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Condensed Benzimidazoles Are a Novel Scaffold for Antioxidant Agents' Search and Development

Vadim A. Kosolapov, Alexander A. Spasov, Vera A. Anisimova and Olga N. Zhukovskaya

Abstract

Taking into account that the imidazole ring has π-electron redundancy, condensed benzimidazole derivatives have attracted our attention as a promising class for the search for antioxidant substances. Synthesis was carried out, and information on the antioxidant activity of imidazo- and tetrahydropyrimido benzimidazoles was provided. Highly active antioxidant substance enoxifol has been revealed. The data on the synthesis and study of the pharmacodynamic, pharmacokinetic, and toxicological properties of the new antioxidant compound enoxifol are presented. The antioxidant activity of the compound is due to its ability to inactivate superoxide, hydroxyl, and peroxyl radicals, thereby reducing the overall oxidation rate due to a decrease in the total initiation rate. It has been shown that enoxifol has hepatoprotector, antihypoxic, cerebroprotective, nootropic, stress-protective, neuropsychotropic, actoprotective, cardioprotective, antiaggregant, and antithrombogenic properties and is able to prevent rheological disorders in diabetes mellitus.

Keywords: benzimidazoles, antioxidant, free radicals, enoxifol

1. Introduction

Currently, the fact that considered the leading role of free radical processes in the pathogenesis of more than 200 diseases has been established. Special attention is paid to damage to the brain tissue due to their particular sensitivity to disruption of oxygen balance and redox balance. The chain character of the reactions of lipid peroxidation (LPO) causes the appearance of a whole cascade of reactive oxygen forms, including superoxide, hydroxyl, and perhydroxyl radicals, singlet oxygen, hydrogen peroxide, and their active metabolites (nitrogen oxide, hypochlorite, etc.). The result is a violation of the integrity and permeability of the cell membrane and the destruction of proteins, lipids, carbohydrates, and nucleic acids, the cell genome suffers, and degenerative changes and neuron death develop. The processes of lipid peroxidation underlie most of the diseases of the central nervous system, which include acute and chronic disorders of cerebral blood circulation, degenerative diseases of the brain and spinal cord, cancer pathology, etc. [1, 2].

Antioxidants, both endogenous and exogenous substances, limit LPO processes. Today, in practical health care, antioxidant preparations of natural origin and

synthetic compounds are used [3]. It should be noted that the group of drugs related to natural antioxidants has found quite wide application in clinical practice. At the same time, there is a clear shortage of synthetic antioxidant drugs. In view of the abovementioned, the emergence of a new effective antioxidant compound should arouse the interest of qualified specialists.

2. The results

A computer prediction of the antioxidant activity of new compounds was carried out in the "Microcosm" software [4], and the high predictive ability of the "Microcosm" information technology regarding the antioxidant activity of imidazo-benzimidazole derivatives has been demonstrated.

Condensed benzimidazole derivatives were synthesized, and experimental screening of the antioxidant activity of new chemical compounds was carried out among the derivatives of 2-(hetaryl) imidazo[1,2-a]benzimidazoles (IMBI) [5], 3-aroyl- and 3-hetaroyl-IMBI [6], 2-methoxyphenyl-substituted 9-dialkylaminoethyl-IMBI [7], 2-methoxy- phenyl- and 2-oxyphenyl-substituted 1-dialkylaminoalkyl-IMBI [8], N-acylmethyl derivatives of 9H-2,3-dihydro-IMBI and 10H-2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazoles [9], 9-dialkylami-noethyl-2-oxy(dioxy)phenyl-IMBI [10], aroylmethyl derivatives of tricyclic benzimidazole systems containing hydroxy groups in aroyl radicals [11], 3-(2,2,2-tri-chloro-1-hydroxyethyl)-IMBI [12], 3-acetyl-2-R-9-dialkylaminoethyl-IMBI [13], 1-dialkyl(alkyl)aminoethyl-2,3-dihydro-IMBI [14], 9-R-2-halogenophenyl-IMBI [15], 10-alkylaminoethyl-2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazoles [16], 3-(n,n-disubstituted)acetamide-1-r-2-aminobenzimidazolium [17], amides of 2,3-dihydroimidazo- and 2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazolyl-acetic acids [18], phenyl- and alkylthiocarbamides of 2,9-disubstituted IMBI [19], and 1-substituted 2-benzylaminobenzimidazoles with phenyl methoxyls [20]. Butylated hydroxytoluene (BHT, CAS Number 128-37-0), also known as dibunol, was chosen as a comparative drug.

It was found that among the derivatives of 2-(hetaryl)-IMBI, high antioxidant activity superior to dibunol was shown by compounds containing in the second position of the tricycle such substituents as 1-methylbenzimidazolyl and 5-bro-mothienyl. The remaining substances of this series showed an average level of activity, similar to or inferior to dibunol [5]. Among the 3-aroyl- and 3-hetaroyl-IMBI, no substances with antioxidant activity were found [6]. Among 2-methoxyphenyl-substituted 9-dialkylaminoethyl-IMBI, 4-methoxy- and 3 methoxyphenyl-IMBI showed the greatest antioxidant effect, which was comparable to or exceeded dibunol [7]. Salts of the compounds 9-dialkylaminoethyl-IMBI with 3,4-dioxy-phenyl substitute exhibited the greatest antioxidant activity that was twofold active than reference drug BHT. Compounds with 4- and 3-oxyphenyl substitutes or 2,4- and 2,5-dioxyphenyl substitutes, as a rule, lacked 3,4-dioxyphenyl-IMBI in activity; however they were similar in it to BHT [10]. Most of aroylmethyl derivatives of tricyclic benzimidazole systems containing hydroxy groups in aroyl radicals also proved to be highly active antioxidant substances that were superior to dibunol [11]. Derivatives of 9-R-2-halogenophenyl-IMBI had a pronounced inhibitory effect on the processes of lipid peroxidation comparable to dibunol [15]. The compounds from the series of 1-substituted 2-benzylaminobenzimidazoles with phenyl methoxyls acted similarly [20]. Amides of 2,3-dihydroimidazo- and 2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazolyl-acetic acids showed moderate AO activity compared to dibunol [18]. It was revealed that 2-methoxy-phenyl- and 2-oxyphenyl-substituted 1-dialkylaminoalkyl-IMBI [8], N-acylmethyl derivatives

of 9H-2,3-dihydro-IMBI and 10H-2,3,4,10-tetrahydropyrimido[1,2-a]benzimid-azoles [9], most of 3-(2,2,2-trichloro-1-hydroxyethyl)-IMBI [12] и 3-acetyl-2-R-9-dialkylaminoethyl-IMBI [13], 1-dialkyl(alkyl)aminoethyl-2,3-dihydro-IMBI [14], and 3-(n,n-disubstituted)acetamide-1-r-2-aminobenzimidazolium [17] showed the weak antioxidant activity compared to the reference drug. Among 10-alkylami-noethyl-2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazoles [16] and phenyl- and alkylthiocarbamides of 2,9-disubstituted IMBI [19], antioxidant action has not been found.

As a result of previous investigations, the 2-(3,4-dihydroxyphenyl)-9-diethyl-aminoethyl-imidazo [1,2-a]benzimidazole derivative of dihydrobromide (RU-185, enoxifol) was identified as an antioxidant compound with antiradical and mem-brane-protective properties [21, 22], having multicomponent mechanisms of action and a wide range of pharmacodynamic effects. For enoxifol, a cardioprotective effect and a positive effect on the microcirculation of blood vessels are characteris-tic. Antioxidant compound reduces platelet aggregation [23–25], reduces increased blood viscosity, and increases red blood cell deformability [26]. Preclinical toxicol-ogy studies have been carried out, and the pharmacokinetics of the compound has been studied [27].
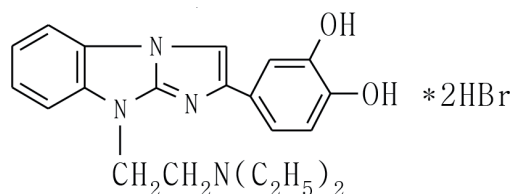

2.1 Synthesis

Synthesis of 2-(3,4-dihydroxyphenyl)-9-diethylamino-ethylimidazo[1,2-a]benzimidazole dihydrobromide (RU-185) starts from saponification of benzimid-azole-2-carbamic acid methyl ester under alkaline conditions to obtain 2-amino-benzimidazole. Subsequent alkylation in acetone in the presence of alkali with *N,N*-diethylaminoethyl chloride formed by the action of thionyl chloride on *N,N*-diethylaminoethanol and condensation with a 3,4-dimetoxiphenacylbromide or or 3,4-dioxiphenacyl chloride, followed by intermolecular cyclization in 48% hydro-bromic acid (boiling temperature 127°C) under reflux, afforded target compound RU-185.

2.1.1 Stage 1: preparation of 2-aminobenzimidazole

benzimidazole-2-carbamic acid methyl ester   2-aminobenzimidaz-ole
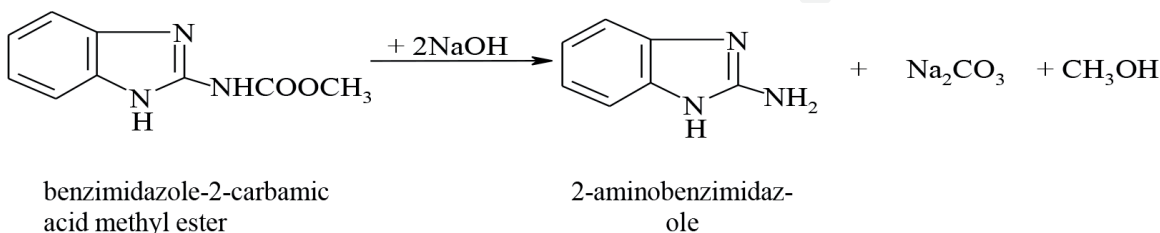
2-Aminobenzimidazole is obtained by saponification of benzimidazole-2-car-bamic acid methyl ester (BMC-2) in aqueous solution of sodium hydroxide. The yield of 2-aminobenzimidazole is 65–75%. At the next stage, 2-aminobenzimidazole is used with a melting point of 223–232°C (within 2 degrees). If necessary, the amine is purified by crystallization from water and acetone or by precipitation from an aqueous hydrochloric acid solution.

2.1.2 Stage 2: preparation of diethylaminoethyl chloride hydrochloride

$$(C_2H_5)_2NCH_2CH_2OH \xrightarrow{SOCl_2} (C_2H_5)_2NCH_2CH_2Cl \cdot HCl + SO_2 \uparrow$$

diethylaminoethanol — diethylaminoethylchloride hydrochloride
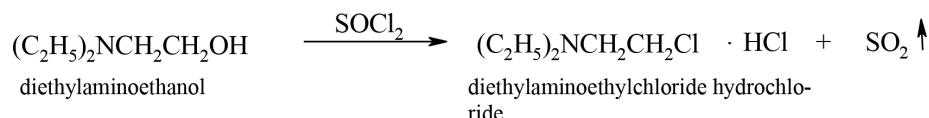
Diethylaminoethyl chloride hydrochloride is obtained by chlorination of diethylaminoethanol with thionyl chloride in toluene or benzene. Purification can be performed by distillation at atmospheric pressure, collecting the fraction with 161–162°C boiling point.

Diethylaminoethanol was distilled in case of poor quality and dried over anhydrous potassium carbonate (1:10 w/v) prior to use. After standing for 6–8 hours with periodic stirring, the desiccant was filtered off. Thionyl chloride (technical) was also distilled at atmospheric pressure before use (boiling point 74–75°C).

2.1.3 Stage 3: synthesis of 2-amino-1-diethylaminoethylbenzimidazole

2-aminobenzimidazole

2-amino-1-diethylaminoethyl-benzimidazole
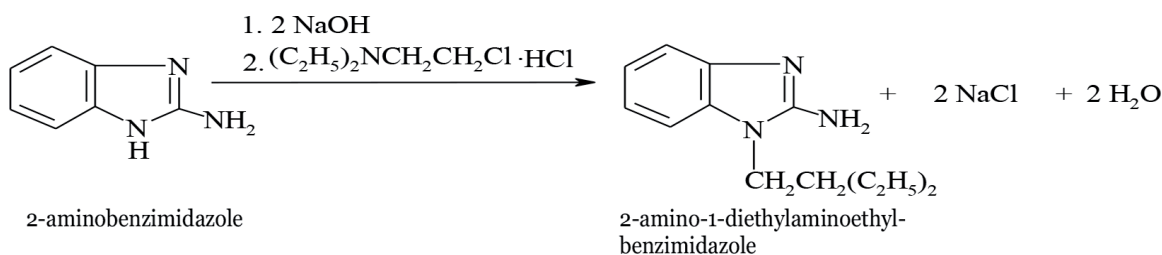
2-Amino-1-diethylaminoethylbenzimidazole is obtained by alkylation of 2-aminobenzimidazole with N,N-diethylaminoethyl chloride in acetone in a presence of a concentrated alkali solution. Upon completion of the reaction, the acetone is distilled off at atmospheric pressure and can be used at the same stage without further purification. Water is added to the residue, and the precipitate of the alkylated amine is filtered off to give 82–88% yield.

2.1.4 Stage 4: 2-(3,4-dihydroxyphenyl)-9-diethylaminoethyl-imidazo[1,2-a]benzimidazole dihydrobromide (RU-185)

Synthesis of compound RU-185 is achieved by treatment of 2-amino-1-diethylaminoethylbenzimidazole with α-chloro-3,4-dihydroxyacetophenone [28] (Method A) or with 3,4-dimethoxyphenacyl bromide. Subsequent cyclization of the formed quaternary 2-aminobenzimidazole salts is achieved in concentrated HBr (Method B).

2-amino-1-diethylaminoethyl-benzimidazole

chloro-3,4-dihydroxyacetophenone or 3,4-dimethoxyphenacyl bromide

quaternary salts of 2-aminobenzimidazole

R = H, CH₃

2-(3,4-dihydroxyphenyl)-9-diethylaminoethyl-imidazo[1,2-a]benzimidazole dihydroxybromide
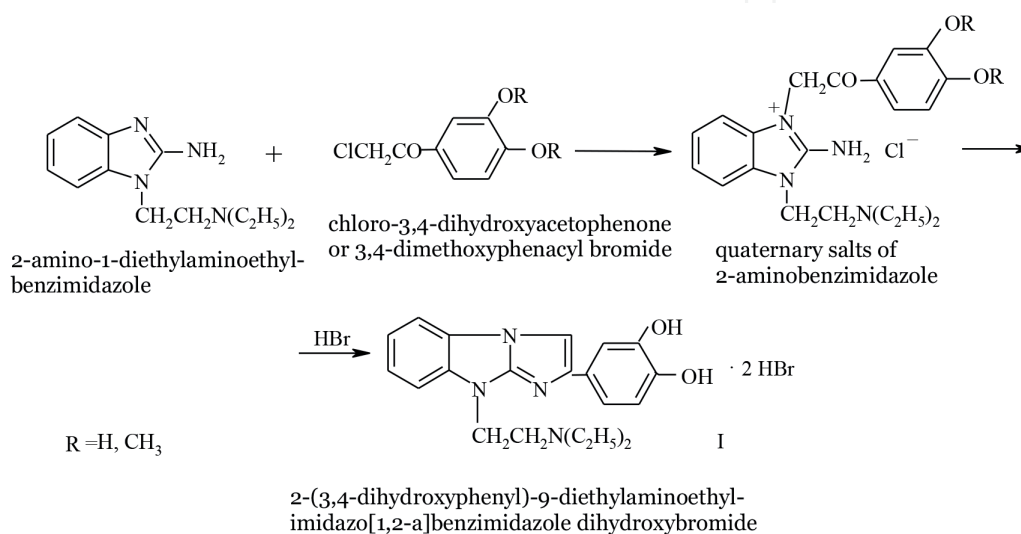
Method A The method consists of boiling of 1-diethylaminoethyl-2-aminobenzimidazole and α-chloroacetopyrocatechol in dry acetonitrile until the quaternization reaction is complete (2–3 hours with TLC control). Then the solvent is evaporated to dryness, and concentrated HBr is added followed by reflux for 1–2 hours. Reaction mixture is left overnight in the refrigerator. The next day, the precipitate formed (large needles) is filtered off. The yield of dihydrobromide (I) is 97%. Recrystallization of the crude product from 80% aqueous ethanol with addition of activated charcoal affords white crystals with a yield of 85%. The product should be protected from direct sunlight. After drying at 110–120°C, the melting point of dihydrobromide is 289–290°C (decomposition).

Method B 3,4-Dimethoxyphenacyl bromide is added to a hot solution of 2-amino-1-diethylaminoethylbenzimidazole in acetone (molar ratio 1:1). Next, the mixture is kept for 6–8 hours at room temperature. The precipitate of 2-amino-(3,4-dimethoxyphenacyl)-1-diethylaminoethylbenzimidazolium bromide is filtered off. The output is 93.5% and melting point 182°C (decomposition, from ethanol).

Next, the resulting bromide is refluxed in conc. HBr (127°C) for 6–8 hours, while as the boiling of the initially formed solution precipitates. After cooling, it is filtered off, washed with acetone, and air dried. The yield is 96%. Crystallization from 80% aqueous ethanol gives (3,4-dihydroxyphenyl)-9-diethylaminoethyl-imidazo[1,2-a]benzimidazole dihydrobromide (I) identical to the product described in method A.

2.2 Pharmacological properties

2.2.1 Antioxidant activity

For several years, the ability of compounds to inhibit oxidative processes in the cells of various tissues, organs, and biological fluids has been thoroughly investigated. The study of the antioxidant properties of enoxifol was carried out by several methods: ascorbate-induced LPO according to the method [29], Fe^{2+}-induced chemiluminescence (CL) of lipids [30], and NADPH-dependent LPO [31]. Superoxide dismutase-like activity has been studied in a quercetin oxidation model [32], interaction with the free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH*) according to the method [33], and interaction with reactive oxygen species on the luminol-dependent CL model [34], the ability to inactivate the superoxide anion radical was estimated by the xanthine-xanthine oxidase-induced lucigenin-dependent CL method [2], and the ability of substances to intercept and inactivate the peroxyl radical was determined by the ABAP-induced CL method [35]. The spectrum of antioxidant activity of enoxifol and the reference compound trolox is shown on **Figure 1**. It was found that enoxifol can react with lipid peroxyl radicals
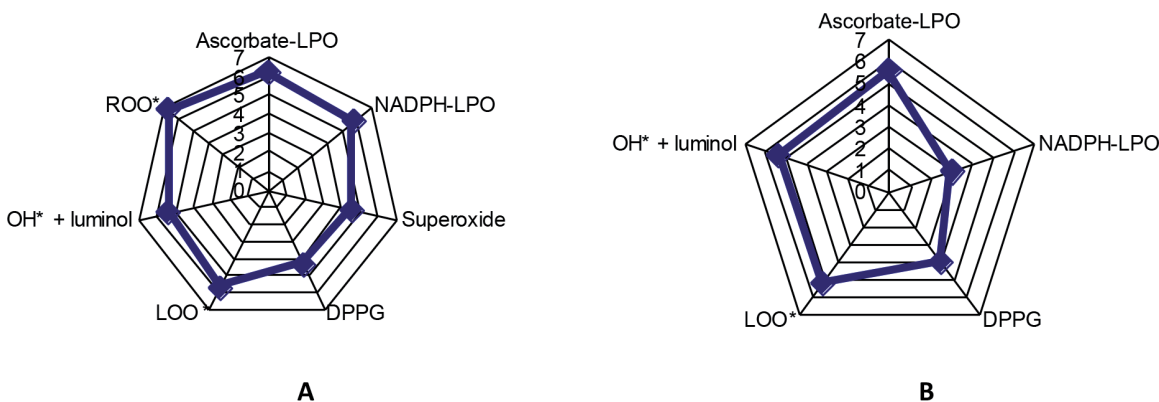
Figure 1.
Antioxidant activity spectrum of enoxifol (A) and trolox (B) (−Lg IC₅₀ (M)).

at the chain termination stage, as indirectly evidenced by its high inhibitory activity during ascorbate, NADPH-induced lipid peroxidation, and CL of lipids, as well as directly inactivate superoxide, hydroxyl, and peroxyl radicals that initiate oxidation, as demonstrated in reactions with the free radical DPPH, quercetin oxidation, Fe^{2+}-induced CL in the presence of luminol, xanthine-xanthine oxidase induced by lucigenin-dependent CL, as well as thermal decomposition of the water-soluble compound 2,2-azobis (2-methylpropionamidine) dihydrochloride (ABAP) with the release of peroxyl radicals, inactivating free radicals and enoxifol, thereby reducing the overall oxidation rate by reducing the total initiation rate [35, 36].

As a result of the analysis of the chemical structure of enoxifol, the structural descriptors were determined, allowing the compound to exhibit antioxidant and antiradical activities. The molecule of enoxifol can manifest these properties due to a fragment of 3,4-dihydroxyphenyl and π-electronic redundancy of the imidazole ring in the structure of imidazobenzimidazoles [37]. Given the relatively low toxicity and high antioxidant potential, the compound was selected for in-depth study on pathological models.

2.2.2 Membranoprotective properties

Membranoprotective activity of enoxifol was studied using the model [38], determining the mechanical strength and resistance of erythrocyte membranes to the hypoosmotic HCl solution. The membrane-stabilizing activity of the compound (inhibition of slow and fast incoming transmembrane ion currents) has been revealed. An effective dose of a compound of 20.5 mg/kg has been established [39].

2.2.3 Hepatoprotective effects

When studying the hepatoprotective properties of enoxifol with a 3-day prophylactic administration in dose 2.25 mg/kg on a model of acute tetrachloromethane hepatitis, it was found that enoxifol significantly improved the absorption and excretory function of the liver and its detoxification abilities Under its influence the development of signs of the cytolytic, hepatodepressive, mesenchymal-inflammatory, and cholestatic syndrome was significantly inhibited, surpassing tocopherol acetate in effectiveness [40].

2.2.4 Antihypoxic action

The antihypoxic properties of enoxifol have been studied in several models of hypobaric, hemic, and tissue hypoxia. In particular, the activity of the compound was established during intrauterine hypoxia and during recovery after acute hypobaric hypoxia. The potential protective effect of the antioxidant compound in hypobaric hypoxia was determined in high- and low-resistant to hypoxia animals in a flow-type pressure chamber at the "height" of 12,500 and 11,000 m, respectively [41]. The most effective doses in these models were 3 and 5 mg/kg [42]. Enoxifol showed a protective effect in modeling intrauterine hypoxia in dose 10 mg/kg during course administration [42].

The activity of the compound in the recovery period after acute hypobaric hypoxia was determined by the change in behavioral reactions, cognitive functions, and the physical condition of the animals. The protective dose of enoxifol was 5 mg/kg. Tissue and hemic hypoxias were created by injecting potassium cyanide and sodium nitrite, respectively [43]. The protective action of enoxifol was judged by the degree of survival of the animals. The range of effective doses was determined from 0.5 to 5 mg/kg [44].

2.2.5 Cerebroprotective properties

The cerebroprotective effect of enoxifol was established on two models of cerebral ischemia of two and four vascular ligature of the carotid and paravertebral arteries, respectively, as well as during the reperfusion period [25, 44]. Antioxidant compound significantly increased the survival rate of animals, decreased blood viscosity by reducing platelet and red blood cell (RBC) aggregation and improving the mechanical properties of RBC, and corrected the behavioral deficit in the postischemic period. Doses of enoxifol in which the cerebroprotective activity was established were 5 and 10 mg/kg.

2.2.6 Nootropic activity

Nootropic activity of enoxifol was established in the model of the conditioned passive avoidance reaction, which allows to evaluate the effect of a studied compounds on learning and memory. As a result, it was found that enoxifol influenced the ability of animals to consolidate information, reduced the deficit in the reproduction of a memorable trace, and intensified the input and initial processing of information in dose 10 mg/kg. The study of the enoxifol effect on the research behavior and emotionality of animals was carried out in the open-field modified test that allows to evaluate the nonassociative behavior of animals in familiar surroundings. Against the background of a 3-day course of administration of the antioxidant compound in dose of 10 mg/kg, psycho-emotional reactivity and anxiety in animals were decreased. With repeated testing, the memories of the arena research were better preserved. Experiments conducted in a Morris maze test confirmed the potential ability of enoxifol in a similar dose to increase mnestic abilities and improve the spatial orientation of animals [45].

2.2.7 Stress-protective effects

The stress-protective effect of enoxifol was studied on models of short-term (1 hour) and prolonged (48 hours) hypokinetic stress [46] and hyperkinetic stress with the paradoxical sleep phase deprivation in rats, in the test of slowly rotating rod [47]. Enoxifol was administered to animals in doses 5 and 10 mg/kg. An antioxidant compound showed an ambiguous therapeutic effect depending on the experimental model used. The antioxidant was not effective in the short-term hypokinetic stress technique. Enoxifol reduced post-stress injuries of internal organs and corrected the targeted behavior of animals in prolonged hypokinetic stress. Enoxifol did not affect the behavioral activity of rats but protected internal organs from pathological abnormalities in hyperkinetic stress [37].

2.2.8 Actoprotective activity

The effect of enoxifol on physical performance and recovery period after physical exertion was studied under normal and complicated conditions in several experimental models. We used the swim to the limit test [48], fatigue development test [49], and efficiency test in anti-orthostatic condition [50]; the model allows to study the recovery process after exercises [51] and the method of research of animals' endurance to training and exhausting loads [49]. Actoprotective properties were studied in models associated with limiting physical loads (swimming to failure), with the rate of fatigue development, and in the test of swimming in complicated anti-orthostatic condition. Enoxifol increased the duration of physical performance under normal and complicated conditions, stabilized some indicators

of energy metabolism (the level of lactate, glycogen, tryptophan, the ratio of pyruvate and lactate), normalized indicators of lipid peroxidation, and had a protective effect on the myocardium of animals during the period of exhaustive physical activity. A number of effective doses amounted to 1, 3, 5, 10, 20, 25, and 50 mg/kg in various physical activity tests [52].

2.2.9 Cardioprotective properties

Cardioprotective activity was studied in isolated atrial rhythm disturbances induced by hydrogen peroxide oxidation [53], in postischemic reperfusion fibrillations of the heart ventricles [54], in the technique of rhythm disturbances caused by calcium chloride and adrenaline intoxication [55], and in the myocardial ischemia provoked by coronary occlusion [31] and the experimental myocardial infarction method [56]. As a result it was found that enoxifol exerted an antiarrhythmic effect, increasing the resistance of cardiomyocyte membranes to LPO products. The compound increased the stability of the myocardium of animals having a non-antioxidant diet to calcium chloride cardiac arrhythmias and increased myocardial tolerance to ischemia. Enoxifol completely prevented ventricular fibrillation of the heart in postischemic myocardial reperfusion. It reduced the extrasystole severity and ventricular fibrillation and reduced the death of animals in the model of coronary artery occlusion and systemic peroxidation syndrome. The novel compound limited area of necrosis in experimental myocardial infarction. It showed activity in doses 7.9, 14, and 20.5 mg/kg [39, 55].

2.2.10 Antithrombotic and antiaggregant effects

Enoxifol had a pronounced antithrombotic effect in arterial thrombosis models induced by application of ferric chloride and electric current on the rat carotid artery and blocked platelet aggregation caused by ADP, collagen, adrenaline, arachidonic acid, thrombin, and an agonist of thromboxane receptors U46619 [24]. The antioxidant compound reduced levels of proaggregant and vasoconstrictor TXA_2, which was confirmed by a decreased level of MDA in the ex vivo pathology caused by thrombin, and decreased the level of total and membrane-bound calcium in platelets, inhibiting calmodulin-dependent PDE cAMP.

2.2.11 Action on hemorheology

The study of the enoxifol action on hemorheology was carried out in the model of "increased viscosity syndrome" according to the method [57]. An improvement in blood flow, membrane plasticity, and inhibition of erythrocyte aggregation under the influence of enoxifol administration in dose 5 mg/kg was found [18]. An increase in the rate of local cerebral blood flow and a direct effect on the tone of cerebral vessels in a similar dose were determined in the method of tissue microcirculation [58]. The study of the enoxifol effect on hemorheology in models of severe forms of streptozotocin diabetes was also performed. Antioxidant compound corrected hemobiological parameters (aggregation, deformability, mechanical properties of erythrocytes), almost normalized indicators of lipid peroxidation, reducing the products of peroxidation, and increased the activity of antioxidant enzymes. The effective dose was 5 mg/kg for the course of the 3-day administration preliminary to diabetes modeling [58].

2.2.12 Pharmacokinetic profile

In the study of the pharmacokinetic properties of enoxifol [27], determination was carried out for 12 hours in the blood and internal organs and in the

urine and feces within 48 hours. It was found that the absolute bioavailability of unchanged enoxifol was 30% and for the total amount of enoxifol and its active metabolites, 99%. When administered orally, enoxifol was well absorbed from the gastrointestinal tract, the maximum concentration in the blood was observed after 1 hour, and after 7 hours the compound was not detected. The excretion of the compound occurs mainly through the intestines, and only one-fifth is excreted unchanged; a small amount of enoxifol is excreted through the kidneys. The half-life for enoxifol was 1.43 hours. After the intravenous administration, the maximum concentration of the compound in the blood is determined in 10 minutes; after 7 hours the antioxidant in the blood was not recorded. The excretion of the infusion form of enoxifol occurs mainly through the kidneys in the unchanged form. In a much smaller amount, enoxifol was eliminated with feces. The half-life was 0.78 hours [27].

2.2.13 Toxicological properties

A study of the drug safety of enoxifol [59] found that the antioxidant compound can be attributed to low-toxic substances. The acute toxicity of enoxifol after oral administration was 1792.56 mg/kg for male and 2260.28 mg/kg for female rats. When administered intravenously, the LD50 was determined for male in dose 109.20 mg/kg and for female rats in dose 126.04 mg/kg. Chronic administration in therapeutic doses (5–25 mg/kg) showed that toxic effects in the central nervous system, liver function, kidney function, and the generative system were not observed. In the high dose (200 mg/kg), slight deviations in the behavioral responses of animals and a slight decrease in the detoxification function of the liver were observed. The accumulation ability of enoxifol wasn't found [59].

3. Conclusion

Summarizing the data obtained, we can conclude that condensed benzimidazole derivatives with π-electron redundancy are a new scaffold for searching antioxidant substances. The highest amount of compounds with high antioxidant activity was found in derivatives of 2-(hetaryl)-aroylmethyl-, and 9-dialkylaminoethyl-IMBI, with oxy- and dioxyphenyl substitutes especially.
The revealed compound enoxifol from the 9-dialkylaminoethyl-IMBI series exhibits pronounced antioxidant, hepatoprotective, antihypoxic, cerebroprotective, nootropic, stress-protective, neuropsychotropic, actoprotective, cardioprotective, antithrombogenic and hemorheological properties. Pharmacokinetic parameters of enoxifol were established, and general and specific toxicities were studied. All mentioned above allows us to consider enoxifol to be the basis of a new effective drug.

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References

[1] Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, et al. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Antioxidants (Basel). 2018;**7**(8):E102. DOI: 10.3390/antiox7080102

[2] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. BioMed Research International. 2014;**2014**:761264. DOI: 10.1155/2014/761264

[3] Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutrition Journal. 2016;**15**(1):71. DOI: 10.1186/s12937-016-0186-5

[4] Vasiliev PM, Spasov AA, Kosolapov VA, Anisimova VA. Computer forecast and experimental verification of the antioxidant activity of new chemical compounds using QSAR-relationships. Bulletin of Volgograd State Medical University. 2005;**2**(14):16-19. [Russian]

[5] Anisimova VA, Spasov AA, Kucheryavenko AF, Panchenko TI, Ostrovskii OV, Kosolapov VA, et al. Synthesis and pharmacological activity of 2-(hetaryl) imidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2002;**36**(10):528-533. DOI: 10.1023/A:1022498224975

[6] Anisimova VA, Spasov AA, Ostrovskii OV, Dudchenko GP, Kosolapov VA, Kucheryavenko AF, et al. Synthesis and pharmacological activity of 3-aroyl- and 3-hetaroylimidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2002;**36**(12):637-641. DOI: 10.1023/A:1023421709976

[7] Anisimova VA, Spasov AA, Kosolapov VA, Ostrovskii OV, Larionov NP, Libinzon RE. Synthesis and pharmacological activity of 2-methoxyphenyl-substituted 9-dialkylaminoethylimidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2005;**39**(9):476-482. DOI: 10.1007/s11094-006-0005-y

[8] Anisimova VA, Tolpygin IE, Spasov AA, Kosolapov VA, Chernikov MV, Stepanov AV, et al. Synthesis and pharmacological activity of 2-methoxy-phenyl- and 2-oxyphenyl-substituted 1-dialkylaminoalkylimidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2005;**39**(11):579-585. DOI: 10.1007/s11094-006-0024-8

[9] Anisimova VA, Tolpygin IE, Spasov AA, Kosolapov VA, Stepanov AV, Kucheryavenko AF. Synthesis and biological activity of N-acylmethyl derivatives of 9H-2,3-dihydroimidazo- and 10H-2,3,4,10-tetrahydropyrimido[1,2-a]benzimidazoles and their reduction products. Pharmaceutical Chemistry Journal. 2006;**40**(5):261-267. DOI: 10.1007/s11094-006-0105-8

[10] Anisimova VA, Spasov AA, Kosolapov VA, Chernikov MV, Stukovina AY, El'tsova LV, et al. Synthesis and biological activity of 9-dialkylaminoethyl-2-oxy(dioxy)phenylimidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2006;**40**(10):521-528. DOI: 10.1007/s11094-006-0185-5

[11] Anisimova VA, Tolpygin IE, Spasov AA, Kosolapov VA, Stepanov AV, Orlova AA, et al. Synthesis and pharmacological activity of aroylmethyl derivatives of tricyclic benzimidazole systems containing hydroxy groups in aroyl radicals. Pharmaceutical Chemistry Journal. 2007;**41**(3):126-130. DOI: 10.1007/s11094-007-0028-z

[12] Anisimova VA, Spasov AA, Kosolapov VA, Tolpygin IE, Porotikov VI, Kucheryavenko AF, et al. Synthesis and pharmacological activity of 3-(2,2,2-trichloro-1-hydroxyethyl) imidazo[1,2-a]benzimidazole dihydrochlorides. Pharmaceutical Chemistry Journal. 2009;**43**(9):491-494. DOI: 10.1007/s11094-009-0340-x

[13] Anisimova VA, Spasov AA, Tolpygin IE, Kosolapov VA, Kucheryavenko AF, Gurova NA, et al. Synthesis and pharmacological activity of salts of 3-acetyl-2-R-9-dialkylaminoethyl-imidazo[1,2-a] benzimidazoles. Pharmaceutical Chemistry Journal. 2010;**44**(3):117-122. DOI: 10.1007/s11094-010-0411-z

[14] Anisimova VA, Tolpygin IE, Chernikov MV, Yakovlev DS, Goryagin II, Gurova NA, et al. Synthesis and pharmacological activity of 1-dialkyl(alkyl)aminoethyl-2,3-dihydroimidazo[1,2-a]benzimidazoles. Pharmaceutical Chemistry Journal. 2010;**44**(5):241-244. DOI: 10.1007/s11094-010-0439-0

[15] Anisimova VA, Tolpygin IE, Minkin VI, Chernikov MV, Yakovlev DS, Stukovina AY, et al. Synthesis and pharmacological activity of 9-R-2-halogenophenylimidazo[1,2-a] benzimidazoles. Pharmaceutical Chemistry Journal. 2010;**44**(7):345-351. DOI: 10.1007/s11094-010-0465-y

[16] Anisimova VA, Tolpygin IE, Spasov AA, Yakovlev DS, Kolobrodova NA, Gurova NA, et al. Synthesis and pharmacological activity of 10-alkylaminoethyl-2,3,4,10-tetrahydropyrimido[1,2-a] benzimidazoles. Pharmaceutical Chemistry Journal. 2012;**46**(6):325-330. DOI: 10.1007/s11094-012-0791-3

[17] Anisimova VA, Spasov AA, Kosolapov VA, Tolpygin IE, El'tsova LV, Kucheryavenko AF, et al. Synthesis and pharmacological activity of 3-(n,n-disubstituted)acetamide-1-r-2-aminobenzimidazolium chlorides. Pharmaceutical Chemistry Journal. 2012;**46**(9):526-530. DOI: 10.1007/s11094-012-0839-4

[18] Anisimova VA, Spasov AA, Kosolapov VA, Tolpygin IE, Tibir'kova EV, Salaznikova OA, et al. Synthesis and pharmacological activity of amides of 2,3-dihydroimidazo- and 2,3,4,10-tetrahydropyrimido[1,2-a] benzimidazolyl-acetic acids. Pharmaceutical Chemistry Journal. 2013;**46**(11):647-652. DOI: 10.1007/s11094-013-0862-0

[19] Anisimova VA, Anisimova VA, Spasov AA, Kuznetsova VA, Kosolapov VA, Yakovlev DS, et al. Synthesis and pharmacological activity of 2,9-disubstituted imidazo[1,2-a]benzimidazole phenyl- and alkylthiocarbamides. Pharmaceutical Chemistry Journal. 2016;**49**(10):653-656

[20] Zhukovskaya ON, Anisimova VA, Spasov AA, Vasil'ev PM, Kosolapov VA, Kucheryavenko AF, et al. 1-Substituted 2-benzylaminobenzimidazoles with phenyl methoxyls: Synthesis, computer prediction, and pharmacological activity. Pharmaceutical Chemistry Journal. 2016;**49**(11):735-742. DOI: 10.1007/s11094-016-1362-9

[21] Anisimova VA, Kosolapov VA, Minkin VI, Petrov VI, Spasov AA. Dihydrobromide 9-(3,4-dihydroxyphenyl)-9-diethylaminoethyl-imidazo[1,2-a] benzimidazole and a pharmaceutical composition based on it. Patent RU 2391979, priority from 12.05.2008. [Russian]

[22] Kosolapov VA. Antioxidant selection strategy, application prospects [abstract of thesis for the secondary doctorate degree]. Volgograd; 2005. p.40 [Russian]

[23] Spasov AA, Ostrovskiĭ OV, Ivakhnenko IV, Kosolapov VA, Anisimova VA. The effect of compounds with antioxidant properties on thrombocyte functional activity. Eksperimental'naia i Klinicheskaia Farmakologiia. 1999;**62**(1):38-40. [Russian]

[24] Spasov AA, Anisimova VA, Kucheryavenko AF, Minkin VI. The agent possessing antithrombogenic activity. Patent RU 2440814, priority from 10.11.2010 [Russian]

[25] Spasov AA, Kosolapov VA, Ostrovskiĭ OV, Drozd VV, Vereshchagin VK, Sen'chukov ID, et al. Anti-ischemic properties of the novel antioxidant enoxifol. Eksperimental'naia i Klinicheskaia Farmakologiia. 2003;**66**(4):17-20. [Russian]

[26] Spasov AA, Stepanov AV, Samokhina MP, Kosolapov VA. The effect of enoxifol on blood viscosity and erythrocyte aggregation in vitro. Bulletin of Volgograd State Medical University. 2003;**59**(9):43-46. [Russian]

[27] Smirnova LA, Kosolapov VA, Lekomtsev AS, Spasov AA, Ozerov AA. Pharmaco-kinetic properties of cerebroprotective and antioxidant drug enoxifol. Bulletin of Volgograd State Medical University. 2002;**58**(8):32. [Russian]

[28] Rubtsov MV, Baychikov AG. Synthetic chemical and pharmaceutical preparations. Moscow: Medicine; 1971. 54 p. [Russian]

[29] Lankin VZ, Gurevich SM, Burlakova EB. The study of ascorbate-dependent lipid peroxidation of tissues using the test with 2-thiobarbituric acid. Proceedings of the Moscow Society of Nature Testers. 1975;**52**:73-78. [Russian]

[30] Farhutdinov RR, Likhovskikh VA. Chemiluminescent methods for the study of free-radical oxidation in biology and medicine. Ufa; 1995. 54 p. [Russian]

[31] Kubatiev AA, Andreev SV. On the ratio of the processes of generation and detoxification of lipid peroxides in normal and hypertrophied hearts. Myocardial Metabolism. M.: Medicine. 1981:251-262. [Russian]

[32] Kostyuk VA, Potapovich AI, Kovaleva ZV. A simple and sensitive method for determining the activity of superoxide dismutase, based on the reaction of oxidation of quercetin. Questions of medical chemistry. 1990;**36**(2):88-91. [Russian]

[33] Glavind G. Antioxidants in animal tissue. Acta Chemica Scandinavica. 1963;**17**(6):1635-1640

[34] Semeshko SG, Farhutdinov RR. Total antioxidant activity of the tear fluid. Clinical and Laboratory Diagnostics. 2002;**24**:33-34. [Russian]

[35] Kosolapov VA, Spasov AA, Anisimova VA. Study of antiradical activity of new compounds by chemiluminescence. Biomeditsinskaya Khimiya. 2005;**51**(3):287-294. [Russian]

[36] Kosolapov VA. Criteria for the use of luminol and lucigenin in chemiluminescence to study the mechanisms of action of antioxidant substances. Bulletin of Volgograd State Medical University. 2003;**9**:54-57. [Russian]

[37] Ostrovsky OV. Pharmacology of antioxidants—Condensed benzimidazole derivatives [abstract of thesis for the secondary doctorate degree]. Volgograd; 1996. p. 38 [Russian]

[38] Godin DV, Al T, Garnott MK. Membrane perturbational effects of antiarrhythmic drugs. Journal of Molecular and Cellular Cardiology. 1979;**2**(3):261-274

[39] Panchenko TI. Antiarrhythmic properties of new antioxidant substances—Condensed benzimidazole derivatives [abstract of thesis for the PhD degree]. Rostov-on-Don; 1990. p. 31 [Russian]

[40] Spasov AA, Anisimova VA, Kosolapov VA, Minkin VI, Petrov VI. Agent with antiarrhythmic and hepatoprotective properties. Patent RU 246972, priority from 13.07.2011 [Russian]

[41] Lukyanova LD, Malyshev AY, Krapivin SV. Antihypoxic effect of succinate-containing oxypyridine derivative on EEG power spectra in rats with different resistance to oxygen deficiency. Bulletin of Experimental Biology and Medicine. 1997;**124**(7): 57-61. [Russian]

[42] Tregubova IA. Effect of antioxidant substances on the mother-fetus system in hypoxic states (experimental study) [abstract of thesis for the PhD degree]. Volgograd; 2000. p. 31 [Russian]

[43] Korablev MV, Lukienko PI. Antihypoxic Drugs. Minsk: Belarus; 1976. p. 126. [Russian]

[44] Spasov AA, Anisimova VA, Kosolapov VA, Tregubova IA, Minkin VI, Petrov VI. An agent with anti-hypoxic, actoprotective, nootropic effect and affecting physical performance. Patent RU 2462245, priority of 07/12/2011 [Russian]

[45] Tregubova IA, Kosolapov VA, Spasov AA, Anisimova VA. Experimental study of the effect of a new antioxidant agent on learning and memory. Bulletin of Experimental Biology and Medicine. 2014;**156**(6):793-795. DOI: 10.1007/s10517-014-2452-x

[46] Malikova LA, Arefolov VA. Protective effect of phenazepam and sodium oxybutyrate on somatic manifestations in immobilization stress.

Bulletin of Experimental Biology and Medicine. 1983;**96**(12):60-62 [Russian]

[47] Van Hulsen ZJM, Coenen AML. The pendulum technique for paradoxical sleep deprivation in rats. Physiology & Behavior. 1980;**25**:807-811

[48] Rylova MA. Methods for studying the chronic effects of harmful environmental factors in the experiment. Leningrad: Medicine; 1964. 324 p. [Russian]

[49] Kiplinger GF. The effects of drugs on the rate of development of fatigue in mice. Texas Reports on Biology and Medicine. 1967;**25**:531-540

[50] Avakian OM, Shirinian EA. New model of dynamic work in small laboratory animals. Biulleten' Eksperimental'noĭ Biologii i Meditsiny. 1977;**84**(9):375 [Russian]

[51] Dawson CA, Horvath SA. Swimming in small laboratory animals. Medicine and Science in Sports. 1970;**2**:51-78

[52] Tsibanev AV. Search for a means of increasing physical performance and recovery processes among imidazo (1,2-a) benzimidazole derivatives, which show antioxidant activity [abstract of thesis for the PhD degree]. Volgograd; 1991. p. 29 [Russian]

[53] Didenko VV. Arrhythmias caused by the induction of lipid peroxidation in an isolated atrium. Moscow; 1985. 15 p. [Russian]

[54] Penkoske PA, Sobel BC, Corr PB. Disparate electrophysiological alterations accompanying dysrhythmia due to coronary occlusion and reperfusion in the cat. Circulation. 1978;**58**:1023-1035

[55] Spasov AA, Anisimova VA, Kosolapov VA, Minkin VI, Petrov VI. The agent with antiarrhythmic and

hepatoprotective properties. Patent RU 2469720, priority from 13.07.11

[56] Harris AS. Circulation. 1950;**1**:1318 [Russian]

[57] Plotnikov MB, Aliev OI, Koltunov AA, Maslov MI. Increased blood viscosity syndrome in SHR rats: Analysis of the model adequacy. Bulletin of Experimental Biology and Medicine. 1998;**126**(8):150-151 [Russian]

[58] Samokhina MP. Hemorheological properties of condensed imidazobenzimidazole derivatives exhibiting an antioxidant effect [abstract of thesis for the PhD degree]. Volgograd; 2003. p. 27. [Russian]

[59] Bugaeva LI, Kuzubova EA, Bukatin MV, Rebrova DN. The effect of enoxifol on the gonadotropic function of male rats. Successes of Modern Natural Science. 2006;**8**:56 [Russian]