Research Results in Pharmacology

Research Results in Pharmacology 11(2): 59–72
UDC: 617.7-073.178
DOI 10.18413/rrpharmacology.11.570

Research Article

The effect of synthesized 5-R-1H-benzo[d]imidazole-2-thiol derivatives on intraocular pressure in normal and pathological conditions

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Academic editor: Oleg Gudyrev ♦ **Received** 31 January 2025 ♦ **Accepted** 17 May 2025 ♦ **Published** 26 June 2025

Citation: Taran AS, Zhukovskaya ON, Naumenko LV, Chebanko AM, Efremova YuV, Bodrug ID, Beloshapka AA, Zaichenko SB, Morkovnik AS, Spasov AA (2025) The effect of synthesized 5-R-1H-benzo[d]imidazole-2-thiol derivatives on intraocular pressure in normal and pathological conditions. Research Results in Pharmacology 11(2): 59–72. https://doi.org/10.18413/rrpharmacology.11.570

Abstract

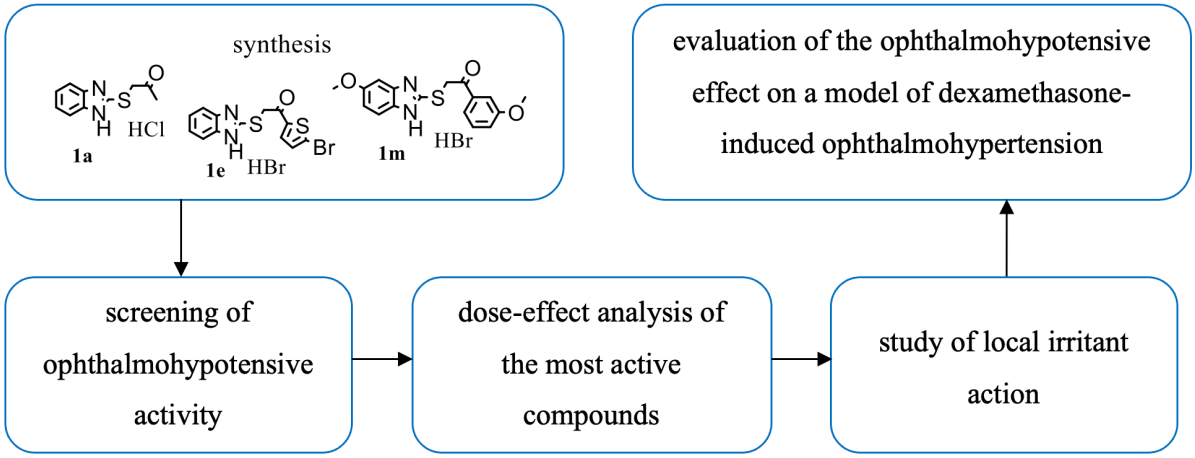
Introduction: Development of new drugs that reduce intraocular pressure and do not have a resorptive effect is a topical task for the treatment of glaucoma. Eighteen derivatives of 5-R-1H-benzo[d]imidazole-2-thiols were synthesized and studied for ophthalmic hypotensive activity in animals with normal intraocular pressure and in animals with dexamethasone-induced ophthalmic hypertension.

Materials and Methods: Ophthalmohypotensive activity was studied by tonometry with the TonoVet veterinary tonometer in 90 intact and experimental sexually mature mongrel rats weighing 250-350g before and after instillation of solutions of the studied compounds. Local irritant effects were determined by performing a conjunctival test using 25 guinea pigs.

Results: The most active compound among the 5-R-1H-benzo[d]imidazole-2-thiol derivatives was compound 1a, which reduced the ophthalmotonus in normotensive animals by 31.37%, exceeding the effect of the reference drugs timolol (-26.84%) and melatonin (-30.95%), and in rats with dexamethasone-induced glaucoma – by 23.74%, similar to melatonin (-23.72%), but inferior to timolol (-29.75%). It was found that substance 1a has no local irritant effect. It was revealed that at the screening concentration (4 mg/mL), compound 1a has a systemic effect, at lower concentrations, the ophthalmohypotensive effect decreases, the latent period increases, and the severity of the resorptive effect decreases.

Conclusion: The most active compound, 1a, was found to have demonstrated in vivo IOP-lowering activity when administered as a single instillation at a concentration of 0.4% (4 mg/mL) in ophthalmonormotensive animals and animals with steroid-induced glaucoma. Compound 1a was shown to have no local irritant effect.

Graphical abstract



Keywords glaucoma, intraocular pressure, benzimidazole, melatonin

Introduction

Glaucoma is characterized by progressive degeneration of the optic nerve with associated visual field loss and eventually blindness if not treated appropriately (Storgaard et al. 2021; Iomdina et al. 2021). Despite the expansion of the glaucoma drug lineup with new drug classes, many barriers and challenges still exist for topical therapy drugs – non-compliance, economics, and side effects such as ocular surface damage, pain, irritation, contact dermatitis, hyperpigmentation, periorbitopathy, and systemic effects such as bronchoconstriction, heart failure, bradycardia, etc. (Shalaby et al. 2020; Wu et al. 2021; Patchinsky et al. 2022).

Ophthalmotonus (intraocular pressure, IOP) is an important indicator for controlling the normal function of the visual organs. Its increase is one of the main causes of glaucoma (Pagano et al. 2023). In all forms of glaucoma (primary, secondary, congenital), elevated intraocular pressure is recognized as a major risk factor for glaucoma development and progression, and IOP lowering is currently the only drug treatment for glaucoma (Storgaard et al. 2021). There is no doubt that IOP reduction and maintenance at the "target pressure" (target IOP) is an important goal in stopping disease progression, but practice shows that IOP compensation does not always lead to glaucoma arrest and stabilization (Katz et al. 2022). The multifactorial nature of glaucoma and the multifaceted definition of "target pressure" requires attention to many different mechanisms. In addition, knowledge of exactly how IOP level affects glaucoma development is constantly improving (Pagano et al. 2023).

Melatonin is a ubiquitous molecule found in living organisms, from bacteria to plants to mammals. It has diverse properties, partly due to its powerful antioxidant nature, but also because of its specific interaction with melatonin receptors, which are present in almost all tissues. Melatonin regulates various physiological functions and contributes to homeostasis throughout the body. The human eye also contains small amounts of melatonin, which is produced by cells in the anterior (cornea, iris, ciliary body and lens) and posterior (vitreous, retina, chorioid, optic nerve) segments. In the eye, melatonin may provide antioxidant protection as well as regulate physiologic functions of ocular tissues, including IOP. Thus, it is possible that exogenous topical administration of sufficiently large amounts of melatonin and its analogues into the eye may be useful in several instances: for the treatment of ocular pathologies such as glaucoma because of the IOP-lowering and neuroprotective effects of melatonin; for the prevention of other dysfunctions such as dry eye and refractive defects (cataract and myopia), mainly because of its antioxidant properties; in diabetic retinopathy due to metabolic effects and neuroprotective effects; in yellow spot degeneration due to antioxidant and neuroprotective properties; and in

uveitis, mainly due to anti-inflammatory and immunomodulatory properties (Rusciano and Russo 2024).

Recently, the synthesis of melatonin analogues with similar pharmacological properties has attracted the interest of researchers. In general, the strategy involves changing groups in different parts of the indole ring or replacing the indole ring with an analogue. Melatonin is a heterocyclic compound based on an indole pharmacophore, and the compounds under study are benzimidazole derivatives (Fig. 1), which gives reason to consider them isosteres (Shirinzadeh et al. 2020). Isosterism is widely used in the rational modification of parent compounds to increase efficacy, selectivity, improve pharmacokinetic properties, eliminate toxicity and obtain new chemical compounds (Kumari et al. 2020).

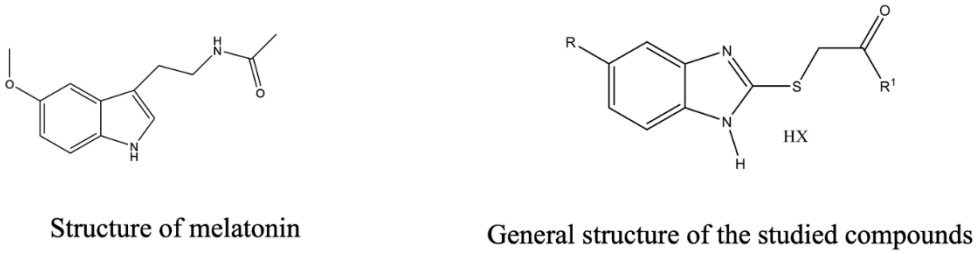


Figure 1. Chemical structures of melatonin and the compounds studied**.**

Currently, nitrogen-containing heterocyclic molecules are of great interest to synthetic chemists and pharmacologists. Among heterocyclic compounds, benzimidazole frameworks significantly predominate as potential drugs. They are accessible, functional, stable, and due to the isostructural pharmacophore of natural active biomolecules, benzimidazole derivatives are of great importance as chemotherapeutic agents in various clinical conditions. Over the past decades, researchers have synthesized many benzimidazole derivatives, with a significant proportion of these compounds exhibiting high biological activity against many diseases with good bioavailability, safety, and stability (Patchinsky et al. 2022).

2-Mercaptobenzimidazole derivatives exhibit a wide range of biological activities: inhibition of cell migration and suppression of CD-44 mRNA expression (Radwan et al. 2023), inhibition of activity against COX in humans (Veerasamy et al. 2021), anthelmintic (Hajnal et al. 2021), anxiolytic activity (Spasov et al. 2020), inhibitory activity against DPP-4 (Zhukovskaya et al. 2019), inhibitory activity against α-glucosidase (Mumtaz A. et al. 2018), antimicrobial and antioxidant activity (Ouasif et al. 2017), antituberculosis activity (Anand et al. 2016), and inhibitory activity against NHE-1 (Zhang et al. 2007).

Previous studies have shown that among melatonin isosteres – benzimidazole derivatives – there are highly active compounds that lower intraocular pressure (Spasov et al. 2020; Naumenko et al. 2021; Kibalova et al. 2023). It was advisable to study the effect of new mercaptobenzimidazole derivatives on the ophthalmotonus of normotensive animals and animals with steroid glaucoma.

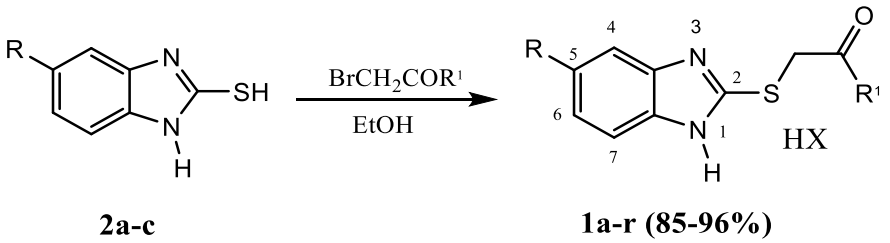
Materials and Methods

Experimental animals

All procedures involving animals in the study were performed in accordance with generally accepted ethical standards for animal manipulations. The animals were kept in accordance with the rules of laboratory practice for preclinical research in the Russian Federation (GOST 351.000.3-96 and 51000.4-96), the Order of the Ministry of Health and Social Development of the Russian Federation of August 23, 2010 № 708n "On Approval of the Rules of Laboratory Practice", and also in compliance with the directives 2010/63/EU of the European Parliament and the Council of the European Union of 22.09.2010 "On the Protection of Animals Used for Scientific Purposes". The experiments were approved by the Biomedical Ethics Commission of Volgograd State Medical University (VolSMU) IRB 00005839 IORG 0004900, OHRP, Certificate No. 2021/056 dated 15.06.2021. All sections of this study comply with the ARRIVE Guidelines for reporting animal studies (Shevchenko et al. 2023). The animals were kept in the vivarium of VolSMU of the Ministry of Health of Russia at a temperature of 24°C and relative humidity of 60% under natural light cycle with free access to food and water.

Chemical synthesis

The synthesis of salts 1-((1H-benzo[d]imidazol-2-yl)thio)propan-2-one and 2-((1H-benzo[d]imidazol-2-yl)thio)-1-(R^1-phenyl)ethan-1-one (1-a-r), containing a phenylethanone-1-group bound to sulfur, is presented in Figure 2.



2a-c **1a-r (85-96%)**

Figure 2. Synthesis of 5-R-1-benzo[*d*]imidazole-2-thiol derivatives. ***Note:*** **1**: **R=H,** R_1=Me **(a),** X=Cl; R_1= 3,5-di-*tert*-butyl-4-hydroxyphenyl **(b)**; $C_6H_3(O_2CH_2)$-3,4 **(c)**; C_6H_4-C_6H_5 **(d)**; 5-bromothienyl-2 **(e)**; **R=Cl**, R_1= 2-thienyl **(f)**; 2-furyl **(1g)**; 3,5-di-*tert*-butyl-4-hydroxyphenyl **(h);** C_6H_4(OMe)-3 **(i)**; $C_6H_3(O_2CH_2)$-3,4 **(j)**, C_6H_3(OMe)-3,4 **(k); R=OMe**, R_1= 3,5-di-*tert*-butyl-4-hydroxyphenyl **(l)**; C_6H_4(OMe)-3 **(m);** C_6H_4-C_6H_5 **(n)**; $C_{10}H_7$ **(o)**; $C_6H_3(O_2CH_2)$-3,4 **(p);** C_6H_4(OMe)-4 **(q);** 2-furyl **(r)**, X=Br. **2:** R=H (a), Cl (b), MeO (c).

In this work, a synthesis between 5R-1H-benzo[d]imidazole-2-thiol derivatives and various phenacyl bromides is presented. Products 1a–r were obtained in good yields and high purity. Both analytical and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR) of all synthesized compounds 1a–r are in complete agreement with the proposed structures. It is known that the ambidentate anion of benzimidazoline-2-thione is alkylated exclusively at the sulfur atom (Bespalov et al. 2015). The IR spectra of compounds 1a-r showed some general characteristics: 3452-3394 cm^{-1} (very weak N-H absorption), 2955-3030 cm^{-1} (C-H) aromatic, 1583-1513 cm^{-1} (C=C) aromatic, (1626-1616) cm^{-1} (C=N) imidazole, 1664–1716 (C=O) cm^{-1}, 593-688 cm-1 (C^2-S). Vibrations of the (C=S) group with ν (C=S) at 1098, 1099, 1104, 1108, characteristic of the thione structure, were absent. The NMR spectra showed the following general data: S-CH_2 - H^1 4.84-5.36 ppm; C^{13} 38.9-43.48 ppm. In the ^{1}H NMR spectra of compounds 1a-r, the signal of the NH group proton merges with the baseline, which is explained by the rather rapid migration of the proton between two nitrogen atoms in the NMR time scale. In the ^{13}C NMR spectra of compounds 1a-r, containing an alkylated sulfur atom and an aromatic benzimidazole fragment, the signal of the C^2 atom is located around 149-150 ppm.

IR spectra (ν/cm^{-1}) of the compounds obtained were recorded on a Varian Excalibur 3100 FT-IR spectrophotometer (Varian, USA), using the method of attenuated total reflection in powder; NMR-spectra were recorded on Bruker Avance 600 N (USA) (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer. The chemical shifts are given relative to the residual signals of protons of the deuterated solvent (2.49 for ^{1}H, 39.7 ^{13}C in DMSO-d6). Melting points were measured on a Fisher-Johns Melting Point Apparatus (Fisher Scientific, USA). Elemental analysis was carried out using a classical method (Gelman et al. 1987). Reaction progress and purity of synthesized compounds were monitored by TLC (plates with Al_2O_3 III degree of activity, eluent $CHCl_3$, visualization with iodine vapors in a moist chamber).

Benzimidazole-2-thiol, 5-chloro-, 5-methoxy-benzimidazole-2-thiols, chloroacetone used in the work were purchased from the company Alfa Aesar (Great Britain).

4-, 3-methoxy-, 3,4-dimethoxy, 3,4-methylenedioxy-phenacyl bromides are obtained by bromination of the corresponding acetophenones in alcohol according to the method (Anisimova et al. 2005); 2-bromo-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)ethanone-1 was obtained by the action of bromine on the corresponding acetophenone in octane or isooctane (Volod'kin et al. 1966).

Acetylthiophene and 2-acetylfuran are brominated with bromine in an ether-dioxane solution similarly to (Shevchuk et al. 1963); 5-bromo-2-acetylthiophene in dry chloroform according to the method of (Smirnov et al. 1971).

General procedure for synthesizing

To a solution of the corresponding 1*H*-benzo[*d*]imidazole-2-thiol 2a-c (5 mmol) in ethanol at room temperature, there was added the corresponding 2-bromo-1-(4-R^1-phenyl)ethan-1-one (5 mmol). The mixture was stirred until it dissolved, after which it was kept for 8-10 hours at 20-25 °C. Salts 1a-r were filtered off and thoroughly washed with acetone. The obtained compounds 1 a-r were dried in air and crystallized from the corresponding solvents.

1-((1H-benzo[d]imidazol-2-yl)thio)propan-2-one hydrochloride (1a·HCl)

Yield 85 %, m.p. 158.5-160º C (MeOH) (dec). IR spectrum, ν/ cm^{-1}: 3420 (NH) , 2941, 3002 (C-H)Ar, 1716 (C=O). 1625, 1589, 1514 (C=C, C=N), 598, 619, 659 (C^2-S). Found (%): C 49.37; H 4.68; Cl 14.48; N 11.41; S 13.07. $C_{10}H_{10}N_2OS \cdot HCl$. Calculated (%): C 49.48; H 4.57; Cl 14.61; N 11.54; S 13.21. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 2.31(c, 3H, Me); 4.85 (c, 2H, S-CH_2); 7.4-7.42 (k, 2H, H_{Ar}, J=3.1); 7.63-7.65 (k, 2H, H_{Ar}, J=4.0). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 28.69, 43.00, 112.93, 124.40, 124.40, 132.76, 150.14, 200.31

2-((1H-benzo[d]imidazol-2-yl)thio)-1-(3,5-di-tert-butyl-4-hydroxyphenyl)ethan-1-one hydrogen bromide (1b .HBr)

Yield 88 %, m.p. 203-204º C (MeCN) (dec). IR spectrum, ν/ cm^{-1}: 3626(OH), 3425 (NH), 2948, 1659 (C=O), 1624, 1586, 1512 (C=C, C=N), 593, 619, 655. Found (%): C 57.75; H 6.24; Br 16.61; N 5.75; S 6.57. $C_{23}H_{28}N_2O_2S \cdot HBr$. Calculated (%): C 57.86; H 6.12; Br 16.74; N 5.87; S 6.71. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 1,42 (c, 18H, 6 CH_3); 5.36 (c, 2H, S-CH_2); 7.45 (dd, 2H, $H^{5,6}$, J=9.24); 7.68 (dd, 2H, $H^{4,7}$, J=9.24); 7.82 (c, 2H, H_{Ph}); 8.01 (c, 1H, OH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 29.89, 34.45, 40.98, 113.07, 124.85, 125.79, 126.25, 132.86, 138.34, 150.55, 159.56, 190.91.

2-((*1H-benzo[d]imidazol-2-yl)thio)-1-(benzo[d][1,3]dioxol-5-yl)ethan-1-one hydrogen bromide (1c ·HBr)*

Yield 82 %, m.p. 219-220º C (EtOH) (dec). IR spectrum, ν/ cm^{-1}: 2975, 1656 (C=O), 1623, 1616, 1597, 641, 665. Found (%): C 48.75; H 3.44; Br 20.19; N 7.08; S 8.01. $C_{16}H_{12}N_2O_3S \cdot HBr$. Calculated (%): C 48.87; H 3.33; Br 20.32; N 7.20; S 8.15. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 5.34 (c, 2H, SCH_2); 6.17 (c, 2H, CH_2); 7.14 (d, 1H, H_{Ph}, J=8.2); 7.44-7.45 (m, 2H, $H^{5,6}$); 7.52 (d, 1H, H_{Ph}, J=1,8); 7.66-7.68 (m, 2H, $H^{4,7}$), 7.72-7.74 (m, 1H, H_{Ph}). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 41.36, 102.27, 107.67, 108.25, 113.05, 124.88, 125.33, 129.15, 132.82, 147.89, 150.39, 152.24, 190.17.

2-((*1H-benzo[d]imidazol-2-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethan-1-one hydrogen bromide (1d ·HBr)*

Yield 95 %, m.p. 228-229º C (EtOH/DMF) (dec). IR spectrum, ν/cm^{-1}: 3392, 3127, 2975, 1667 (C=O), 1625, 1602, 1583, 593, 616. Found (%): C 59.19; H 4.15; Br 18.65; N 6.47; S 7.41. $C_{21}H_{16}N_2OS$ HBr. Calculated (%): C 59.30; H 4.03; Br 18.79; N 6.59; S 7.54. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 5.44 (c, 2H, CH_2); 7.44-7.45 (m, 3H, H_{Bif}); 7.52 (t, 2H, $H^{5,6}$, J=7.65), 7.68 (k, 2H, $H^{4,7}$, J=3.1); 7.78 (t, 2H, H_{Bif}, J= 3.6); 7.92 (d, 2H, H_{Bif}, J=8.46); 8.16 (d, 2H, H_{Bif}, J=8.46). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 41.44, 113.09, 124.8, 126.96, 125.98,128.54, 129.04, 129.22, 132.98, 133.48, 138.53, 145.39, 150.35, 191.68.

2-((*1H-benzo[d]imidazol-2-yl)thio)-1-(5-bromothiophen-2-yl)ethan-1-one hydrogen bromide (1e ·HBr)*

Yield 85 %, m.p. 229-230º C (EtOH/DMF) (dec). IR spectrum, ν/ cm^{-1}: 3130, 3048, 2974, 2918, 1646, 1590, 1522, 1505, 618, 596. Found (%): C 35.85; H 2.43; Br 36.68; N 6.33; S 14.63. $C_{13}H_9BrN_2OS_2$ HBr. Calculated (%): C 35.96; H 2.32; Br 36.81; N 6.45; S 14.77. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 5.16 (c, 2H, CH_2); 7.4 (dd, 2H, $H^{5(6)}$, H^3thioph, J= 3.1, J= 3.12), 7.49 (d, 1H, $H^{6(5)}$, J= 4.1); 7.64 dd, 2H, $H^{3,4}$, J= 3.2, J= 3.18); 8.02 (d, 1H, H^4thioph, J= 2.04). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 39.9, 113.24, 123.04, 124.45, 132.58, 133.79, 135.55, 142.79, 149.65, 184.56.

2-((5-chloro-1H-benzo[d]imidazole-2-yl)thio)-1-(thiophen-2-yl)ethan-1-one hydrogen bromide (1f·HBr)

Yield 87 %, m.p. 223-225º C (EtOH) (dec). IR spectrum, ν/ cm^{-1}: 3130, 3048, 2974, 2918, 1646, 1590, 1522, 1505, 618, 596. Found (%): C 39.97; H 2.70; Br+Cl 29.46; N 7.08; S 16.30.$C_{13}H_9ClN_2OS_2$ HBr. Calculated (%): C 40.07; H 2.59; Br + Cl 29.60; N 7.19; S 16.44. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm, J (Hz): 5.15 (c, 2H, CH_2); 7.31-7.33 (m, 2H, H^6, H^4 thioph); 7.58 (d, 1H, H^7, J=8.64); 7.64 (d, 1H, H^4, J=1.86); 8.10-8.11 (k, 1H, H^3 thioph, J=5.88); 8.16-8.17 (k, 1H, H^5 thioph, J=4.8). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm, J (Hz): 40.0, 113.26, 114.57, 123.66, 127.87, 128.90, 134.28, 134.68, 136.0, 135.50, 141.30, 151.51.

2-((5chloro-1H-benzo[d]imidazole-2-yl)thio)-1-(furan-2-yl)ethan-1-one hydrogen bromide (1g .HBr)

Yield 86 %, m.p. 218.5-220 ºC (EtOH) (dec). IR spectrum, ν/cm^{-1}: 3126, 3029, 2916, 1665, 1633, 1557, 1501, 650, 621. Found (%): C 41.68; H 2.82; Br + Cl 30.74; N 7.37; S 8.44. $C_{13}H_9ClN_2O_2S$

HBr. Calculated (%): C 41.79; H 2.70; Br + Cl 30.87; N 7.50; S 8.58. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 4.98 (c, 2H, S-CH$_2$); 6.78 (k, 1H, H^4$_{furf,}$ J=1.58); 7.33 (dd, 1H, H^6, J= 1.68, J=1.74); 7.57 (d, 1H, H^3$_{furf,}$ J=6.42); 7.63-7.64 (m, 2H, H^7, H ^{5}$_{furf}$); 8.08 (c, 1H, H^4). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 36.9, 112.81, 113.25, 114.57, 119.91, 123.68, 127.90, 134.22, 136.44, 148.55, 150.27, 151.45, 180 59.

2-((5chloro-1H-benzo[d]imidazole-2-yl)thio)-1-(3,5-di-tert-bityl-4hydrpxyphenyl)ethan-1-one hydrogen bromide (1h .HBr)

Yield 89 %, m.p. 216-217º C (dec). IR spectrum, ν/cm^{-1}: 3624(OH), 3421 (NH), 1665 (C=O), 2941, 3002 (C-H)$_{Ar}$, 1716 (C=O). 1625, 1589, 1514 (C=C, C=N), 598,619, 659. Found (%): C 53.86; H 5.62; Br + Cl 22.41; N 5.34; S 6.12. $C_{23}H_{27}ClN_2O_2S$ HBr**.** Calculated (%): C 53.97; H 5.51; Br + Cl 22.54; N 5.47; S 6.26. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 1,42 (c, 18H, 6 CH$_3$); 5.35 (c, 2H, CH$_2$); 7.45 (dd, 1H, H^6, J=9.24); 7.68 (dd, 2H, H^{4,7}, J=9.24); 7.82 (c, 2H, H$_{Ph}$); 8.01 (c, 1H, OH). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 31.6, 34.40, 38.2, 115.8, 116.6. 123.3, 124.1, 128.5, 129.2, 136.0, 137.0, 140.3, 147.1, 159.0, 194.1.

2-((5-chloro-1H-benzo[d]imidazole-2-yl)thio)-1-(3-methoxyphenyl)ethan-1-one hydrogen bromide (1i .HBr)

Yield 91 %, m.p. 214-216º C (EtOH/DMF) (dec). IR spectrum, ν/cm^{-1}: 3003, 2959, 1681 (C=O), 1665, 1633, 1597, 1585, 1249, 1038, 621, 654, 653. Found (%): C 46.54; H 3.52; Br + Cl 27.75; Br + Cl 27.75; N 6.65; S 7.42. $C_{16}H_{13}ClN_2O_2S$ HBr. Calculated (%): C 46.65; H 3.41; Br + Cl 27.88; N 6.77; S 7.56 . ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.75 (c, 3H, OCH$_3$); 5.03(c, 2H, S-CH$_2$); 7.23 (dd, 2H, H^6, H$_{ph}$, J= 8.7, J= 8,28); 7.43-7.46 (m, 3H, H$_{ph}$), 7,51 (c, 1H, H^7), 7.58 (d, 1H, H^4, J=7.38). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 40.89, 56.16, 113.64, 114.05, 115.39, 120.85, 121.73, 124.24, 128.55, 131.02, 135.76, 136.87, 137.99, 152.29, 160.14, 193.61.

1-(benzo[d][1,3]dioxol-5-yl)-2-((5-chloro-1H-benzo[d]imidazole-2-yl)thio)ethan-1-one hydrogen bromide (1j .HBr)

Yield 93 %, m.p. 231-232 ºC (EtOH) (dec). IR spectrum, ν/cm^{-1}: 2975, 1656 (C=O), 1623, 1616, 1597, 641, 665. Found (%): C 44.82; H 2.94; Br + Cl 26.84; N 6.43; S 7.29. $C_{16}H_{11}ClN_2O_3S$ HBr. Calculated (%): C 44.93; H 2.83; Br + Cl 26.97; N 6.55; S 7.43 . ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 5.18 (c, 2H, S-CH$_2$); 6.16 (c, 2H, O-CH$_2$-O); 7.11(d, 1H, H$_{Ph}$, J=8.2); 7.32-7.34 (dd, 1H, H^6); 7.51 (d, 1H, H^7, J=1,8); 7.58 (d, 1H, H$_{ph}$, J=8.6), 7.64 (d, 1H, H$_{Ph}$, J=1,9); 7.71-7.72 (m, 1H, H^4). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 40.53, 102.2, 107.90, 108.18, 113.19, 114.22, 123.69, 125.21, 125.84, 126.42, 127.62, 147.86, 151.85, 152.09, 190.48.

2-((5-chloro-1H-benzo[d]imidazole-2-yl)thio)-1-(3,4-dimethoxyphenyl)ethane-1-one hydrogen bromide (1k .HBr)

Yield 95 %, m.p. 229-230.5 ºC (EtOH) (dec). IR spectrum, ν/cm^{-1}: 3106, 3004, 2886, 1664, (C=O), 1615, 1593, 1583, 1513, 1260, 1060, 621, 589. Found (%): C 45.91; H 3.74; Br + Cl 25.87; N 6.18; S 7.08. $C_{17}H_{16}ClN_2O_3S$ HBr. Calculated (%): C 46.02; H 3.63; Br + Cl 26.00; N 6.31; S 7.22. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.75 (c, 3H, OCH$_3$); 3.80 (c, 3H, OCH$_3$); 5.03 (c, 2H, S-CH$_2$); 7.04 (d, 1H, H$_{ph}$, J=8.58); 7.26 (dd, 1H, H^6, J=2.4, J=1.92); 7.41 (d, 1H, H$_{ph}$, J= 1.98); 7.48 (d, 1H, H$_{ph}$, J= 8.64); 7.54 (d, 1H, H^7, J= 1.92); 7.67 (dd, 1H, H^4, J=2.04, J=1.98). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 40.71, 56.34, 56.60, 111.21, 111.77, 113.97, 115.35, 124,41, 125.50, 128.27, 128.81, 135.28, 137.47, 149.27, 152,49, 154.53, 192.08.

1-(3,5-di-tert-butyl-4-hydroxyphenyl)-2-((5-methoxy-1H-benzo[d]imidazo-2-yl)thio)ethane - 1-one hydrogen bromide (1l .HBr)

Yield 94 %, m.p. 205-207 ºC (MeCN) (dec). IR spectrum, ν/cm^{-1}: 3624 (OH), 3331(NH), 2948, 1666 (C=O), 1632, 1624, 1586, 1521 (C=C, C=N), 621, 655(C^2-S). Found (%): C 56.69; H 6.06; Br 15.62; N 5.40; S 6.18. $C_{24}H_{30}N_2O_3S$ HBr. Calculated (%): C 56.80; H 6.16; Br 15.75; N 5.52; S 6.32. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 1.33 (c, 18H, 6 CH$_3$); 3.75 (c, 3H, OCH$_3$); 5.09 (c, 2H, S-CH$_2$); 6.98 (m, 3H, H$^{4,6.7}$),7.48 (d, 1H, OH, J= 8.94); 7.75 (c, 2H, H$_{Ph}$). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 30.53, 35.12, 41.13, 56.57, 96.85, 114.82, 114.99, 126.66, 126.83, 128.60, 135.01, 139.19, 149.55, 158.08, 160.52, 192.39.

2-((5-methoxy-1H-benzo[d]imidazole-2-yl) thio)-1-(3- methoxyphenyl)ethan-1-one hydrogen bromide (1m .HBr)

Yield 96 %, m.p. 213-214 (EtOH/DMF) (dec). IR spectrum, ν/cm^{-1}: 3003, 2959, 1681 (C=O), 1665, 1633, 1597, 1585, 1249, 1038, 621, 654, 653. Found (%): C 49.78; H 4.30; Br 19.39; N 6.72; S 7.69. $C_{17}H_{16}N_2O_3S$ HBr. Calculated (%): C 49.89; H 4.19; Br 19.52; N 6.84; S 7.83. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.83, 3.84 (c, 6H, 2OCH$_3$), 5.36 (c, 2H, S-CH$_2$), 7.05-7.07 (dd, 1H, H^6, J= 2.34, J= 2.34); 7.12 (d, 1H, H^4, J= 2.28), 7.30 (t, 1H, H$_{Ph}$, J=4.11), 7.52 (t, 1H, H$_{Ph}$, J=7.95), 7.54 (t, 1 H, H$_{Ph}$, J=1.89), 7.56 (d, 1H, H^7, J=8.8), 7.66 (d, 1H, H$_{Ph}$, J=7.95). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 41.54, 55.49, 55.83, 96.01, 113.14, 113.92, 114.34, 120.04, 120.92, 127.15, 130.05, 133.68, 136.00, 148.76, 157.43, 159.43.

1-([1,1'-biphenyl]-4-yl)-2-((5-methoxy-1H-benzo[d]imidazole-2-yl)thio)ethan-1-one hydrogen bromide (1n .HBr)

Yield 94 %, m.p. 221-222 (EtOH/DMF) (dec). IR spectrum, ν/cm^{-1}: 3392, 3127, 2975, 1667 (C=O), 1625, 1602, 1583, 593, 616. Found (%): C 57.92; H 4.32; Br 17.42; N 6.03; S 6.90. $C_{22}H_{18}N_2O_2S$ HBr. Calculated (%): C 58.03; H 4.21; Br 17.55; N 6.15; S 7.04. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): .82 (c, 3H, OCH$_3$); 5.52 (c, 2H,S- CH$_2$); 7.04 (dd, 1H, H^6, J=2.4, J=2.34); 7.11 (d, 1H, H^4, J=0.84); 7.43-7.45 (k,1H, H^7, J= 12); 7.51 (t, 2H, H$_{Bif}$, J= 7.62); 7.56 (d, 1H, H$_{Bif}$, J= 8.94); 7,76 (c,1H, H$_{Bif}$); 7.78 (d, 1H, H$_{Bif}$, J=1.4); 7,88 (c, 1H, H$_{Bif}$); 7.89 (c, 1H, H$_{Bif}$); 8,16 (c, 1H, H$_{Bif}$); 8.17 (c, 1H, H$_{Bif}$). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 41.78, 55.75, 95.92, 113.84, 114.09, 126.92, 126.97, 128.50, 129.02, 129.22, 133.52, 133.94, 138.56, 145.33, 148.70; 157.25, 191.96.

2-((5-methoxy-1H-benzo[d]imidazole-2-yl)thio)-1-(naphthalene-1-yl)ethan-1-one hydrogen bromide (1o .HBr)

Yield 90 %, m.p. 228-229 (EtOH/DMF) (dec). IR spectrum, ν/cm^{-1}: 3040, 3005, 2907, 2815, 1662, 1636, 1592, 1571, 660, 641. Found (%): C 55.84; H 3.88; Br 18.48; N 6.41; S 7.33. $C_{20}H_{16}N_2O_2S$ HBr. Calculated (%): C 55.95; H 3.99; Br 18.61; N 6.53; S 7.47. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.75 (c, 3H, OCH$_3$); 5.19 (c, 2H,S- CH$_2$); 6.99 (d, 1H, H^6, J=8.8); 7.05(c, 1H, H^4); 7.47 (d, 1H, H$_{Ar}$, J=8.9); 7.54 (t, 2H, H^7, H$_{Ar}$, J=4.29); 7.61 (t, 1H, H$_{Ar}$, J=7.71); 7.95 (t, 1H, H$_{Ar}$, J=4.4); 8.14 (d, 1H, H$_{Ar}$, J=8.3); 8.2 (d, 1H, H$_{Ar}$, J=7.1);8.4 (c, 1H, H$_{Ar}$). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): ^{13}C: 43.48, 56.58, 96.86, 11.86, 114.96, 125.56, 125.74, 127.54, 128.80, 129.15, 129.43, 130.22, 130.58, 132.83, 134.18, 134..82, 135,15, 149.18, 158.06, 196.39.

1-(benzo[d][1,3]dioxol-5-yl)-2-((5-methoxy-1H-benzo[d]imidazol-2-yl)thio)ethan-1-one hydrogen bromide (1p .HBr)

Yield 89 %, m.p. 217-218 (EtOH) (dec). IR spectrum, ν/cm^{-1}: 2975, 1656 (C=O), 1623, 1616, 1597, 641, 665. Found (%): C 48.13; H 3.68; Br 18.75; N 6.50; S 7.43. $C_{17}H_{14}N_2O_4S$ HBr. Calculated (%): C 48.24; H 3.57; Br 18.88; N 6.62; S 7.57. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.81 (c, 3H, OCH$_3$); 5.19 (c, 2H, S-CH$_2$); 6.17 (c, 2H, O-CH$_2$O); 6.99 (dd, 1H, H^6, J= 2.4, J=2.4); 7.08 (d, 1H, H$_{Ph}$, J=2.34); 7.11(d, 1H, H^4, J= 8.16); 7.50-7.51 (dd, 2H, H^7, H$_{Ph,}$ J=3.66, J=3.48); 7.72 (dd, 1H, H$_{Ph,}$ J=1.8, J=1.74). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 40.83, 55.73, 96.20, 102.22, 107.65, 108.20, 113.49, 114.02, 125.24, 128.63, 129.29, 134.89, 147.87, 148.75, 152.13, 156.96, 190.48.

2-((5-methoxy-1H-benzo[d]imidazole-2-yl)thio)-1-(4-methoxyphenyl)ethan-1-one hydrogen bromide (1q .HBr)

Yield 92 %, m.p. 221.5-222 (EtOH) (dec). IR spectrum, ν/cm^{-1}: 3003, 2959, 1681 (C=O), 1665, 1633, 1597, 1585, 1249, 1038, 621, 654, 653. Found (%): C 49.78; H 4.28; Br 19.39; N 6.71; S 7.69. $C_{17}H_{16}N_2O_3S$· HBr. Calculated (%): C 49.89; H 4.19; Br 19.52; N 6.84; S 7.83. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.83 (c, 3H, OCH$_3$); 3.86 (c, 3H, OCH$_3$); 5.31 (c, 2H, S-CH$_2$); 7.06 (m, 1H, H^6); 7.12 (m, 2H, H^4, H$_{ph}$); 7.58 (d, 1H, H^7, J=8.94); 8.04 (d, 2H, H$_{ph}$, J= 8.9). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 41.29, 55.67, 55.82, 95.99, 113.88, 114.11, 114.34, 127.04, 127.49, 130.89, 133.59, 149.01, 157.44, 163.87, 190.49.

1-(furan-2-yl)-2-((5-methoxy-1H-benzo[d]imidazole-2-yl)thio)ethan-1-one hydrogen bromide (1r..HBr)

Yield 90 %, m.p. 208-210 (EtOH) (dec). IR spectrum, ν/cm^{-1}: 3126, 3029, 2916, 1665, 1633, 1557, 1501, 650, 621. Found (%): C 45.43; H 3.64; Br 21.51; N 7.47; S 8.54. $C_{14}H_{12}N_2O_3S$ ·HBr. Calculated (%): C 45.54; H 3.55; Br 21.64; N 7.59; S 8.68. ^{1}H NMR spectrum (600 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 3.75 (c, 3H, OCH$_3$); 4.83 (c, 2H, CH$_2$); 6.72 (m, 1H, H^4 $_{furf}$); 7.00 (dd, 1H, H^6, J=1.62, J=2,34); 7.08 (d, 1H, H^4, J= 2.28); 7.49 (d, 1H, H^3 $_{furf}$, J= 8.94); 7.55 (d, 1H, H^7, J= 3.6); 7.94 (c, 1H, H^5 $_{furf}$). ^{13}C NMR spectrum (150 MHz, DMSO-d$_6$), δ, ppm, J (Hz): 39.65,

56.59, 96.83, 113.88, 114.93, 115.23, 121.45, 128.59, 134.98, 148.77, 149.62, 150.61, 158.17, 181.43.

Influence of compounds on intraocular pressure

The study of the effect of compounds on IOP level was performed on adult intact rodent rats and rats with dexamethasone-induced ophthalmic hypertension by tonometry using a veterinary tonometer TonoVet (Finland) (Du Sert et al. 2020). The study of compounds was performed according to the method of Marcus et al. (Pease et al. 2006), according to which one drop of the solution of the compound under study was instillated into the right (tested) eye of the laboratory animal, and one drop of the solvent - deionized water - was instilled into the left eye. IOP was measured in both eyes. The left eye served to assess the possible resorptive effect of the tested compounds.

The investigated melatonin isosters – mercaptobenzimidazole derivatives – were instilled at a concentration of 0.4% once in a volume of 50 μL. IOP was measured at four time points (0, 1, 2, 3 h), where 0 h was the baseline value. The concentration dependence of ophthalmohypotensive activity was evaluated for the most active compounds. The presence of ophthalmohypotensive activity was evaluated by the maximum IOP reduction from the initial data. 0.5% solution of timolol (Grotex LLC, Russia) (a drug used in clinical practice) and 0.4% solution of melatonin (SigmaAldrich, USA) were used as comparison drugs. To model steroidal ophthalmic hypertension in rats, bilateral local instillation of 0.1% dexamethasone solution (Elfa Research and Production Center JSC, Russia) was performed for 1 month (Marcus et al. 2018).

Local irritant action

Local irritant action of the most active compounds was studied on guinea pigs. For this purpose, a conjunctival test was performed (Marcus et al. 2019). 50 μL of 0.4% aqueous solution was instilled into the right (test) eye of guinea pigs using an automatic pipette; the left (contralateral) eye served as a control. Experimental groups included 3 animals each. Reactions were recorded after 15, 30, 60 minutes, 24 hours and evaluated in scores.

Statistical processing

Statistical processing and graphing were performed in Prism 8.0 program (GraphPad Inc.) with automatic calculation of arithmetic mean and standard deviation. Student's t-test was used to evaluate the change in intraocular pressure relative to the initial values.

Results and Discussion

As a result of this work, it was found that the comparison drug timolol 2 hours after instillation reduces IOP by 26.84%, and melatonin maximally lowers IOP after 3 hours by 30.95%, after which ophthalmotonus gradually begins to return to the initial values (Table 1). Both drugs decreased IOP in the contralateral eye, which indicates the presence of systemic action after absorption into the bloodstream.

According to the results obtained, all the tested compounds can be divided into groups: highly active (ophthalmohypotensive effect > 25%) – **1a**, **1e**, **1m**; compounds with moderate ophthalmic hypotensive activity (15-25%) – **1b**, **1h**, **1i**, **1f**, **1l**, **1o**, **1p** and low-activity (ophthalmohypotenchive effect <15%) – **1c**, **1d**, **1j**, **1g**, **1k**, **1r**, **1n**, **1q**.

Thus, the most active compounds **1a**, **1m** were superior to timolol in their ophthalmic hypotensive activity (-31.37%, -29.95% and -26.84%, respectively), and compound **1e** was not inferior to the comparison drug timolol (-26.87% and 26.84%, respectively). After instillation of compound **1a**, the maximum IOP-lowering effect developed after 60 minutes and amounted to - 31.37%, which exceeds the ophthalmic hypotensive effect of melatonin (-30.33%), but after 2 hours, IOP returned to the initial values. Similar dynamics was observed in the control eye, which indicates undesirable systemic action of the substance.

In the study of compound **1e**, the maximum reduction of IOP by 26.87% was observed 2 hours after instillation and insignificantly changed by 3 hours. Reduction of ophthalmotonus in the control eye may be a manifestation of resorptive effect.

The maximum IOP decrease after instillation of compound **1m** was observed after 2 hours and amounted to -29.95% of the initial values and insignificantly changed by 3 hours. The dynamics of IOP decrease in the control eye is the basis for judgment about the resorptive effect of the compound under study.

The following substances showed average ophthalmohypotensive effect. Instillation of compound **1p** resulted in decrease of ophthalmotonus by 23.16% only after 180 minutes. At the same time IOP decrease was also observed in the control eye.

Table 1. Effect of 0.5% solution of timolol, melatonin and new derivatives of mercaptobenzimidazole on IOP of normotensive rats at a single instillation at a concentration of 0.4%

Compound	Concentration, %	Max Δ IOP, %	Effect on the contralateral eye
Timolol	0.5	-26.84±0.94*	+
Melatonin	0.4	-30.33±2.91*	+
1a	0.4	**-31.37±1.01***	+
1b	0.4	-17.29±6.75	-
1c	0.4	-13.1±2.56	+
1d	0.4	-11.92±2.39	-
1e	0.4	**-26.87±3.85***	+
1h	0.4	-19.09±5.00	+
1i	0.4	-17.78±3.40*	+
1j	0.4	-3.7±3.7	+
1f	0.4	-15.87±4.88	+
1g	0.4	-12.12±9.10	-
1k	0.4	-12.73±6.39	-
1l	0.4	-19.7±6.48	-
1m	0.4	**-29.95±6.79***	+
1r	0.4	-13.15±3.52	-
1n	0.4	-13.13±3.33	+
1o	0.4	-17.41±8.99	+
1p	0.4	-23.16±2.15*	+
1q	0.4	-11.11±5.56	-

***Note**:* "-" – absence of effect; "+" – presence of effect; *** –** differences are statistically significant relative to baseline values (Student's t-test, $p<0.05$).

Substance **1l** 60 minutes after instillation into the test eye reduced IOP by 13.8%, and after 120 minutes – by 19.7%. By the third hour, the index of ophthalmotonus remained at the level of -19.7% relative to the initial values. The compound had no significant effect on the control eye.

After instillation of compounds **1h** and **1i**, the maximum IOP-lowering effect developed after 3 hours and amounted to -19.09% and -17.78%, respectively. Compound **1o** reduced IOP by 17.41% after 60 minutes, after 120 minutes the pressure increased and amounted to -10.37% relative to the initial data. When studying these compounds in the contralateral eye, a decrease in ophthalmotonus was also observed, which may indicate a systemic effect.

Compounds **1b** and **1f** gradually decreased the ophthalmotonus of the test eye after instillation, reaching a maximum after 3 hours (-17.29% and -15.87, respectively). No IOP changes were observed in the contralateral eye.

The other compounds showed low ophthalmohypotensive activity. The results revealed that compounds **1c**, **1r** and **1n** reduced IOP by 13.1%, 13.15% and 13.13%, respectively. Compounds **1r** and **1n**, unlike **1c**, had a systemic effect.

Substances under laboratory codes **1d**, **1g** and **1k** caused a decrease in ophthalmotonus by -11.92%, -12.12% and -12.73% 120 minutes after instillation, respectively, subsequently IOP returned to the initial values. The substance under the laboratory code **1q** showed a weak ophthalmohypotensive effect after 60 minutes (-11.11%); by the third hour, there was an increase in pressure to the initial values. None of four substances had any significant effect on the contralateral eye.

Instillation of compound **1j** hardly caused any ophthalmohypotensive effect. The maximum IOP decrease amounted to -3.7% relative to the initial values.

According to the screening results, for the most active compounds, concentration dependence, effect on ophthalmotonus in rats with dexamethasone-induced glaucoma, evaluation of their local irritant effect, and study of the ophthalmohypotensive effect of the most promising compound were carried out.

The study of the dose-dependent effect of the test substance for compounds **1a**, **1e** and **1m** was carried out at concentrations of 0.1%, 0.2%, 0.4%.

When substance **1a** was instilled at a concentration of 0.4%, the maximum IOP reduction was observed after 60 minutes, -28.53% relative to baseline values. In lower concentrations (0.2% and 0.1%), the maximum ophthalmohypotensive effect developed only by 2 hours (-15.56 % and -16.5%, respectively). The studied compound **1a** at a concentration of 0.4% caused a decrease in IOP in the contralateral eye, which was not detected when 0.2% and 0.1% solutions were instilled. Thus, the decrease in concentration leads to an increase in the latency

period and a decrease in the ophthalmohypotensive effect, as well as a decrease in the manifestations of resorptive action (Fig. 3).

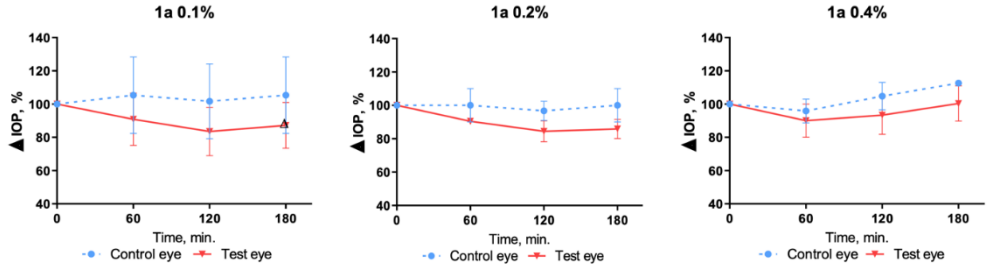


Figure 3. Effect of compound **1a** on the intraocular pressure level of normotensive rats by a single instillation of 30 μL into the test eye at concentrations of 0.1%, 0.2%, 0.4%.

In the study of compound **1e** at a concentration of 0.4%, IOP was significantly reduced by 27.39% 2 hours after instillation. At instillation of 0.2% solution after 60 minutes, the pressure decreased only by 18.06% relative to the initial values. The investigated compound in 0.1% concentration had no effect on ophthalmotonus (-6.67% relative to the initial data – not significant). The investigated compound **1e** at a concentration of 0.4% caused a decrease in ophthalmotonus in the contralateral eye, and, at lower concentrations, there were no manifestations of systemic effect. Thus, a decrease in the concentration leads to a decrease in the ophthalmohypotensive effect, as well as a decrease in the manifestations of resorptive action (Fig. 4).

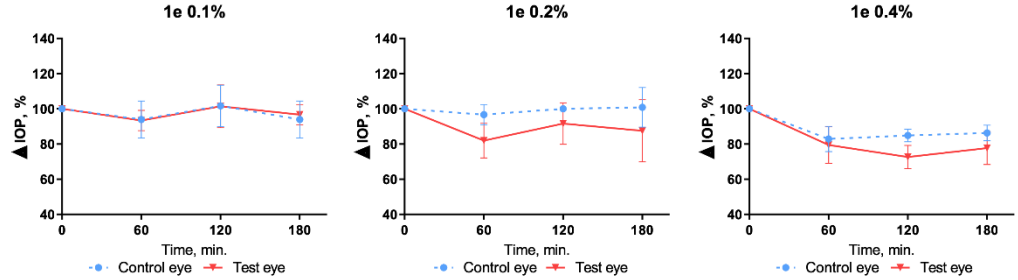


Figure 4. Effect of compound **1e** on the intraocular pressure level of normotensive rats by a single instillation of 30 μL into the test eye at concentrations of 0.1%, 0.2%, 0.4%.

Instillation of compound **1m** at a concentration of 0.4% resulted in the development of maximum IOP-lowering effect after 120 minutes (-22.46% relative to baseline values). At concentrations of 0.2% and 0.1%, the reduction in ophthalmotonus was -17.68% and -15.00%, respectively. The compound **1m** in any of three concentrations had no effect on the control eye (Fig. 5).

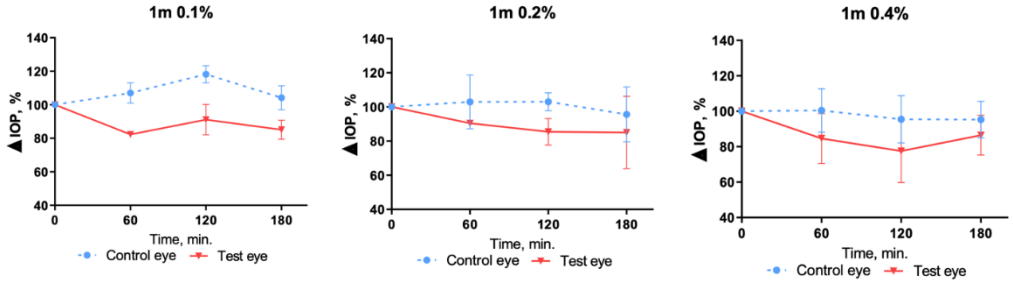


Figure 5. Effect of compound **1m** on the intraocular pressure level of normotensive rats by a single instillation of 30 μL into the test eye at concentrations of 0.1%, 0.2%, 0.4%.

The next stage was to study the local irritant effect of the compounds that showed the highest ophthalmohypotensive effect in the study on normotensive rats. Thus, during the conjunctival test in guinea pigs by instillation aqueous solutions of compounds **1a**, **1e** and **1m** at a

concentration of 0.4%, there was no reddening of the lacrimal duct, sclera and conjunctiva, which may indicate the absence of irritant effect.

At the next stage, the most active compounds were studied on the model of dexamethasone-induced glaucoma (Fig. 6). Instillation of timolol in animals with ophthalmic hypertension caused a decrease in ophthalmotonus after 3 hours by 29.76% relative to baseline values. Similar dynamics was observed in the control eye, which confirms the undesirable resorptive effect of this drug.

Instillation of melatonin to animals with dexamethasone-induced glaucoma also resulted in IOP reduction by 23.72% by 3 hours, no systemic effect was detected, as no IOP change was observed in the control eye.

After instillation of compound **1a** the maximum ophthalmohypotensive effect developed after 180 minutes and amounted to -23.74%, which does not exceed the effect of the comparison drug timolol, but corresponds to the activity of melatonin. In the control eye similar dynamics was observed, which may indicate undesirable systemic action of the substance.

In the study of compound **1e** on animals with ophthalmohypertension, a decrease in ophthalmotonus was observed 1 hour after instillation by 7.85%, which is not significant. The substance had no effect on the control eye.

There was almost no ophthalmohypotensive effect after instillation of substance **1m**.

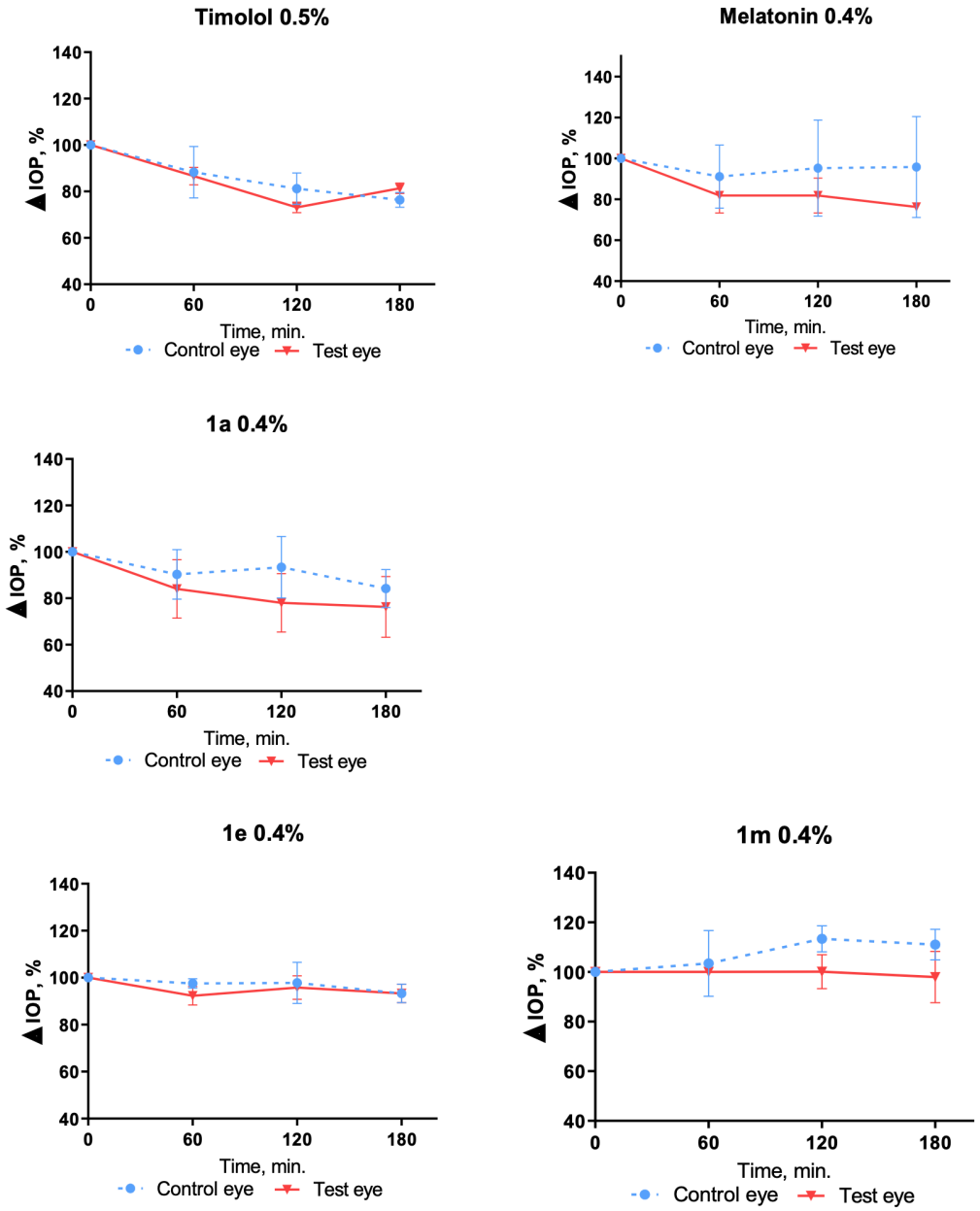


Figure 6. Effect of 0.5% solution of timolol and compounds **1a**, **1e**, **1m** at a concentration of 4 mg/mL on IOP of rats with dexamethasone-induced glaucoma by a single instillation of 30 μL into the test eye.

Conclusion

Thus, according to the results of our studies, 2-mercaptobenzimidazole derivatives have shown to be a promising class for the search of new compounds that reduce IOP.

At a single instillation of 30 μL of solutions of the studied compounds in the concentration of 4mg/mL, the most active compounds were compounds **1a** and **1m**, which were superior to timolol in their ophthalmohypotensive activity. Compound **1e** is comparable to the comparison drug in its ophthalmic hypotensive activity.

The study of the dependence of IOP-lowering effect on the concentration (0.4%, 0.2% and 0.1%) of the administered compounds showed that with decreasing concentration, there was an increase in the latent period of the effect development and a decrease in the investigated activity.

The most active compounds **1a**, **1e** and **1m** had no local irritant effect in the conjunctival test when 30 μL of 0.4% solutions were instilled once in guinea pigs.

Compound **1a** also showed high activity in a steroid-induced glaucoma model (dexamethasone), reducing IOP by 23.74%, with a single instillation of 30 μL of solution at a dose of 4 mg/mL, which is similar to melatonin (-23.72%) but inferior to the comparison drug timolol (-29.76%), making it most promising for further study.

Additinal information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The chemical section was financially supported by the Ministry of Science and Higher Education of the Russian Federation (state task in the field of scientific activity, Southern Federal University, grant FENW-2023-0011).

Pharmacological studies were carried out within the framework of project No. 22-15-2002 with financial support from the Russian Science Foundation and the Volgograd Region Administration.

Data availability

All of the data that support the findings of this study are available in the main text.

Acknowledgments

This work was carried out using the equipment of the Common Use Center of the Southern Federal University, Rostov-on-Don, Russia.

Ethics Statements

The experiments were approved by the Biomedical Ethics Commission of Volgograd State Medical University (VolSMU) IRB 00005839 IORG 0004900, OHRP, Certificate No. 2021/056 dated 15.06.2021.

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