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Research Article

Antiplatelet activity of new derivatives of benzimidazole containing sterically hindered phenolic group in their structure

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Abstract

Introduction: Cardiovascular diseases are currently the leading cause of global disability and mortality. According to the centers for disease control and prevention, the average life expectancy of a person would be 10 years longer but for a high prevalence of cardiovascular diseases, and if antiplatelet drugs and special therapy were used.

Materials and methods: Antiplatelet activity of the novel benzimidazole derivatives containing a sterically hindered phenolic group in their structure has been investigated in vitro, using a model of ADP-induced platelet aggregation of rabbit's plasma. The compounds exhibiting high antiplatelet activity and acetylsalicylic acid, as a reference drug, were examined for antioxidant properties in an ascorbate-dependent model of lipid peroxidation.

Results: It was established that the compounds with high antiplatelet activity demonstrated the pronounced antioxidant action. The compound RU-1144 (1-(3,5-ditretbutyl-4-hydroxyphenyl) -1-hydroxypropyl)-phenyl-pyrimidobenzimidazole hydrochloride), in *in vitro* experiments, had a pronounced antiplatelet activity, surpassing the reference drug acetylsalicylic acid by 21.8 times; in the study of antioxidant activity, the leader compound was inferior to the reference drug dibunol by 1.7 times. By inhibiting intravascular platelet aggregation *in vivo*, this compound exceeded acetylsalicylic acid by 1.5 times and was slightly inferior to clopidogrel by 1.4 times.

Discussion: Benzimidazole derivatives with a hindered phenolic substituent in their structure exhibited antiplatelet and antioxidant properties. It was established that the compounds with high antiplatelet activity demonstrated the pronounced antioxidant action.

Conclusion: The chemical class of benzimidazole derivatives with a hindered phenolic substituent in their structure is promising for the search for new antiaggregant and antioxidant drugs.

Keywords

benzimidazole, antiplatelet activity, acetylsalicylic acid, antioxidant activity.

Introduction

One of the most relevant causes of deaths all over the world are cardiovascular diseases and their consequences. Even with the constantly improving quality of life and development of the pharmaceutical industry, the number of people suffering from cardiovascular diseases is growing every day. The process of blood clotting plays one of the most important roles in the pathogenesis of ischemic disorders in various organs and tissues of the human body and, thus, making the use of antiplatelet agents and other therapies for their treatment and prevention of vital importance (Gaba et al. 2018). The basic pathologies accompanied by inflammatory and atherosclerotic complications are the processes of macro- and microthrombogenesis, which can be the consequence of hyperreactivity of platelets and contribute to an increased risk of atherothrombotic events (Szabó et al. 2017). Recently active forms of oxygen and nitrogen have been identified as the main causal agents of this pathology. Reactive oxygen species, such as active forms of oxygen and nitrogen, play an important role in regulating platelet responses to collagen and are collagen-dependent on thrombus formation. But at the same time, they play a vital role in the lipid peroxidation, which can lead to an abnormally increased thrombogenic potential of blood. As a result, the rate of damaging the vascular endothelium and of atherosclerotic complications is increasing. Currently, the search for new antioxidant drugs to prevent the excessive formation of free radicals that are involved in the pathogenesis of many pathological conditions, such as hypoxic, ischemic and reperfusion injuries of organs, especially the brain, myocardium, and also ageing processes, is highly relevant (Spasov et al. 2013, Jang et al. 2015, Bisht et al. 2017, Kattoor et al. 2017).

The literature review (Baldisserotto et al. 2020), as well as the results of the studies previously conducted at the Department of Pharmacology and Bioinformatics at Volgograd State Medical University showed that there were heterocyclic nitrogen-containing compounds capable of blocking the aggregation of platelets and reducing lipid peroxidation (Kucheryavenko et al. 2014, Kucheryavenko 2016).

There have been also studies that proved that the main mechanism of antiplatelet action of the class of benzimidazole derivatives was the inhibition of thromboxane synthesis; the similar results were also obtained by foreign researchers (Chang et al. 2017, Houston et al. 2017, Baldisserotto et al. 2020).

Oxidative stress is proved to contribute to the development of cardiovascular diseases (Fuentes et al. 2019) and may play an important role in platelet activation. This may be due to the direct effect of oxidative stress on platelets, as well as to its indirect effect that causes the destruction of labile vessels, vascular agents originating from the endothelium, such as nitric oxide. Oxidative stress caused by platelets through several intracellular sources, which also affect vascular tone, is important for blood flow and blood clotting in blood vessels (Li et al. 2014, Kattoor et al. 2017).

Considering that oxidative stress is caused by the interaction of platelets and blood vessels (Sies 2015, Fuentes et al. 2019), it is of importance to search for and develop combination antiplatelet and antioxidant drugs (Reinisch et al. 2001).

It is known that in the treatment of pathological conditions of the cardiovascular system, combination therapy is used, not only when using antiplatelet agents of various groups, but also in combination with antioxidant drugs to prevent hypoxic conditions associated with heart attacks, strokes, etc. (Szabó et al. 2017, Gaba et al. 2018). That is why the dual use of antiplatelet and antioxidant drugs is highly recommended nowadays in the prevention of pathogenetic development of thrombosis (Anjum et al. 2018).

The chemical class of substituted heterocyclic benzimidazoles is considered to be the base structure of new drugs based on it, which was shown by a wide range of biological activity shown before (Spasov et al. 1997, Anisimova et al. 2002, 2006, Spasov et al. 2009, Kucheryavenko et al. 2014, Kucheryavenko 2016). The previous studies determined the ability of benzimidazole derivatives containing spatially hindered phenol in their structure to inhibit oxidative stress. In this connection, it appeared interesting to study antiplatelet activity in these compounds, since the creation of drugs combining antiplatelet, antioxidant activities may be promising for the treatment of pathologies associated with increased blood thrombogenic potential.

Some other previous studies determined the ability of benzimidazole derivatives containing spatially hindered phenol in their structure to exhibit pronounced antioxidant activity (Baldisserotto et al. 2020). That is why, in addition to studying an antiplatelet activity, these compounds were studied in the ascorbate-dependent lipid peroxidation (LPO) test.

However, the well-known antiplatelet drugs very often do not have a required activity, and also have a lot of side effects of varying severity (Guthrie 2011). That is why the need for searching for new inhibitors of the platelet aggregation process with a more pronounced activity and fewer side effects, remains highly relevant.

Materials and methods

This paper reports on an experimental study of the effect of 24 new benzimidazole derivatives containing spatially hindered phenols (Research Institute of Physical and Organic Chemistry of Southern Federal University) on platelet aggregation and ascorbate-dependent lipid peroxidation *in vitro*. The experiments were performed on 10 rabbits, weighing 3–3.5 kg, and 40 white outbred male rats kept in the vivarium (temperature 22–24 °C, relative humidity 40–50%) with natural illumination on a standard diet, following the rules of good laboratory practice when conducting preclinical studies in the Russian Federation, as well as the rules and international recommendations of *The European Convention for the Protection of Vertebrate*

Animals Used in experimental Studies (1997). All the procedures with the animals were carried out in accordance with the standards set forth in the eighth edition of *Guide for the Care and Use of Laboratory Animals* and ARRIVE (Animal Research: Reporting of *In Vivo* Experiments).

The antiplatelet activity was studied on the model of ADP-induced platelet aggregation according to the method described in (Born 1962) in modification of Gabbasov V.A. (Gabbasov 1989) on a laser platelet aggregation analyzer Biola 220 LA (Russia). The studies were performed on platelet-rich rabbit plasma, which had been obtained by the method of VA Lyusov. and Belousova Yu.B. (1971). Adenosine-5-diphosphoric acid (ADP) (Sigma, USA), at a final concentration of 5 μM, was used as an aggregation inducer. The tested compounds and the reference preparation – acetylsalicylic acid – were studied at a concentration of 100 μM. The level of aggregation was evaluated by a degree of aggregation, defined as the maximum increment of light transmission after the addition of the inductor. To evaluate the activity of the compounds, Δ% inhibition of the functional activity of platelets was determined. For the most active substances, a dose-dependent antiplatelet activity was studied to calculate IC_{50} (inhibitory concentration, inhibiting platelet aggregation by 50%), using the regression analysis method in Microsoft Excell.

The antioxidant activity of substances was studied in experiments *in vitro* on the model of ascorbate-dependent lipid peroxidation (Lankin 1975). The compounds were studied in a concentration range of 1×10^{-7}-1×10^{-5} M. As a substrate, 4% rat liver homogenate was used. The reaction was initiated with 50 mM ascorbic acid (Chemapol, Czech Republic). The oxidation rate was judged by the accumulation of malondialdehyde in the reaction with thiobarbituric acid (Fluka, Switzerland). The optical density of the coloured product was measured at a wavelength of 532 nm on a PD-303 UV spectrophotometer (APEL, Japan) in a cuvette with an optical path length of 10 mm. The activity of the substances was evaluated in% in relation to the sample without the compound. Dibunol (Merck, Germany) was used as a reference drug. The calculation of IC_{50} (inhibitory concentration, suppressing LPO by 50%) was performed using regression analysis in Microsoft Excell.

The effect of the substance on the functional activity of platelets in an *in vivo* test, with biological material being examined *ex vivo* according to the Born (1962) method, modified by Gabbasov (1989) on a Biola LA-220 laser platelet aggregation analyzer. For the test, blood was sampled from the abdominal aorta of 72 outbred adult male rats, weighing 250.0–300.0 g (anaesthetized with a solution of chloral hydrate (400 mg/kg)). Two hours before the study, the compounds and comparison drugs were administered intragastrically, using a metal atraumatic intragastric probe. All the investigated samples were dissolved in distilled water. The control group of the animals was injected with a solvent in an equivalent volume. Next, platelet-rich plasma was obtained, and the studies were carried out according to the method for studying the functional activity of platelets *in vitro* described above.

The method for analyzing the relationship between the antiplatelet and antioxidant activities of hindered phenols was carried out by the probabilistic histogram method (Mandel 1988). For this, all the studied substances were divided into classes with different levels of activity. To determine the boundaries of the class of the compounds with a high antiplatelet activity, a cluster analysis of data was performed, according to the Δ% indicator in the studied concentrations: highly active – $\Delta\% \geq 50\%$; moderately active – $\Delta\% \geq 25\%$ and low-active – $\Delta\% \geq 20\%$.

The toxicity study of the most active compounds was carried out following the requirements and instructions of the Federal Service for Supervision of Healthcare and Social Development (Makarov et al. 2012). Acute toxicity was determined on 75 white nonlinear male mice, weighing 20–22 grams, with intraperitoneal administration. The deaths of animals were recorded within two weeks. The toxicological indicator – LD_{50} was calculated according to the Litchfield-Wilcoxon's method.

In the last stage of the experiment, the dependence of the antiplatelet activity of benzimidazole derivatives having hindered phenolic substituent in their chemical structure was determined. Statistical processing of the experimental data was carried out using the Mann-Whitney criterion by means of GraphPad 5.0 and Microsoft Excell 2007 statistical software package.

Results

While searching for compounds with antiplatelet and antioxidant activity, 13 highly active compounds were identified among 26 new benzimidazole derivatives having a shielded phenolic substituent in their structure, which statistically significantly exceed acetylsalicylic acid. The antiplatelet effect of 2 substances was comparable to that of the reference drugs, the other 12 compounds were inferior to it by activity (Table 1).

In addition to the study of antiplatelet activity, these compounds were studied in the ascorbate-dependent lipid peroxidation test. Regarding the inhibition of lipid peroxidation, among the 26 tested compounds, 12 highly active substances were revealed that were comparable to dibunol (Table 1).

Among the most active 13 compounds, in relation to the inhibition of platelet aggregation *in vitro*, a dose-effect relation was studied for calculating IC_{50}, presented in Table 2. As you can see, the first three compounds showed the greatest activity.

The next study was on the correlation dependence of antiplatelet and antioxidant activities. In the group of the compounds with high antiplatelet activity, a positive correlation was observed towards the second type of activity (Table 3). The correlation coefficient for this group was positive and amounted to 0.73. When comparing other groups, this indicator did not confirm the correlation dependence.

Table 1. Effect of Benzimidazole Derivatives Having a Hindered Phenolic Substituent on ADP-induced (5 μM) rabbit platelet aggregation and on lipid peroxidation (LPO) *in vitro* (M ± m) (n = 6).

№	Tested compound	Inhibition of platelet aggregation (Δ%) at a concentration of 100 μm (Mean ± SEM)	Antioxidant activity at a concentration of 100 μm (Mean ± SEM)
1	RU-873	91.9 ± 4.31*#	61.8 ± 3.41*
2	RU-1144	91.9 ± 2.53*#	87.6 ± 6.52*@
3	RU-1263	86.5 ± 3.72*#	80.7 ± 2.34*
4	RUP-4b	86.1 ± 2.85*#	36.8 ± 5.21*@
5	RUP-7b	84.4 ± 6.36*#	35.4 ± 4.54*@
6	RUS-193	84.3 ± 4.39*#	87.0 ± 6.65*@
7	RU-871	82.0 ± 6.33*#	73.8 ± 2.23*
8	RU-1261	80.0 ± 8.11*#	67.9 ± 5.13*
9	RU-1249	77.7 ± 6.61*#	77.2 ± 7.85*
10	RU-903	69.9 ± 8.34*	76.1 ± 2.12*
11	RUP-6b	69.8 ± 7.91*	36.3 ± 4.65*@
12	RU-1180	67.7 ± 5.83*	88.8 ± 2.51*@
13	RUP-5b	65.9 ± 6.25*	43.9 ± 8.75*@
14	RUP-3b	45.3 ± 1.73*	31.1 ± 4.89*@
15	RUS-191	40.5 ± 4.52*	48.0 ± 3.98*@
16	RU-1250	36.4 ± 5.53*	0
17	RUP-2b	35.0 ± 1.85*#	46.8 ± 7.98*@
18	RUS-190	34.1 ± 4.89*#	19.5 ± 6.71*@
19	RU-1265	27.9 ± 5.37*#	87.6 ± 7.12*@
20	RUP-2	27.3 ± 4.33*#	61.7 ± 3.67*
21	RUCH-6	26.0 ± 1.91*#	20.4 ± 6.81*@
22	RU-1260	19.4 ± 7.02*#	65.4 ± 5.45*
23	RU-1251	13.7 ± 4.80*#	0
24	RU-887	12.8 ± 3.43#	0
25	RUS-198	9.1 ± 3.53#	30.1 ± 5.15*@
26	RUCH-2	3.7 ± 0.46*#	49.6 ± 6.49*@
27	Acetylsalicylic acid	53.1 ± 4.40*	–
28	Dibunol	–	85.8 ± 2.78*

Note: * – (p≤0.05) changes are statistically significant in relation to the control, Mann-Whitney test; # – (p < 0.05) changes are statistically significant in relation to the effect of the reference drug acetylsalicylic acid; @ – (p < 0.05) changes are statistically significant in relation to the effect of the reference drug dibunol; n – number of the animals tested.

Table 2. Inhibiting Activity (IC_{50}) of New Benzimidazole Derivatives and Acetylsalicylic Acid (Mean ± SEM) (n = 6).

№	Tested compound	Inhibition of platelet aggregation, Δ% (Mean ± SEM)			IC_{50}, μM
		Tested concentration, μM			
		100	10	1	
1.	RU-1263	86.5 ± 3.72†	45.4 ± 3.96†	38.3 ± 4.40†	5.3
2.	RU-1144	91.0 ± 2.53†	52.8 ± 0.96†	29.9 ± 4.60†	5.5
3.	RU-1261	80.0 ± 8.11†	53.5 ± 1.77†	32.8 ± 5.54†	5.9
4.	RU-871	82.0 ± 6.33†	49.5 ± 2.72†	25.5 ± 1.33†	8.3
5.	RUP-7b	84.4 ± 6.36†	49.9 ± 1.00†	15.1 ± 2.73†	10
6.	RU-873	91.9 ± 4.31†	31.0 ± 2.56†	18.6 ± 1.05†	12
7.	RUP-4b	86.1 ± 2.85†	38.0 ± 4.36†	19.2 ± 3.13†	12
8.	RUP-6b	69.8 ± 7.91†	44.0 ± 2.22†	22.7 ± 7.39†	16
9.	RU-903	69.9 ± 8.34†	36.0 ± 5.19†	29.5 ± 5.40†	17
10.	RUS-193	84.3 ± 4.39†	28.9 ± 5.70†	5.9 ± 0.97	18
11.	RU-1249	77.7 ± 6.61†	28.5 ± 2.44†	15.4 ± 2.16†	20
12.	RUP-5b	65.9 ± 6.25†	40.7 ± 3.04†	18.7 ± 4.59†	22
13.	RU-1180	67.7 ± 5.83†	34.7 ± 3.46†	26.4 ± 1.10†	23
14.	Aspirin	53.1 ± 5.40†	26.8 ± 1.77†	5.6 ± 1.25	120

Note: † – (p≤0.05) changes are statistically significant in respect to the control, the Mann-Whitney test; n – number of the animals tested.

Table 3. Ranking of Tested Substances by Correlation Indicators Between Antiplatelet and Antioxidant Activities on the Models of ADP-induced Platelet Aggregation and Ascorbate-dependent Lipid Peroxidation *in Vitro*.

Activity types	Correlation coefficient		
	Highly active	Moderately active	Low-active
Antiplatelet activity Antioxidant activity	0.730582	-0.65583	0.61985

The substances that showed the highest antiplatelet activity were selected to study the IC_{50} antioxidant activity, compared with that of dibunol (Table 4). The study showed that all the substances were inferior in this activity to the reference drug; however, the sample under code RU-1144 turned out to be the closest in this value to IC_{50}

To determine the leader compound. *In vivo* studies were performed to inhibit platelet aggregation of the three compounds under codes RU-1144, RU-1261, and

Table 4. Antioxidant Activity of Compounds RU-1144, RU-1261, RU-1263 on the Model of Ascorbate-dependent Lipid Peroxidation (*in Vitro* Experiments) (Mean ± SEM, n = 6).

Code	Inhibition of antioxidant activity (Δ%, Mean ± SEM)					IC_{50}
	1×10^{-5}	1×10^{-6}	5×10^{-6}	2.5×10^{-6}	1×10^{-6}	
RU-1144	87.6 ± 6.52*	79.71 ± 0.7*	61.13 ± 1.1*	48.53 ± 1.6*	35.36 ± 2.8	2.12×10^{-6}
RU-1261	67.9 ± 5.13*	59.71 ± 0.7*	44.71 ± 1.4*	24.20 ± 1.9*	6.25 ± 1.4	5.53×10^{-6}
RU-1263	80.7 ± 2.34*	76.38 ± 0.8*	53.30 ± 2.6*	31.09 ± 1.8*	17.33 ± 2.7*	4.13×10^{-6}
Dibunol	85.8 ± 2.78*	74.1 ± 3.12*	–	–	45.3 ± 1.8*	1.23×10^{-6}

Note: * – ($p < 0.05$) statistical reliability of differences in comparison with baseline

RU-1263, which showed the highest antiplatelet activity *in vitro,* in order to calculate the ED_{50} index. As a result, it was shown that, by this indicator, compound RU-1144 was superior to the other two substances and the reference drug acetylsalicylic acid, and was also comparable to clopidogrel (Fig. 1).

The presence of data from the *in vitro* studies and acute daily toxicity made it possible to further calculate the conditinoal range of the therapeutic effect (conditional therapeutic index (CTI)) (Table 5). Tested compound RU-1144 exceeded acetylsalicylic acid by CTI by 3.7 times.

Next, the dependence of antiplatelet activity on the chemical structure of the compounds was studied.

All the tested compounds that were studied are conjugates of 2,6-di-tret-butylphenol and a fused heterocyclic nucleus. The structure of the latter makes it possible to isolate 6 scaffolds: *1H*-benzimidazoles and salts of *1H*-benzimidazolium-3, N9-2,3-dihydroimidazobenzimidazoles, 3,5-dihydrotriazinobenzimidazoles, 2,3,4,10-tetrahydropyrimido-benzimidazoles, 2,3- dihydroimidazobenzimidazoles and 4H-triazole-benzimidazoles (Fig. 2).

The highest level of activity was more specific for 2,3,4,10-tetrahydropyrimidobenzimidazole derivatives. All 5 compounds of this group at a concentration of 100 μM blocked platelet aggregation by more than 70% and exceeded the reference drug acetylsalicylic acid. The most active of all the tested compounds of this class were RU-873 and RU-1144, which are hydrochlorides. RU-871 hydrobromide and RU-1249 succinate are 10% less active. The introduction of methyl substituents in the 7,8-dimethyl-2,3,4,10-tetrahydropyrimido-benzimidazole hydrobromide molecule RU-903 led to a loss of 20% of the activity. 2,3-dihydroimidazobenzimidazole hydrochloride RU-1180, which is the closest homolog of the leader compound RU-1144, also had rather a high activity (67.7%).

Besides, a high activity (65.9–86.19% in relation to the suppression of ADP-induced platelet aggregation) was noted in the compounds of the 1H-benzimidazolium salt group, especially those that are dihydrobromides (RUP-4b, RUP-5b, RUP-6b, RUP-7b) and hydrochlorides containing propyl (RU-1261) or propenyl (RU-1262) radicals in the N1 position. The other 1H-benzimidazolium hydrobromides showed a significantly less activity or were hardly active (RUCh-2, RU-1260). Other substituents (alkyl, benzyl, amino) were found in the cluster of both active and inactive compounds. The limited number of derivatives available for the study did not make it possible to make a conclusion about the contribution of each of them

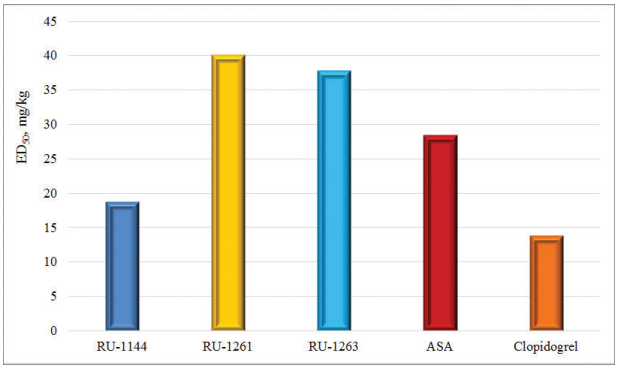

Figure 1. ED_{50} of antiplatelet activity of the compounds, acetylsalicylic acid (ASA) and clopidogrel with a single intragastric administration to male rats on the model of ADP-induced (5 μM) platelet aggregation.

Table 5. Antiplatelet Activity (IC_{50}), Acute Daily Toxicity (LD_{50}), and the Conventional Therapeutic Index (CTI) of New Benzimidazole Derivatives and Acetylsalicylic Acid.

№	Code	ED_{50}, mg/kg	LD_{50} mg/kg	CTI LD_{50}/ED_{50}
1	RU -1144	18.8	749.2	39.9
2	Aspirin	28.5	310.0	10.9

to the level of antiplatelet activity, which seem to indicate the presence of non-additive interactions among radicals.

The only derivative of 2,3-dihydrothiadiazinobenzimidazole RUS-193, where the hindered phenolic substituent is in the second position, showed a pronounced antiaggregant activity (84.3%). The least active (suppression of platelet aggregation by 40% or less at a concentration of 100 μM) are 1H-benzimidazole derivatives, containing a phenolic substituent in the N1 position, and 3,5-dihydrotriazinobenzimidazole derivatives RUS-190 and RUS-191, 4H-triazole-benzimidazole RUS- 198. It can be concluded that the increased electron density in the 3rd ring of the condensed heterocyclic system is an unfavorable factor.

Thus, the highest number of highly active compounds belongs to the group of derivatives of 2,3,4,10-tetrahydropyrimidobenzimidazole and 1H-non-imidazolium salts, whereas the representatives of the other scaffolds had no pronounced activity. An exception is active 2,3-dihydrothiadiazinobenzimidazole RUS-193, which, due to its planar structure, is very different from the other tested derivatives.

The group of substances derived from 1H-benzimidazoles is the largest in terms of the number of representatives, most of which showed a pronounced antiplatelet ac-

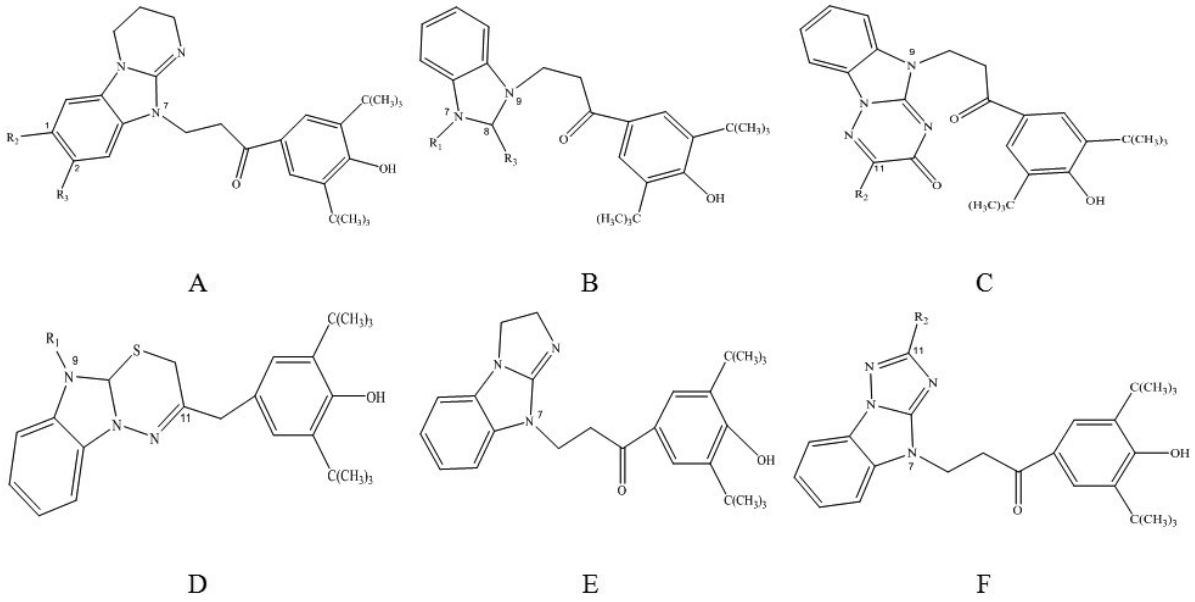

Figure 2. General formula of derivatives of N-7-ditretbutyl-4-hydroxyphenyl pyrimidobenzimidazoles (**A**), N-9-ditretbutyl-4-hydroxyphenyl benzimidazoles (**B**), N-9-ditretbutyl-4-hydroxyphenyl triazinobenzimidazoles (**C**), 11- 2,6-ditretbutyl-1-hydroxyphenyl-2,3-thiadiazinobenzimidazoles (**D**); N-7-ditretbutyl-4-hydroxyphenyl-N9-2,3-dihydroimidazobenzimidazoles (**E**) and N-7-ditretbutyl-4-hydroxyphenyl-triazolobenzimidazoles (**F**).

tivity. Most of the representatives of this group had a salt residue represented by hydrobromide in their structure. However, the compounds with dihydrobromides in the structures showed a higher activity. The tested samples of this group, in which the hindered phenol substituent was in the first position, did not show pronounced activity and were inferior to the reference drug. Compound 1-methyl-2- (3,5-di-tert-butyl-4-hydroxyphenyl) -propane-1-one-3-amine, in which the hindered phenol substituent was in the second position, while in positions 1 and 3 were methyl and amino substituents, did not have a pronounced effect on its antiplatelet activity either. The most active were the compoundes that in the second position had 1- 3,5-ditertbutyl-4-hydroxyphenyl)-propane-1-one. The activity of these compounds in the test of ADP-induced platelet aggregation was highest relative to the other compounds. The introduction of the methyl derivative into position 3 and N-ethyl piperidine or N, N-diethyl aminoethyl into position 1 led to a sharp increase in this type of activity. Benzyl in the 3rd position also increased the activity of the compounds. The other compounds of this group showed a low antiplatelet activity. Thus, the inclusion of benzyl into position 3 and 4-ethyl morpholine into position 1 of the structure reduced the activity. The only benzimidazole derivative, having the hindered phenolic substituent in the 3rd position and methyl and amine – in positions 1 and 2, showed no pronounced antiplatelet activity either. Moreover, the inclusion of benzyl and propenyl-1 into the structure of the compounds in the first position did not lead to an increase in an antiplatelet activity.

The next group of compounds represented by the common structure of triazinobenzimidazoles, where the hindered phenolic substituent was in the first position, and methyl or propanoic acid residue – in the second has no high antiplatelet properties.

The last two groups of substances, where the main structures were N9-2,3-dihydroimidazobenzimidazoles and triazolobenzimidazoles, and in the first position of each derivative there was a hindered phenolic substituent represented by 1-(3,5-ditertbutyl-4-hydroxyphenyl)-propane-1-on, showed a very low antiplatelet activity. However, a compound derived from N9-2,3-dihydroimidazobenzimidazole, having 2,6-di-tretbutyl-4-(1-hydroxypropyl)-phenyl in the first position and a hydrochloride salt as hydrochloride in the first position, showed a pronounced activity towards inhibiting ADP-induced platelet aggregation.

Discussion

Activation of platelet hemostasis and oxidative stress are among the main reasons for an increased blood thrombogenic potential.

The study of 26 new benzimidazole derivatives with a sterically hindered phenol in their structures showed that more than half of all compounds had a potential antiplatelet effect *in vitro*, which significantly exceeded the activity of the reference drug, acetylsalicylic acid. Earlier studies revealed a pronounced antioxidant activity in benzimidazole derivatives with a sterically hindered phenol in their structures (Venkatesan and Rao 2000, Wright et al. 2001). That is why besides studyinh the antiplatelet activity, these compounds were studied in the test of ascorbate-dependent lipid peroxidation (LPO). When searching for the most active compounds with antioxidant

activity, it was found that 12 benzimidazole derivatives were more active than dibunol, the other compounds were less active or inactive.

Moreover, in order to fully take into account the cooperative effect of the above factors on the formation of blood plate aggregates in the vascular bed and to evaluate more accurately the antiplatelet effect of the most active compounds, an *in vivo* study was performed. As a result, it was shown that substances under codes RU-1144, RU-1261 and RU-1263 had a high antiplatelet activity, but the most active compound in terms of ED_{50} was connection RU-1144. The tested compound RU-1144 in terms of CTI exceeds acetylsalicylic acid by 3.7 times.

The 26 compounds selected in this study belonged to the following 6 scaffold groups: 1H-benzimidazoles and salts of 1H-benzimidazolium-3, N^9-2,3-dihydroimidazobenzimidazoles, 3,5-dihydrotriazinobenzimidazoles, 2,3,4,10-tetrahydropyrimidobenzimidazoles, 2,3-dihydrothiadiazinobenzimidazoles and 4H-triazole-benzimidazoles.

All pyrimidobenzimidazole derivatives having ditretbutyl-4-hydroxyphenyl radical in position R1 had a pronounced antiplatelet activity and, in terms of Δ% of platelet aggregation inhibition, at a concentration of 100 μM, exceeded the reference drug acetylsalicylic acid. Also, the study propved the high antioxidant activity of these compounds, which was comparable to that of dibunol.

Derivatives of 1H-benzimidazole showed an antiplatelet activity of various intensity. Most compounds of this group were derivatives containing 1- (3,5-ditretbutyl-4-hydroxyphenyl) propane-1-on in the R2 position, which to various extents effected both antiplatelet and antioxidant activities. When this radical was moved to the R1 position, the compounds lost these two types of activities, and when the hindered phenolic substituent was moved to the R3 position, the antioxidant activity increased, whereas the antiplatelet activity decreased. Thus, only 6 compounds showed a pronounced antiplatelet activity superior to that of the reference drug acetylsalicylic acid. What these compounds had in common was having a hindered phenolic substituent represented by 1-(3,5-ditretbutyl-4-hydroxyphenyl) propane-1-on in position R2.

Triazinobenzimidazole derivatives were represented by 2 substances, having 1-(3,5-ditretbutyl-4-hydroxyphenyl) -propane-1-on in the R1 position, though no reliable data were obtained about this radical influencing in any was the activities under study, due to a small sampling of the substances.

When studying the group of derivatives of 2,3-thiazinobenzimidazoles, which contain a hindered phenolic substituent in position R2, only one active compound was found. Therefore, it was impossible to make any conclusions about this radical influencing the studied types of activities.

Thus, scaffolds based on N-7-ditretbutyl-4-hydroxyphenylpyrimidobenzimidazoles and N-9-ditretbutyl-4-hydroxyphenyl benzimidazoles exhibited pronounced antiplatelet and antioxidant activities, which were superior to those of following scaffolds: N-9-ditretbutyl-6,6-4-ditretbutyl-1-hydroxyphenyl-2,3-thiadiazinobenzimidazoles, N9-2,3-dihydroimidazobenzimidazoles, N-7-ditretbutyl-4-hydroxyphenyl-N9-2,3-dihydroimidazobenzimidazoles and N-7-ditretbutyl-4-hydroxyphenyl-triazolobenzimidazoles. With dislocating the ditretbutyl radical from positions N-7; N-9 and C-11, there was a decrease in antiplatelet and antioxidant activities in the above groups.

Thus, the ability of benzimidazole derivatives, having a ditretbutyl radical in their structures, to inhibit platelet aggregation processes and to prevent oxidative stress, makes them promising for further study of their antiplatelet activity.

Conclusions

Thus, as a result of studying 26 new benzimidazole derivatives with spatially hindered phenol in their structure, it was shown that three compounds under the codes RU-1144, RU-1261 and RU-1263 showed high antiaggregant and antioxidant activity.

Scaffolds based on N-7 ditretbutil-4-hydroxyphenyl of pyrimidinemethanol and N-9 ditretbutil-4-hydroxyphenyl of benzimidazoles showed pronounced antiplatelet and antioxidant activity, surpassing scaffolds: N-9 ditretbutil-4-hydroxyphenyl triazenoimidazole With-11-2,6-ditertbutyl-1-hydroxyphenyl-2,3-mediasentiment, N9-2,3-dihydroimidazole, N-7 ditretbutil-4-hydroxyphenyl-N9-2,3-dihydroimidazole and N-7 ditretbutil-4-hydroxyphenyl triazolopyrimidines. When the ditretbutyl radical is shifted to the position in N-7; N-9 and C-11 are represented in the groups there was a decrease in antiplatelet and antioxidant activity.

Compound RU-1144 exhibits a pronounced antiplatelet effect, combined with a high antioxidant activity, which makes it attractive for further in-depth study as a drug with a multi-target mechanism of action for the treatment and prevention of thrombosis.

Conflict of interest

The authors declare no conflict of interest.

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