EFFECT OF 3-SUBSTITUTED 1,4-BENZODIAZEPIN-2-ONES ON BRADYKININ-INDUCED SMOOTH MUSCLE CONTRACTION

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Biochemical properties of 3-substituted 1,4-benzodiazepine determined by the characteristics of their chemical structure. Influence of 3-substituted 1,4-benzodiazepin-2-ones on maximal normalized rate and amplitudes of isometric smooth muscle contraction in rats was investigated. Compounds MX-1775 and MX-1828 demonstrated the similar inhibition effect on bradykinin-induced contraction of smooth muscle like competitive inhibitor des-arg^9-bradykinin-acetate to bradykinin B_2-receptors. MX-1626 demonstrated unidirectional changes of maximal normalized rate and force of smooth muscle that proportionally depended on bradykinin concentration in the range 10^{-10}-10^{-6} M. MX-1828 has statistically significant decrease of normalized rate of smooth muscle contraction for bradykinin concentrations 10^{-10} and 10^{-9} M by 20.7 and 8.6%, respectively, but for agonist concentration 10^{-6} M, this parameter increased by 10.7% and amplitude was reduced by 29.5%. Compounds MX-2011, MX-1785 and MX-2004 showed no natural effect on bradykinin-induced smooth muscle contraction. Compounds MX-1775, MX-1828, MX-1626 were selected for further research of their influence on kinin-kallikrein system and pain perception.

K e y   w o r d s: smooth muscle contraction, bradykinin, 3-substituted 1,4-benzodiazepin-2-ones, maximal normalized rate, force of contraction.

Various peripheral mediators contribute to development and maintenance of inflammatory and neuropathic pain by few mechanisms. Activation or excitation of nociceptive nerve endings or fibers by these substances implicates generation of action potentials which were disseminated on the central nervous system and may induce pain perception. Sensitization of nociceptors occurs in response to various stimuli of external and internal origin, including temperature, mechanical and chemical influences. Bradykinin (BK) is one of the most potent pain-producing agents formed under inflammatory conditions. Multitude of its sensitizing and excitatory effects on peripheral nociceptors supporting its role as a prototype of peripheral pain mediators have been described [1, 2, 6, 10]. A number of bradykinin effects mediated by products of the arachidonic acid-cyclooxygenase cascade tes-

tify to the existence of mutual interactions between the pain mediators [12]. The synthesis of bradykinin and related kallidin is carried out in two ways: intravascularly in plasma and extravascularly in tissues. Prekallikrein formed through clotting factor XII (Hagemann) by formation of kallikrein predecessors that is activated by contact with negatively charged surfaces. Plasma kallikrein acts on high-molecular-weight kininogen that leads to bradykinin and kallidin producing which act preferentially on B_2-bradykinin receptors. The tissue prekallikrein is transformed to kallikrein upon inflammation or tissue damage. Today two types of bradykinin and related kinin receptors - B_1 and B_2, have been described [7, 9, 14, 16]. B_2-type present in the neurons of the brain stem, basal nuclei, cortex, thalamus and hypothalamus. Immune labels of these receptors were found in the endothelium of the upper sagit-

tal sinus dura matter and ependyma of the third and lateral ventricles. B_1-kinin receptors are localized in neurons of the thalamus, hypothalamus and spinal cord [13]. B_1 and B_2 receptors are important mediators of cardiovascular homeostasis, inflammation and nociception. While the B_2-type is constitutively expressed in many tissues including smooth muscle of intestine and stomach [15], B_1-type synthesis is induced only in inflammatory conditions. However, B_1 is one of the central nociceptive mediators feeling that suggest permanent presence of the receptor in the brain and spinal cord [4]. Bradykinin interaction with these receptors leads to activation of G-proteins and specific changes in the levels of $[Ca^{2+}]_{cyt}$, involving in various systems such as phospholipase C, prostaglandins, protein kinases and phospholipase A_2 [8]. Natural and artificially synthesized receptor blockers are important for investigations of action mechanisms of agonists and antagonists of the kinin-kallikrein system. Search for high affinity and selective non-peptide antagonists that demonstrate prolonged effect and do not decompose in a body by peptidases is very important task for pharmacology and medicine.

B2-receptors activation in smooth muscle of stomach can run it contraction as described above. Therefore, the model bradykinin-induced contraction of the stomach smooth muscles is simple and informative to investigate the effect of different substances on the function of kallikrein-kinin system.

1,4-Benzodiazepine derivatives are synthetic inhibitors that act as highly efficient analgesics. Biochemical properties of 3-substituted 1,4-benzodiazepine are determined by features of their chemical structure. Using radicals with different chemical and physical properties to obtain derivates of basis molecules we created some compounds with affinity for specific biological targets [11]. These substances can mimic the β-bend that is important for their biochemical activity [5]. In addition, such compounds are well sustained by patients.

Previously synthesized in A. V. Bogatsky Physico-Chemical Institute of NAS of Ukraine 3-substituted 1,4-benzodiazepin-2-ones exhibiting analgesic activity at doses ranging from 0.007 to 6.6 mg/kg were studied in this paper, some representatives of these series see in Table. These substances are perspective analgesics that have sedative, spasmolytic, anti-inflammatory and low toxicity properties.

The existing today compounds that act through opioid receptors cause addiction. Nonsteroidal anti-inflammatory drugs have many side effects, including contribution to the development of stomach ulcers. Compounds, used in our investigation, are deprived these effects.

In view of their low toxic and high analgesic activity, the aim of the study was to investigate influence of 3-substituted-1,4-dihydro benzodiazepines on bradykinin-induced smooth muscle contraction, as most simple and informative model for interactions of these compounds with kallikrein-kinin system.

Materials and Methods

Tenzometric investigations were carried out using specimens ring stomach muscles of white non-bread male rats which were kept in the standard conditions in vivarium of Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv. All manipulations with animals were carried out in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" and the Law of Ukraine "On Protection of Animals from Cruelty". Animal weight was 240-260 g. Rats were rapidly decapitated; the stomach was isolated and washed with Krebs solution. Muscle layer from antral part of the stomach was separated from the serosa and mucosa and cut into strips (size – 1.5-2 × 10 mm).
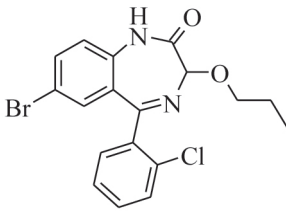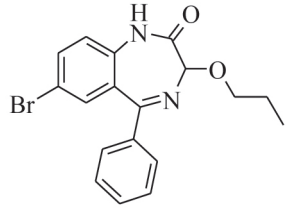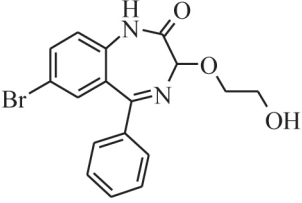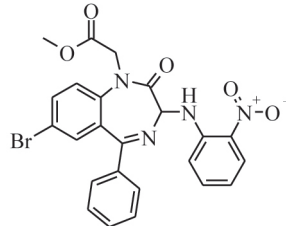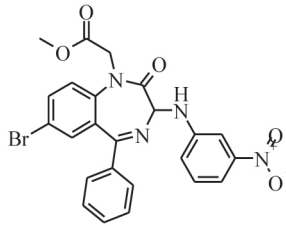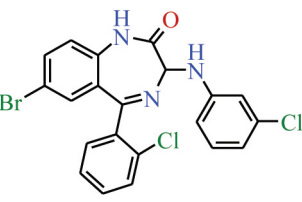
We used the next solutions:

• Krebs solution (mM/l): NaCl – 120.4; KCl – 5.9; $NaHCO_3$ – 15.5; NaH_2PO_4 – 1.2; $MgCl_2$ – 1.2; $CaCl_2$ – 2.5; glucose-11.5; pH 7.4.

• High-K solution where 80 mM K^+ was used as initial inducer of contraction for test measurements. It was prepared by replacing sodium ions equimolar number of potassium ions in the Krebs solution.

Bradykinin solutions of specified concentrations were prepared by dilution of 1 mM bradykinin solution. Action of derivatives of 3-substituted 1,4-benzodiazepine on smooth muscles was evaluated using experimental model of bradykinin-induced contraction of smooth muscle strips. Contraction of smooth muscles caused by application of bradykinin in concentration range 10^{-10}-10^{-5} M. The final concentration of 3-substituted derivatives of 1,4-benzodiazepine in the incubation solution was 10^{-6} M. The bradykinin-induced contraction of smooth muscle strips on background applications competitive inhibitor of bradykinin receptor des-arg9- [leu8]-bradykinin acetate (10^{-6} M) was studied for additional evaluation of the biological effects of derivatives of 3-substituted 1,4-benzdiazepines.

Structural formulas and molecular weights of 3-substituted 1,4-benzdiazepines and analgesic activity (by the method of "acetic acid writhing")

N	Name	Mm	Structure	Analgesic activity ED_{50}, mg/kg
1	MX-2011	407.70		0.03 ± 0.01
2	MX-2004	373.25		0.05 ± 0.02
3	MX-1775	375.22		0.94 ± 0.17
4	MX-1775	375.22		$0.12 \pm 0.03*$
5	MX-1828	523.35		$0.25 \pm 0.12*$
6	MX-1626	475.18		0.90 ± 0.30

* [11].

As derivatives of 3-substituted 1,4-benzodiazepines we used the following compounds: MX-1775, MX-2011, MX-2004, MX-1785, MX-1626, MX-1828, the structural formulas of which are presented in Table. All compounds were synthesized in A. V. Bogatsky Physico-Chemical Institute, NAS of Ukraine, Odesa.

Effectiveness of 3-substituted 1,4-benzodiazepines on bradykinin-induced contraction we evaluated in percent by comparison with amplitude (mN) and maximal normalized rate (Vn) of contraction upon impact of bradykinin only.

Every substance of 3-substituted 1,4-benzodiazepines was tested on 10 separated smooth muscle strips.

Data processing on dynamics of contraction was performed according to the method of T. Burdyha and S. Kosterin [3]. Statistical analysis of experimental data was carried out using Shapiro-Wilk test for control of normality of distribution data. If the data did not have the normal distribution, the comparison was carried out by Kruskal-Wallis independent sample criterion. In case of normal distribution, comparison of control and experimental samples we used ANOVA (Scheffe test) ($P < 0.05$).

Results and Discussion

We analyzed the influence of 3-substituted 1,4-benzodiazepines on amplitude and rate of bradykinin-induced smooth muscle contraction. The concentration range of bradykinin was 10^{-10}-10^{-6} M.

The presence of MX-1775 in solution led to the statistically significant increase of Vn by 11.9% and decrease of amplitude by 13.1% (Fig. 1, *A*, *B*) for bradykinin-induced contraction (10^{-6} M). Maximum normalized rate of contraction decreased by 9.1% for agonist concentration 10^{-7} M, but force of contraction was not changed. The reducing concentration of BK led to proportional statistically significant increase of Vn as: 10^{-8} M BK – 13.2%, 10^{-9} M BK – 15.8% and 10^{-10} M BK – 20.7%. It demonstrates the inhibiting effect of this compound on the bradykinin-induced contraction. The contraction force was increased by 9.4% for the bradykinin concentration 10^{-8} M.

The presence of MX-1626 in incubation solution contributes to decreased Vn for all range of agonist concentrations. Next statistically significant changes were revealed for different bradykinin concentrations: 10^{-6} M – 27.1% 10^{-7} M – 23.2%, 10^{-8} M – 11.8%, 10^{-9} M – 22.2%, 10^{-10} M – 17.0% (Fig. 1, *A*). However, the force of contraction linearly decreased for this range of BK concentration. There were no differences for 10^{-6} M BK, but statistically significant decrease of amplitude by 22.2% was observed for 10^{-10} M BK (Fig. 1, *B*).

MX-2004 had no effect on Vn of contraction of smooth muscles induced by BK for all the concentration range (Fig. 1, *A*). However, the force of contractions for bradykinin concentrations 10^{-6} and 10^{-7} M was statistically significantly reduced by 8.2%, 16.6%, respectively upon influence of MX-2004 (Fig. 1, *B*).
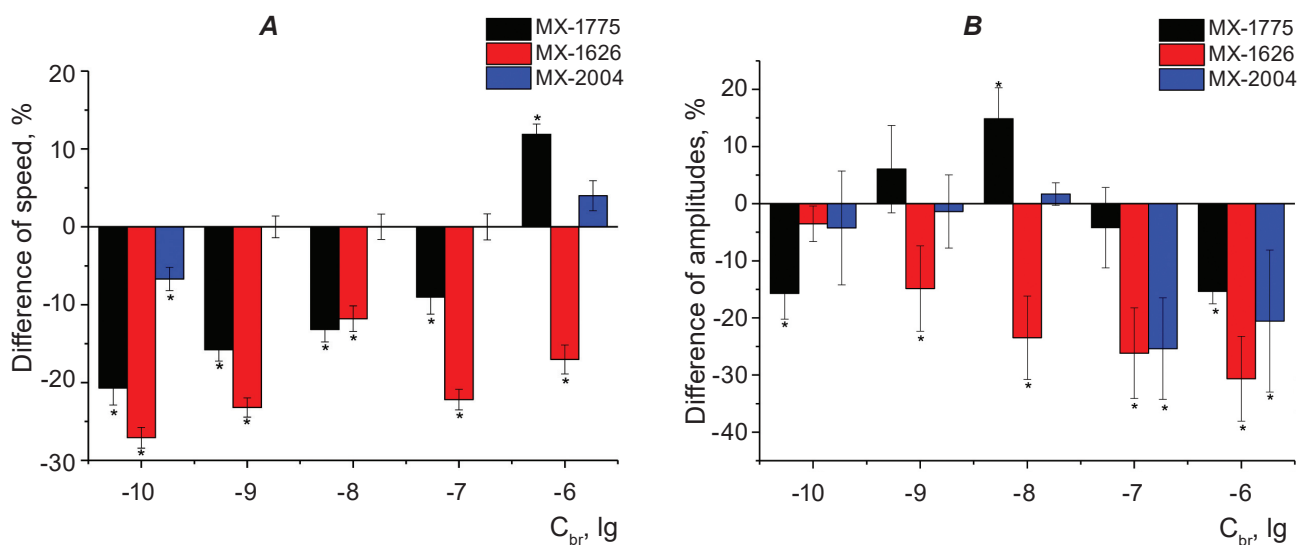

Fig. 1. Influence of 3-substituted 1,4-benzdiazepines on Vn (A) and force (B) smooth muscle contraction of rats stomachs (*P < 0.05). (Comparison with amplitude (mN) and maximal normalized rate (Vn) of contraction upon impact of bradykinin only (line "0"))
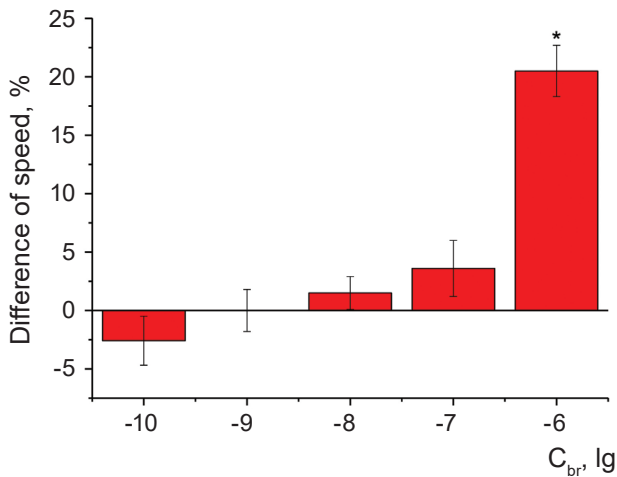
Fig. 2. Influence of des-arg⁹-bradykinin acetate on Vn smooth muscle contraction of rats stomachs (*P < 0.05). (Comparison with amplitude (mN) and maximal normalized rate (Vn) of contraction upon impact of bradykinin only (line "0"))

MX-1775 and MX-1626 statistically significant changed the Vn smooth muscle contraction (Fig. 1) like as inhibitor B_2-receptor des-arg⁹-bradykinin acetate (Fig. 2). These effects may evidence of interactions of these compounds with bradykinin receptors or it signal transduction pathways.

MX-1828 statistically significant decreased the normalized rate of smooth muscle contraction for bradykinin concentrations 10^{-10} and 10^{-9} M by 20.7 and 8.6%, respectively. Nevertheless, for agonist

concentration 10^{-6} M, this parameter increases by 10.7% (Fig. 3, *A*) and amplitude is reduced by 29.5% (Fig. 3, *B*).

MX-2011 statistically significant decreased Vn smooth muscle contraction by 20-25% for almost all used BK concentrations, but concentration 10^{-9} M had weak effectiveness – 14.9% (Fig. 3, *A*). The force of smooth muscle contraction was decreased for bradykinin concentrations of 10^{-6} and 10^{-7} M with effects – 16.7 and 28.1%, respectively, upon influence of MX-2011 (Fig. 3, *B*).

MX-1785 demonstrated the reduced Vn smooth muscle contraction by 10-15% for bradykinin concentrations of 10^{-10}-10^{-7} M. This parameter is not changed for 10^{-6} M BK (Fig. 3, *A*). Force smooth muscles contractions are decreased by 13.46% and 16.07% when BK concentration was 10^{-6} and 10^{-7} M, respectively. Contraction force was increased by 17% for BK concentration 10^{-8} M in presence of MX-1785 (Fig. 3, *B*).

We have been analyzing insufficient number derivatives 3-substituted 1,4-benzodiazepines for demonstration of biochemical effect which depends on their structure. Preliminary assumptions are following: substances MX-2011 and MX-2004 are similar in their effect due to the presence chlorine atom. Maybe similar effects of MX-1785 and MX-1828 caused by structural factors.

So, analysis data show that two 3-substituted 1,4-benzodiazepines MX-1775 and MX-1828 demon-
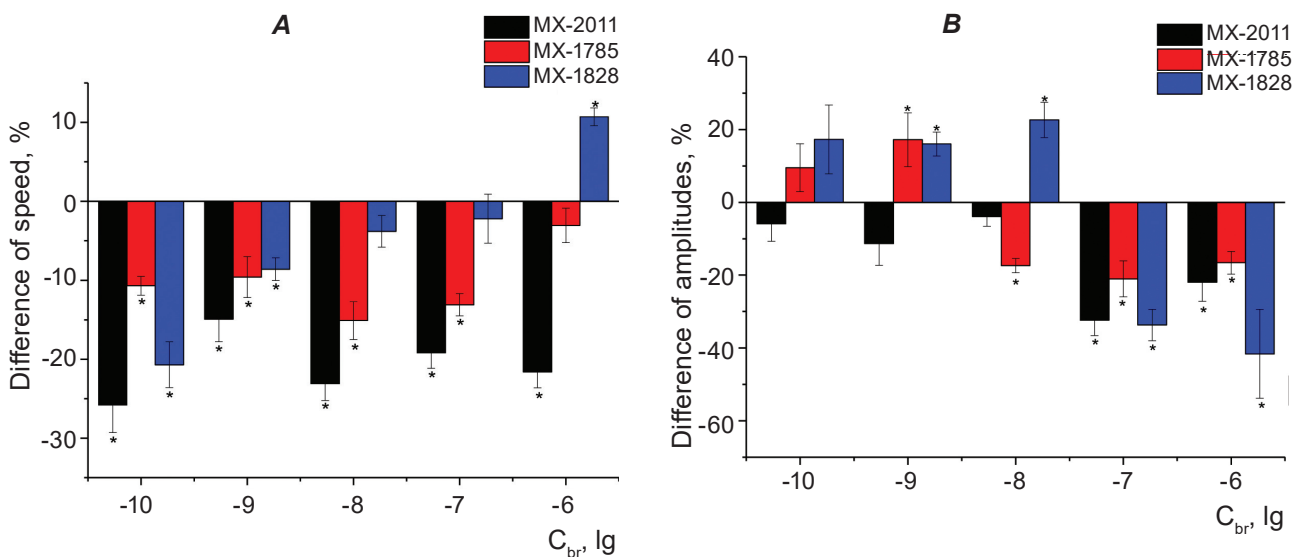

Fig 3. Influence of 3-substituted 1,4-benzdiazepines on Vn (A) and force (B) smooth muscle contraction of rats stomachs (*P < 0.05). (Comparison with amplitude (mN) and maximal normalized rate (Vn) of contraction upon impact of bradykinin only (line "0"))

strate the similar inhibition effect on bradykinin-induced contraction of smooth muscle like competitive inhibitor des-arg[9]-bradykinin-acetate to bradykinin B$_2$-receptors. These substances caused the increase of Vn of bradykinin-induced contractions at 10^{-6} M like as the competitive inhibitor of B$_2$-receptors des-arg[9]-bradykinin-acetate. MX-1626 deserves special attention, this substance demonstrates almost unidirectional changes of Vn and force smooth muscle that proportionally depend on BK concentration in range 10^{-10}-10^{-6} M. Compounds MX-2011, MX-1785 and MX-2004 show no natural effect on bradykinin-induced smooth muscle contraction.

So, MX-1775, MX-1626, MX-1828 can be selected for further research of their biochemical properties, influence on kinin-bradykinin system and pain perception.

ВПЛИВ 3-ЗАМІЩЕНИХ 1,4-БЕНЗДІАЗЕПІН-2-ОНІВ НА БРАДИКІНІНІНДУКОВАНЕ СКОРОЧЕННЯ ГЛАДЕНЬКИХ М'ЯЗІВ

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Біохімічні властивості 3-заміщених 1,4-бенздіазепінів визначаються особливостями їхньої хімічної структури. Проаналізовано вплив 3-заміщених 1,4-бенздіазепін-2-онів на максимальну нормовану швидкість та амплітуду ізометричного скорочення гладеньких м'язів щурів. Сполуки MX-1775 та MX-1828 виявляли інгібуючий ефект на брадикінініндуковане скорочення гладеньких м'язів, схожий до такого, як у конкурентного інгібітора B$_2$-брадикінінових рецепторів – des-arg[9]-bradykinin-acetate. MX-1626 показав односпрямовані зміни максимальної нормованої швидкості та сили скорочення гладеньких м'язів, які пропорційно залежа-

ли від діапазону концентрацій брадикініну 10^{-10}–10^{-6} М. MX-1828 статистично вірогідно зменшував максимальну нормовану швидкість скорочення за концентрацій брадикініну 10^{-9} та 10^{-10} М на 20,7 і 8,6% відповідно, але за 10^{-6} М цей параметр зростав на 10,7% за зменшення амплітуди на 29,5%. MX-2011, MX-1785 та MX-2004 не виявляли закономірності впливу на брадикінініндуковане скорочення. Сполуки MX-1775, MX-1828, MX-1626 можуть бути вибрані для подальших досліджень щодо їх впливу на кінін-калікреїнову систему та больову чутливість гладеньких м'язів.

К л ю ч о в і   с л о в а: скорочення гладеньких м'язів, брадикінін, 3-заміщені 1,4-бенздіазепін-2-они, максимальна нормована швидкість, сила скорочення.

ВЛИЯНИЕ 3-ЗАМЕЩЕННЫХ 1,4-БЕНЗДИАЗЕПИН-2-ОНОВ НА БРАДИКИНИНИНДУЦИРОВАННОЕ СОКРАЩЕНИЕ ГЛАДКИХ МЫШЦ

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Биохимические свойства 3-замещенных 1,4-бенздиазепинов определяются особенностями их химической структуры. Было проанализировано влияние 3-замещенных 1,4-бенздиазепин-2-онов на максимальную нормированную скорость и амплитуду изометрического сокращения гладких мышц крыс. Соединения MX-1775 и MX-1828 проявляли ингибирующий эффект на брадикинининдуцированное сокращение гладких мышц, подобный такому как у конкурентного ингибитора B$_2$-брадикининовых рецепторов – des-arg[9]-bradykinin-acetate. MX-1626 показал однонаправленные изменения максимальной нормированной скорости и силы сокращения гладких мышц, которые пропорционально зависели от диапазона концентраций брадикинина 10^{-10}–10^{-6} М. MX-1828 статистически достоверно уменьшал максимальную

нормированную скорость сокращения при концентрациях брадикинина 10^{-9} и 10^{-10} М на 20,7 и 8,6% соответственно, но при $10-6$ М данный показатель возрастал на 10,7% при уменьшении амплитуды на 29,5%. MX-2011, MX-1785 и MX-2004 не проявляли закономерности влияния на брадикинининдуцированное сокращение. Соединения MX-1775, MX-1828, MX-1626 могут быть отобраны для дальнейших исследований их влияния на кинин-каликреиновую систему и болевую чувствительность гладких мышц.

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