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Research Article

Analgesic activity of imidazo[1,2-a]indole derivative and its involvement of TRPV1 and κ-opioid receptors

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Abstract

Introduction: Various analgesics, primarily nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, are used to relieve pain. On the account that opioids can cause respiratory depression, psychological and physical dependence, as well as addiction, and NSAIDs can cause gastro- and enteropathy, nephrotoxicity, bronchospasm, bleeding, and cardiovascular dysfunction, the search for new molecules exerting an analgesic effect through other cellular targets is relevant. **The aim of this study** was to evaluate the analgesic effect of a new imidazo[1,2-a]indole derivative in various pain models and its effect on TRPV1 and κ-opioid receptors.

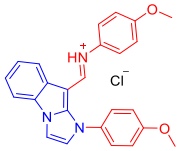
Materials and Methods. The median lethal dose (LD_{50}) of the imidazo[1,2-a]indole derivative (lab code SV-1010) was determined in experiments on mice and rats. The analgesic effect of SV-1010 was studied by means of the hot plate test (in mice), tail-flick test (in rats), paw pressure test (in rats), abdominal constriction test (in mice), formalin test (in rats), and neuropathic pain test (in rats). In addition, the effect of SV-1010 on TRPV1 ion channels was examined (in a Chinese hamster ovary cell line with an induced expression of a rat TRPV1 channel), and the potential for κ-opioid receptor activation was tested (by means of molecular docking). SV-1010 and the reference drugs diclofenac, indomethacin, ketorolac, and pregabalin were administered intragastrically.

Results and Discussion: SV-1010 is less toxic than diclofenac, indomethacin, and ketorolac, exhibits a pronounced analgesic effect, but exceeds diclofenac, indomethacin, ketorolac, and pregabalin by the potency and therapeutic index. The treatment sites for SV-1010 to exert its analgesic effect are the supraspinal, supraspinal + peripheral, spinal, and peripheral levels of pain sensitivity. Of note is the high selectivity of SV-1010 to blocking TRPV1 ion channel and activating κ-opioid receptors.

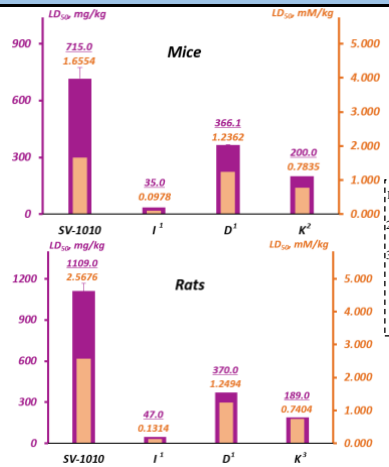
Conclusion: Significantly lower acute toxicity (when compared to such of diclofenac, indomethacin, and ketorolac), high analgesic activity, and a wider therapeutic index (when compared to such of diclofenac, indomethacin, ketorolac, and pregabalin) make SV-1010 promising for further preclinical study.

Graphical Abstract

SV-1010, diclofenac, indomethacin, ketorolac, and their acute toxicity after intragastric (i.g.) administration

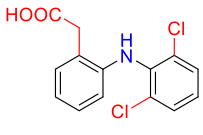


4-Methoxy-N-((1-(4-methoxyphenyl)-1*H*-imidazo[1,2-*a*]indol-9-yl)methylene)benzene-ammonium chloride
(Laboratory code **SV-1010**)

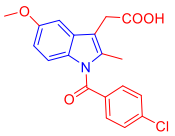


[1]Buslama et al. 2017;
[2]Mandema and Stanski 1996;
[3]Sosnov et al. 2018.

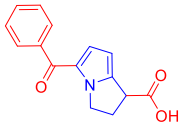
Reference drugs



Diclofenac (D)
[sodium salt of (2-[2', 6'dichlorophenyl) amino] phenylacetic acid)



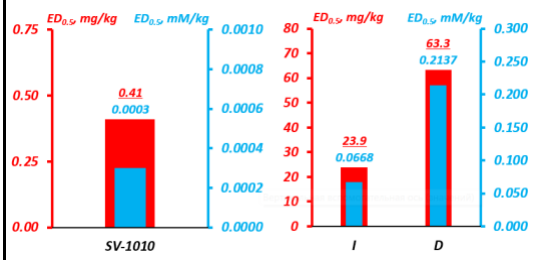
Indomethacin (I)
[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indolyl-3-acetic acid]



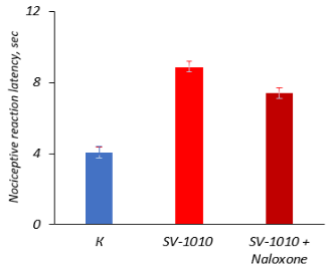
Ketorolac (K)
(±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol

Analgesic effect of SV-1010, diclofenac (D), indomethacin (I) and ketorolac (K) with intragastrical administration

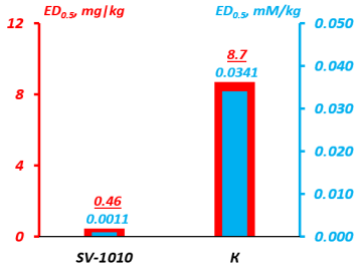
hot plate test



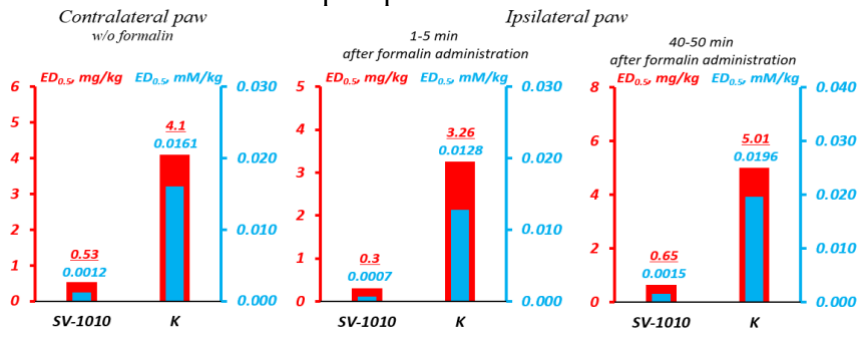
hot plate test + naloxone



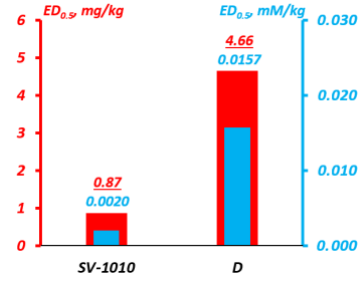
tail-flick test



paw pressure test



abdominal constriction test



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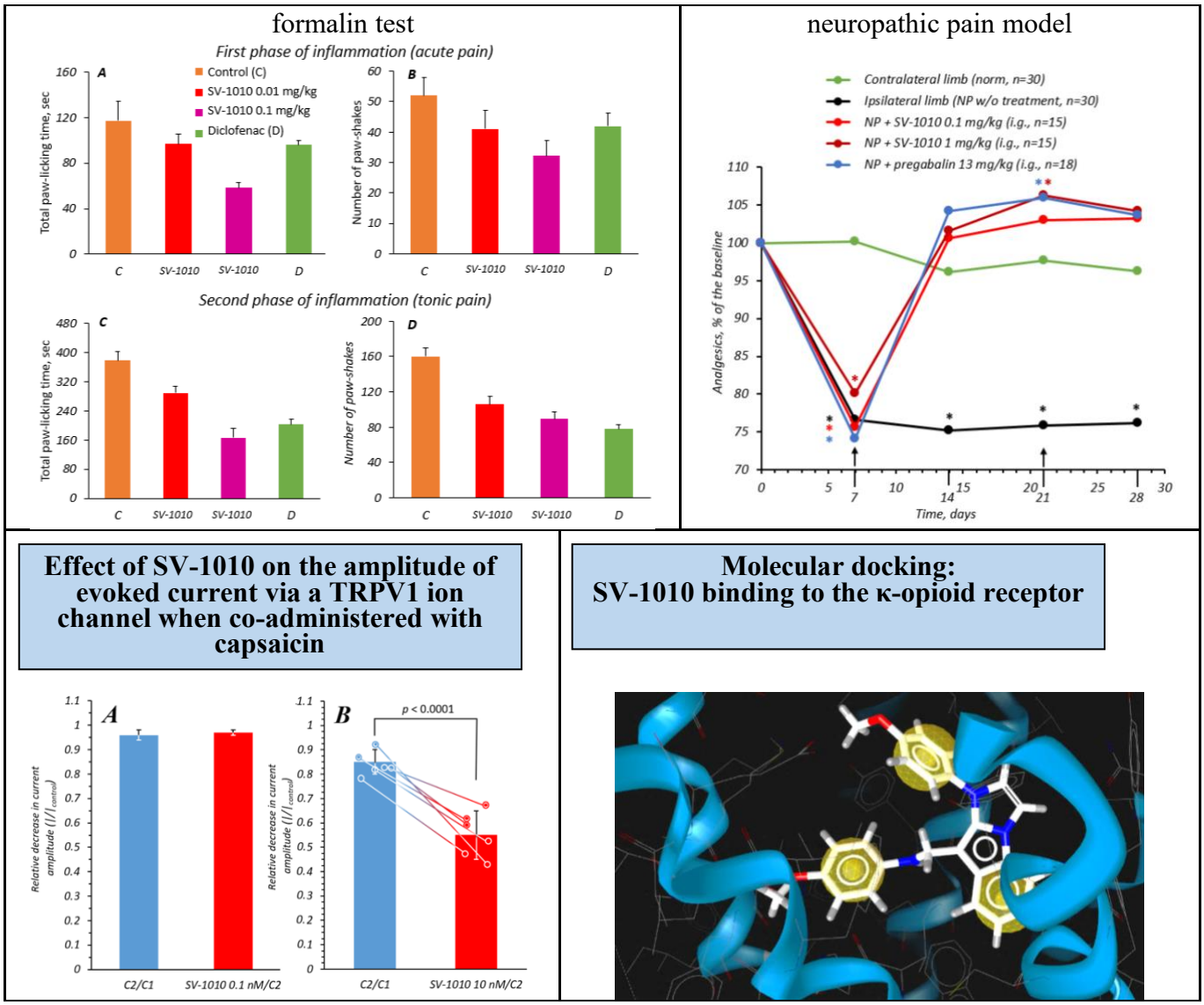

Keywords

analgesic effect, imidazo[1,2-a]indole derivative, TRPV1 ion channels, κ-opioid receptors

Introduction

Pain syndromes represent a major category among the spectrum of diseases, greatly diminishing patients' well-being and their quality of life. Modern strategies to prevent and relieve pain are aimed at interrupting pain mechanisms at different levels of the nervous system – somatic, or cerebrospinal, and autonomic, or vegetative (Papalia et al. 2023; Titova and Bezdolny 2025). Of note is that autonomic-mediated pain is a component of neuropathic pain, controlled by two divisions of the autonomic nervous system – parasympathetic and sympathetic.

Available analgesics affect the nociceptive system at different levels. Currently, analgesics are represented by opioids, non-steroidal anti-inflammatory drugs (NSAIDs), α2-adrenergic receptor agonists, some anticonvulsant drugs (ACDs), serotonin reuptake blockers, drugs acting on GABAergic structures, etc. (Kirkpatrick et al. 2016; Mashkovsky 2021).

NSAIDs and opioid analgesics are most often used to relieve pain: the former exert an analgesic effect when nociceptors are irritated under inflammatory conditions, but have a number of serious side effects: gastro-, entero-, nephro- and cardiotoxicity, and teratogenicity (Lanas and Chan 2017; Karateev et al. 2018; Velz et al. 2018; Horoshun and Lazareva 2022; Minhas et al.

2023), the latter relieve severe pain of any origin, but depress breathing, cause addiction, mental and physical dependence (Arbukh et al. 2017; Rizzi et al. 2017, Listos et al. 2019; Koob 2020).

By means of chemoproteomic and chemoreactome profiling, we earlier demonstrated that an imidazo[1,2-*a*]indole derivative (lab code SV-1010) acts on metabotropic glutamate receptors, which mediate their effect both through G proteins (Pereira and Goudet 2019) and through activating intracellular signaling cascades leading to the modification of other proteins, such as ion channels (Carlton et al. 2009). Glutamate receptors are located in cells of the nervous and immune systems and are closely linked with other receptor structures, in particular with non-selective cation channels TRPV1 (transient receptor potential cation channel subfamily V member 1), localized on primary afferents and playing the role of the main integrators of pain stimuli. TRPV1 is activated by heat (t > 43°C), acidification of the extracellular pH to under 6.0, lipid-based inflammatory mediators, and other substances (toxins) of plant and animal origin (Alawi and Keeble 2018).

Currently, one of the priority areas for pharmacologists worldwide, in collaboration with chemists and clinicians, is the search for molecules that modulate and antagonize TRP-ion channels in order to develop painkillers with few side effects.

The capacity of imidazo[1,2-*a*]indole derivative to inhibit the amplitude of the current in TRPV1 evoked by the selective agonist capsaicin made it possible to suggest that this compound had the analgesic effect, which was experimentally confirmed in various pain sensitivity tests.

The aim of this study was to evaluate the analgesic activity of a new imidazo[1,2-*a*]indole derivative in various pain models and its effect on TRPV1 and κ-opioid receptors.

Materials and Methods

Compounds under study

Chemical substance 4-methoxy-N-((1-(4-methoxyphenyl)-1*H*-imidazo[1,2-*a*]indol-9-yl)methylene)benzene ammonium chloride under lab code SV-1010 (synthesized at the Southern Federal University, Rostov-on-Don, Russia), diclofenac (chemical substance, batch 23390D017, Armavir Biofactory, Russia), indomethacin (enteric-coated tablets, weighing 25 mg (Ozon LLC, Russia), ketorolac [film-coated tablets (Renewal Pharmaceutical Manufacturing Company, Russia)], pregabalin [hard-gelatin capsules (Moscow Endocrine Plant, Russia)], naloxone (naloxone hydrochloride dihydrate; Moscow Endocrine Plant, Russia), capsaicin (Sigma Aldrich, USA).

Animals

Experiments were conducted on 249 male albino mice weighing 18–32 g and 272 male rats weighing 210–320 g, including 20 mongrel and 252 Wistar rats, maintained under standard vivarium conditions on a standard diet according to good laboratory practice (GOST 33216-2014). The studies complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg edition, 2006), the Law of the Russian Federation "On the Protection of Animals from Cruel Treatment" dated June 24,1998, the Rules of Laboratory Practice for Preclinical Trials in the Russian Federation (GOST 3 51000.3-96 and GOST P 53434-2009), the provisions of the Helsinki Declaration of the World Medical Association on the Humane Treatment of Laboratory Animals (Report of the AVMA Panel on Euthanasia JAVMA, 2001), Guidelines for the Care and Use of Laboratory Animals (interstate standard GOST 33216-2014 dated July 1, 2016), Guidelines for the Conduct of Preclinical Trials of Medicinal Products (Mironov 2012) and The Eurasian Economic Commission's Guidelines for working with laboratory (experimental) animals in preclinical (non-clinical) studies (recommendation No. 33 of the Board of the Eurasian Economic Commission, November 14, 2023, Moscow). The experiments were approved by the Ethics Committee of Kuban State Medical University of the Ministry of Health of the Russian Federation (minutes No. 119 of April 13, 2023).

Experimental Design

To study SV-1010 and the reference drugs diclofenac, indomethacin, ketorolac, and pregabalin in the selected study area, the following groups of animals were formed:

– acute toxicity (determination of the median lethal dose – LD_{50}) of SV-1010 when intragastrically (i.g.) administered to mice in a wide range of doses (500, 600, 700, 800, and 900 mg/kg) and to rats (1000, 1100, 1200, and 1300 mg/kg), respectively, to Groups 5 and 4, containing 5 animals each;

– analgesic effect in the hot plate test – 12 groups of 10 mice each: Group 1 – control-1 (potato starch gel, i.g.), Groups 2-4 – SV-1010 (0.01, 0.1 and 1 mg/kg, i.g.), Group 5 – control-2 (potato starch gel, i.g.), Groups 6-8 – diclofenac (30, 60 and 90 mg/kg, i.g.), Group 9 – control-3 (potato starch gel, i.g.), and Groups 10-12 – indomethacin (10, 20 and 30 mg/kg, i.g.);

– analgesic effect in the hot plate test using naloxone – 3 groups of 7 mice each: Group 1 – control (potato starch gel, i.g.), Group 2 – SV-1010 (0.1 mg/kg, i.g.), and Group 3 – SV-1010 (0.1 mg/kg, i.g.) + naloxone (1 mg/kg, subcutaneously (sc));

– analgesic effect in the tail-flick test – 7 groups of 10 rats each: Group 1 – control (potato starch gel, i.g.), Groups 2-4 – SV-1010 (0.01, 0.1 and 1 mg/kg, i.g.), and Groups 5-7 – ketorolac (2.5, 5 and 10 mg/kg, i.g.);

– analgesic effect in the paw pressure test – 7 groups of 8-10 rats each: Group 1 – control (potato starch gel, i.g.), Groups 2-4 – SV-1010 (0.01, 0.1 and 1 mg/kg, i.g.), and Groups 5-7 – ketorolac (2, 4 and 6 mg/kg, i.g.);

– analgesic effect in the abdominal constriction test – 8 groups of 10 mice each: Group 1 – control-1 (acetic acid, intraperitoneally (i.p.)), Groups 2-4 – SV-1010 (0.01, 0.1 and 1 mg/kg, i.g.), Group 5 – control-2 (acetic acid, i.p.), and Groups 6-8 – diclofenac (2.5, 5 and 7.5 mg/kg, i.g.);

– analgesic effect in the formalin test – 4 groups of 10 rats each: Group 1 – control (2% formalin, subplantarly (s.p.)), Groups 2-3 – SV-1010 (0.01 and 0.1 mg/kg, i.g.), and Group 4 – diclofenac (7 mg/kg, i.g.);

– analgesic effect in the neuropathic pain test in rats – 4 groups: Group 1 – with neuropathy (NP) without treatment (n=30); Groups 2 and 3 – NP + SV-1010 at doses of 0.1 and 1 mg/kg, i.g. (n=15 in each group); Group 4 – pregabalin at a dose of 13 mg/kg (equivalent to 150 mg/day in humans), i.g. (n=18).

The effect of SV-1010 on κ-opioid receptors was determined using molecular docking.

The effect of SV-1010 on TRPV1 ion channels was studied using electrophysiology in a continuous cell line with an induced expression of the channel under study, in a whole-cell patch-clamp technique.

The study design is shown in Figure 1.

Experimental study models

Lethal dose determination

The median lethal dose (LD_{50}, mg/kg) of SV-1010 and the reference drugs diclofenac, indomethacin, and ketorolac was determined through i.g administration (via a metal probe) of potato starch gel used as a vehicle to male mice and male rats. The animals were observed for 2 weeks (Arzamastsev et al. 2000).

Since the results of the experiments to determine acute toxicity were recorded in an alternative form, LD_{50} was calculated using the Prozorovsky method (1962).

The parameters of acute toxicity for the reference drugs: diclofenac (for male rats), indomethacin, and ketorolac – were obtained from (Buzlama et al. 2017; Sosnov et al. 2018; Mandema and Stanski 1996).

Study of analgesic activity of SV-1010

The analgesic activity of SV-1010 in a hot plate test, which reflects the spinal level of pain sensitivity, was determined in mice using the method described by Voronina and Guzevatykh (2012). This test is basic for studying the analgesic activity: it is based on behavioral responses to thermal pain and is used to identify the compounds that inhibit superficial and acute pain somatically.

SV-1010, diclofenac, and indomethacin were administered i.g. to mice using 1% potato starch gel (12 hours after prior food deprivation) 2 hours before the experiment. Control animals received 1% potato starch gel i.g. in volumes equivalent to such in the experimental groups of mice.

The hot plate test was performed using a II TC Life Science unit (USA). Animals were placed on a plate heated to 55.0° ± 0.5°C, and then the latency time of the pain response was recorded, characterized by withdrawing and licking hind paws. To reduce the risk of thermal damage to pads, the experiment time did not exceed 30 seconds. The analgesic activity was expressed as the mean latency time in a group and the percentage of pain response inhibition (PRI), which was calculated using formula (1):

$$PRI = \left(\frac{T_{expt}}{T_{ctrl}} \times 100\right) - 100, \qquad (1)$$

where T_{Ctrl} is the latency time of the pain response in control, and T_{Expt} is the latency time of the pain response in an experimental group.

To evaluate the comparative efficacy of SV-1010 and the reference drugs, the median effective dose ($ED_{0.5}$) (Raevsky 1976; Galenko-Yaroshevsky et al. 2021), which characterizes their analgesic activity, and the therapeutic index (TI), which reflects the therapeutic effect range ($LD_{50}/ED_{0.5}$), were calculated (graphically).

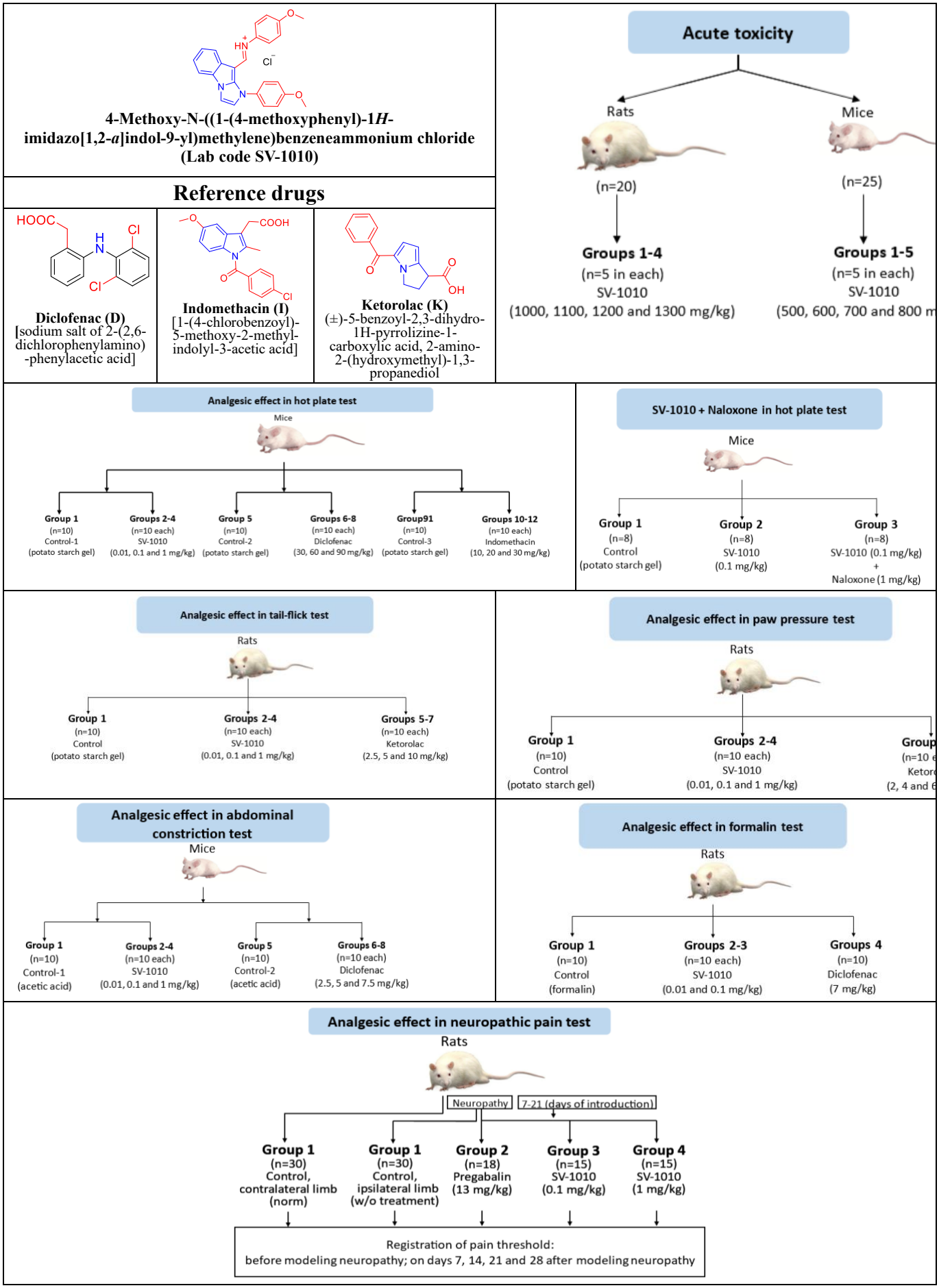

Figure 1. Study design flowchart.

Study of the analgesic activity of SV-1010 in a hot plate test against the background of naloxone hydrochloride dehydrate

The test was conducted using the method described above. SV-1010 (0.1 mg/kg, i.g.) was administered in combination with naloxone (1 mg/kg, sc) 1 hour and 15 min before the experiment, respectively.

The analgesic activity of SV-1010 in a tail-flick test was studied in rats using the method described by Voronina and Guzevatykh (2012). This method allows determining the effect of SV-1010 on pain sensitivity primarily at the spinal level. For measurements, a IITC Life Science analgesia meter (USA) was used. Light-thermal irritation was applied to the rat tail kept in a restrainer, followed by recording the latency of the tail flick.

The tested substances were administered i.g. (12 hours after prior food deprivation) in a 1% starch gel 1 hour before the test.

The analgesic activity was assessed based on the mean latency time in a group and the percentage of pain response suppression, which was calculated using equation (1). For the experiments, highly sensitive animals were selected, with pain response latency of up to 4 seconds. The comparative efficacy of SV-1010 and the reference drug was assessed using the $ED_{0.5}$ and TI.

The analgesic activity of SV-1010 in a paw pressure test (*Randall-Selitto test)* against the background of formalin-induced paw edema in rats was assessed using the method described by Voronina and Guzevatykh (2012). This test allows evaluating the effect of the tested substance on the supraspinal and peripheral levels of pain sensitivity.

The analgesic activity of SV-1010 and the reference drug ketorolac in the mechanical paw pressure test was studied using a IITC Life Science algesiometer (USA).

To enhance sensitivity to mechanical stimulation in the right paw, acute exudative inflammation was simulated by a subplantar injection of 0.1 mL of 2% formalin solution (GOST 1625-86 FM, Russia). With increased paw pressure, there was consistently observed a spinal limb-withdrawal reflex, a complex of supraspinal motor responses in order to release the paw, and vocalization.

The tested substances were administered i.g. (12 hours after prior food deprivation) in a 1% starch gel 1 hour before the test. The animals in the control group were administered with the equivalent volumes of a 1% starch gel i.g.

The paw pressure test was performed on both paws of rats: the left (contralateral) paw – without formalin administration, and the right (ipsilateral) paw – with formalin-induced edema. On the contralateral paw, the measurements were taken once, and on the ipsilateral paw – twice: 1-5 minutes and 40-50 minutes after formalin administration. The vocalization threshold was recorded. The analgesic effect criterion was a significant reduction in the intensity of pain responses by the force applied to the paw, measured in grams. The comparative efficacy of SV-1010 and the reference drug was assessed using the $ED_{0.5}$ and TI.

The analgesic activity of SV-1010 was studied in the abdominal constriction test, which reflects the peripheral level of pain sensitivity, mediated by inflammatory mediators, in particularly bradykinin, which stimulate nociceptors at the site of injury. The test was performed in mice, using the method described by Voronina and Guzevatykh (2012) by i.g. administration of a 0.6% acetic acid solution at a rate of 0.1 mL per 10 g of body weight. The pain response with writhes (extended hind legs, contraction and relaxation of abdominal muscles, and back-arching) was recorded for 15 minutes.

The test substances were administered i.g. (12 hours after prior food deprivation) in a 1% potato starch gel 1 hour before the experiment. Control animals were administered i.g. with a 1% potato starch gel in volumes equivalent to those of the tested substances.

The analgesic effect of SV-1010 and diclofenac was assessed by a reduced number of writhes when compared to those in control animals. Besides, the latency of writhing onset (in seconds) was recorded as an additional criterion for characterizing the intensity of the analgesic activity.

The analgesic effect – pain response inhibition (PRI, %) – was assessed by a reduced number of writhes in comparison with those in the control group and calculated using equation (2):

$$PRI = \frac{(C_{Ctrl} - C_{Expt})}{C_{Ctrl}} \times 100, \qquad (2)$$

where C_{Ctrl} is the number of writhes in control and C_{Expt} is the number of writhes in an experimental group. The comparative efficacy of SV-1010 and the reference drug was assessed using the $ED_{0.5}$ and TI.

The analgesic activity of SV-1010 in rats in a formalin test was assessed using the method described by Voronina and Guzevatykh (2012). Rats were injected subplantarly with 0.1 mL of a 2% formalin solution (GOST 1625-86 FM, Russia) into the right paw. The formalin test consists of two phases: the first (lasting 5 min) reflects acute pain and is associated with peripheral nociceptive sensitization; the second (lasting 1 hour or more) reflects tonic pain

associated with activation of the central nociceptive structures (Chaika et al. 2015; Gregory et al. 2013).

The tested substances were administered to rats i.g. (12 hours after prior food deprivation) in a 1% potato starch gel, using a gavage 1 hour before the experiment. Control animals were administered with a 1% potato starch gel i.g. in the equivalent volumes corresponding to those of the tested compound.

The analgesic effect was assessed based on the total duration and number of licking the paw injected with formalin, as well as the total number of paw shakes within 60 minutes.

The analgesic activity of SV-1010 on neuropathic pain induced by sciatic nerve axotomy in rats was determined in a paw pressure test according to the method described by Erofeeva et al. (2021).

NP was modeled by sciatic nerve axotomy of the rat left hind limb. Anesthesia was induced with Telazol (Zoetis Inc., USA) at a dose of 25 mg/kg and 1% lidocaine solution at a dose of 80-110 μL per animal. According to the topographic location of the sciatic nerve, a 1.5-cm long incision was made on the lateral thigh, then the muscles were moved apart, and a 1-cm long section of the sciatic nerve was excised. Then the wound was closed with Vicryl Plus 4/0 absorbable suture (Jonson & Jonson International, Belgium). The sutured wound surface was disinfected with chlorhexidine (Yuzhpharm, Russia). Additionally, to prevent further infection, the animals were administered subcutaneously with the antibiotic ceftriaxone (BZMP OJSC, Belarus) at a dose of 200 mg/kg.

SV-1010 and pregabalin were administered i.g. in a 1% potato starch gel, using a gavage, daily for 2 weeks starting on day 7 after NP modeling. Control animals were administered i.g. with a 1% potato starch gel in the volumes equivalent to those of the tested compound.

Before NP modeling, as well as on days 7, 14, 21, and 28 after surgery, the pain response threshold (PRT) to mechanical stimulation was recorded, using a Randall-Selitto algesimeter (IITC Life Science, USA). PRT was measured three times at 5-7-minute intervals, and then its average value was calculated. The analgesic effect was assessed as a percentage change in PRT from the baseline value.

Effect of SV-1010 on vanilloid receptors

The effect of SV-1010 on TRPV1 was determined using a continuous Chinese hamster ovary (CHO) cell line expressing the rat TRPV1 receptor. A continuous cell line from a cryobank was thawed and then cultured for at least 2 weeks in a nutrient medium (DMEM: F12 /10% fetal bovine serum /1% PS (penicillin/streptomycin)) supplemented with a mixture of B/Z antibiotics (blasticidin S (5 μg/mL) and zeocin (250 μg/mL)) in sterile flasks at 37°C in the presence of 5% CO_2, with cells being regularly passaged every 3-4 days. Twenty-four hours before the experiments, the cells were seeded on glasses (4 × 4 mm) pre-coated with poly-D,L-lysine to enable cells to adhere to glass surface, and TRPV1 expression was induced by adding tetracycline at a dose of 1 μg/mL. Glasses with cells were placed in an experimental chamber filled with a standard washing solution (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, 10 HEPES titrated to pH 7.4 with NaOH. A glass micropipette was moved to the cell using a micromanipulator until ultra-tight contact with the plasma membrane was formed, and then the membrane was broken by applying "negative" pressure (suction, about 70-80 mmHg), after which ion currents from the whole cell were recorded. The potential of the cell was recorded using an EPC-800 patch-clamp amplifier (HEKA, Lambrecht, Germany). The microelectrodes were filled with a solution (in mM): 135 CsCl, 5 NaCl, 1 $MgCl_2$, 10 EGTA, 10 HEPES, 10 D-glucose, pH 7.4. Voltage ramp recording from the whole cell was performed at a frequency of 1.25 Hz, with a resting potential of -70 mV and a potential range of -100 mV to 100 mV within 200 ms. A single recording of the full voltage spectrum was recorded at a frequency of 0.5 kHz and processed with a low-pass filter at a frequency of 3 kHz.

To activate currents through the TRPV1 receptor, a solution of its agonist, capsaicin in a concentration of 50 nM without SV-1010 was used, using an SF-77B Perfusion Fast Step System (Warner Instruments, Holliston, MA, USA). The time to apply the activating solution was set to 12 sec, after which the bath solution was automatically replaced with a standard washing solution. Ion currents were recorded for a stepwise voltage-ramp membrane potential on a cellular membrane with 800-msec intervals in whole-cell configuration. The greatest response of TRPV1 ion channels was observed at a potential of +100 mV, for which the change in response amplitude was analyzed depending on the composition of the activating solution.

Consecutive applications of capsaicin can lead to the channel desensitization effect manifested in the reduction of amplitude therefore each response was normalized to the previous one on the same cell. The current amplitude after the application of SV-1010 at concentrations of 0.1 and 10 nM mixed with the agonist was compared with the current amplitude after the application of the agonist alone, each time normalizing the value to the amplitude of the previous

measurement. Recordings from at least three independent cells were analyzed. The data obtained were presented as the mean value with standard deviation. The significance of differences was determined using Student's t-test.

Docking of SV-1010, a partial κ-receptor agonist butorphanol, and a highly selective κ-receptor agonist U-50488 to the specific binding site of the κ-opioid receptor

Ten conformations of each compound were constructed using MarvinSketch 17.1.23 (https://chemaxon.com/marvin), which were then optimized using MOPAC2012 (http://openmopac.net), and the best one with the lowest energy was selected. Docking to the X-ray model of a human κ-opioid receptor dimer [PDB code 4DJH] (Wu et al. 2010) was performed using AutoDock Vina 1.1.1 software (Trott and Olson 2010). Each compound was added 5 times to each of the two dimer sites, and the lowest docking energy ΔE was calculated. The ensemble docking procedure is described in-depth in (Vasiliev et al. 2016).

The binding constants K were calculated through the docking energy ΔE using formula (3):

$$K = e^{-\Delta E/RT}, \qquad (3)$$

where R is the gas constant, equal to 8.314 J/(moL K); T is the temperature; the default value in docking is 300 K.

An analysis of the binding mechanism of SV-1010 to the κ-opioid receptor site was conducted using LigandScout 4.2.1 softaware (http://www.inteligand.com/).

Statistical analysis

The obtained results (calculation of arithmetic means – M, standard errors of the mean – m, LD_{50}, and $ED_{0.5}$) were statistically processed using Statistica Version 6.0 software (Stat Soft Inc., USA), as well as special software applications developed at the Department of Pharmacology, Kuban State Medical University, Russia. Hypotheses about the mean values were tested using Student's t-test. Differences were considered significant at $p < 0.05$.

Results

Determination of the median lethal dose (LD_{50})

The studies revealed that the LD_{50} of SV-1010 when administered i.g. to male mice was 715.0 (657.0 ÷ 773.0) mg/kg, and when administered to male rats – 1109.0 (1014.3 ÷ 1203.7) mg/kg. LD_{50} values of diclofenac, indomethacin, and ketorolac administered i.g. to male mice were 366.1 (311.2 ÷ 420.9) mg/kg (Buzlama et al. 2017), 35.0 (25.0 ÷ 42.0) mg/kg (Buzlama et al. 2017), and 200.0 mg/kg (Mandema and Stanski 1996) mg/kg, and to male rats – 370.0 (247.0 ÷ 533.0) mg/kg (Buzlama et al. 2017), 47.0 mg/kg (Buzlama et al. 2017), and 189.0 mg/kg (Sosnov et al. 2018), respectively.

Study of the analgesic activity of SV-1010

Analgesic effect of SV-1010 in the hot plate test

A comparative study of the analgesic effects of SV-1010, indomethacin, and diclofenac in the hot plate test in mice revealed that in control, the latency of pain response to thermal stimulation of animals' paws was 11.2 seconds, whereas with SV-1010 administered at doses of 0.01, 0.1, and 1 mg/kg, it was 12.8, 18.5, and 18.9 sec, which means that the pain response reduced by 14.6, 65.1, and 68.8%, respectively. Under the influence of indomethacin at doses of 10, 20 and 30 mg/kg, the latency of pain response was 13.4, 16.9 and 18.9 sec versus 11.6 sec in control, which means that this indicator increased by 15.1, 45.1 and 62.4%, respectively, and when administering diclofenac at doses of 30, 60 and 90 mg/kg, the latency was 14.2, 17.1 and 18.1 sec versus 11.1 sec in control, which means that the latency increased by 27.8, 53.9 and 62.6%, respectively (Table 1).

When calculating $ED_{0.5}$, for SV-1010, it was found to be 0.41 mg/kg, and for indomethacin and diclofenac – 23.9 and 63.3 mg/kg, respectively, i.e., SV-1010 is 58.3 times more active than indomethacin, and 154.4 times more active than diclofenac (Table 1).

When comparing TI of SV-1010, indomethacin, and diclofenac, TI for SV-1010 was 1743.9 RU (relative units), and for indomethacin and diclofenac – 1.46 and 5.78 RU, respectively, i.e., by TI, SV-1010 exceeds indomethacin by 1194.5 times and diclofenac – by 301.7 times (Table 1).

Analgesic effect of SV-1010 in the hot plate test against the background of naloxone hydrochloride dihydrate

To clarify the involvement of the opioidergic system in the analgesic effect of SV-1010, its analgesic effect was studied against the background of a non-selective opioid receptor antagonist, naloxone, in a hot plate test.

Table 1. Analgesic effect of SV-1010 (0.01, 0.1 and 1 mg/kg), indomethacin (10, 20 and 30 mg/kg) and diclofenac (30, 60 and 90 mg/kg) when administered i.g. to mice in the hot plate test

Animal group	Dose, mg/kg	Latency of pain response, sec	Pain response inhibition, %	$ED_{0.5}$, mg/kg	TI
Control-1 PSG [1]		11.20 ± 0.43 (10.22 ÷ 12.18)			
SV-1010 [2]	0.01	12.83 ± 0.42 (11.88 ÷ 13.78)	14.6	0.41	1743.9
SV-1010 [3]	0.1	18.49 ± 0.44 (17.49 ÷ 19.49)	65.1		
SV-1010 [4]	1.0	18.91 ± 0.42 (17.96 ÷ 19.86)	68.8		
Control-2 PSG [5]		11.62 ± 0.42 (10.67 ÷ 12.57)			
Indomethacin [6]	10	13.38 ± 0.40 (12.45 ÷ 14.31)	15.1	23.9	1.46
Indomethacin [7]	20	16.86 ± 0.43 (15.88 ÷ 17.84)	45.1		
Indomethacin [8]	30	18.87 ± 0.42 (17.92 ÷ 19.82)	62.4		
Control-3 PSG [9]		11.11 ± 0.43 (10.13 ÷ 12.09)			
Diclofenac [10]	30	14.20 ± 0.41 (13.27 ÷ 15.13)	27.8	63.3	5.78
Diclofenac [11]	60	17.10 ± 0.41 (16.17 ÷ 18.03)	53.9		
Diclofenac [12]	90	18.06 ± 0.43 (17.08 ÷ 19.04)	62.6		

***Note*:** 1. PSG – potato starch gel, $ED_{0.5}$ – median effective dose causing 50% of pain response inhibition, TI – therapeutic index. 2. In round brackets – confidence limits at $p < 0.05$, in square brackets – numbers of animal groups.

Naloxone, when administered subcutaneously, was found to reduce analgesia caused by a single i.g. administration of SV-1010 (119.5% versus 83.2%), i.e., the reduction in pain response in the group of combined administration of SV-1010 and naloxone was 36.3% (Table 2).

Table 2. Analgesic effect of SV-1010 (0.1 mg/kg) against the background of naloxone (1 mg/kg) in mice in the hot plate test

Animal group	Latency of pain response, sec	Pain response inhibition, %
Control	4.05 ± 0.24 (3.46 ÷ 4.64)	
SV-1010 0.1 mg/kg	8.89 ± 0.51 (7.63 ÷ 10.15)	119.5
SV-1010 0.1 mg/kg + Naloxone 1 mg/kg	7.42 ± 0.33 (6.61 ÷ 8.23)	83.2

Analgesic effect of SV-1010 in the tail-flick test

Under the test conditions, SV-1010 administered i.g. at doses of 0.01, 0.1, and 1 mg/kg produced a statistically significant analgesic effect: the latency of pain response was 1.98, 2.27, and 2.61 sec versus 1.55 sec in control, and the pain response inhibition was 27.7, 46.5, and 68.4%, respectively. For ketorolac at doses of 2.5, 5, and 10 mg/kg administered i.g., the latency of pain response was 1.83, 2.25, and 2.52 sec versus 1.55 sec in control. And the pain response inhibition was 18.1%, 45.2%, and 62.6%, respectively (Table 3).

After calculating $ED_{0.5}$, for SV-1010, it was found to be 0.46 mg/kg, while for ketorolac it was 8.7 mg/kg, which means that SV-1010 is 18.9 times more active than ketorolac (Table 3).

When comparing TI of SV-1010 and ketorolac, TI for SV-1010 was found to be 2410.9 RU, while for ketorolac – 21.7 RU, which means that by TI, SV-1010 exceeds ketorolac by 111.1 times (Table 3).

Table 3. Comparative analgesic effects of SV-1010 and ketorolac two hours after i.g. administration in rats in the tail-flick test

Animal group	Dose, mg/kg	Latency of pain response, sec	Pain response inhibition, %	$ED_{0.5}$, mg/kg	TI
Control PSG [1]		1.55 ± 0.04 (1.49 ÷ 1.61)			
SV-1010 [2]	0.01	1.98 ± 0.03 (1.90 ÷ 2.06)	27.7	0.46	2410.9
SV-1010 [3]	0.1	2.27 ± 0.03 (2.20 ÷ 2.34)	46.5		
SV-1010 [4]	1.0	2.61 ± 0.03 (2.53 ÷ 2.69)	68.4		
Ketorolac [5]	2.5	1.83 ± 0.03 (1.76 ÷ 1.90)	18.1	8.70	21.7
Ketorolac [6]	5.0	2.25 ± 0.03 (2.19 ÷ 2.31)	45.2		
Ketorolac [7]	10.0	2.52 ± 0.03 (2.44 ÷ 2.60)	62.6		

Note: 1. PSG – potato starch gel, $ED_{0.5}$ – median effective dose causing 50% of pain response inhibition, TI – therapeutic index. 2. In round brackets – confidence limits at $p < 0.05$, in square brackets – animal group numbers.

Analgesic effect of SV-1010 in the paw pressure test (Randall-Selitto test)

In the experiments to study the analgesic effect of SV-1010 at doses of 0.01, 0.1 and 1 mg/kg and ketorolac at doses of 2, 4 and 6 mg/kg when administered i.g. under conditions of acute exudative inflammation of the rat's right (ipsilateral) paw caused by subplantar administration of a 2% formalin solution in the paw pressure test, it was found that SV-1010 at the selected doses caused a statistically significant increase in the pressure force on the contralateral paw (without formalin administration) by 334.1, 418.9 and 484.2 g, respectively, versus 287.5 g in control (1 hour after i.g. administration of potato starch gel), whereas ketorolac at the selected doses caused an increase by 346.7, 436.4 and 502.0 g versus 287.5 g in control, i.e. the increase in the pressure force on the contralateral paw for SV-1010 when compared to control was 16.2, 45.7, and 68.4%, and for ketorolac – 20.6, 51.8, and 74.6%, respectively (Table 4).

One to five minutes after subplantar administration of formalin to the ipsilateral paw of rats, under the influence of SV-1010 at doses of 0.01, 0.1, and 1 mg/kg, the pressure force was 185.0, 226.2, and 260.0 g versus 146.4 g in control, and under the influence of ketorolac at doses of 2, 4, and 6 mg/kg, it was 190.3, 244.0, and 267.2 g versus 146.4 g in control, which means that the increase in the pressure force on the ipsilateral paw for SV-1010 compared to control was 26.4, 54.5, and 77.6%, whereas for ketorolac it was 30.0, 66.7, and 82.5%, respectively (Table 4).

Forty-five minutes after subplantar administration of formalin to the ipsilateral paw of rats, under the influence of SV-1010 at doses of 0.01, 0.1, and 1 mg/kg, the pressure force was 240.1, 286.2, and 309.3 g versus 195.5 g in control, and under the influence of ketorolac at doses of 2, 4, and 6 mg/kg it was 229.1, 289.3, and 301.7 g versus 195.5 g in control, which means that the increase in pressure force on the ipsilateral paw for SV-1010 compared to control was 22.8%, 46.4%, and 58.2%, respectively, whereas for ketorolac it was 17.2%, 48.0%, and 54.3%, respectively (Table 4).

Thus, when comparing the data regarding the potency of SV-1010 and ketorolac on contralateral and ipsilateral paws, it is of note that both compounds are almost comparable in this indicator. However, the analgesic effect of SV-1010 appears at significantly lower doses than under the influence of ketorolac.

Calculation of $ED_{0.5}$ showed that for SV-1010, $ED_{0.5}$ for the contralateral paw was 0.53 mg/kg, whereas for ketorolac it was 4.1 mg/kg, i.e., the former is 7.7 times more active than the latter (Table 5).

When comparing TI of SV-1010 and ketorolac, TI for the former was found to be 2092.5 RU, whereas for the latter – 46.1 RU, which means that by TI, SV-1010 exceeds ketorolac by 45.4 times (Table 5).

$ED_{0.5}$ for the ipsilateral paw, 1-5 minutes after subplantar administration of formalin, was 0.3 mg/kg for SV-1010, whereas for ketorolac it was 3.26 mg/kg, i.e., the former is 10.9 times more active than the latter (Table 5).

When comparing TI of SV-1010 and ketorolac, this indicator for the former was found to be 3696.7 RU, whereas for ketorolac – 58.0 RU, i.e., by TI, SV-1010 exceeds ketorolac by 63.7 times (Table 5).

Table 4. Effect of SV-1010 (0.01, 0.1 and 1 mg/kg) and ketorolac (2, 4 and 6 mg/kg) when administered i.g on pain sensitivity in rats under conditions of acute exudative inflammation of the paw induced by subplantar administration of formalin (2% solution) into the right paw in the paw pressure test

Compounds and doses	Number of animals	Pressure force on contralateral paw[1], g	Pressure force on ipsilateral paw, g	
			1-5 min after formalin administration	40-50 min after formalin administration
Control	10	287.5 ± 17.3 (248.3 ÷326.7)	146.4 ± 16.2 (109.7 ÷ 183.1)	195.5 ± 15.6 (160.2 ÷ 230.8)
SV-1010 0.01 mg/kg	10	334.1 ± 18.4 (292.5 ÷ 375.7) [16.2]	185.0 ± 15.4 (150.2 ÷ 219.8) [26.4]	240.1 ± 15.9 (204.1 ÷ 276.1) [22.8]
SV-1010 0.1 mg/kg	10	418.9 ± 18.4 (377.3 ÷ 460.5) [45.7]	226.2 ± 17.3 (187.0 ÷ 265.4) [54.5]	286.2 ± 18.4 (244.6 ÷ 327.8) [46.4]
SV-1010 1 mg/kg	8	484.2 ± 19.0 (439.4 ÷ 529.0) [68.4]	260.0± 22.5 (206.7 ÷ 313.3) [77.6]	309.3 ± 19.1 (264.1 ÷ 354.5) [58.2]
Ketorolac 2 mg/kg	10	346.7 ± 16.8 (308.8 ÷ 384.6) [20.6]	190.3 ± 14.9 (156.5 ÷ 224.1) [30.0]	229.1 ± 14.3 (196.8 ÷ 261.4) [17.2]
Ketorolac 4 mg/kg	9	436.4 ± 19.0 (392.5 ÷ 480.3) [51.8]	244.0 ± 20.2 (197.3 ÷ 290.7) [66.7]	289.3 ± 21.4 (239.9 ÷ 338.7) [48.0]
Ketorolac 6 mg/kg	10	502.0 ± 18.4 (460.4 ÷ 543.6) [74.6]	267.2 ± 19.5 (223.1 ÷ 311.3) [82.5]	301.7 ± 16.8 (263.8 ÷ 339.6) [54.3]

***Note*:** [1] One hour after administration of potato starch gel (control), SV-1010, and ketorolac. In round brackets – confidence limits at p = 0.05, in square brackets – analgesic effect as a percentage (%) relative to control.

In the ipsilateral paw, 40-50 minutes after subplantar formalin administration, $ED_{0.5}$ for SV-1010 was 0.65 mg/kg, whereas for ketorolac it was 5.01 mg/kg, i.e., the former was 7.7 times more active than the latter (Table 5).

Table 5. Comparative analgesic activity by ED0.5 (mg/kg) and TI of SV-1010 and ketorolac in the paw pressure test

Compaunds	$ED_{0.5}$, mg/kg		
	Contralateral paw	Ipsilateral paw	
		1-5 min after formalin administration	40-50 min after formalin administration
SV-1010	0.53 (2092.5)	0.30 (3696.7)	0.65 (1706.2)
Ketorolac	4.1 (46.1)	3.26 (58.0)	5.01 (37.7)

***Note*:** In round brackets – confidence limits at p = 0.05, in square brackets – TI.

A comparison of TI of SV-1010 and ketorolac revealed that TI for SV-1010 was 1706.2 RU, whereas for ketorolac it was 37.7 RU, which means that by TI, SV-1010 exceeds ketorolac by 45.3 times (Table 5).

Analgesic effect of SV-1010 in the abdominal constriction test

When studying the analgesic effect of SV-1010, it was found that in control, acetic acid at a concentration of 0.6% administered i.p induced a pain response in the form of writhes, the number of which over a 15-minute interval was 46.3, and the latency of their appearance was 220.8 sec. Administration of SV-1010 i.g. at doses of 0.01, 0.1 and 1 mg/kg caused a statistically significant inhibition of visceral pain response. Moreover, the average number of writhes was 33.0, 26.3 and 22.3 versus 46.3 in control: the pain response decreased by 28.7, 43.2 and 51.8%, respectively; the latency of the writhe onset was 263.1, 287.4 and 309.0 sec, i.e., it increased by 19.2, 30.2, and 39.9%, respectively (Table 6).

Diclofenac, a comparison drug, at doses of 2.5, 5, and 7.5 mg/kg induced a statistically significant reduction in the pain response: the average number of writhes was 28.9, 22.9, and 20.4, versus 49.3 in control, which means that the pain response reduced by 41.4, 53.5, and 58.6%, respectively; The latency to the writhe onset was 319.5, 389.5, and 393.2 sec, which means that it increased by 42.4, 73.7, and 75.3%, respectively (Table 6).

Calculation of $ED_{0.5}$ showed that for SV-1010 it was 0.87 mg/kg, and for diclofenac – 4.66 mg/kg, i.e., the former is 5.36 times more active than the latter (Table 6).

Table 6. Effect of SV-1010 and diclofenac on pain sensitivity in mice in abdominal constriction test (M ± m, n=10)

Animal group	Dose, mg/kg	Number of writhes within 15 min	Latency of writhe onset, sec	Pain response inhibition, %	$ED_{0.5}$, mg/kg	TI
SV-1010 i.g. + acetic acid i.p.						
Control – PSG i.g. + acetic acid i.p [1]		46.3 ± 1.7 (44.6 ÷ 48.0)	220.8 ± 8.2 (202.2 ÷ 239.4)			
SV-1010 [2]	0.01	33.0 ± 1.6 (29.3 ÷ 36.7)	263.1 ± 11.1 (237.9 ÷ 288.3)	28.7	0.87	1274.7
SV-1010 [3]	0.1	26.3 ± 1.8 (22.3 ÷ 30.5)	287.41 ± 9.5 (265.9 ÷ 308.9)	43.2		
SV-1010 [4]	1.0	22,3 ± 1,3 (19,3 ÷ 25,3)	309,0 ± 12,0 (281,8 ÷ 336,2)	51.8		
Diclofenac i.g. + acetic acid i.p						
Control – PSG i.g. + acetic acid i.p [5]		49.3 ± 1.9 (44.9 ÷ 53.7)	224.3 ± 9.5 (202.5 ÷ 246.1)			
Diclofenac [6]	2.5	28.9 ± 1.4 (25.7 ÷ 32.1)	319.5 ± 14.3 (287.2÷ 351.8)	41.4	4.66	78.6
Diclofenac [7]	5.0	22.9 ± 1.6 (19.2 ÷ 26.6)	389.5 ± 11.5 (365.8 ÷ 413.2)	53.5		
Diclofenac [8]	7.5	20.4 ± 1.4 (17.2 ÷ 23.6)	393.2 ± 11.5 (367.3 ÷ 419.1)	58.6		

***Note*:** 1. PSG – potato starch gel, i.p. – intraperitoneally, i.g. – intragastrically, $ED_{0.5}$ – median effective dose causing 50% of pain response inhibition, TI – therapeutic index. 2. In round brackets – confidence limits at $p < 0.05$, in square brackets – animal group numbers.

When comparing TI of SV-1010 and diclofenac, this indicator for the former was found to be 1274.7 RU, and for diclofenac – 78.6 RU, which means that by TI, SV-1010 exceeds diclofenac by 16.2 times (Table 6).

Analgesic effect of SV-1010 in the formalin test

SV-1010 at a dose of 0.01 mg/kg during the first phase of inflammation reduced the number of paw shakes by 17.6%, while the number of paw licks increased by 17.5%. In the second phase of inflammation the number of paw shakes decreased by 33.8%, while the number of paw licks – by 3.4% (Table 7). It is of note that SV-1010 at the above dose statistically significantly reduced the number of paw shakes, whereas it did not significantly change the number of paw licks.

With an increase in the dose of SV-1010 to 0.1 mg/kg during the first phase of inflammation, the number of paw shakes decreased by 38.1%, and the number of paw licks decreased commensurately (by 42.3%). During the second phase of inflammation, the number of paw shakes and paw licks, like in the first phase, decreased by 44.3% and 49.5%, respectively (Table 7).

As for the reference drug diclofenac (7 mg/kg), under its influence, in both the first and second phases of inflammation, the number of paw shakes decreased by 19.2% and 51.3%, whereas the number of paw licks slightly increased by 18.2% in the first phase and slightly decreased by 2.4% in the second phase, respectively, which shows that diclofenac's mechanism of action is similar to that of SV-1010 at a dose of 0.01 mg/kg (Table 7).

Analgesic effect of SV-1010 in neuropathic pain

A study of the analgesic effect of SV-1010 in neuropathic pain induced by sciatic nerve axotomy using the Randall-Selitto test revealed that in control, mechanical hyperalgesia in the ipsilateral limb was observed in 12 of 30 animals (40%) on the 4th day of control, and in 100% of rats after 7 days, which manifested in a decrease in PRT from 141.7 g to 108.5 g (a 23.4% decrease) when compared to the pre-surgery levels. And mechanical hyperalgesia was observed for up to 28 days. PRT in the contralateral limb did not change significantly throughout the entire study period (Table 8).

SV-1010 administered at doses of 0.1 and 1 mg/kg resulted in the inhibition of mechanical hyperalgesia in 100% of rats by day 14, which manifested in an increase in PRT in the ipsilateral limb to 140.3 g and 141.2 g, slightly exceeding the pre-surgery levels of 139.5 g and 139.0 g, respectively, and was observed throughout the entire study period (Table 8).

Pregabalin, a comparison drug, at a dose of 13 mg/kg, had a similar analgesic effect to SV-1010: it also inhibited mechanical hyperalgesia by day 14 in 100% of cases, PRT in the ipsilateral limb increased to 146.6 g, almost reaching the pre-surgery level of 140.7 g and remaining unchanged throughout the 28-day observation period (Table 8).

Table 7. Effect of SV-1010 and diclofenac on pain sensitivity in rats in the formalin test (M ± m, n = 10)

Animal group and dose of compound	Total duration of pain response, sec	1st phase of inflammation			2nd phase of inflammation воспаления		
		Number of paw licks	Total time of paw licks, sec	Number of paw shakes	Number of paw licks	Total time of paw licks, sec	Number of paw shakes
Control – potato starch gel i.g. + formalin s.p. [1]	495.8	13.7 ± 1.0 (11.5 ÷ 15.9)	117.2 ± 7.4 (100.3 ÷ 134.1)	52.0 ± 2.6 (46.7 ÷ 57.9)	20.8 ± 1.5 (17.4 ÷ 24.2)	378.6 ± 10.3 (355.3 ÷ 401.9)	160.2 ± 4.0 (151.1 ÷ 169.3)
SV-1010 (0.01 mg/kg) i.g.+ formalin s.p. [2]	385.1	16.1 ± 1.3 (13.4 ÷18.8)	96.6 ± 3.9 (87.8 ÷ 105.5)	41.1 ± 2.6 (35.2 ÷ 47.0)	20.1 ± 1.4 (16.9 ÷ 23.3)	288.5 ± 8.5 (269.2 ÷ 307.8)	106.0 ± 3.9 (97.2 ÷ 114.8)
SV-1010 (0.1 mg/kg) i.g. + formalin s.p. [3]	224.2	7.9 ± 1.5 (4.5 ÷11.3)	58.4 ± 2.1 (53.7 ÷ 63.1)	32.2 ± 2.2 (27.3 ÷ 37.1)	10.5 ± 1.3 (7.6 ÷ 13.4)	165.8 ± 11.1 (140.6 ÷ 191.0)	89.3 ± 3.5 (91.5 ÷ 97.1)
Diclofenac (7 mg/kg) i.g. + formalin s.p. [4]	298.6	16.2 ± 1.3 (13.5 ÷ 18.9)	95,8 ± 1,6 (92,1 ÷ 99,5)	42.0 ± 1.8 (37.8 ÷ 46.2)	20.3 ± 1.4 (17.1 ÷ 23.5)	202.8 ± 6.3 (188.6 ÷ 217.0)	78.0 ± 2.2 (73.1 ÷ 82.9)

Note: 1. s.p. – subplantarly, i.g. – intragastrically. 2. In round brackets – confidence limits at p = 0.05, in square brackets – animal group numbers.

Of note is that SV-1010, either at doses of 0.1 mg/kg or 1 mg/kg, caused no visible side effects, whereas pregabalin induced hypodynamia, drowsiness, and gait instability (ataxia) in animals, which are typical in the treatment of patients with epilepsy and post-traumatic neuropathic pain (Table 8).

Table 8. Comparative analgesic effect of SV-1010 (0.1 and 1 mg/kg) and pregabalin (13 mg/kg) in the model of neuropathic pain induced by sciatic nerve axotomy in the Randall-Selitto test in rats

Drug and dose	Number of animals	Pressure on paw, g				
		Baseline	Day 7	Day 14	Day 21	Day 28
Contralateral paw						
Control	30	139.8 ± 1.9 (135.9 ÷143.7)	140.1 ± 2.0 (136.0 ÷ 144.2)	134.3 ± 1.7 (130.9 ÷ 137.7)	136.6 ± 1.8 (133.0 ÷ 140.2)	134.5 ± 1.5 (131.4 ÷ 137.6)
Ipsilateral paw						
Control	30	141.7 ± 2.2 (137.2 ÷ 146.2)	108.5 ± 1.7 (105.0 ÷ 112.0)	106.6 ± 1.5 (103.5 ÷ 109.7)	107.4 ± 1.7 (103.9 ÷ 110.9)	108.0 ± 1.8 (104.4 ÷ 111.6)
SV-1010 0.1 mg/kg	15	139.5 ± 2.8 (133.6 ÷ 145.4) [100]	105.5 ± 2.9 (99.2 ÷ 111.8) [75.6/-24.4]	140.3 ± 2.8 (134.2 ÷ 146.4) [100.6/0.6]	143.7 ± 3.1 (139.4 ÷ 148.0) [103.0/3.0]	144.0 ± 2.3 (139.0 ÷ 149.0) [103.2/3.2]
SV-1010 1 mg/kg	15	139.0 ± 2.9 (132.7 ÷ 145.3) [100]	111.3± 2.7 (105.5 ÷ 117.1) [80.1/-19.9]	141.2 ± 2.3 (136.2 ÷ 146.2) [101.6/1.6]	147.7 ± 2.6 (142.1 ÷ 153.3) [106.3/6.3]	144.9 ± 2.8 (139.0 ÷ 150.8) [104.2/4.2]
Pregabalin 13 mg/kg	18	140.7 ± 1.9 (136.6 ÷ 144.8) [100]	104.3 ± 2.2 (99.7 ÷ 108.9) [74.1/-25.9]	146.6 ± 2.3 (141.8 ÷ 151.4) [104.2/4.2]	149.1 ± 2.5 (143.8 ÷ 154.4) [106.0/6.0]	145.9 ± 2.3 (141.0 ÷ 150.8) [103.7/3.7]

Note: In round brackets – confidence limits at p = 0.05, in square brackets – PRT in % of the baseline.

So, SV-1010 (0.1 mg/kg) has a pronounced analgesic effect in a model of neuropathic pain induced by partial sciatic nerve axotomy in rats at a dose 130 times lower than the reference drug pregabalin (13 mg/kg), at the same time producing a comparable analgesic effect with the latter, without any visible side effects.

Effect of SV-1010 on vanilloid receptors – TRPV1

SV-1010 at a concentration of 10 nM was found to statistically significantly reduce the evoked current amplitude (by 50% of the baseline) through the TRPV1 channel. A further decrease in the concentration did not lead to any significant blocking effect on the TRPV1 channel. Therefore, SV-1010 causes a very pronounced inhibition, but not blockade, of the TRPV1 ion channel. The resulting effect was reversible; however, after the first washout, the current from further activation was not fully restored. After repeated application of capsaicin, the TRPV1 channel response reached the baseline level (Figs 2 and 3).

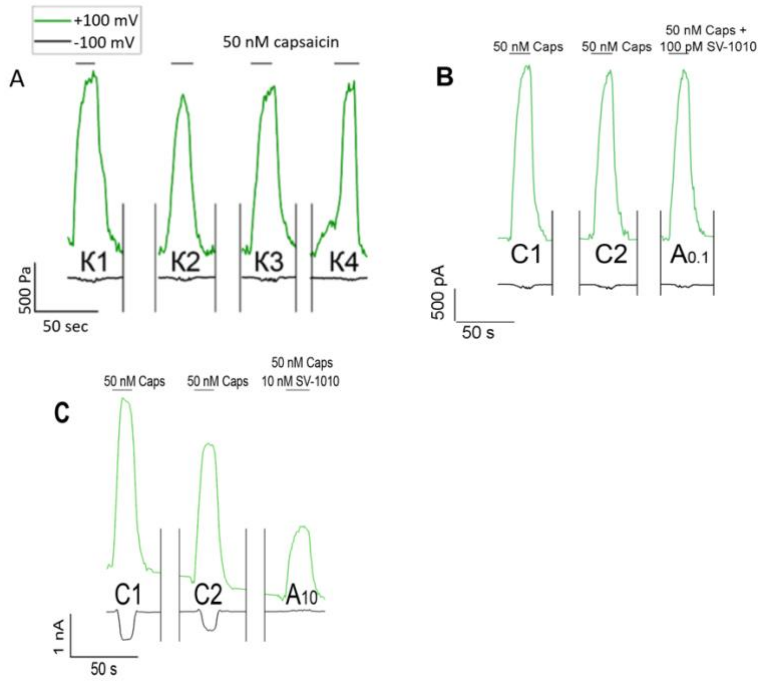

Figure 2. Primary data from consecutive measurements of current in a single cell at potentials of +100 and -100 mV under conditions of consecutive single applications of the agonist alone (**A**), two-time applications of the agonist and a combination of the agonist and SV-1010 at a concentration of 100 pM (**B**), or at a concentration of 10 nM (**C**). Capsaicin at a concentration of 50 nM was used as an agonist; each application lasted for 12 sec, after which the cell was washed with a buffer solution for at least 2 min until the current returned to the baseline level.

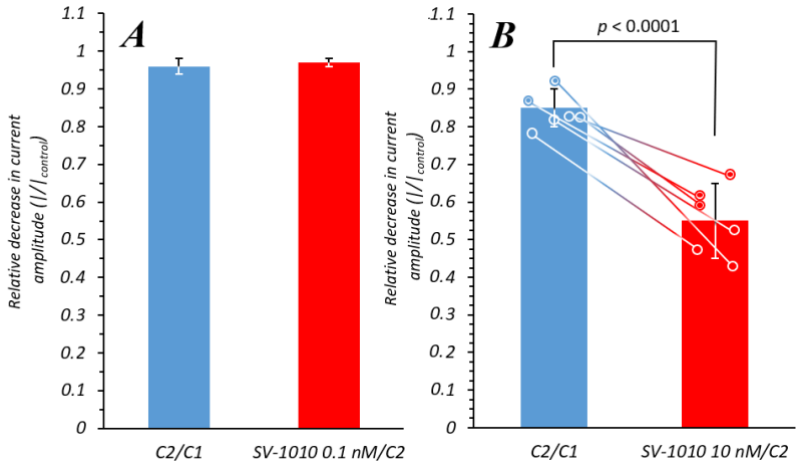

Figure 3. Effect of SV-1010 at concentrations of 0.1 nM (**A**) and 10 nM (**B**) when applied together with the selective agonist capsaicin on the amplitude of the evoked current through the rat TRPV1 ion channel. ***Note:*** SV-1010 at a concentration of 0.1 nM + 50 nM capsaicin have no effect on the amplitude of evoked currents (**A**), but at a concentration of 10 nM + 50 nM capsaicin, they inhibit the amplitude of these currents.

Effect of SV-1010 on κ-opioid receptors

According to the calculated values of the binding constant K (Table 9), the affinity of SV-1010 for the κ-opioid receptor is 78.6 times higher than that of the partial κ-agonist butorphanol and 6.3 times higher than that of the selective κ-agonist U-50488.

Table 9. Docking results of SV-1010, butorphanol, and U-50488 at the specific binding site of the κ-opioid receptor

Compound	Docking energy ΔE, kcaL/moL	Binding constant K, nM
SV-1010	-10.30	31.0
Butorphanol	-7.70	2437.3
U-50488	-9.20	196.5

According to the analysis of the SV-1010 binding mechanism to the κ-opioid receptor site, the key binding amino acids are ILE730, VAL667, MET579, ILE726, TRP723, ILE460, and TYR464. Binding is mediated by three hydrophobic interactions between the benzene fragments of the SV-1010 structure and the above-mentioned amino acids of the κ-opioid receptor site (Fig. 4).

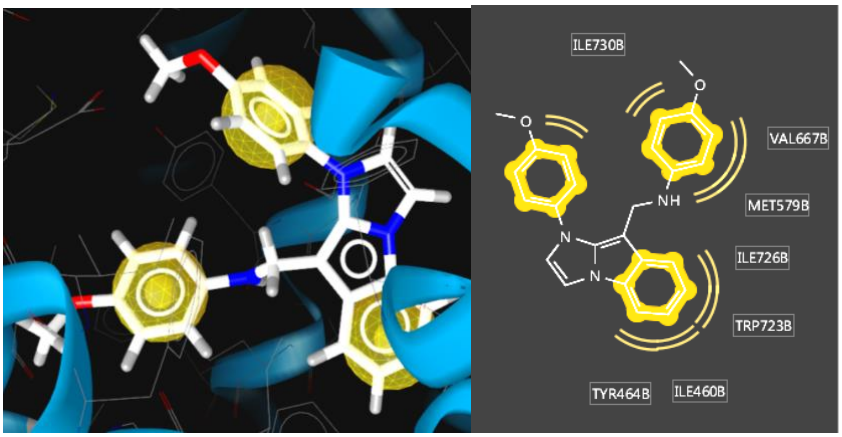

Figure 4. Analysis of the SV-1010 binding mechanism to the κ-opioid receptor site. ***Note:*** **A** – Location of the 3D model of SV-1010 at the κ-opioid receptor binding site; **B** – Key amino acids mediating SV-1010 binding to the κ-opioid receptor site.

Discussion

The studies showed that SV-1010 has relatively low acute toxicity. In terms of LD_{50}, this substance was 1.95, 20.4, and 3.6 times less toxic (in mice) and 3.0, 23.6, and 5.9 times less toxic (in rats) than the reference drugs – diclofenac, indomethacin, and ketorolac, respectively.
SV-1010 has a pronounced analgesic effect in all the pain models used (hot plate, tail-flick, paw pressure, abdomen constriction, formalin test, neuropathic pain), significantly exceeding, both in terms of potency and TI, the reference drugs used – diclofenac, indomethacin, ketorolac, and pregabalin, which points to a complex mechanism of action of the studied compound. First, it is of note that in the chemical structure of SV-1010 there are similar molecular fragments characteristic of indomethacin (with both containing an indole framework in their structures) and ketorolac (with both having two fused five-membered rings with a bridging nitrogen atom) (Fig. 5).

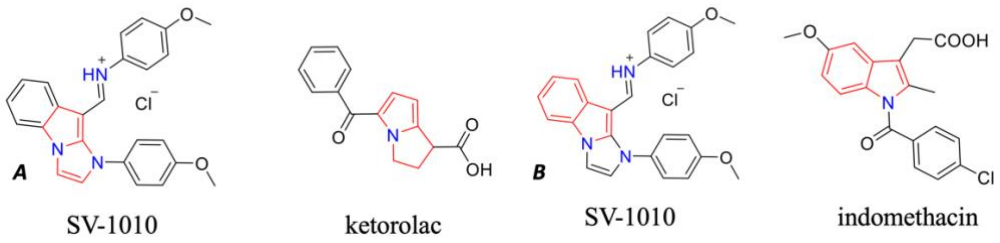

Figure 5. Comparison of the structure of SV-1010 with those of ketorolac (**A**) and indomethacin (**B**).

Studies of the analgesic effect of SV-1010 in the hot plate test revealed its high analgesic activity. The most pronounced analgesic effect of SV-1010 was observed at doses of 0.1 and 1 mg/kg administered i.g., which points to a possible effect of this compound on the supraspinal level of pain sensitivity, suggesting its action on the TRPV1 receptor, which is activated by thermal stimuli.

When combining SV-1010 with naloxone, there was a decrease in the analgesic effect of the former, indicating the involvement of the central opioidergic system in the analgesic effect of this compound.

The tail-flick test revealed that SV-1010 had the most pronounced analgesic effect at a dose of 1 mg/kg i.g., which points to its influence on the spinal level of pain sensitivity.

When studying the analgesic activity of SV-1010 in the paw pressure test (normal and inflamed) using the Randall-Selitto method, it was found that SV-1010 at a dose of 1 mg/kg i.g. increased the pain threshold in the contralateral paw (without formalin administration) by 68.4% compared to control, indicating pain modulation at the supraspinal and peripheral levels of pain sensitivity. Under conditions of formalin-induced inflammation (tactile allodynia test), it exerted an analgesic effect on the ipsilateral paw after 1-5 minutes and 40-50 minutes.

Abdomen constriction test, which is associated with chemical irritation of the peritoneum, an increased production of prostaglandins and kinins (mainly bradykinin), as well as the involvement of opioid receptors, mainly κ-receptors (Bondarenko et al. 2011; Aliforenko et al. 2023; Ghorbanzadeh et al. 2016), showed that SV-1010 most significantly increased the latency of the onset of writhes and reduced their number at a dose of 1 mg/kg when administered i.g. to mice, affecting the peripheral level of pain sensitivity.

Docking of SV-1010 and the comparative compounds butorphanol and U 50488 at the specific binding site of the κ-opioid receptor revealed that SV 1010 exhibits high affinity for the κ-opioid receptor, significantly exceeding (in terms of the binding constant) the selective agonist U-50488 – by 6.3 times, and the non-selective agonist butorphanol – by 78.6 times. This indicates that a high analgesic activity of SV-1010 in the abdomen constriction test is, to some extent, due to its effect on κ-opioid receptors. It is not unlikely that the analgesic effect of SV-1010 in this test also depends on the inhibitory effect of this compound on kinins and prostaglandins, which requires further study.

In the formalin test, which measures pain responses associated with skin incisions, somatic traumas, and chemical injuries, SV-1010 was as effective as diclofenac. This test has a predictive value when it comes to the mechanism of action of new painkillers, as it has been experimentally and clinically proven that opioids can block phases I and II of nociception, NSAIDs – only phase II, and local anesthetics – only phase I. We showed that SV-1010, administered i.g. at a dose of 0.1 mg/kg, exhibits antinociceptive activity in both phases I and II of the formalin test, which may be due to blockade of pain receptors and inhibition of pain impulse transmission along nerve conductors to the dorsal horns of the spinal cord along with inhibiting the somatic reflex. Moreover, this compound affects the perception of pain impulses in the cortex of the cerebral hemispheres, which indicates that SV-1010 considerably inhibits the formation of a pain response (blockade of the nociceptive pathway through TRPV1 inhibition), which reflects in paw licking. It is of note that in terms of the number of paw shakes, which is an alternative indicator of the pain response to formalin (Chaika et al. 2015) and which, according to Wheeler-Aceto and Cowan (1991), is more reliable than paw licking, SV-1010 at a dose of 0.1 mg/kg in experiments in rats in the formalin test has a more significant analgesic effect in the first phase of inflammation (acute pain) than diclofenac at a dose of 7 mg/kg, and is comparable to the latter when used at a dose of 0.01 mg/kg. In the second phase of inflammation (tonic pain), SV-1010 at doses of 0.01 and 0.1 mg/kg is inferior to diclofenac.

In a model of neuropathic pain due to damage or dysfunction of the central nervous system, as well as peripheral components of the somatosensory nervous system (Bouhassira 2019; Bannister et al. 2020), SV-1010 at a dose of 0.1 mg/kg administered i.g. has a pronounced analgesic effect, significantly exceeding pregabalin (13 mg/kg intravenously) in terms of dose ratio (130-fold). The analgesic effect of SV-1010 under the accepted experimental conditions may be due to its effect on opioid structures. Besides, SV-1010 had an inhibitory effect on capsaicin receptors – TRPV1.

Recently, Ivanova et al. (2023) demonstrated that NMDA-subtype glutamate receptors are involved in analgesia induced by activation of TRPV1 receptor ion channels. It can be assumed that NMDA may also be involved in the analgesic effect of SV-1010, as we previously found out in studies using AI – chemoreactome and chemoproteomic analysis (Galenko-Yaroshevsky et al. 2024); however, this requires further experimental confirmation.

As known, the mechanism of analgesic effect of NSAID primarily involves two components – antiphlogistic properties, which reduce the mechanical pressure of inflamed tissue on pain receptors, and a reduced algogenic effect of pain mediators (kinins, prostaglandins, histamine, serotonin, leukotrienes, etc.) formed at the site of inflammation (Mironov 2012). The analgesic effect of SV-1010 identified in the present study is part of its complex analgesic mechanism, which can be further explored when studying the anti-inflammatory activity of this compound.

Conclusion

Given that SV-1010 has lower acute toxicity (when compared to diclofenac, indomethacin, and ketorolac), high analgesic activity, and a broad therapeutic index (when compared to diclofenac, indomethacin, ketorolac, and pregabalin), as well as it has a unique mechanism of action

(primarily combined antagonism towards TRPV1 and towards κ-opioid receptors), the data obtained make it possible to recommend SV-1010 for further preclinical study, which will open up the prospect for using the molecule and its analogs for the practical development of highly effective analgesic drugs.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

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Ethics statement

The experiments were approved by the Ethics Committee of Kuban State Medical University of the Ministry of Health of the Russian Federation (minutes No. 119 of April 13, 2023).

Data availability

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

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