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Research Article

Study of the biotransformation of a new sydnonimine derivative with predominant cerebral vasodilatory activity

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Abstract

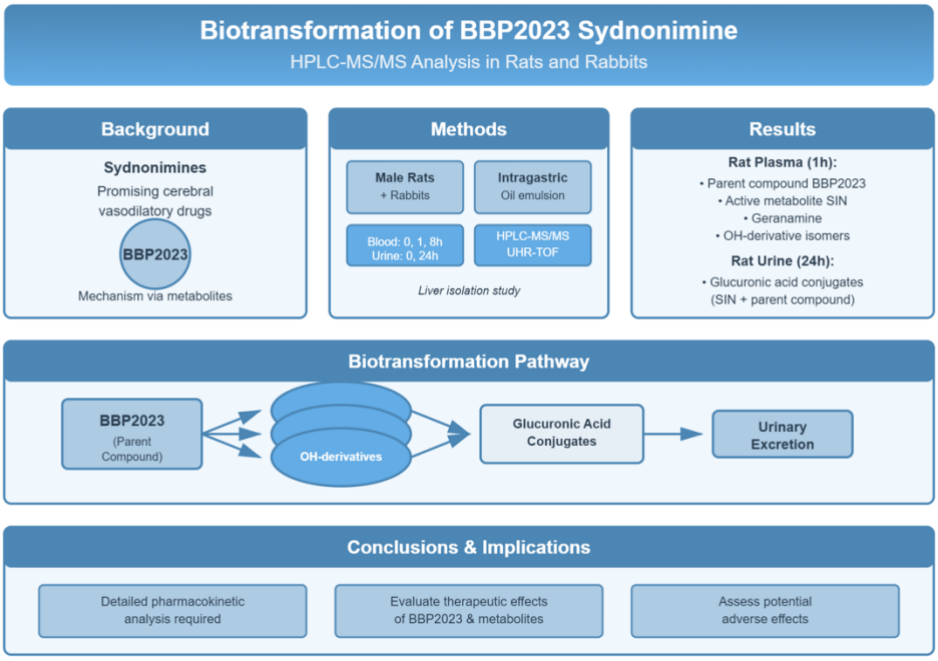
Introduction: Sydnonimines are a promising group of drugs for development of novel substances with predominant cerebral vasodilatory activity. One of them is experimental compound with laboratory code BBP20233. The mechanism of action of BBP2023 compound is tied to its metabolites. This study aims for identification of biotransformation products of BBP2023 using HPLC-MS/MS method.

Materials and Methods: Identification of BBP2023 biotransformation products was performed in male rats and male rabbits after intragastric administration of BBP2023 in form of oil emulsion. Blood samples were collected before and 1 and 8 hours after administration. Urine samples from rats were collected before and 24 hours after administration. Additionally, complete liver isolation was performed in another group of rats to investigate liver's role in BBP2023 metabolism. Identification of products was performed using HPLC-MS/MS method as well as UHR-TOF method.

Results and Discussion: Analysis of rat plasma samples collected 1 hour after administration revealed the presence of parent compound, active metabolite SIN, geranamine and various isomers of OH-derivatives. Analysis of rat urine samples collected 24 hours after administration showed the presence of glucuronic acid conjugates of SIN and parent compound. Similar results were obtained from rabbit plasma samples, although with less variety of hydroxylated derivative isomers.

Conclusion: Investigated features of BBP2023 biotransformation require detailed analysis of pharmacokinetics for both BBP2023 and its metabolites to determine their role in therapeutic effect and possible adverse effects.

Graphical Abstract



Keywords

biotransformation, pharmacokinetics, sydnonimines, vasodilators, chromatography-mass spectrometry

Introduction

Cardiovascular diseases, including acute myocardial infarction (AMI), coronary artery disease (CAD), chronic heart failure (CHF), and acute cerebrovascular accidents (stroke) are the leading causes of disability and mortality worldwide. Modern therapeutic strategies rely on combined treatment with antiplatelet agents and thrombolytics, alongside neuroprotectors and vasodilators. However, their use is limited by risks of hypotension, tolerance, or a narrow therapeutic window. Moreover, in stroke, the choice of centrally acting vasodilators is justified by the need to maintain cardiac output and minimize tonic activity in peripheral vessels. Central vasodilators traditionally include calcium channel blockers (nimodipine), selective cerebral vasodilators (vinpocetine), and antihistamine agents (cinnarizine). Nimodipine, used in subarachnoid hemorrhages, exhibits selective action on cerebral arteries but carries a risk of systemic hypotension (Rass et al. 2023). Cinnarizine, despite improving cerebral blood flow, may cause extrapyramidal side effects (Gaitatzis and Sander 2013), while vinpocetine's efficacy in stroke remains debated due to conflicting clinical trial data (Kim et al. 2019). Historically, the search for centrally acting vasodilators included psychotropic compounds from the sydnonimine group, such as feprosidinine and mesocarb, developed in the 1970s. These drugs combined anxiolytic activity with moderate vasodilatory effects but were limited by non-selectivity and side effects (sedation, tolerance) (Savolainen 1982). Two main drug classes exist: α_2-adrenoceptor agonists and imidazoline receptor agonists. Among modern drugs with fewer side effects, only Moxonidine (Physiotens, Moxonitex) stands out. This underscores the need for compounds combining vasodilatory, antihypoxic, and cerebroprotective effects, making nitric oxide (NO) donors – particularly sydnonimines, with their unique dual activation mechanism – promising candidates (Fumanal et al. 2025). Donors of nitric oxide (NO), mediator of vascular homeostasis, which is critical for treating ischemic conditions due to its vasodilatory, antiplatelet, and antiproliferative effects (Dendorfer 1996). NO, synthesized by endothelial cells via eNOS synthase, activates soluble guanylate cyclase (sGC), increasing cyclic guanosine monophosphate (cGMP) and relaxing smooth muscle cells (Feelisch et al. 1989; Schrammel et al. 1998). However, in endothelial dysfunction (common in CAD and CHF), endogenous NO production declines, requiring exogenous compensation (Granik and Grigoriev 2004). Traditional organic nitrates, though effective, are limited by tolerance due to depletion of sulfhydryl (SH) groups

(Bohn et al. 1992). Historically, nitrates/nitrites like glyceryl trinitrate (nitroglycerin) entered cardiology for rapid angina relief, though their NO-release mechanism remained unknown until the late 20th century (Ignarro 1999; Zetterström 2009). Paradoxically, compounds initially developed for industrial use became antianginal therapy cornerstones, with NO identified as their key mediator only in the 1980s – a Nobel Prize-winning discovery (1998) for its role in vascular tone, platelet aggregation, and cell proliferation (Furchgott and Zawadzki 1980). Unlike classical nitrates (which require thiol-dependent biotransformation to NO), sydnonimines like molsidomine employ two activation pathways: non-enzymatic spontaneous NO release and enzymatic biotransformation into active metabolites. This duality avoids SH-group dependence, minimizing tolerance risk and ensuring prolonged effects, positioning them as promising alternatives to nitrates (Samarskaya 2015). Molsidomine's effects arise from hepatic metabolism into SIN-1, a compound that spontaneously generates NO and superoxide anions, activating sGC and elevating cGMP (confirmed in vitro/in vivo) (Schrammel et al. 1998).

Thus, sydnonimines represent a promising chemical group for developing novel cerebrovasodilatory drugs (Rehse et al.1993; Janero and Ewing 2000; Fershtat and Zhilin 2021), with one lead candidate being the experimental compound with laboratory code BBP2023. The central vasodilatory action of this compound likely involves specific metabolites, whose identification is a mandatory preclinical step (Liese et al. 2006; Popov et al. 2023, 2024). Subsequent quantification of these biotransformation products in tissues is essential to fully study pharmacokinetics (including distribution and elimination) and establish pharmacodynamic correlations for the drug candidate.

The aim of the study: Identification of biotransformation products of a lead sydnonimine compound as a basis for developing vasodilators with predominantly central action.

Materials and Methods

Study object

Screening of pharmacological activity among sydnonimine drug candidates revealed compounds exhibiting predominantly central vasodilatory effects. One such lead compound was the test substance with laboratory code BBP2023 (Fig. 1).

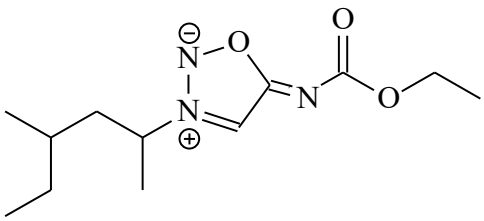


Figure 1. Structure of compound BBP2023 (N-(ethoxycarbonyl)-3-(4-methylhexan-2-yl)sydnonimine).

Pharmacological phase of the study

Animal containment conditions

The metabolism of compound BBP2023 was studied *in vivo* using male Wistar rats (rodents) weighing 250–270 g and male Soviet Chinchilla rabbits (non-rodents) weighing 3.3–3.5 kg (bred by SMC STEZAR LLC, Russia).

The animals were housed in the vivarium of Tver State Medical University of the Ministry of Health of Russia in steel cages with mesh lids, equipped with feeders and water bottles. Sterile wood shavings were used as bedding. The rats and rabbits were kept under controlled environmental conditions (temperature 20–26°C, relative humidity 30–70%). The animal rooms maintained a 12-hour light cycle and 8–10 air changes per hour. Rats were fed complete feed PK-120 (Provimi LLC, Russia), while rabbits received feed KK 90-2-235 (Soyuzpisheprom Association LLC, Russia). Food and filtered tap water were provided *ad libitum*. Cages were cleaned daily, and drinking water was replaced twice daily. Wet cleaning of the housing areas was performed daily. In the evening before the experiment, food was withheld.

Study design

BBP2023 was administered to rats (N=3) as a single intragastric dose of a 10% oil emulsion at 1/100 LD_{50} (11.0 mg/kg). Rat blood samples (0.2 mL) were collected using a syringe (1 mL, SC-Sanguis Counting Kontrollblutherstellungs-und Vertriebs, Germany) containing 10 µL of 2M citrate buffer solution (pH=1.9) via a catheter (Sci-Cat, Russia) inserted into the jugular vein at

the following time points: before administration and at 1 and 8 hours post-administration. Additionally, 24-hour urine samples were collected using metabolic chambers (Open Science NPC LLC, Russia) before and after administration.

To investigate the liver's role in BBP2023 biotransformation, an additional experiment with complete liver isolation was performed in rats (N=3). Under general anesthesia induced by a combination of zolazepam and tiletamine (Zoletil® 100 mg/mL, Virbac, France) and xylazine (Xylanit® 20 mg/mL, NITA-PHARM LLC, Russia), a midline laparotomy was performed. Intestinal loops were exteriorized, wrapped in sterile saline-soaked gauze, and retracted to the left. An Adson retractor was placed, followed by ligation and transection of the portal vein (*V. portae*) and celiac trunk (*Truncus celiacus*) using USP 3/0 PET sutures (Volot LLC, Russia). Critical to the procedure was ligating the portal vein before its hepatic branches. After vascular isolation, BBP2023 (in 5% aqueous DMSO) was injected into the infrahepatic segment of the inferior vena cava (*V. cava inferior*) at 11.0 mg/kg using an insulin syringe. A hemostatic sponge (FNPTS Belkozin JSC, Russia) was applied to the puncture site. Following hemostasis, the laparotomy was closed in layers. A 0.5 mL blood sample was collected from the jugular vein 1-hour post-administration.

Rabbits (N=3) also received a single intragastric dose of BBP2023 oil emulsion, adjusted to 6.0 mg/kg based on interspecies dose conversion factors. Rabbit blood samples (0.5 mL) were obtained from the marginal ear vein at the same time points.

Plasma was immediately separated from each blood sample by centrifugation at 3000g and +4°C for 10 minutes. Plasma samples were processed by adding 100 µL of biological material to 400 µL of 0.1% formic acid (PanReac Applichem, Spain) in methanol (J.T. Baker, Netherlands) pre-cooled to -28°C. The mixtures were vortexed (V-1 plus, Biosan, Latvia) for 15 seconds. Precipitates were removed by centrifugation at 16,000g and -10°C for 15 minutes. The resulting supernatant was either used directly for chromatographic analysis or diluted 5-fold with 0.1% formic acid in 50% aqueous acetonitrile for direct infusion into the mass spectrometer ion source via a syringe pump (10 µL/min).

Urine samples were processed by adding 100 µL of biological material to 400 µL of 0.1% formic acid (PanReac Applichem, Spain) in methanol (J.T. Baker, Netherlands) pre-cooled to -28°C. The mixtures were vortexed (V-1 plus, Biosan, Latvia) for 15 seconds. Precipitates were removed by centrifugation at 16,000g and -10°C for 15 minutes. The resulting supernatant was either used directly for chromatographic analysis or diluted 5-fold with 0.1% formic acid in 50% aqueous acetonitrile for direct infusion into the mass spectrometer ion source via a syringe pump (10 µL/min).

Ethical expertise

This study was approved by the Local Ethical Committee of Tver State Medical University of the Ministry of Health of the Russian Federation (Meeting minutes No. 5 dated June 19, 2024). All experiments were conducted in compliance with the Good Laboratory Practice Rules approved by Order No. 708n of the Russian Ministry of Health (August 23, 2010) and Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes.

Bioanalytical study

Identification of BBP2023 metabolism products was carried out using a triple quadrupole mass spectrometer AB Sciex QTrap 3200 MD (Sciex, Singapore) equipped with an electrospray ionization (ESI) source, as well as an ultra-high resolution time-of-flight mass spectrometer (UHR-TOF) Bruker MaXis 4G (Bruker, Germany). Analyte separation was performed using an Agilent Technologies 1260 Infinity II high-performance liquid chromatograph (Agilent Technologies, USA) on a Poroshell InfinityLab 120 EC-C18 analytical column (4.6×100 mm, 2.7 µm; Agilent Technologies, USA) coupled with a Zorbax Eclipse Plus C18 guard column (4.6×12.5 mm, 5 µm; Agilent Technologies, USA). Elution was conducted with a mixture of deionized water (A) and acetonitrile (B) containing 0.1% formic acid in gradient mode (Table 1).

At the first stage of screening, the structures of potential BBP2023 metabolites were theoretically predicted based on known pathways of xenobiotic biotransformation, and the exact monoisotopic molecular masses of the predicted products were calculated. At the second stage, mass spectrometric analysis of BBP2023 was performed to identify characteristic product ions (Product Ion mode). At the third stage, considering that potential metabolites might produce fragment ions with *m/z* values matching those of BBP2023 fragment ions, a reverse screening of molecular ions was conducted using the corresponding product ions (Precursor Ion mode) in chromatographic analysis of the previously obtained supernatant. At the fourth stage, potential metabolites identified through chromatography were subjected to mass spectrometric analysis in Product Ion mode with direct plasma extract infusion into the ion source to indirectly confirm

the proposed chemical structures. Additionally, chromatographic analysis of predicted metabolites was performed based on neutral mass loss detection (Neutral Loss mode).

Table 1. Chromatographic parameters for detection of putative BBP2023 biotransformation products

Chromatography column	**Agilent InfinityLab Poroshell 120 EC-C18 4.6×100 mm, 2.7 µm**			
Protective column	Zorbax Eclipse Plus C18 4.6×12.5 mm, 5 µm			
Eluent A	Deionized water+0.1% formic acid			
Eluent B	Acetonitrile + 0.1% formic acid			
Gradient	Time, min	Flow rate, mL/min	% A	%B
	0.0	0.4	90	10
	1.0		90	10
	4.0		5	95
	8.0		5	95
	8.01		90	10
	12.0		90	10
Column thermostat temperature, °C	30			
Sample volume, mcL	10			
Total analysis duration, min	12			
Injector wash procedure	Via flush port, 3 seconds, 50% aqueous methanol solution			

To confirm the presence of identified BBP2023 biotransformation products, blood plasma extracts from both intact rats and animals administered the test compound were directly infused into the ultra-high-resolution mass spectrometer's ion source, and first-order mass spectra were recorded. The agreement between the *m/z* values of metabolites detected in the experimental samples and the theoretically calculated values, along with the absence of corresponding signals in the intact material screening, confirmed the accuracy of biotransformation product identification.

To verify the chemical structures of the identified metabolites and support subsequent pharmacokinetic studies, these compounds were synthesized and further utilized as analytical standards.

Statistical processing

Statistical analysis of raw chromatographic-mass spectrometric data was performed using AB Sciex Analyst 1.3.6 (AB Sciex, USA) and ESI Compass DataAnalysis Ver. 4.0 SP3 (Bruker, Germany). To compare two rat groups (with liver isolation and without liver isolation), Student's *t*-test for independent sets was used.

Results and Discussion

Considering data on general xenobiotic biotransformation pathways and the metabolism of known sydnonimine compounds, the first research stage involved calculating monoisotopic mass values of predicted BBP2023 metabolites (Table 2).

Table 2. *m/z* values of proposed BBP2023 biotransformation products protonated molecules

Proposed metabolite designation	**Molecular mass, Da**	**Proposed metabolite designation**	**Molecular mass, Da**
BBP2023	256,1661	geranamine (1,3-dimethylamylamine)	116,1361
BBP2023 + OH	272,1610	BBP2023 + OH + glucuronic acid	448,1931
BBP2023 + 2OH	288,1595	BBP2023 – $(CO)OC_2H_5$ + OH + glucuronic acid (SIN + OH + glucuronic acid)	376,1719
BBP2023 + 3OH	304,1509	geranamine (1,3-dimethylamylamine) + OH	132,1310
BBP2023 – $(CO)OC_2H_5$ (SIN)	184,1449	geranamine (1,3-dimethylamylamine) + OH + glucuronic acid	307,1631
BBP2023 – $(CO)OC_2H_5$ + OH (SIN + OH)	200,1399	BBP2023 + OH + sulfuric acid	352,1178
BBP2023 – $(CO)OC_2H_5$ + 2OH (SIN + 2OH)	216,1348	BBP2023 + glutathione	561,2342
BBP2023 – $(CO)OC_2H_5$ – NO (SIN – NO)	155,1469	BBP2023 – $(CO)OC_2H_5$ – NO+ OH + glucuronic acid (SIN – NO + OH + glucuronic acid)	346,1740

At the next stage, the BBP2023 compound was directly introduced into the mass spectrometer's electrospray ionization source as a 50 ng/mL solution in 50% acetonitrile with 0.1% formic acid added. The MS/MS screening of BBP2023 under positive ionization revealed

several distinct signals. Based on the known fragmentation mechanisms of molecules, a hypothesis was formulated regarding the structure of the detected product ions (Fig. 2).

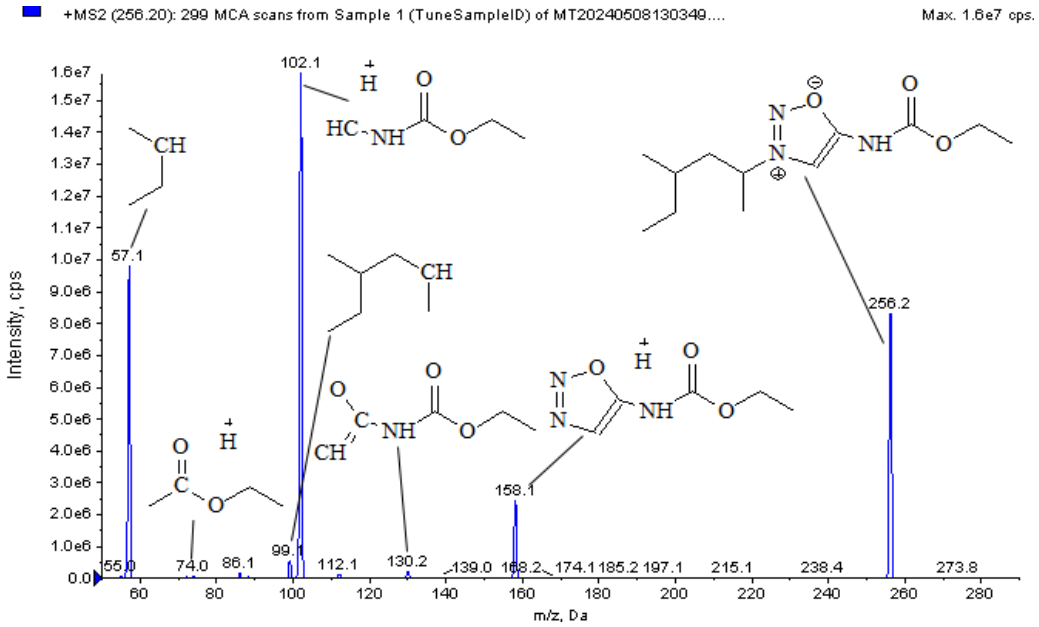


Figure 2. Second-order mass spectrum of BBP2023 (positive ionization mode) and proposed product ion structures. ***Note:*** mass spectrometer – AB Sciex QTrap 3200 MD, injection – direct via syringe pump; ion source – electrospray; BBP2023 solution with concentration of 50 ng/mL in 50% acetonitrile with addition of 0.1% formic acid, injection rate – 10 μL/min.

For the most prominent signals, optimal detection conditions were selected to ensure maximum sensitivity (Table 3). These parameters were subsequently employed in the chromatographic screening for potential BBP2023 biotransformation products in biological samples using the precursor ion detection mode based on corresponding product ions. Following the identification of putative metabolites, their detection conditions were further optimized.

Table 3. Parameters of mass spectrometric detection for chromatographic screening of potential BBP2023 metabolites in biological material using Precursor Ion mode

Ion source		**Turbo Spray**			
Ionization method		Electrospray (ESI)			
Ionization mode		Positive			
Ion source temperature, °C		350.0			
Ion source voltage, V		5500.0			
Gas		Nitrogen			
Curtain gas pressure, *psi*		20.0			
Nebulizer gas pressure, *psi*		30.0			
Heater gas pressure, *psi*		40.0			
Ion inlet voltage, V		5			
Scan time, sec		0.5			
Product ions, *m/z*	**Precursor ion search range, *m/z***	**DP, V**	**CEP, V**	**CE, eV**	**CXP, V**
102.1	180 – 460	45.0	21.0	20.0	2.5
57.1	100 – 460		19.0	24.0	2.6
158.1	200 – 500		28.0	15.0	3.0
86.1	100 – 500		24.0	15.0	2.7

Note: DP – declustering potential; CEP – collision cell entrance potential; CE – collision energy; CXP – collision cell exit potential.

Chromatographic screening of rat plasma samples collected 1 hour after BBP2023 administration, performed in precursor ion detection mode for the product ion with *m/z* 102.1 Da, revealed several distinct peaks. Mass spectrometric analysis of these signals demonstrated that peaks with retention times (RT) of 6.44, 6.67, and 6.74 minutes (Fig. 3) likely correspond to oxidation products of BBP2023 at the hydrocarbon radical (di- and monohydroxy derivatives).

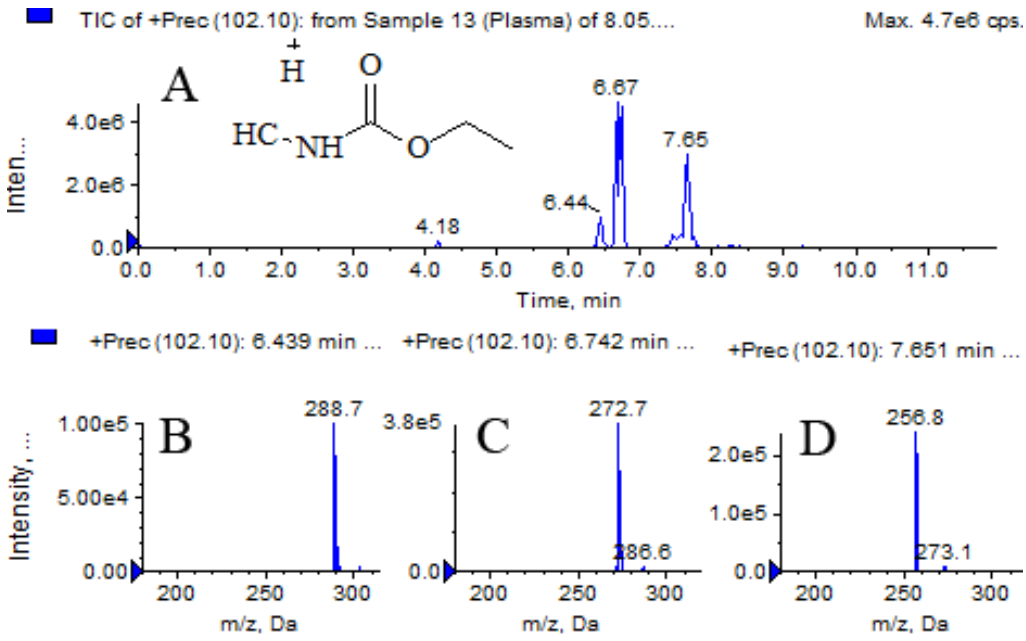


Figure 3. Proposed biotransformation products of BBP2023 identified via precursor ion chromatographic screening (*m/z* 102.1 Da). ***Note:*** R – hydrocarbon radical C_7H_{15}.

When detecting precursor ions for the product ion with *m/z* 57.1 Da (hydrocarbon radical fragment), chromatographic signals corresponding to geranamine (*m/z* 116.4 Da, RT 6.58 min) and a metabolite representing BBP2023 without its ethoxycarbonyl group (SIN, *m/z* 184.8 Da, RT 6.76 min) were identified. Additionally, the total ion chromatogram revealed a signal matching the parent compound BBP2023 (*m/z* 256.9 Da, RT 7.65 min). The corresponding chromatogram, precursor ion mass spectrum, and proposed metabolite structures are shown in Figure 4.

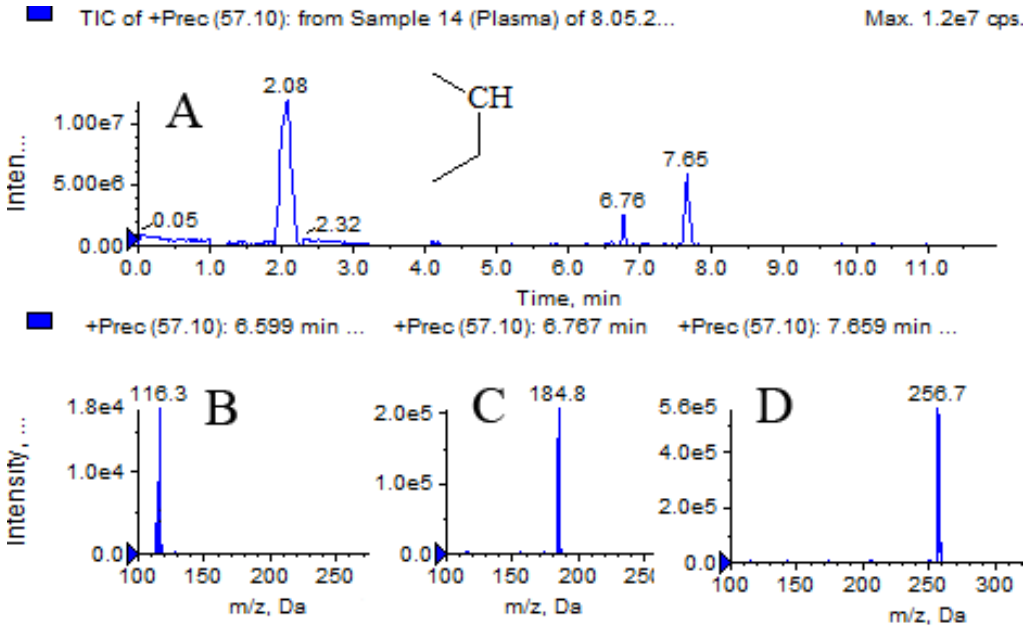


Figure 4. Proposed biotransformation products of BBP2023 identified via Precursor Ion chromatographic screening (m/z 57.1 Da). ***Note:*** R – hydrocarbon radical C_7H_{15}.

The study established that the product ion with *m/z* 158.1 Da is formed by BBP2023 metabolites generated through hydrocarbon radical oxidation (Fig. 5). The total ion chromatogram of precursor ions corresponds to the product ion chromatogram with *m/z* 102.1 Da.

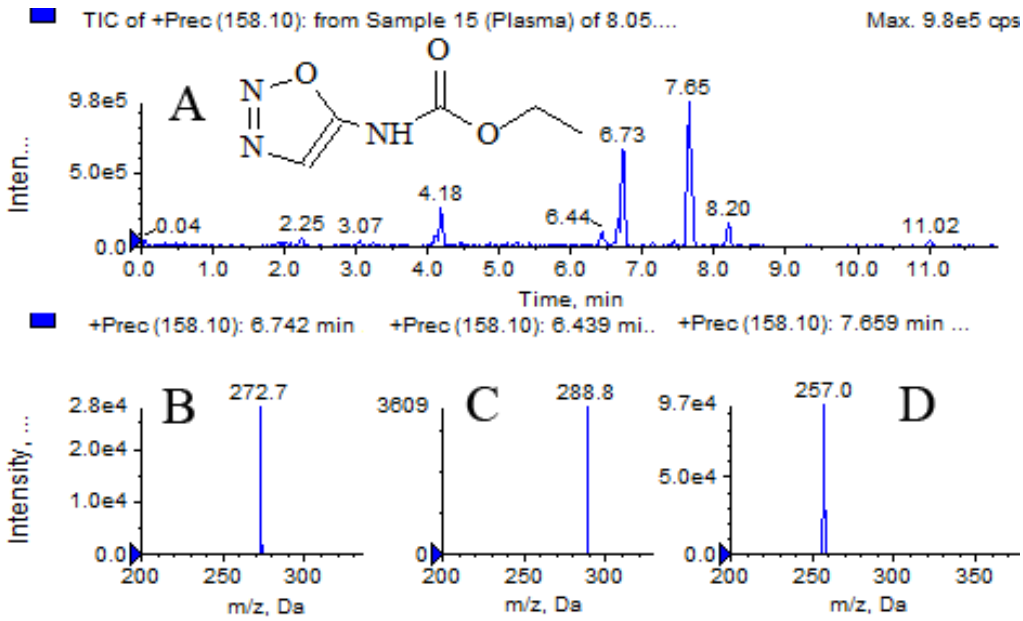


Figure 5. Proposed biotransformation products of BBP2023 identified via Precursor Ion chromatographic screening (m/z 158.1 Da). ***Note:*** R – hydrocarbon radical C_7H_{15}.

Analysis of the total ion chromatogram of precursor ions obtained for the product ion with *m/z* 86.1 Da revealed signals corresponding to the SIN metabolite (*m/z* 184.9 Da, RT 6.75 min) as well as its mono- and dihydroxy derivatives at the hydrocarbon radical (*m/z* 200.5 Da and 216.4 Da, RT 6.47 min). Additionally, it was established that another fragment ion also having *m/z* 86.1 Da is formed during fragmentation of hydroxyl derivatives of geranamine (*m/z* 132.9 Da, RT 4.40 and 4.65 min). The results of the corresponding chromatographic-mass spectrometric analysis are presented in Figure 6.

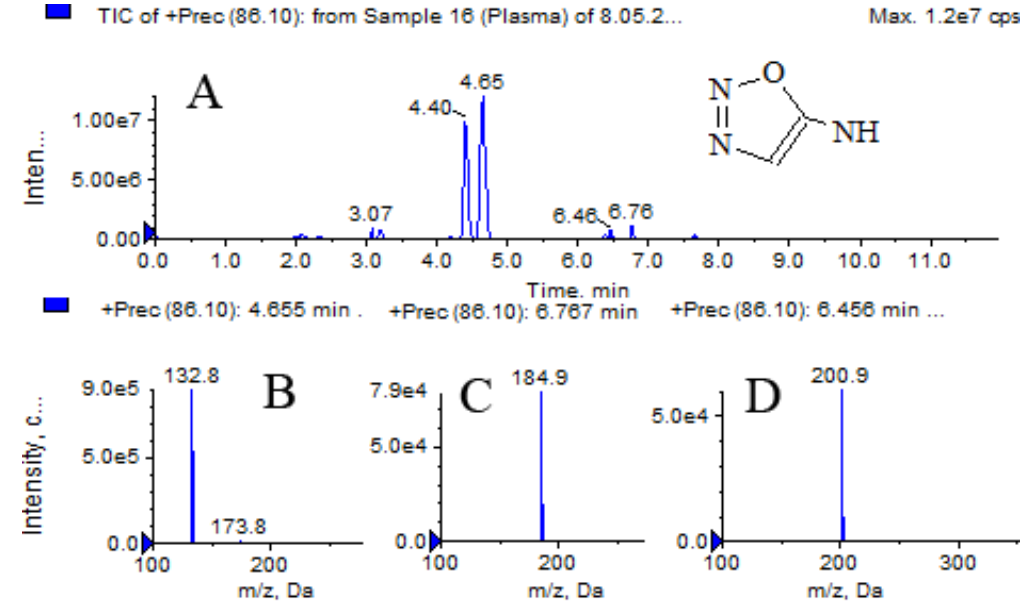


Figure 6. Proposed biotransformation products of BBP2023 identified via Precursor Ion chromatographic screening (*m/z* 86.1 Da). ***Note:*** R – hydrocarbon radical C_7H_{15}.

Thus, the screening chromatographic study revealed the following biotransformation products: BBP2023 compound lacking the ethoxycarbonyl group (SIN); mono- and dihydroxy derivatives of this metabolite and the parent compound at the hydrocarbon radical; geranamine, and its hydroxyl derivatives.

To confirm the structure of the identified BBP2023 biotransformation products, product ion mass spectra analysis was performed by directly introducing plasma extracts from experimental rats administered the test compound into the mass spectrometer ion source. Additionally, a comparative analysis of first-order mass spectra was conducted using plasma extracts from both experimental and intact animals, obtained with an ultra-high resolution time-of-flight mass spectrometer (UHR-TOF).

In the analysis of second-order mass spectra of hydroxylated BBP2023 derivatives (*m/z* 272.2 Da), a signal at m/z 73.3 Da was detected instead of 57.1 Da. Considering the fragmentation patterns of BBP2023 (Fig. 2), this finding indicates oxygen attachment at the terminal region of the hydrocarbon radical (Fig. 7A). Figure 7B shows the product ion mass spectrum of dihydroxylated BBP2023 derivatives (*m/z* 288.2 Da). The detected signal at *m/z* 131.2 Da instead of 99.1 Da confirms the presence of two hydroxyl groups in the radical structure. The presence of the metabolite representing the parent compound without the ethoxycarbonyl group (SIN, Fig. 7C) is confirmed by the second-order mass spectrum of the precursor ion at m/z 184.1 Da. Characteristic signals include fragments of the hydrocarbon radical (*m/z* 57.1 and 99.1 Da) and sydnonimine (*m/z* 86.1 Da). Notably, the absence of signals at *m/z* 130.1 and 158.1 Da in the product ion spectrum verifies the lack of the ethoxycarbonyl group in the metabolite structure. Figure 7D displays the fragmentation mass spectrum of geranamine (*m/z* 116.1 Da), where the detected product ions at *m/z* 57.1 and 99.1 Da indicate an identical hydrocarbon radical structure. Figures 7E and 7F present second-order mass spectra of monohydroxylated derivatives of SIN and geranamine, respectively. The hydroxyl group in their hydrocarbon radicals is evidenced by the signal at *m/z* 115.1 Da replacing 99.1 Da.

The presence of these BBP2023 biotransformation products was further verified by analyzing precursor ion mass spectra acquired through ultra-high resolution mass spectrometry. The exact matches between experimental and theoretical *m/z* values of protonated molecules, along with the absence of corresponding signals in intact samples, confirm the origin of these compounds (Fig. 8).

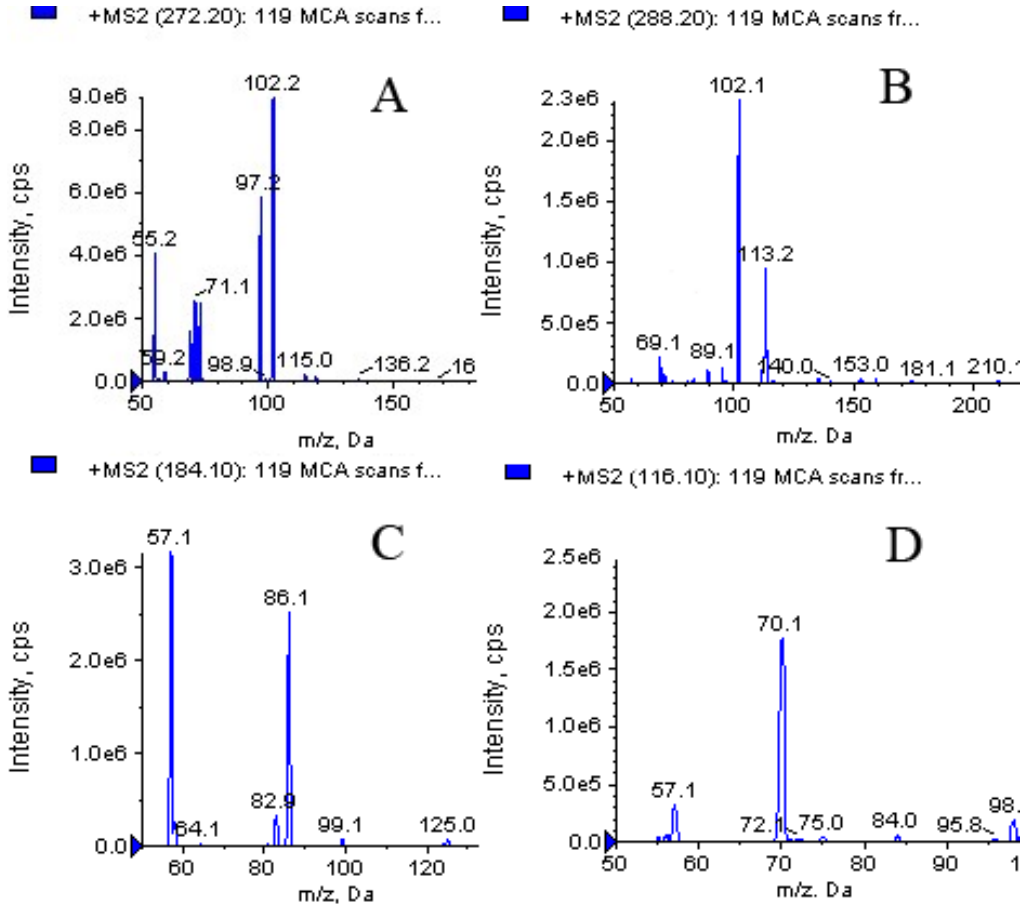


Figure 7. Product ion mass spectra of identified BBP2023 biotransformation products (A – monohydroxylated derivative of BBP2023; B – dihydroxylated derivative of BBP2023; C – SIN; D – geranamine; E – monohydroxylated derivative of SIN; F - monohydroxylated derivative of geranamine).

According to additional screening of potential BBP2023 metabolites based on theoretically calculated monoisotopic molecular masses, conjugates of the parent compound and its SIN metabolite with glucuronic acid were detected in the urine of experimental rats (*m/z* 448.1931 Da and 376.1719 Da, respectively). Additionally, a metabolite formed after nitric oxide elimination from SIN was identified in blood plasma (*m/z* 155.1469 Da). The ultra-high resolution mass spectra of molecular ions of the detected metabolites are presented in Figure 9.

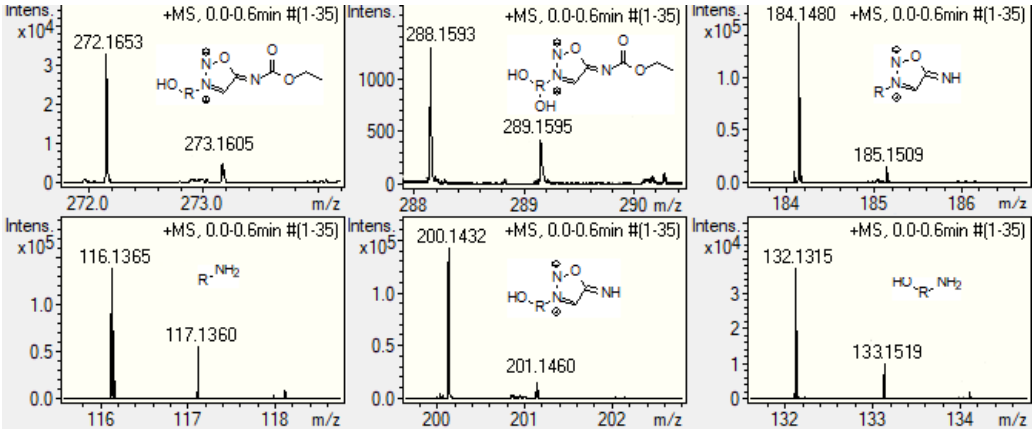


Figure 8. Ultra-high resolution (UHR-TOF) mass spectra of precursor ions from identified biotransformation products of compound BBP2023. ***Note:*** R – hydrocarbon radical C_7H_{15}.

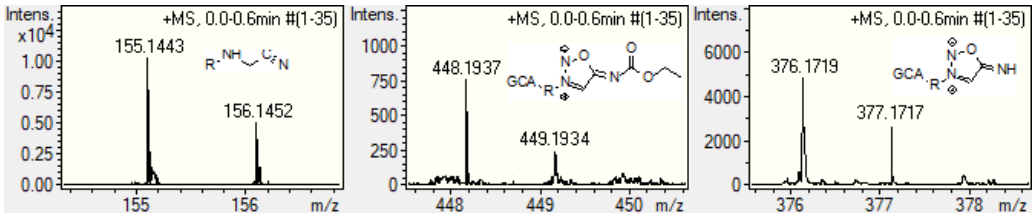
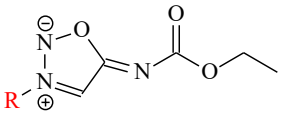
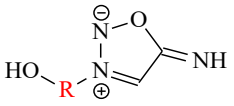
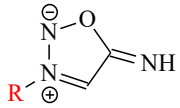
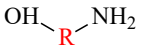
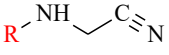
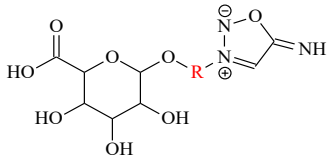
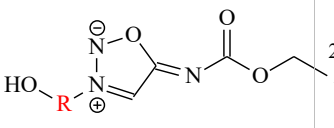
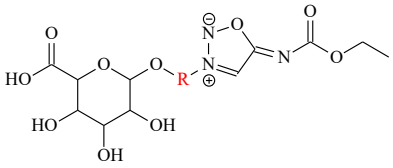
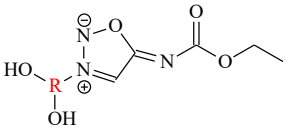


Figure 9. Ultra-high resolution (UHR-TOF) mass spectra of precursor ions from identified biotransformation products of compound BBP2023 based on additional screening results. ***Note:*** R – hydrocarbon radical C_7H_{15}.

At the next stage, optimal mass spectrometric detection conditions were established for the identified BBP2023 biotransformation products using multiple reaction monitoring (MRM) mode. The structural formulas of BBP2023 metabolites and the MRM transitions employed for detection during chromatographic analysis are presented in Table 4.

Table 4. Structures of identified BBP2023 metabolites and MRM transitions used for their detection via chromatography

Structure	MRM transition	Structure	MRM transition
	256.2/57.1 256.2/102.1		200.1/73.1 200.1/86.1
	184.1/57.1 184.1/86.1		132.1/114.1 132.1/86.1
	115.2/57.1 115.2/95.0		376.2/86.1 376.2/115.1 376.2/183.1
	272.2/102.1 272.2/73.1		
	116.1/57.1 116.1/99.1		448.2/255.2 448.2/158.1 448.2/102.1
	288.2/102.1 288.2/89.1		

***Note*:** R – hydrocarbon radical C_7H_{15}.

According to chromatographic analysis of rat plasma samples collected 1 hour after intragastric administration of compound BBP2023 at a dose of 1/100 LD_{50} (11.0 mg/kg), the presence of the parent compound, active metabolite SIN, geranamine, as well as various isomers of their hydroxylated derivatives was confirmed (Fig. 10). Given that geranamine and its hydroxylated derivatives, – which, according to literature, exhibit pleiotropic pharmacological effects – were identified among the biotransformation products, further preclinical studies should evaluate the effects of BBP2023 on vascular tone, blood pressure, and heart rate in large animals, particularly beagle dogs (Granik and Grigoriev 2004).

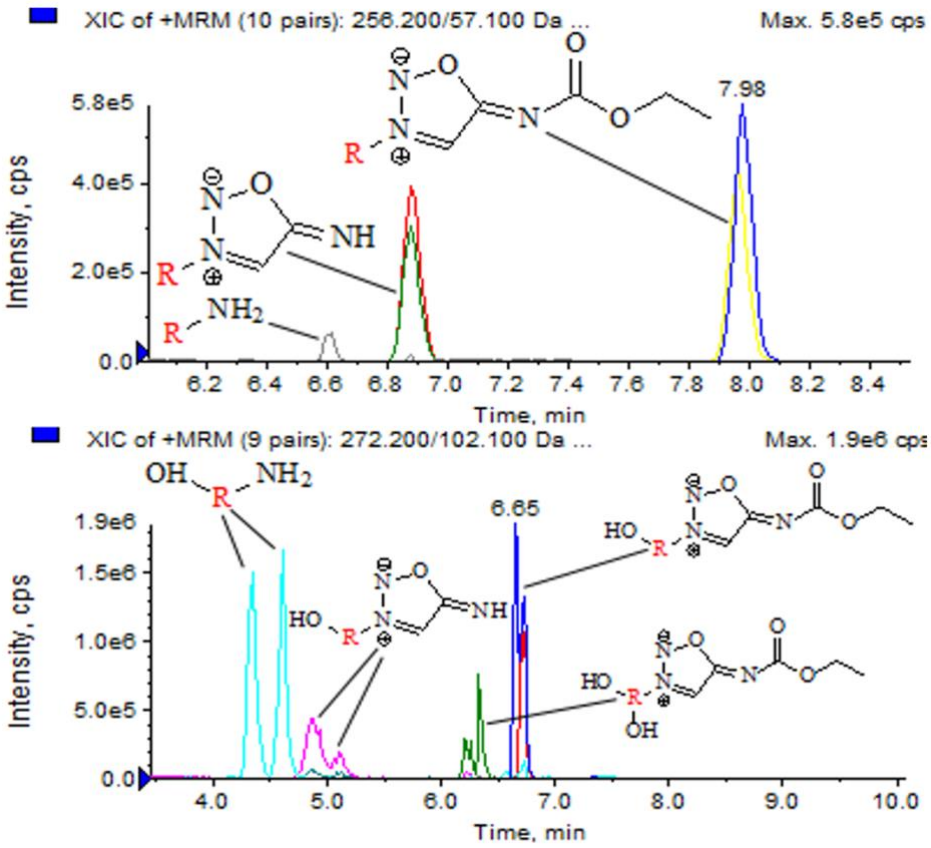


Figure 10. Chromatographic fragments of rat plasma sample collected 1 hour after intragastric administration of compound BBP2023 at a dose of 1/100 LD_{50} (11.0 mg/kg). ***Note:*** R – hydrocarbon radical C_7H_{15}.

Chromatographic analysis of rat urine sample collected within 24 hours after intragastric administration of compound BBP2023 at a dose of 1/100 LD_{50} (11.0 mg/kg) revealed the presence of various isomers of glucuronic acid conjugates of metabolite SIN and parent compound BBP2023 (Fig. 11).

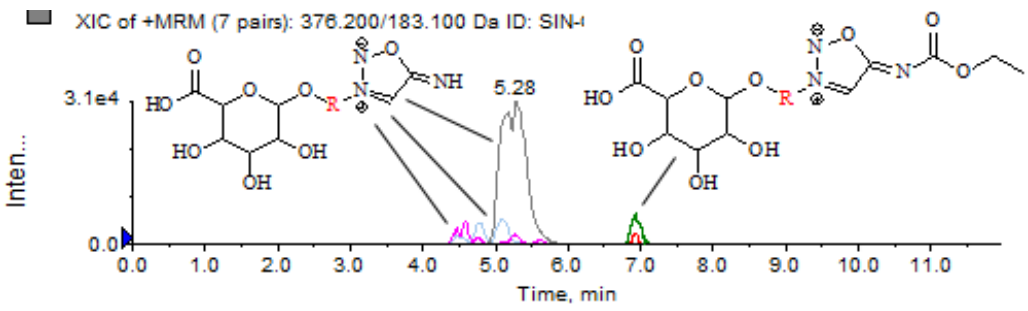


Figure 11. Chromatogram of rat 24-hour urine sample following intragastric administration of compound BBP2023 at a dose of 1/100 LD_{50} (11.0 mg/kg). ***Note:*** R – hydrocarbon radical C_7H_{15}.

Chromatographic analysis of rat plasma samples collected 8 hours after compound administration revealed no distinct peaks corresponding to the parent compound or its ethoxycarbonyl-lacking derivative. However, signals from hydroxylated biotransformation

products showed high intensity. Analysis of chromatograms from control rat plasma samples demonstrated no peaks with retention times or MRM transitions matching BBP2023 metabolites. Similar results were obtained in rabbit plasma analysis, though the compound's metabolism in these animals exhibited less diversity of hydroxylated derivative isomers.

Analysis of plasma from rats receiving BBP2023 after hepatic vascular isolation confirmed detectable signals of the parent compound, with peak areas 2.8-fold greater on average than in sham-operated controls.

Compound BBP2023 has certain potential advantages compared to other known sydnonimines. For example, molsidomine pharmacodynamics based on nitrous oxide release, followed by blood vessels dilation (mostly veins and major coronary arteries), which enhances collateral blood flow, and platelets aggregation inhibition. On the other hand, presence of geranamine as one of the BBP2023 metabolites can lead to brain vessels dilation, thus enhancing cerebral blood flow (Fig. 12), which can be used in treatment of cerebral vascular disorders.

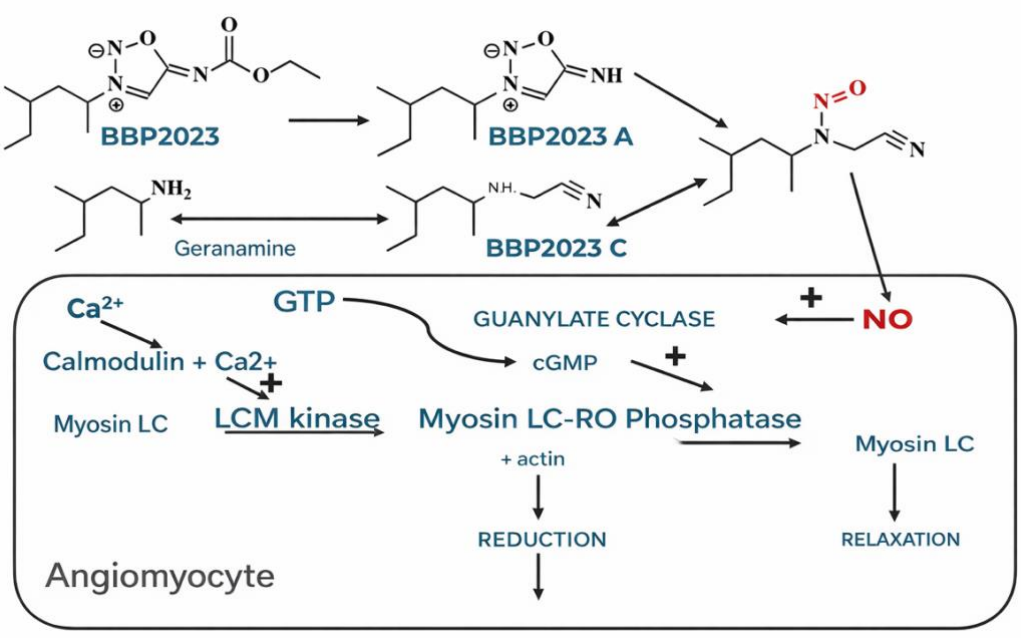


Figure 12. Structures of BBP2023, its metabolites, and the most probable mechanism of BBP2023 vasodilatory activity.

Study limitations

None.

Conclusion

Thus, the experimental results revealed the presence of a metabolite representing the BBP2023 compound lacking the ethoxycarbonyl group. Additionally, a product formed after the elimination of nitric oxide from the aforementioned molecule was detected. This finding may indirectly indicate the presumed pharmacodynamics of the investigated drug candidate. It was established that one of the biotransformation products of BBP2023 is geranamine. Furthermore, screening identified mono- and di-hydroxylated derivatives of the aforementioned metabolites and the parent compound, as well as esters of these compounds with glucuronic acid. Some of the detected metabolites were synthesized and used as standards for chromatographic analysis. The matching retention times of these compounds confirm the correct identification of these metabolites during the screening stage. The absence of chromatographic signals corresponding to BBP2023 biotransformation products was observed in both control samples and plasma from rats subjected to hepatic vascular isolation. These results indicate the liver's direct involvement in the formation of the identified BBP2023 metabolites.

The identified features of BBP2023 biotransformation, including the formation of active hydroxylated forms and glucuronic acid conjugates, justify the need for a detailed analysis of the experimental pharmacokinetics of both the parent compound and its metabolites to assess their roles in therapeutic effects and potential side effects.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Ethics statement

This study was approved by the Local Ethical Committee of Tver State Medical University of the Ministry of Health of the Russian Federation (Meeting minutes No. 5 dated June 19, 2024). All experiments were conducted in compliance with the Good Laboratory Practice Rules approved by Order No. 708n of the Russian Ministry of Health (August 23, 2010) and Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes.

Data availability

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

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All authors have made equivalent and equally significant contributions to the preparation of this publication. All authors confirm that their authorship complies with the ICMJE international criteria (all authors have made substantial contributions to research conception, study execution, and article preparation; all have read and approved the final version prior to publication).