Research Results in Pharmacology

Research Results in Pharmacology 10(3): 73–84
UDC: 615.457:547.856.1
DOI 10.18413/rrpharmacology.10.486

Research Article

Synthesis and pharmacological properties of novel guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione

Alexander A. Ozerov, Daria V. Merezhkina, Natalia A. Gurova, Lyudmila V. Naumenko, Denis A. Babkov, Victor S. Sirotenko, Roman A. Litvinov, Alena S. Taran, Nadezhda V. Stepanova, Umida M. Ibragimova, Alexander A. Spasov, Vadim A. Kosolapov

Volgograd State Medical University, 1, Pavshikh Bortsov Sq., Volgograd, 400131, Russia

Corresponding author: Vadim A. Kosolapov (vad-ak@mail.ru)

Academic editor: Mikhail Korokin ♦ **Received** 28 May 2024 ♦ **Accepted** 10 August 2024 ♦ **Published** 23 September 2024

Citation: Ozerov AA, Merezhkina DV, Gurova NA, Naumenko LV, Babkov DA, Sirotenko VS, Litvinov RA, Taran AS, Stepanova NV, Ibragimova UM, Spasov AA, Kosolapov VA (2023) Synthesis and pharmacological properties of novel guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione. Research Results in Pharmacology 10(3): 73–84. https://doi.org/10.18413/rrpharmacology.10.486

Abstract

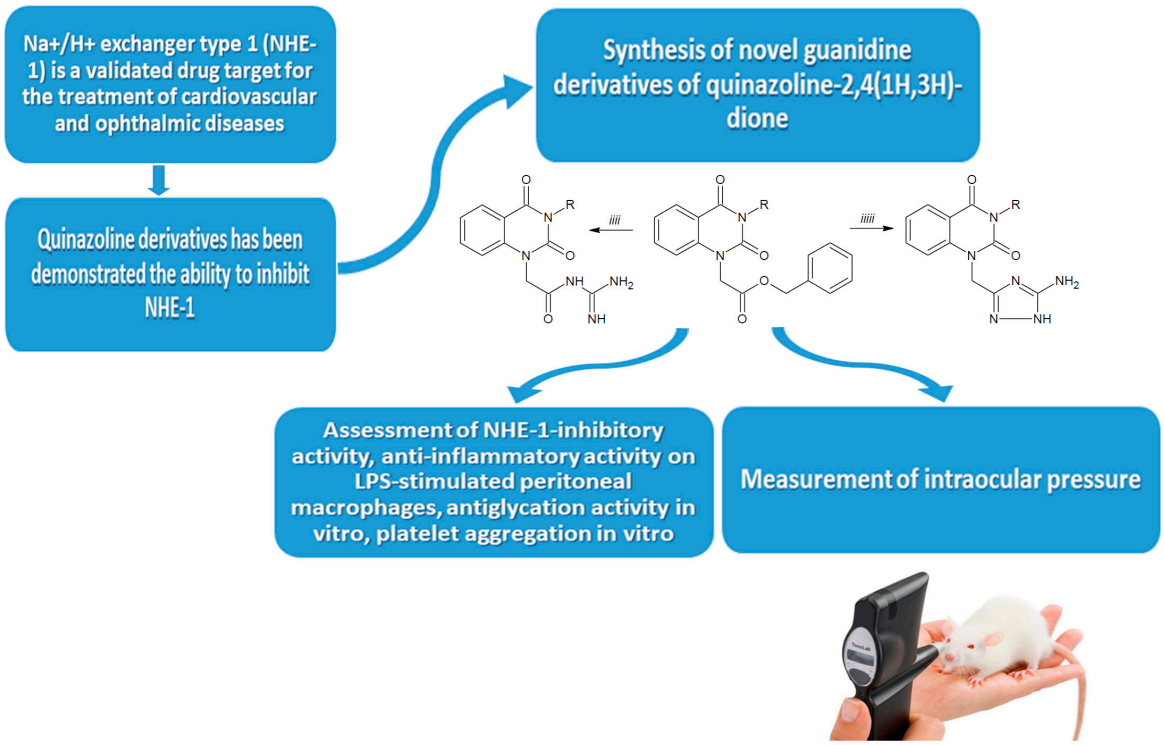
Introduction: Na^+/H^+ exchanger type 1 (NHE-1) is a validated drug target for the treatment of cardiovascular and ophthalmic diseases due to the cytoprotective, anti-ischemic and anti-inflammatory properties of NHE-1 inhibitors. This article presents data on the synthesis and pharmacological activity studies of novel guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione **6-11** and reference drugs amiloride, rimeporide, zoniporide, dexamethasone, aminoguanidine, and acetylsalicylic acid.

Materials and Methods: Pharmacological properties were assessed using pH-dependent platelets deformation assay, anti-inflammatory activity assay on LPS-stimulated peritoneal macrophages, antiglycation assay, analysis of platelet aggregation *in vitro* and measurement of intraocular pressure *in vivo*.

Results: Several compounds combine NHE-1 inhibition with antiglaucomic and antiplatelet activity. Compound **11** significantly inhibits pro-inflammatory activation of murine macrophages (IC_{50} 15.64 μM) and effectively suppresses the formation of glycated proteins (38.1±2.6% in C 1 mM).

Conclusion: The investigated compounds represent a promising scaffold for development of agents for the treatment of cardiovascular pathologies, glaucoma, excessive inflammation, and late diabetic complications including retina diabetics and thrombosis.

Graphical abstract



Keywords

Na^+/H^+-exchanger, NHE-1, guanidine derivatives of quinazoline, antiglaucomic action, antiglycation, antiplatelet activity

Introduction

The ability of quinazoline derivatives to inhibit Na^+/H^+-exchanger type 1 (NHE-1) has been demonstrated earlier (Spasov et al. 2021). The interest in studying this activity was due to the fact that NHEs are the most widely studied acid-base regulators in various mammalian cells. They play a key role in the regulation of cytoplasmic acid-base homeostasis together with HCO_3^- transport systems, including Na^+/HCO_3^- cotransporters (NBCs) and Cl^-/HCO_3^- anion exchangers (AEs). In numerous cell types, NHE-1 is a major contributor to alkalinization, preventing the detrimental effects of over-acidification. In myocardial ischemia-reperfusion injury, there is an excessive entry of Na^+ ions into the cell, as well as extracellular Ca^{2+}, releasing intracellular Ca^{2+} from the depot. As a result of calcium overload in the cell, free radicals are formed, NO synthesis increases, cytotoxic edema occurs, and oxidative stress develops, leading to cell death. In addition to this, NHE-1 is also involved in the regulation of cardiomyocyte volume, growth, proliferation, apoptosis and differentiation, as well as a number of physiological activities (Pedersen and Counillon 2019; Yeves and Ennis 2020). NHE-1, activated in immunocompetent cells, leads to aggravation of the inflammatory response (Shi et al. 2013). The level of intracellular pH is also an important factor influencing the processes of phagocytosis, release of cytokines and chemokines, and generation of reactive oxygen species (De Vito 2006). Inhibition of NHE-1 reduces the formation of superoxide radicals and a number of proinflammatory cytokines, such as IL-6, IL-1β and TNF-α, induced in microglia during inflammation (Liu et al. 2010).

NHE inhibitor properties have been identified for many heterocyclic structures, but compounds containing guanidine moiety are more selective against NHE-1. They include amiloride and its derivatives, benzoylguanidine derivatives such as NOE-642 (cariporide), eniporide (EMD-85131), sabiporide (BIIB-722), bicyclic guanidine derivatives, containing quinoline (zoniporide (CP-597396)), MS 31038), indole (SM 20220, SM 20550, SMP-300), benzoxazinone (KB-R9032), dihydrobenzofuran (BMS 284640), tetrahydronaphthalene (T-162559), cycloheptapyridine (TY-12533) and others (Yeves and Ennis 2020). NHE-1 inhibitors have been found among a number of compounds of guanidine

derivatives of quinazoline (Vishnevskaya et al. 2022). We have previously synthesized a number of guanidine derivatives of quinazoline and demonstrated their ability to inhibit NHE-1 and cause some other pharmacological effects such as anti-inflammatory, antiplatelet, intraocular pressure lowering and antiglycating (Spasov et al. 2022; Taran et al. 2023).

Platelet function and platelet-endothelium interactions are involved in the microcirculation including ocular, which is supported by clinical and experimental evidence (Ma et al. 2019). Previous researchers have observed the involvement of platelets in coagulation and inflammation in the endothelium of the Schlemm's canal in glaucoma (Watanabe et al. 2010), and also that patients with glaucoma had significantly lower platelet counts and significantly higher levels of platelet PDW (platelet distribution width) and MPV (mean platelet volume) indexes, compared to controls (Ma et al. 2019). Glaucoma is one of the leading causes of blindness, affecting more than 76 million patients worldwide. In glaucoma, there is an obstruction in the outflow of aqueous fluid, resulting in an increase in intraocular pressure (IOP) (Al-Humimat et al. 2021). IOP reflects the balance between inflow through the ciliary epithelium and outflow, which mainly exits through the trabecular meshwork and in Schlemm's canal of the eye. NHE-1 is expressed at the basolateral margin of the non-pigmented ciliary epithelium, which is related to glaucoma (Freddo et al. 2022).

Non-enzymatic glycosylation (glycation) is a pathobiochemical process universal to all living organisms (Ansari and Rasheed 2010). Glycation is one of the aspects of pathogenesis of late complications of diabetes mellitus, diseases of the senior age, and diseases of degenerative nature (Vasan et al. 2003). It is also available for pharmacologic regulation to attenuate the clinical manifestations of the mentioned conditions and their treatment. Antiglycation agents have different leading mechanisms of antiglycation action. *In vitro* studies provide insight into the potential of compounds to exhibit antiglycation effects.

Here, we expanded our previous study to continue the synthesis of guanidine quinazoline derivatives and to study their possible effect on pH-dependent platelet deformation, inflammation, protein glycation, platelet aggregation, and late complications of diseases of degenerative nature.

Materials and Methods

Chemistry

All reagents were obtained from Panreac Quimica SLU (Spain) and Acros Organics BVBA (Belgium) at the highest grade available and used without further purification. Yields refer to spectroscopically (NMR) homogeneous materials. The melting points were determined in glass capillaries on a Mel-Temp 3.0 apparatus (USA). Thin-layer chromatography (TLC) was performed on Merck TLC Silica gel 60 F254 plates by eluting with corresponding solvent that was developed using a VL-6.LC UV lamp (Vilber). The NMR spectra were recorded using a Bruker Avance 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer in DMSO-d_6 with tetramethylsilane as an internal standard. HRMS were recorded on Bruker micrOTOF (time-of-flight mass analyzer) mass spectrometer equipped with an electrospray ionization source (ESI).

Benzyl (2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetate (2)

A mixture of quinazoline-2,4(1*H*,3*H*)-dione (**1**) (10.00 g, 61.7 mmol), 100 mL of hexamethyldisilazane, and ammonium chloride (0.10 g) was boiled until a clear solution formed, and the excess silylating agent was removed under vacuum. To the residue, benzyl bromoacetate (14.00 g, 61.4 mmol) was added, heated at a temperature of 160-170 °C for 2 hr., while removing the generated trimethylbromosilane under vacuum. After cooling, 50 mL of 95% ethanol and 5 mL of concentrated ammonium hydroxide were added, boiled for 15 min, cooled, and the resulting precipitate was filtered, crystallized from 95% ethanol, resulting in 18.1 g of compound **2**. White solid; yield 95%; mp 232-235 °C; R_f 0.77 (ethyl acetate); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 11.76 (1H, s, NH), 8.03 (1H, d, J = 8 Hz, H-5), 7.71 (1H, t, J = 8 Hz, H-7), 7.34-7.37 (6H, m, phenyl, H-8), 7.29 (1H, t, J = 8 Hz, H-6), 5.20 (2H, s, CH_2), 5.00 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.1, 161.6, 150.3, 140.8, 135.5, 135.3, 128.4, 128.1, 127.8, 127.6, 122.9, 115.4, 114.5, 66.5, 43.8.

Benzyl (3-methyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetate (3)

A mixture of compound 2 (5.00 g, 16.1 mmol), methyl iodide (1.10 mL, 17.7 mmol), and anhydrous finely ground K_2CO_3 (5.00 g, 36.1 mmol) was stirred in a DMF solution (75 mL) at room temperature for 48 hr. The reaction mass was filtered, evaporated to dryness in vacuo; the residue was treated with water (100 mL); the solid residue was filtered off, dried at room temperature, and recrystallized from 95% ethanol, resulting in 3.5 g of compound 3. White solid; yield 67%; mp 152-155 °C; R_f 0.61 (EtOH-$CHCl_3$, 1:35); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.07 (1H, d, J = 8 Hz, H-5), 7.72 (1H, t, J = 8 Hz, H-7), 7.41 (1H, d, J = 8 Hz, H-8), 7.35-7.37 (5H, m, phenyl), 7.31 (1H, t, J = 8 Hz, H-6) 5.21 (2H, s, CH_2), 5.05 (2H, s, CH_2), 3.32 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.1, 161.0, 150.6, 139.5, 135.5, 135.2, 128.4, 128.1, 127.9, 127.8, 123.1, 114.6, 114.3, 66.5, 44.8, 28.1.

Benzyl (3-allyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetate (4)

Compound 4 was obtained similarly from compound 2 using allyl bromide as alkylating agent. White solid; yield 67%; mp 125-128 °C; R_f 0.73 (EtOH-$CHCl_3$, 1:35); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.08 (1H, d, J = 8 Hz, H-5), 7.73 (1H, t, J = 8 Hz, H-7), 7.42 (1H, d, J = 8 Hz, H-8), 7.29-7.36 (6H, m, phenyl, H-6), 5.85-5.91 (1H, m, CH), 5.20 (2H, s, CH_2), 5.04-5.13 (4H, m, CH_2, =CH_2), 4.56 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.0, 160.5, 150.1, 139.6, 135.5, 135.4, 132.2, 128.4, 128.1, 128.0, 127.9, 123.2, 116.6, 114.6, 114.4, 66.6, 44.8, 43.1.

Benzyl (3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetate (5)

Compound **5** was obtained similarly from compound **2** using benzyl chloride as alkylating agent. White solid; yield 69%; mp 119-121 °C; R_f 0.79

(EtOH-$CHCl_3$, 1:35); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.11 (1H, d, J = 8 Hz, H-5), 7.74 (1H, t, J = 8 Hz, H-7), 7.45 (1H, d, J = 8 Hz, H-8), 7.23-7.37 (11H, m, 2 phenyl, H-6), 5.21 (2H, s, CH_2), 5.17 (2H, s, CH_2), 5.09 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.0, 160.9, 150.52, 139.6, 136.9, 135.5, 135.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.5, 127.2, 123.3, 114.6, 114.5, 66.6, 44.9, 44.3.

N-Carbamimidoyl-2-(3-methyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetamide (6)

A mixture of compound **3** (2.00 g, 6.17 mmol), guanidine hydrochloride (0.60 g, 6.31 mmol), and KOH (0.35 g, 6.25 mmol) was refluxed in 95% ethanol solution (50 mL) for 10 min. The hot reaction mass was filtered and cooled. The solid residue was filtered off, dried at room temperature, and twice recrystallized from ethanol, resulting in 1.27 g of compound **6**. White solid; yield 75%; mp 252-255 °C; R_f 0.57 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.01 (1H, d, J = 8 Hz, H-5), 7.67 (1H, t, J = 8 Hz, H-5), 7.22 (1H, t, J = 8 Hz, H-6), 7.16 (1H, d, J = 8 Hz, H-8), 4.43 (1H, s, CH_2), 3.39 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 170.9, 161.3, 158.9, 150.5, 140.4, 134.8, 127.4, 122.1, 114.8, 114.3, 47.1, 28.0; HRMS (ESI) Calcd. for $C_{12}H_{14}N_5O_3$ $[M+H]^+$: 276.1091, Found: 276.1093.

N-Carbamimidoyl-2-(3-allyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetamide (7)

Compound **7** was obtained similarly from compound **4**. White solid; yield 91%; mp 96-100 (dec.) °C; R_f 0.58 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.02 (1H, d, J = 8 Hz, H-5), 7.69 (1H, t, J = 8 Hz, H-7), 7.57 (4H, s, NH), 7.23 (1H, t, J = 8 Hz, H-6), 7.19 (1H, d, J = 8 Hz, H-8), 5.84-5.93 (1H, m, CH), 5.10-5.16 (2H, m, =CH_2), 4.56 (2H, d, J = 5 Hz, CH_2), 4.46 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.1, 160.8, 158.7, 150.0, 140.6, 125.0, 132.56, 127.5, 122.3, 116.7, 114.9, 114.35, 47.1, 43.0; HRMS (ESI) Calcd. for $C_{14}H_{16}N_5O_3$ $[M+H]^+$: 302.1248, Found: 302.1248.

N-Carbamimidoyl-2-(3-benzyl-2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)acetamide (8)

Compound **8** was obtained similarly from compounds **5**. White solid; yield 88%; mp 132-135 °C; R_f 0.50 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.88 (1H, d, J = 8 Hz, H-5), 7.53 (1H, t, J = 8 Hz, H-7), 7.08-7.25 (5H, m, NH, H-6), 6.97 (1H, d, J = 8 Hz, H-8), 5.00 (2H, s, CH_2), 4.56 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.7, 162.1, 157.4, 150.7, 139.6, 136.2, 135.4, 128.2, 127.6, 127.4, 127.3, 127.23, 123.0, 114.1, 46.8, 44.4; HRMS (ESI) Calcd. for $C_{18}H_{18}N_5O_3$ $[M+H]^+$: 352.1404, Found: 352.2209.

1-[(5-Amino-1H-1,2,4-triazol-3-yl)methyl]-3-methylquinazoline-2,4(1H,3H)-dione (9)

A mixture of compound **3** (2.00 g, 6.17 mmol), aminoguanidine carbonate (0.65 g, 6.19 mmol), and KOH (0.35 g, 6.25 mmol) was refluxed in 95% ethanol solution (50 mL) for 1 hr. The hot reaction mass was filtered and cooled. The solid residue was filtered off, dried at room temperature, and twice recrystallized from ethanol, resulting in 1.29 g of compound **9**. White solid; yield 77%; mp 359-362 °C; R_f 0.45 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.03 (1H, d, J = 8 Hz, H-5), 7.67 (1H, t, J = 8 Hz, H-7), 7.22 (1H, t, J = 8 Hz, H-6), 7.17 (1H, d, J = 8 Hz, H-8), 4.37 (2H, s, CH_2), 3.33 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.8, 161.5, 150.5, 140.7, 134.7, 127.3, 122.0, 115.1, 114.3, 47.7, 28.0; HRMS (ESI) Calcd. for $C_{12}H_{13}N_6O_2$ $[M+H]^+$: 273.1095, Found: 273.0940.

1-[(5-Amino-1H-1,2,4-triazol-3-yl)methyl]-3-allylquinazoline-2,4(1H,3H)-dione (10)

Compound **10** was obtained similarly from compound **4**. White solid; yield 63%; mp 273-275 °C; R_f 0.63 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.01 (1H, d, J = 8 Hz, H-5), 7.66 (1H, t, J = 8 Hz, H-7), 7.22 (1H, t, J = 8 Hz, H-6), 7.18 (1H, d, J = 8 Hz, H-8), 5.84-5.91 (1H, m, CH), 5.10-5.15 (2H, m, =CH_2), 4.55 (2H, d, J = 5 Hz, CH_2), 4.36 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.7, 160.9, 150.0, 140.9, 134.8, 132.7, 127.4, 122.0, 116.6, 115.3, 114.3, 47.7, 42.9; HRMS (ESI) Calcd. for $C_{14}H_{15}N_6O_2$ $[M+H]^+$: 299.1251, Found: 299.1140.

1-[(5-Amino-1H-1,2,4-triazol-3-yl)methyl]-3-benzylquinazoline-2,4(1H,3H)-dione (11)

Compound **11** was obtained similarly from compound **5**. White solid; yield 59%; mp 258-261 °C; R_f 0.50 (EtOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.05 (1H, d, J = 8 Hz, H-5), 7.69 (1H, t, J = 8 Hz, H-7), 7.20-7.37 (7H, m, phenyl, H-8, H-6), 5.17 (2H, s, CH_2), 4.47 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 170.5, 161.3, 160.0, 150.4, 140.7, 137.3, 135.1, 128.3, 127.7, 127.6, 127.2, 122.3, 115.1, 114.4, 47.4, 44.2; HRMS (ESI) Calcd. for $C_{18}H_{17}N_6O_2$ $[M+H]^+$: 349.1408, Found: 349.0927.

Preparation of compounds samples

Test compounds were dissolved in 99% DMSO (40 mM concentration) and stored at –25 °C. Serial dilutions were prepared *ex tempore* in a medium suitable for the specific study. Final concentration in samples: DMSO <0.25% (added to control samples in equal concentrations).

Animals

All animal procedures were performed in accordance with the ethical standards for animal manipulation adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and considering the International Recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental Research (1997). The study was approved by the Regional Ethical Committee of Volgograd State Medical University (Registration No. IRB 00005839 IORG 0004900, OHRP), Certificate No. 2022/097, 21.01.2022. All sections of this study comply with the ARRIVE Guidelines for Reporting Animal Research (Percie du Sert et al. 2020). For *in vitro* pH-dependent platelets deformation method, 20 male rabbits of the Chinchilla breed weighing 3-4 kg purchased from Scientific Center of Biomedical Technologies of the Federal Medical and Biological Agency (veterinary certificate №17069) were used. For *in vivo* study of the effect on intraocular pressure, 60 mongrel male rats (220-400g, purchased from Scientific Center of Biomedical Technologies of Federal Medical and Biological Agency (veterinary certificate №18424) were used. For *in vitro* study of anti-inflammatory activity, 9 male C57bl/6j mice (21-24 g obtained from Rappolovo

facility, Saint Petersburg. Russia) were used. The experiments for analysis of platelet aggregation *in vitro* were performed on 6 male rabbits of the Chinchilla breed weighing 2.5-3 kg purchased from Scientific Center of Biomedical Technologies of the Federal Medical and Biological Agency (veterinary certificate №17069).

pH-dependent platelets deformation method for indirect NHE-1 activity measure

NHE-1-inhibitory activity was studied *in vitro* by changing the shape of rabbit platelets by measuring changes in light transmission (Rosskopf et al. 1991). Blood samples were collected from the marginal vein of the rabbit ear into a tube containing 3.8% sodium citrate in a 1:10 ratio. To obtain platelet-rich plasma (PRP), blood was centrifuged at 1000 rpm for 12 min. PRP with Krebs' solution (600 µL, pH 7.4; 37 °C) was used for calibration. In a control series of experiments, 600 µL of sodium propionate solution (135 mM sodium propionate, 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose; pH 6.7; 37°C) was added to 200 µL of PRP to lower the intracellular pH. Changes in platelet shape were recorded using a laser platelet aggregation analyzer ALAT-2 (Biola LLC, Russia). All substances at a concentration of 1×10^{-8} M in a volume of 10 µL were added to 200 µL of PRP in a cuvette 5 minutes before adding sodium propionate solution, incubated under constant stirring (800 rpm, 37°C). The following NHE-1 inhibitors were used as comparison drugs: zoniporide, rimeporide, and amiloride (Sigma, USA). The dose choice was related to the activity of zoniporide as the most effective NHE-1 inhibitor. The activity of the substances was determined by the ability of the compounds to change the platelet shape (light transmission) in comparison with the control (Δ%). For the most active compounds, the dose-dependent effect was studied in the wide concentration range. The IC_{50} value was calculated as the concentration of a compound that inhibits platelet NHE-1 by 50%.

Measurement of intraocular pressure

Rats were randomly assigned to 9 experimental and 1 intact control groups of 6 animals each. At 9:00 a.m. the initial IOP was measured in animals of all groups with a veterinary tonometer ICARE Tono Vet (Finland) without corneal anesthesia (for early diagnosis of glaucoma in veterinary medicine) (Pease et al. 2006). After that, 50 µL of 0.4% water solution of the tested compounds was dropped into the right (tested) eye of the laboratory animal, and the same volume of deionized water was placed into the left eye. Intraocular pressure was measured in both eyes. The left eye, in turn, serves to assess the possible systemic effects of the investigated compounds (Marcus et al. 2018) IOP was measured at four time points (0, 1, 2, 3 hr), where 0 hr. was the baseline IOP value. The presence of IOP-lowering activity was assessed by the maximum IOP reduction from the baseline IOP values of each animal (in %). An IOP-reducing drug used in clinical practice, timolol (0.5% solution), as well as NHE inhibitors zoniporide and amiloride (at a concentration of 0.2%) were used as a reference drugs.

Study of anti-inflammatory activity on LPS-stimulated peritoneal macrophages

Peritoneal macrophages (PM) were isolated from the peritoneal exudate of 9 male C57bl/6j mice (21-24 g, obtained from Rappolovo facility, Saint Petersburg, Russia). To accumulate PMs, 1 mL of 3% proteose peptone (microbiological grade, Obolensk, Russia) solution was injected intraperitoneally (Zhang et al. 2015). After 3 days, mice were euthanized by cervical dislocation. Cells from peritoneal exudate were obtained after aseptically flushing the abdomen with 5 mL of sterile Hanks' solution (+4-6 °C) without calcium and magnesium ions. The total number of cells was counted, and their viability was assessed in a Goryaev's counting chamber (Russia) by staining with 0.4% trypan blue (Sigma-Aldrich, USA). The proportion of live cells exceeded 95%. Cell concentration was adjusted to 1.0×10^6 cells/mL in complete DMEM medium (Gibco) supplemented with 2 mM L-glutamine (Gibco), 10% heat-inactivated fetal bovine serum (BioClot, Germany), 100 U/mL penicillin. and 100 mg/mL streptomycin (Gibco). 200 µL/well were cultured in 96-well clear plates (SPL Life Sciences Co. Ltd., Korea). They were left for 2 hr at 37°C in a humidified atmosphere with 5% CO_2, followed by washing the wells to remove non-adherent cells. After 24 hr of incubation, 20 µL of supernatant was removed and 20 µL of solutions of the tested compounds were added 30 min before *E. coli* O127:B8 LPS (Sigma-Aldrich, USA, same batch for all experiments) in final concentration of 100 ng/mL.

Accumulation of nitrite-anion (a stable end product of NO degradation produced by iNOS) in supernatants after 20-hr incubation of cells with lipopolysaccharide was determined using the standard Griess reagent. The Griess method is based on diazotization of nitrite-anion in acidic medium with sulfonamide and interaction of the diazo compound with *N*-(1-naphthyl)ethylenediamine to form a dyed derivative. Briefly, 50 µL of supernatants sampled 22 hr after PM incubation with test and control compounds were mixed with 50 µL of 1% sulfonamide in 2.5% H_3PO_4 and 50 µL of 0.1% *N*-(1-naphthyl)ethylenediamine in 2.5% H_3PO_4. After incubation at 23 °C for 10 min in a thermostated shaker, the optical density was determined at a wavelength of 550 nm. The optical density was converted to µM NO using a standard curve (0-100 µM $NaNO_2$ diluted in DMEM medium) (Weissman and Gross 2001).

Cytotoxicity

Increase in the content of lactate dehydrogenase enzyme in culture medium is a marker of increased cell membrane permeability and cell death. To determine its content in supernatants, we used a method based on spectrophotometric detection of NADH loss in a mixture containing pyruvate. Supernatant aliquots of 10 µL were taken 24 hr after PM incubation with test and control compounds and LPS were mixed with 250 µL of 0.194 nM NADH solution dissolved in 54 mM phosphate buffer solution, pH 7.5. After that, 25 µL of 6.48 mM pyruvate solution was added to the mixture. The optical density was determined at 340 nm wavelength for 20 min. Conversion of optical density to cell viability was performed using a standard curve (intact cells – 100%, Triton X-100 – 0%) (Cummings and Schnellmann 2021).

Determination of antiglycation activity

Current study utilized a bovine serum albumin glycation model with glucose (Nikitin et al. 2022). According to the mechanistic view of glycation, an effective model reproduces glycation reactions from its initial stages to the final stage of AGEs formation, integrating all potential mechanisms of action of compounds. The reaction medium was comprised of a 0.36 M glucose solution and 1 mg/mL BSA (fraction V) dissolved in phosphate buffer solution (PBS, pH 7.4, 0.137 M NaCl; 0.0027 M KCl; 0.05 M Na_2HPO_4; 0.0018 M KH_2PO_4; 0.02% sodium azide). The selected concentrations differ from physiological ones, but are intended to accelerate the glycation process; at the same time, the PBS composition used includes, in addition to the most significant phosphate ingredients that provide buffer capacity, also other physiologically significant ions. We hypothesize that such an ionic composition of the medium is intended to provide additional stimulation of the glycation reaction and stabilize the number of intermediate products. The samples under investigation were dissolved in 99% DMSO. Following their addition to the reaction medium, the final concentration of the substances was 1000 μM. Aminoguanidine hydrochloride (a recognized antiglycator) was employed as a reference substance, alongside amiloride, zoniporide, and rimeporide (NHE-1 inhibitors). Control samples contained a volume of solvent equivalent to the test substances. The samples were then incubated for a duration of 24 hours at a temperature of 60 °C. Data were gathered using a spectrofluorimetric method, employing the M200 PRO spectrofluorimeter (Tecan GmbH, Grödig, Austria). Advanced Glycation End-products (AGEs) were determined through specific fluorescence at excitation/emission wavelengths of 370/440 nm. To avoid false positive results from compounds known to suppress the fluorescence of AGEs due to interference, logarithmic normalization of the acquired data was performed according to Formula 1:

$$Fly(log) = 10^{(log10(Exp) - log10(Blank))} - 1 \quad (1),$$

where Fly(log) represents the normalized fluorescence intensity of AGEs, log10(Exp) and log10(blank) are the decimal logarithms of the actual fluorescence levels of the glycated and respective unglycated samples (both containing the compound under study and control samples).

The activity of other compounds (both non-fluorescent and fluorescent at the wavelengths used) was expressed using Formula 2:

$$Fly(lin) = Exp - Blank \quad (2),$$

where Fly(lin) is the fluorescence intensity of AGEs, Exp and blank are the actual fluorescence levels of glycated and corresponding unglycated samples (both containing the tested compound and control samples).

Determination of activity, expressed as % suppression of AGEs fluorescence, was performed using Formula 3:

$$\% = 100 - (Fly(Exp) \times 100 / Fly(Contr)) \quad (3),$$

where Fly(Exp) and Fly(Contr) are the AGEs fluorescence intensities of experimental and control samples, respectively (log-normalized or non-log-normalized).

Analysis of platelet aggregation

The effect of tested samples on the functional activity of platelets *in vitro* was studied according to G.Born's method in modification of Gabbasov (1989) on a two-channel laser analyzer of platelet aggregation Biola LA-220. The studies were performed on platelet-rich plasma of rabbits. Venous blood was collected from the auricular marginal vein of rabbits and stabilized with 3.8% sodium citrate solution in the ratio 9:1 followed by centrifugation at 1500 rpm for 10 min on a MultiCentrifuge CM 6M centrifuge (Elmi, Latvia). This method is based on the optical detection of a degree of change in the light transmission of PRP, when adding substances that stimulate aggregation processes (under conditions of constant stirring). To obtain a control sample, 300 μL of PRP with a magnetic stirrer is added into the glass cuvette of the aggregometer and after the aggregogram recording is switched on, at 10 seconds of the process recording, the inducer of platelet aggregation ADP is added to the cuvette at a final concentration of 5 μM (Kwon et al. 2016). To study the antiaggregant activity of the tested compounds, 10 μL of the test sample solution at a certain concentration is added to a cuvette with 300 μL of platelet-rich plasma. The sample is incubated in a specialized cell of the aggregometer at a constant temperature of 37 °C for 5 min., after which the sample is transferred to the recording cell and the aggregogram is recorded. At the 10th second of the process, the aggregation inducer ADP is added to the cuvette at a final concentration of 5 μM. The aggregogram is recorded for 5 minutes, while stirring the sample with a magnetic stirrer (800 rpm).

When recording the process of platelet aggregation, curves representing the increasing light transmission of the experimental sample were obtained. The aggregation level was estimated by the value of the aggregogram amplitude at the 5th minute of the process. The inhibitory effect on platelet aggregation (InPA) of the studied compounds was calculated using Formula 4:

$$InPA = 100 - (B/A) \times 100\% \quad (4),$$

where A – degree of platelet aggregation in rabbit blood without the studied compounds; B – stage of platelet aggregation after incubation of platelet-rich plasma with the studied compounds.

The studied compounds and the comparison drug acetylsalicylic acid were studied at a concentration of 100 μM.

Statistical analysis

Statistical analysis was performed in Prism 8.0 program (GraphPad software, San Diego, CA, USA). Nonparametric Mann-Whitney U-test was used for pairwise comparisons, and one-factor analysis of variance with Dunnett's post-hoc test was used for multiple comparisons. IC_{50} were obtained using nonlinear 3-parametric regression.

Results

Synthesis

The synthetic route to target compounds is shown in Fig. 1. Trimethylsilyl derivative of quinazoline-2,4(1*H*,3*H*)-dione **1** was readily alkylated with 1 molar equivalent of

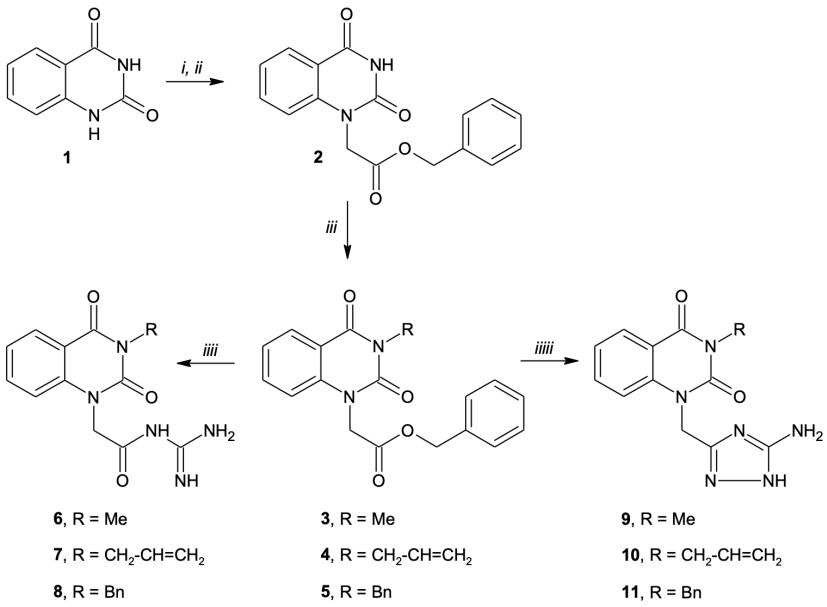


Figure 1. Synthesis of the target compounds: (i) $(Me_3Si)_2NH$, NH_4Cl, reflux, 72 hr., ~100%; (ii) $BrCH_2C(O)OBn$, 160-170 °C, 2 hr., 95%; (iii) MeI, CH_2=CH-CH_2-Br, or BnCl, K_2CO_3, DMF, 25 °C, 48 hr., 67-69%; (iiii) $NH_2C(NH)NH_2 \cdot HCl$, KOH, 95% EtOH, reflux, 10 min, 75-98%; (iiiii) $NH_2C(NH)NHNH_2 \cdot 1/2H_2CO_3$, KOH, 95% EtOH, reflux, 1 hr., 59-77%.

benzyl bromoacetate at 160-170 °C without any solvent to get benzyl (2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)acetate (**2**). After alkylation with methyl iodide, allyl bromide, or benzyl chloride corresponding 1,3-disubstituted quinazoline-2,4(1*H*,3*H*)-dione derivatives **3-5** were obtained in 67-69% yield. The next step involved treatment of esters **3-5** with guanidine generated *in situ* from 1 molar equivalent of guanidine hydrochloride and potassium hydroxide in boiling 95% ethanol, which leads to rapid cleavage of the ester bond and formation of *N*-acyl derivatives of guanidine **6-8** in 75-88% yield. When aminoguanidine was used as a nucleophilic reagent, which was similarly obtained *in situ* from aminoguanidine carbonate and potassium hydroxide in boiling 95% ethanol, the reaction is accompanied by cyclization to form 5-amino-1,2,4-triazole and leads to quinazoline-2,4(1*H*,3*H*)-dione derivatives **9-11** in 59-77% yield.

pH-dependent platelets deformation (NHE-1 inhibitory activity)

An *in vitro* study performed on rabbit platelets showed that at a concentration of 10 nM, the studied compounds exhibited moderate NHE-1-inhibitory activity (Table 1). Compounds **6**, **8** and **9** were superior to rimeporide in their activity but slightly inferior to zoniporide. Compound **7** was nearly inferior to rimeporide; compounds **10** and **11** were 1.8 and 1.4 times inferior to rimeporide, respectively, but had an effect superior to the comparison drug amiloride.

Since the activity of some compounds at a concentration of 10 nM was close to zoniporide, it was of interest to study the dose-dependent effect and calculate the IC_{50}. It was shown that compound **6**, although inferior to zoniporide in terms of IC_{50} by 4.4 times, was significantly superior to other reference drugs. Compound **9** showed a moderate effect, but was superior to amiloride and rimeporide. Compounds **7** and **8** did not show a significant dose-dependent effect, therefore IC_{50} of compound **7** is inferior to all the studied compounds, and for compound **8,** this value was not calculated (Table 1).

Table 1. Effect of new quinazoline-2,4(1*H*,3*H*)-dione derivatives on pH-dependent platelets deformation and intraocular pressure

No.	Compound	pH-dependent platelets deformation (NHE-1 inhibitory activity)		Maximum IOP change, % (m±SEM, n=6, %)
		% Inhibition, 10 nM (m±SEM, n=6, %)	IC_{50} (M)	
1	**6**	-42.47±6.69* ###	1.23×10^{-7}	-7.35±8.68
2	**7**	-32.45±5.12* ###	3.54×10^{-5}	-19.14±8.21
3	**8**	-36.85±5.84* ###	n.d.	-29.50±5.78$
4	**9**	-38.52±6.24* ###	6.05×10^{-7}	-13.75±7.02
5	**10**	-19.26±4.03* # ## ###	n.d.	-7.69±4.44
6	**11**	-24.89±3.09* ###	n.d.	6.3±7.54$$$,$$
7	Amiloride	-5.39±1.82* # ##	1.38×10^{-6}	-13.7±5.16
8	Rimeporide	-34.23±5.91* ###	1.04×10^{-6}	n.s.
9	Zoniporide	-48.05±7.09* ###	2.82×10^{-8}	-44.10±10.67$
10	Timolol	n.s.	n.s.	-30.76±4.69$

Note: n.s. – not studied; n.d. – not determined due to maximum effect being below 50%; * –statistically significant vs. control ($p<0.05$, Mann-Whitney U-test); # – statistically significant vs. comparison drug zoniporide ($p<0.05$, Mann-Whitney U-test); ## – statistically significant vs. comparison drug rimeporide ($p<0.05$, Mann-Whitney U-test); $ – statistically significant vs. baseline ($p\leq0.05$, Kruskal-Wallis with Dunn's post-test); $$ – statistically significant vs. comparison drug group Timolol ($p\leq0.05$, Kruskal-Wallis with Dunn's post-test); ### – statistically significant vs. comparison drug amiloride (p<0.05, Mann-Whitney U-test); $$$ – statistically significant vs. comparison drug Zoniporide ($p\leq0.05$, Kruskal-Wallis with Dunn's post-test).

Effect on intraocular pressure

The results of the study revealed that all the compounds affect intraocular pressure in varying degrees. All studied substances can be conditionally divided into highly active (reducing IOP by more than 25%), moderately active (15-25%), and inactive (less than 15%) (Table 1). Thus, the most active is compound **8** (-29.50%). Compound **7** (-19.14%) can be classified as a medium active ophthalmohypotensive compound. The remaining compounds **6**, **9** and **10**, are inactive, as the IOP reduction varies in the range of -7-15%, and thus have no significant effect. Compound **11** caused an increase of ophthalmotonus by 6.3% during instillation, which is an undesirable effect.

Anti-inflammatory activity

Anti-inflammatory activity of the target compounds was evaluated in a phenotypic cell-based screen. Briefly, murine peritoneal macrophages were incubated with test compounds to allow permeation and stimulated with *E. coli* LPS. Nitric oxide accumulation in cell supernatants was considered as a marker of inflammatory response. Lactate dehydrogenase release from cells was followed in parallel to assess cytotoxicity of the compounds. Data obtained revealed that guanidine derivatives **6-8** demonstrate marginal NO-inhibitory properties, while compounds **9-11** show at least 4-fold inhibition (Table 2). All the compounds did not affect macrophage viability to a significant extent in high micromolar concentrations after 24-hr incubation. Reference compounds dexamethasone and amiloride show submicromolar activity, which is consistent with literature data and our previous works (Spasov et al. 2021, 2022).

Table 2. Effect of quinazoline-2,4(1*H*,3*H*)-dione derivatives on LPS-stimulated mouse macrophages

No.	Compound	NO synthesis vs. LPS control		Cytotoxicity by LDH-test, CC_{50} (μM)
		% Activity, 100 μM (m±SD)	IC_{50} (μM)	
1	**6**	45.36±3.12	n.d.	>100
3	**7**	50.11±10.65	n.d.	>100
5	**8**	31.92±4.91	n.d.	>100
2	**9**	15.29±4.71*	n.d.	>100
4	**10**	24.35±9.11	36.50	>100
6	**11**	3.71±7.03**	15.64	>100
7	Dexamethasone	2.56±9.17*	0.003	>100
8	Amiloride	5.67±5.96*	0.62	>100
9	Rimeporide	n.s.	n.s.	>100
10	Zoniporide	n.s.	15.56	>100

Note: n.s. – not studied; n.d. – not determined; * – statistically significant vs. LPS control (p<0.05, Kruskal-Wallis test with Dunn's post-test); ** – statistically significant vs. LPS control (p<0.005, Kruskal-Wallis test with Dunn's post-test).

Antiglycation activity

During the study of the compounds' antiglycation activity, the ability of a compound to inhibit the glycation reaction at any of its stages is tested, from the earliest stages to the latest ones. This is notable because compounds with any mechanism of antiglycation effect can express their activity within this test system. Table 3 demonstrates that compounds **8** and **11** exhibit superior antiglycation properties compared to the reference drug aminoguanidine. The remaining compounds did not exhibit this activity. There is an increase in activity as the radical in the R position changes from *Me* to *Bn* in the series of compounds **6-8** and **9-11**, respectively. Therefore, the substituents significantly influence the observed activities. It can also be noted that compounds comprising a 2-aminotriazole moiety exhibit higher activity than those containing a non-cyclic fragment.

Table 3. Antiglycation and antiaggregant activity of quinazoline-2,4(1*H*,3*H*)-dione derivatives

No.	Compound	Antiglycation activity	Antiplatelet activity
		% Inhibition, 1000 μM (m±SEM, n=6)	Δ% Inhibition, 100 μM (m±SEM, n=6)
1	**6**	7.0±2.0	23.4±7.2*
2	**7**	16.4±1.1*	27.7±7.2*
3	**8**	25.4±1.2*	19.1±3.1*
4	**9**	0.6±4.2	21.1±7.0*
5	**10**	8.2±1.2	32.8±8.0*
6	**11**	38.1±2.6*	14.0±2.7
7	Amiloride	64.3±0.8*	66.1±0.6*
8	Rimeporide	5.7±2.4	n.s.
9	Zoniporide	-2.2±3.0	n.s.
10	Aminoguanidine	21.6±1.8*	n.s.
11	Acetylsalicylic acid	n.s.	56.5±2.0

Note: n.s. – not studied; * – statistically significant vs. control (p<0.05, 1-way ANOVA, Tukey's post-test).

Antiplatelet activity

All the studied compounds significantly contributed to the inhibition of platelet aggregation (Table 3). However, the comparison drug acetylsalicylic acid at a concentration of 100 μM contributed to a more pronounced inhibition of platelet function, on average exceeding the activity of the tested substances by 2.6 times.

Discussion

Guanidine derivatives of quinazoline are promising scaffolds for the development of active NHE-1 inhibitors on their basis. NHE-1 inhibition can have a positive effect in ischemia-reperfusion of the heart and brain, heart failure, myocardial hypertrophy, the course of diabetes

mellitus and its complications, the development of glaucoma, kidney diseases, and the immune system (Pedersen and Counillon 2019; Wichaiyo and Saengklub 2022; Chen et al. 2022; Correale et al. 2023; Cao et al. 2023; Metwally et al. 2023). Using method from (Rosskopf et al. 1991), we could suppose the presence of sodium-hydrogen exchanger 1 inhibiting activity of our substances. The study of new guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione revealed a moderate inhibitory activity in substances **6** and **9**. Other substances of this series were also noninferior to the comparison agents, which in general proves the contribution of the guanidine grouping to the NHE-1-inhibitory activity of quinazoline series substances.

In addition, we studied the antiglaucomic activity of the synthesized compounds. NHE inhibitors are known to reduce intraocular pressure (Shahidullah et al. 2009). Topical application of three direct Na^+/H^+ exchanger blockers resulted in IOP reduction in mice: dimethylamiloride (DMA), ethylisopropylamiloride (EIPA), and compound BIIB723. These effects were mediated locally rather than systemically, as the addition of DMA in one eye did not affect IOP in the contralateral eye (Avila et al. 2002). The antiglaucoma properties of guanidine derivatives of quinazoline have been shown previously (Taran et al. 2023). In our studies, high ophthalmohypotensive activity of substance **8** comparable to timolol was established. The other guanidine derivatives of quinazoline showed an average level of activity or were inactive.

Studies have shown that neuroinflammation is closely related to the pathogenesis of glaucoma (Soto and Howell 2014). Inhibition of certain proinflammatory pathways appears to be neuroprotective, and macrophages and monocytes play an important role in the regulation of outflow and IOP (Wang et al. 2020). The study of anti-inflammatory activity of new substances revealed clear and consistent structure-activity relationships. That is, guanidine derivatives **6-8** demonstrate marginal NO-inhibitory properties, while cyclic analogs **9-11** comprising the 2-aminotriazole moiety show at least 4-fold inhibition. The influence of the R^3-substituent in both series indicates that benzyl contributes to activity the most, while allyl and especially methyl groups are inferior. Thus, the presence of bulky aromatic fragments is beneficial for anti-inflammatory properties with compound **11** being the most active.

It is known that diabetic retinopathy is one of the most frequent causes of vision loss up to its complete loss. In addition to cataract, diabetes mellitus increases the risk of glaucoma in patients, and agents with antiglycation activity are able to reduce intraocular pressure, which provides an antiglaucoma effect (Khodjaev et al. 2020; Kumaksheva et al. 2021). Aminoguanidine derivatives are characterized by antiglycation activity, which is associated with the ability to form triazines and hydrazones with di- and monocarbonyl compounds (García-Díez et al. 2020). Therefore, it was of interest to consider compounds with a guanidine group for the presence of antiglycation activity, which was detected in substances **8** and **11**, while the other derivatives were significantly inferior to the drug aminoguanidine.

There is evidence that quinazoline derivatives have antiplatelet properties (Eskandariyan at al. 2014). In addition, it has been found that NHE-1 inhibitors have the ability to inhibit the functional activity of platelets (Wichaiyo and Saengklub 2022). In this regard, it was of interest to study the antiplatelet properties of guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione. It was found that the greatest antiplatelet activity was exhibited by compound **10**, which was inferior to acetylsalicylic acid. The guanidine-containing inhibitor of NHE amiloride at a concentration of 100 μM contributed to the reduction of platelet aggregation by an average of 66.1%, being superior to acetylsalicylic acid as a reference drug. This may provide a rationale for the directed synthesis of highly effective antiaggregant compounds based on quinazoline-2,4(1*H*,3*H*)-dione.

Upon comparing the antiglycation activity of compounds **8** and **11**, an interesting observation arises – these compounds, despite having a higher potential for antiglycation action, have a lesser impact on platelet aggregation compared to other compounds (antiplatelet activity is not statistically confirmed for compound **11** according to Table 2). Presumably, the introduction of the Bn substituent in the R position is responsible for the manifestation of both properties. Furthermore, it can be observed that the ability of the compounds to influence NO production also increases as the R group changes from Me to Bn.

Conclusion

Guanidine derivatives of quinazoline-2,4(1*H*,3*H*)-dione are a promising scaffold for further development of agents based on them for the treatment of cardiovascular pathologies, glaucoma, excessive inflammation, protein glycation disorders, and thrombosis.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; nor in the decision to publish the results.

Acknowledgement

This research was carried out with financial support from the Ministry of Science and Higher Education of the Russian Federation (Agreement on the provision of grants from the federal budget in the form of subsidies under paragraph 4 of Article 78.1 of the Budget Code of the Russian Federation, Moscow, October 1, 2020 No, 075-15-2020-777).

Data Availability statement

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

Abbreviations: The following abbreviations are used in this manuscript:
NHE Na^+/H^+ exchanger
IOP Intraocular pressure
PDW Platelet distribution width
TLC Thin-layer chromatography
PM Peritoneal macrophages
LPS Lipopolysaccharide
AGEs Advanced Glycation End-products
DMSO Dimethyl sulfoxide
PRP Platelet-rich plasma
InPA Inhibitory effect on platelet aggregation

References

- Al-Humimat G, Marashdeh I, Daradkeh D, Kooner K (2021) Investigational rho kinase inhibitors for the treatment of glaucoma. Journal of Experimental Pharmacology 13: 197–212. https://doi.org/10.2147/JEP.S259297 [PubMed] [PMC]
- Ansari NA, Rasheed Z (2010) Non-enzymatic glycation of proteins: from diabetes to cancer. Biomedical Chemistry [Biomeditsinskaya Khimiya] 56(2): 168–178. https://doi.org/10.18097/pbmc20105602168 [PubMed] [in Russian]
- Avila MY, Seidler RW, Stone RA, Civan MM (2002) Inhibitors of NHE-1 Na+/H+ exchange reduce mouse intraocular pressure. Investigative Ophthalmology & Visual Science 43(6): 1897–1902. [PubMed]
- Cao Y, Redd MA, Fang C, Mizikovsky D, Li X, Macdonald PS, King GF, Palpant NJ (2023) New drug targets and preclinical modelling recommendations for treating acute myocardial infarction. Heart, Lung and Circulation 32(7): 852–869. https://doi.org/10.1016/j.hlc.2022.12.015 [PubMed]
- Chen S, Coronel R, Hollmann MW, Weber NC, Zuurbier CJ (2022) Direct cardiac effects of SGLT2 inhibitors. Cardiovascular Diabetology 21(1): 45. https://doi.org/10.1186/s12933-022-01480-1 [PubMed] [PMC]
- Correale M, Tricarico L, Croella F, Alfieri S, Fioretti F, Brunetti ND, Inciardi RM, Nodari S (2023) Novelties in the pharmacological approaches for chronic heart failure: new drugs and cardiovascular targets. Frontiers in Cardiovascular Medicine 10: 1157472. https://doi.org/10.3389/fcvm.2023.1157472 [PubMed] [PMC]
- Cummings BS, Schnellmann RG (2021) Measurement of cell death in mammalian cells. Current Protocols 1(8): e210. https://doi.org/10.1002/cpz1.210 [PubMed]
- De Vito P (2006) The sodium/hydrogen exchanger: a possible mediator of immunity. Cellular Immunology 240(2): 69–85. https://doi.org/10.1016/j.cellimm.2006.07.001 [PubMed]
- Eskandariyan Z, Esfahani Zadeh M, Haj Mohammad Ebrahim Tehrani K, Mashayekhi V, Kobarfard F (2014) Synthesis of thioether derivatives of quinazoline-4-one-2-thione and evaluation of their antiplatelet aggregation activity. Archives of Pharmacal Research 37(3): 332–339. https://doi.org/10.1007/s12272-013-0192-5 [PubMed]
- Freddo TF, Civan M, Gong H (2022) Aqueous humor and the dynamics of its flow: Mechanisms and routes of aqueous humor drainage. Albert and Jakobiec's Principles and Practice of Ophthalmology. Cham: Springer International Publishing, 1989–033 pp.. https://doi.org/10.1007/978-3-030-42634-7_163
- Gabbasov ZA, Popov EG, Gavrilova IY, Pozin EY, Markosyan RA (1989) A new highly sensitive method of analysis of thrombocyte aggregation. Laboratornoe Delo 10: 15–18. [PubMed] [in Russian]
- García-Díez G, Ramis R, Mora-Diez N (2020) Theoretical study of the copper complexes with aminoguanidine: Investigating secondary antioxidant activity. ACS Omega 5(24): 14502–14512. https://doi.org/10.1021/acsomega.0c01175 [PubMed] [PMC]
- Khodjaev NS, Sidorova AV, Smirnova EDA, Eliseeva MA, Starostina AV (2020) Therapy of neovascular glaucoma. National Journal of Glaucoma [Natsional'nyy Zhurnal Glaukoma] 19(2): 76–87. [in Russian]
- Kumaksheva TN (2021) Diabetic retinopathy-modern aspects of development, diagnosis and treatment (literature review). Proceedings of the All-Russian Scientific Conference with International Participation. In 3 parts. Part 2. Saransk, 06-11 December; pp. 371–378. [in Russian]
- Kwon HW, Shin JH, Lim DH, Ok WJ, Nam GS, Kim MJ, Kwon HK, Noh JH, Lee JY, Kim HH, Kim JL, Park HJ (2016) Antiplatelet and antithrombotic effects of cordycepin-enriched WIB-801CE from Cordyceps militaris ex vivo, in vivo, and in vitro. BMC Complementary Medicine and Therapies 16(1): 508. https://doi.org/10.1186/s12906-016-1463-8 [PubMed] [PMC]
- Liu Y, Kintner DB, Chanana V, Algharabli J, Chen X, Gao Y, Chen J, Ferrazzano P, Olson JK, Sun D (2010) Activation of microglia depends on Na+/H+ exchange-mediated H+ homeostasis. The Journal of Neuroscience 30(45): 15210–15220. https://doi.org/10.1523/JNEUROSCI.3950-10.2010 [PubMed] [PMC]
- Ma Y, Han J, Li S, Zhang A, Cao W, Sun X (2019) Association between platelet parameters and glaucoma severity in primary open-angle glaucoma. Journal of Ophthalmology 2019: 3425023 https://doi.org/10.1155/2019/3425023 [PubMed] [PMC]
- Marcus AJ, Iezhitsa I, Agarwal R, Vassiliev P, Spasov A, Zhukovskaya O, Anisimova V, Mohd Ismail N (2018) Data on the effects of imidazo[1,2-a]benzimidazole and pyrimido[1,2-a]benzimidazole compounds on intraocular pressure of ocular normotensive rats. Data in Brief 18: 523–554. https://doi.org/10.1016/j.dib.2018.03.019 [PubMed] [PMC]
- Metwally SAH, Paruchuri SS, Yu L, Capuk O, Pennock N, Sun D, Song S (2023) Pharmacological inhibition of NHE1 protein increases white matter resilience and neurofunctional recovery after ischemic stroke. The International Journal of Molecular Sciences 24(17): 13289. https://doi.org/10.3390/ijms241713289 [PubMed] [PMC]
- Nikitin E, Mironova E, Shpakovsky D, Gracheva Y, Koshelev D, Utochnikova V, Lyssenko K, Oprunenko Y, Yakovlev D, Litvinov R, Seryogina M, Spasov A, Milaeva E (2022) Cytotoxic and luminescent properties of novel organotin complexes with chelating antioxidant ligand. Molecules 27(23): 8359. https://doi.org/10.3390/molecules27238359 [PubMed] [PMC]
- Pease ME, Hammond JC, Quigley HA (2006) Manometric calibration and comparison of TonoLab and TonoPen tonometers in rats with experimental glaucoma and in normal mice. Journal of Glaucoma 15(6): 512–519. https://doi.org/10.1097/01.ijg.0000212276.57853.19 [PubMed]
- Pedersen SF, Counillon L (2019) The SLC9A-C mammalian Na^+/H^+ exchanger family: Molecules, Mechanisms, and Physiology. Physiological Reviews 99(4): 2015–2113. https://doi.org/10.1152/physrev.00028.2018 [PubMed]
- Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biology 18(7): e3000411. https://doi.org/10.1371/journal.pbio.3000411 [PubMed] [PMC]
- Rosskopf D, Morgenstern E, Scholz W, Osswald U, Siffert W (1991) Rapid determination of the elevated Na(+)-H+ exchange in platelets of patients with essential hypertension using an optical swelling assay. Journal of Hypertension 9(3): 231–238. [PubMed]
- Shahidullah M, Mandal A, Delamere NA (2009) Responses of sodium-hydrogen exchange to nitric oxide in porcine cultured nonpigmented ciliary epithelium. Investigative Ophthalmology & Visual Science 50(12): 5851–5858. https://doi.org/10.1167/iovs.09-3453 [PubMed] [PMC]
- Shi Y, Kim D, Caldwell M, Sun D (2013) The role of Na(+)/h (+) exchanger isoform 1 in inflammatory responses: maintaining H(+) homeostasis of immune cells. Advances in Experimental Medicine and Biology 961: 411–418. https://doi.org/10.1007/978-1-4614-4756-6_35 [PubMed]
- Soto I, Howell GR (2014) The complex role of neuroinflammation in glaucoma. Cold Spring Harbor Perspectives in Medicine 4(8): a017269. https://doi.org/10.1101/cshperspect.a017269 [PubMed] [PMC]
- Spasov A, Ozerov A, Kosolapov V, Gurova N, Kucheryavenko A, Naumenko L, Babkov D, Sirotenko V, Taran A, Borisov A, Sokolova E, Klochkov V, Merezhkina D, Miroshnikov M, Ovsyankina N, Smirnov A, Velikorodnaya Y (2022) Guanidine derivatives of quinazoline-2,4(1H,3H)-Dione as NHE-1 inhibitors and anti-inflammatory agents. Life 12(10): 1647. https://doi.org/10.3390/life12101647 [PubMed] [PMC]
- Spasov A, Ozerov A, Vassiliev P, Kosolapov V, Gurova N, Kucheryavenko A, Naumenko L, Babkov D, Sirotenko V, Taran A, Litvinov R, Borisov A, Klochkov V, Merezhkin D, Miroshnikov M, Uskov G, Ovsyankina N (2021) Synthesis and multifaceted pharmacological activity of novel quinazoline NHE-1 inhibitors. Scientific Reports 11(1): 24380. https://doi.org/10.1038/s41598-021-03722-w [PubMed] [PMC]
- Taran AS, Naumenko LV, Govorova YA, Gurova NA, Spasov AA, Ozerov AA, Merezhkina DV (2023) Effect of guanidine derivatives of quinazoline on Na+/H+-exchanger and intraocular pressure. Bioorganic Chemistry 49(2): 207–215. https://doi.org/10.31857/S0132342323020215
- Vasan S, Foiles P, Founds H (2003) Therapeutic potential of breakers of advanced glycation end product-protein crosslinks.

Archives of Biochemistry and Biophysics 419(1): 89–96. https://doi.org/10.1016/j.abb.2003.08.016 [PubMed]

- Vishnevskaya VV, Gurova NA, Spasov AA, Ozerov AA, Ovsyankina NV (2022) Effect of NHE-1 inhibitors zoniporide and BMA-1321 on myocardial contractility in animals with chronic heart failure. Experimental and Clinical Pharmacology 4(85): 3–8. https://doi.org/10.30906/0869-2092-2022-85-4-3-8
- Wang B, Kasper M, Laffer B, Meyer Zu Hörste G, Wasmuth S, Busch M, Jalilvand TV, Thanos S, Heiligenhaus A, Bauer D, Heinz C (2020) Increased hydrostatic pressure promotes primary M1 reaction and secondary M2 polarization in macrophages. Frontiers in Immunology 11: 573955. https://doi.org/10.3389/fimmu.2020.573955 [PubMed] [PMC]
- Watanabe Y, Hamanaka T, Takemura T, Murakami A (2010) Involvement of platelet coagulation and inflammation in the endothelium of Schlemm's canal. Investigative Ophthalmology & Visual Science 51(1): 277–283. https://doi.org/10.1167/iovs.08-3279 [PubMed]
- Weissman BA, Gross SS (2001) Measurement of NO and NO synthase. Current Protocols in Neuroscience Chapter 7: Unit 7.13. https://doi.org/10.1002/0471142301.ns0713s05 [PubMed]
- Wichaiyo S, Saengklub N (2022) Alterations of sodium-hydrogen exchanger 1 function in response to SGLT2 inhibitors: what is the evidence? Heart Failure Reviews 27(6): 1973–1990. https://doi.org/10.1007/s10741-022-10220-2 [PubMed]
- Yeves AM, Ennis IL (2020) Na^+/H^+ exchanger and cardiac hypertrophy. Hipertensión y Riesgo Vascular 37(1): 22–32. https://doi.org/10.1016/j.hipert.2019.09.002 [PubMed]
- Zhang X, Goncalves R, Mosser DM (2015) The isolation and characterization of murine macrophages. Current Protocols in Immunology 111: 14.1.1-14.1.16. https://doi.org/10.1002/0471142735.im1401s111 [PubMed] [PMC]

Author Contributions

- **Alexander A. Ozerov**, Doctor of Chemical Sciences (DSc), Chairman of the Department of Pharmaceutical & Toxicological Chemistry, Head of the Department of Synthesis and Pharmaceutical Technologies of Scientific Center for Innovative Medicines, Volgograd State Medical University, Professor, Volgograd, Russia; e-mail: dds.ozerov@gmail.com; **ORCID ID** https://orcid.org/0000-0002-4721-0959. The author advised on the design of the study, took part in the synthesis of novel compounds and edited the text of the article.

- **Daria V. Merezhkina**, Teaching Fellow of the Department of Pharmaceutical & Toxicological Chemistry, Employee of the Department of Synthesis and Pharmaceutical Technologies of Scientific Center for Innovative Medicines, Volgograd State Medical University, Volgograd, Russia; e-mail: merezhkinad@mail.ru, **ORCID ID** https://orcid.org/0000-0002-9848-7149. The author was engaged in conducting the synthesis of novel compounds.

- **Natalia A. Gurova**, Doctor Habil. of Medical Sciences (DSc), Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: gurova.vlgmed@mail.ru; **ORCID ID** https://orcid.org/0000-0002-0670-1444. The author took part in conducting experimental work and analyzing the material.

- **Lyudmila V. Naumenko**, Doctor Habil. of Medical Sciences (DSc), Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: milanaumenko@mail.ru; **ORCID ID** https://orcid.org/0000-0002-2119-4233. The author took part in conducting experimental work and analyzing the material.

- **Denis A. Babkov**, Doctor of Pharmaceutical Sciences (DSc), Assistant Professor of the Department of Pharmacology and Bioinformatics, Head of Scientific Center for Innovative Medicines, Volgograd State Medical University, Volgograd, Russia; e-mail: denis.a.babkov@gmail.com; **ORCID ID** https://orcid.org/0000-0002-9645-3324. The author took part in conducting experimental work and analyzing the material, and translation.

- **Victor S. Sirotenko,** Candidate of Pharmaceutical Sciences (PhD), Assistant Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: sirotenko.viktor@yandex.ru; **ORCID ID** https://orcid.org/0000-0003-2249-020X. The author took part in conducting experimental work and analyzing the material.

- **Roman A. Litvinov**, Candidate of Medical Sciences (PhD), Assistant Professor of the Department of Pharmacology and Bioinformatics, Senior scientist of laboratory for metabotropic drugs of Scientific Center for Innovative Medicines, Volgograd State Medical University, Volgograd, Russia; e-mail: litvinov.volggmu@mail.ru; **ORCID ID** https://orcid.org/0000-0002-0162-0653. The author took part in conducting experimental work and analyzing the material.

- **Alena S. Taran**, Candidate of Medical Sciences (PhD) Assistant Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: alena-beretta-taran@mail.ru; **ORCID ID** https://orcid.org/0000-0001-8477-254X. The author was engaged in conducting the experimental work.

- **Nadezhda V. Stepanova**, Teaching Fellow of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: ovsyankina99@mail.ru; **ORCID ID** https://orcid.org/0000-0002-6629-3729. The author was engaged in conducting the experimental work.

- **Umida M. Ibragimova**, Teaching Fellow of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia; e-mail: iumida@list.ru; **ORCID ID** https://orcid.org/0000-0001-9141-4417. The author was engaged in conducting the experimental work.

- **Alexander A. Spasov**, Doctor of Medical Sciences (DSc), Chairman of the Department of Pharmacology and Bioinformatics, Scientific advisor of Scientific Center for Innovative Medicines, Volgograd State Medical University, Academician of RAS, Professor, Volgograd, Russia; e-mail: aspasov@mail.ru, **ORCID ID** https://orcid.org/0000-0002-7185-4826. The author advised on the research idea, the concept and design of the study, analyzed the results and edited the text of the article.

- **Vadim A. Kosolapov**, Doctor Habil. of Medical Sciences (DSc), Professor of the Department of Pharmacology and Bioinformatics, Head of laboratory for metabotropic drugs of Scientific Center for Innovative Medicines, Volgograd State Medical University, Professor, Volgograd, Russia; e-mail: vad-ak@mail.ru; **ORCID ID** https://orcid.org/0000-0002-6702-1207. The author advised on the design of the study, analyzed the results and edited the text of the article.