881	0.0900	+2
$0.030 \le M(Q)_3 < 0.066$	0.6863	0.3137	0.0634	+2
$0.069 \le Min(Q^+)_3 < 0.095$	0.6179	0.3821	0.0996	+2
$Max(Q^+)_{CS} \ge 0.240$	0.7394	0.2606	0.0570	+2
$Q(Ac_R) < -0.213$	0.6863	0.3137	0.0634	+2
$0.018 \le Min(Q^+)_3 < 0.043$	0.1478	0.8522	0.0071	−4
$-0.006 \le M(Q)_3 < 0.030$	0.3986	0.6014	0.0492	−3
$Max(Q^+)_3 < 0.156$	0.2882	0.7118	0.0372	−3
$0.014 \le Min(Q^+)_{CS} < 0.021$	0.2537	0.7463	0.0124	−3
$-0.384 \le Q(Het_1)_3 < -0.299$	0.3598	0.6402	0.0890	−2
$-0.185 \le Q(N_4)_{CS} < -0.139$	0.2118	0.7882	0.0538	−2

[a]$M(Q)_i$ – mean charge; $M|Q|_i$ – mean absolute charge; $Max(Q^+)_i$ – maximal positive charge; $Max(Q^-)_i$ – maximal negative charge; $Min(Q^+)_i$ – minimal positive charge; $Min(Q^-)_i$ – minimal negative charge; $Q(Het)$ – charge of heteroatom; $Q(N_1)_{CS}$ – charge of N_1; $Q(N_9)_{CS}$ – charge of N_9; $Q(N_4)_{CS}$ – charge of N_4; $Q(Benz)_{CS}$ – overall charge of benzene ring; $Q(Imid_1)_{CS}$ – overall charge of inner imidazole ring; $Q(Imid_2)_{CS}$ – overall charge of outer imidazole ring; $Q(Ac_R)$ – overall charge of acid residue with protonation of R; $Q(Ac_{Cond})$ – overall charge of acid residue with protonation of heterocycle.

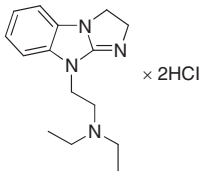


Fig. 4: Diabenol – compound RU-254.

evidence is the increase of glycogen content in insulin-dependent tissues along with increased glycolysis and reduced glycogenolysis, which was observed in rat alloxan-induced diabetes model.

Moreover, diabenol exhibits marked antithrombogenic action that was revealed in platelet-dependent thrombosis models [35]. In particular, diabenol efficiently blocked ADP-induced platelet aggregation, which is a key mechanism of thrombosis in T2DM, exceeding the effect of gliclazide. Inhibition of platelet aggregation induced with arachidonic acid and low concentrations of collagen was also observed along with a decrease in malonic dialdehyde formation [36]. This could lead to assumption that diabenol inhibits production of TXA_2 in platelets.

Experiments have shown the hemorheological activity of diabenol. We observed the charge increase of erythrocyte membranes, which causes a decrease in aggregation of cells during blood circulation, and increased microviscosity of erythrocyte membranes, which increases their plasticity. Erythrocyte aggregation induced with La^{3+} was reduced to a similar extent as with reference membranotropic drug pentoxifylline [37].

No toxic and cancerogenic effects were observed during the chronic administration of diabenol in NMRI mice with low cancer incidence, HER-2/neu transgenic mice, and LIO rats. Importantly, diabenol in NMRI mice retards the development of age-related extral functions disorders and increases life span of animals. Drug inhibited the occurrence of spontaneous tumors, reduced incidence of malignant lymphomas, hindered emergence and development of a colon cancer induced by 1,2-dimethylhydrazine in rats. Rats treated with diabenol developed colon adenocarcinoma (induced by carcinogen) with exophytic type of growth, higher

degree of differentiation and less invasion depth. That is, tested dosage of diabenol has experimentally proven antitumor and geroprotective effects [38].

Taking into account results of experimental studies we advanced diabenol to the clinical trials. During phase 1–2 it was established that hypoglycemic and hemorheological activities contribute to antidiabetic action of diabenol rendering it more efficient than the reference drug gliclazide [39]. Diabenol effect manifested in conditions of insulin resistance, dysfunction of pancreatic β-cells, increased blood viscosity syndrome, i.e. typical complex of T2DM condition. It was found that diabenol restores physiological profile of insulin secretion, reduces HbA_{1c} level, inhibits platelet aggregation and normalize lipid metabolism.

Phase 3 clinical trial was designed as randomized controlled comparative study of the efficacy, tolerability, and safety. Glidiab (gliclazide) was chosen as a reference drug. Upon chronical administration diabenol reduced fasting and postprandial blood glucose, reduced glycated hemoglobin level by 1.1%, and increased postprandial plasma insulin level by the end of the 3rd month [39]. Also, diabenol decreased platelets aggregation, increased erythrocytes deformability and reduced their aggregation, thus normalizing coagulation hemostasis. A significant decrease in blood viscosity was noted at a different shear rates that simulate blood flow characteristics of large and small diameter vessels. Diabenol decreased thrombogenic potential of blood by inhibition of platelet aggregation induced by ADP and arachidonic acid, which confirms earlier experimental data. During the investigation it was found that diabenol also affects coagulation hemostasis, normalizing both extrinsic and intrinsic pathways of blood clotting.

According to the clinical monitoring of patients, it could be concluded that administration of diabenol during 3 months had no adverse or toxic effects evident from biochemical blood and urine analysis but resulted in the sufficient hypoglycemic effect. Diabenol could be recommended for including in clinical practice as a hypoglycemic agent for the treatment of type 2 diabetes complicated with angiopathies.

Conclusions

A class of cyclic guanidine derivatives is a valuable and promising source of a potential antidiabetic agents. Experimental evaluation with subsequent computer-aided lead selection allowed us to identify the novel clinical candidate RU-254 – diabenol, which was found to stimulate insulin secretion resulting in a sustainable hypoglycemia in diabetic animals. It also inhibits platelet aggregation and demonstrates antitumor and geroprotective properties. Pronounced antidiabetic activity and tolerability of diabenol was confirmed in phase 3 clinical trial.

Acknowledgements: This work was supported by the Russian Science Foundation (project № 14-25-00139).

References

[1] I. I. Dedov, V. I. Kalashnikov, S. A. Terekhin, K. V Melkozerov. *Angiol. i Sosud. khirurgiia = Angiol. Vasc. Surg.* **18**, 9 (2012).
[2] *Diabetes and Cardiovascular Disease: Time to Act*, International Diabetes Federation, Brussels, Belgium, 90 (2001).
[3] L. Niskanen, A. Turpeinen, I. Penttila, M. I. Uusitupa. *Diabetes Care* **21**, 1861 (1998).
[4] J. E. Manson, G. A. Colditz, M. J. Stampfer, W. C. Willett, A. S. Krolewski, B. Rosner, R. A. Arky, F. E. Speizer, C. H. Hennekens. *Arch. Intern. Med.* **151**, 1141 (1991).
[5] I. I. Dedov. *Vestn. Ross. Akad. meditsinskikh Nauk / Ross. Akad. meditsinskikh Nauk* **2012**, 7 (2012).
[6] A. A. Spasov, I. N. Yozhitsa, L. I. Bugaeva, V. A. Anisimova. *Pharm. Chem. J.* **33**, 232 (1999).
[7] M. L. Mohler, Y. He, Z. Wu, D. J. Hwang, D. D. Miller. *Med. Res. Rev.* **29**, 125 (2009).
[8] P. V Bharatam, D. S. Patel, L. Adane, A. Mittal, S. Sundriyal. *Curr. Pharm. Des.* **13**, 3518 (2007).
[9] D. Kaiser, E. Oetjen. *Br. J. Pharmacol.* **171**, 2940 (2014).
[10] A. Kosasayama, Y. Watanabe, K. Higashhi, F. Ishikawa. *Chem. Pharm. Bull. (Tokyo).* **27**, 831 (1979).
[11] A. Kosasayama, T. Konno, K. Higashi, F. Ishikawa. *Chem. Pharm. Bull.* **27**, 841 (1979).
[12] A. Kosasayama, K. Higashi, F. Ishikawa. *Chem. Pharm. Bull.* **27**, 880 (1979).

[13] S. Caprio, T. K. Ray, G. Boden, G. A. Reichard, C. R. Shuman, R. H. Smith, M. A. Mozzoli, V. K. Dayal, R. D. Hoeldtke, O. E. Owen. *Diabetologia* **27**, 52 (1984).
[14] O. E. Owen, R. H. Smith, S. Caprio, M. A. Mozzoli, A. K. Rao, G. Litwack, T. K. Ray, G. Boden. *Metabolism* **34**, 567 (1985).
[15] V. A. Anisimova, M. V. Levchenko, G. V. Kovalev, A. A. Spasov, G. P. Dudchenko, S. G. Antonyan, N. V. Bessudnova, R. E. Libinzon. *Pharm. Chem. J.* **21**, 201 (1987).
[16] V. A. Anisimova, A. A. Spasov, I. E. Tolpygin, M. V. Chernikov, D. S. Yakovlev, I. I. Goryagin, N. A. Gurova, O. A. Salaznikova, L. V. Naumenko, V. A. Kosolapov, L. V. Eltsova, N. A. Kolobrodova. *Pharm. Chem. J.* **44**, 241 (2010).
[17] V. A. Anisimova. *Pharm. Chem. J.* **36**, 637 (2002).
[18] V. A. Anisimova, I. E. Tolpygin, A. A. Spasov, V. A. Kosolapov, A. V. Stepanov, A. A. Orlova, L. V. Naumenko. *Pharm. Chem. J.* **41**, 126 (2007).
[19] V. A. Anisimova, A. A. Spasov, V. A. Kosolapov, I. E. Tolpygin, L. V. El'tsova, A. F. Kucheryavenko, L. V. Naumenko, N. A. Gurova, K. V. Lenskaya, D. S. Yakovlev, D. V. Mal'tsev, T. M. Mitina, O. Y. Grechko. *Pharm. Chem. J.* **46**, 526 (2012).
[20] V. A. Anisimova, I. E. Tolpygin, A. A. Spasov, D. S. Yakovlev, N. A. Kolobrodova, N. A. Gurova, O. A. Salaznikova, L. V. Naumenko, V. A. Kosolapov, L. V. El'tsova, T. M. Mitina, M. P. Voronkova, K. V. Lenskaya. *Pharm. Chem. J.* **46**, 325 (2012).
[21] V. A. Anisimova, A. A. Spasov, V. A. Kosolapov, I. E. Tolpygin, E. V. Tibir'kova, O. A. Salaznikova, V. A. Kuznetsova, N. A. Gurova, K. V. Lenskaya, D. S. Yakovlev, D. V. Mal'tsev, N. A. Kolobrodova, T. M. Mitina, O. Y. Grechko. *Pharm. Chem. J.* **46**, 647 (2013).
[22] K. M. Merz, D. Ringe, C. H. Reynolds. *Drug Design: Structure- and Ligand-Based Approaches*, pp. 286, Cambridge University Press, Cambridge (2010).
[23] A. L. Gorelik, V. A. Skripkin. *Metody raspoznavaniya*, pp. 262, Vysshaya shkola, Moscow (2004).
[24] J. Gasteiger. *Handbook of Chemoinformatics*, pp. 1295–1299, Wiley-VCH Verlag GmbH, Weinheim, Germany (2008).
[25] P. M. Vassiliev, A. A. Spasov, K. V. Lenskaya, V. A. Anisimova. *Vestn. VolgGMU* **51**, 28 (2014).
[26] I. D. Mandel. *Cluster Analysis*, pp. 176, Finansy i statistika, Moscow (1988).
[27] K. V. Lenskaya, P. M. Vasilev, A. A. Spasov, V. A. Anisimova. *J. New Med. Technol. eJournal* **9**, 2 (2015).
[28] K. V. Lenskaya, P. M. Vassiliev, A. A. Spasov, V. A. Anisimova. *Vestn. VolgGMU* **54**, 98 (2015).
[29] G. A. Korn, T. M. Korn. *Mathematical Handbook for Scientists and Engineers: Definitions, Theorems, and Formulas for Reference and Review*, pp. 1130, North Chelmsford, Dover Publications (2000).
[30] K. V. Lenskaya, N. I. Cheplyaeva, P. M. Vassiliev, A. A. Spasov, V. A. Anisimova. *Proc. XX Nat. Congr. 'Man Drug'* **20**, 372 (2013).
[31] P. Broto, G. Moreau, C. Vandycke. *Eur. J. Med. Chem.* **19**, 71 (1984).
[32] B. V. Ioffe. *Refraktometricheskie metody khimii*, pp. 352, Khimia (1983).
[33] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart. *J. Am. Chem. Soc.* **107**, 3902 (1985).
[34] G. P. Dudchenko, A. A. Spasov, N. A. Gurova. *Vestn. VolgGMU* **6**, 46 (2000).
[35] A. A. Spasov, A. F. Kucheryavenko, M. V. Chepurnova, K. V. Lenskaya. *Reg. krovoobr. mikrocircul.* **10**, 95 (2011).
[36] A. F. Kucheryavenko, A. A. Spasov, V. I. Petrov, V. A. Anisimova. *Bull. Exp. Biol. Med.* **156**, 796 (2014).
[37] A. F. Kucheryavenko, A. A. Spasov, A. V. Smirnov. *Bull. Exp. Biol. Med.* **159**, 41 (2015).
[38] I. G. Popovich, M. A. Zabezhinski, P. A. Egormin, M. L. Tyndyk, I. V. Anikin, A. A. Spasov, A. V. Semenchenko, A. I. Yashin, V. N. Anisimov. *Int. J. Biochem. Cell Biol.* **37**, 1117 (2005).
[39] M. I. Balabolkin, A. A. Spasov, V. I. Petrov, V. A. Anisimova. *Proc. IV Nat. Diabet. Congr.* **4**, 88 (2008).