Antiulcerogenic activity of borneol derivatives*

M. S. Borisova,[a]★ O. I. Yarovaya,[a,b] M. D. Semenova,[a] T. G. Tolstikova,[a,b] and N. F. Salakhutdinov[a,b]

[a]*N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 9 ul. Lavrent'eva, 630090 Novosibirsk, Russian Federation. Fax: +7 (383) 330 9752. E-mail: mborisova@nioch.nsc.ru*

[b]*Novosibirsk State University, 2 ul. Pirogova, 630090 Novosibirsk, Russian Federation*

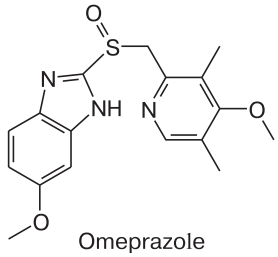
Antiulcerogenic activity of borneol and its N-heterocyclic esters was investigated using indomethacin-induced gastric ulcer model. A considerable antiulcerogenic effect comparable to that shown by Omeprazole and Famotidine was found for two investigated compounds in the indomethacin-induced gastric ulcer model. Bornyl 2-piperazinoacetate, with a non-substituted piperazine moiety, showed maximum antiulcerogenic activity in the indomethacin-induced gastric ulcer model and demonstrated a significant gastroprotective effect in the ethanol-induced gastric ulcer model comparable to the effect of Omeprazole.

Key words: borneol derivatives, antiulcerogenic activity.

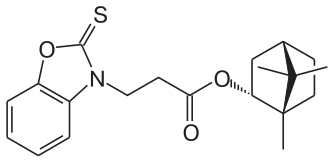
Gastric ulcer is a common chronic recurrent disease and it is referred to as a disease associated with urbanization. This disease attacks the able-bodied population, decreases their working efficiency and quality of life, and also leads to the development of different hazardous complications such as bleeding, perforation and cancer. So far, pharmaceutical market provides a significant number of antiulcer drugs. The basic medicines are the common inorganic antacids and antisecretory drugs. Nevertheless, the increase in the incidence of digestive disorders, malnutrition of different types, and allergic diseases, which are for the most part represented by food allergy and allergic reactions to different types of drugs, stimulate research and development of novel efficient agents for treatment of the pathological conditions of this type. Natural compounds with a broad spectrum of activities and low toxicity are regarded as promising medicinal molecules for synthetic preparation.[1] One of these is borneol, which is a natural bicyclic alcohol existing as a pair of D- and L-isomers. Both enantiomers are components of essential oils of different plants (sage, valerian, lavender, chamomile, etc.).

Borneol is used in pharmaceutical industry, perfume and cosmetics manufacturing, wood industry, agriculture and other industries. In traditional oriental medicine, borneol has long since been used as a remedy against gastrointestinal diseases. Recent investigations have established that borneol and its derivatives show antimicrobial,[2] antiviral[3,4] and anti-inflammatory[5] activities. Antiulcer action of borneol-containing essential oils was examined and presented in multiple works.[6,7] Borneol was shown to promote drugs crossing through the blood-brain barrier, thus improving the efficiency of these drugs.[8,9]

Earlier we demonstrated antiulcerogenic activity of a number of (−)-borneol esters with heteroaromatic moieties connected to the bicyclic scaffold with an ester linker. For example, a considerable antiulcer activity exceeding that of a reference drug Omeprazole was found experimentally in the indomethacin-induced ulcer test[10] for compound **1** containing 2-thiobenzoxazole moiety.



Omeprazole



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The aim of the present work is the search for the antiulcerogenic agents using a series of borneol esters with saturated N-heterocyclic moieties.

Experimental

The study was performed on 96 female Wistar rats weighing 180—220 g, which were provided by the Institute of Cytology

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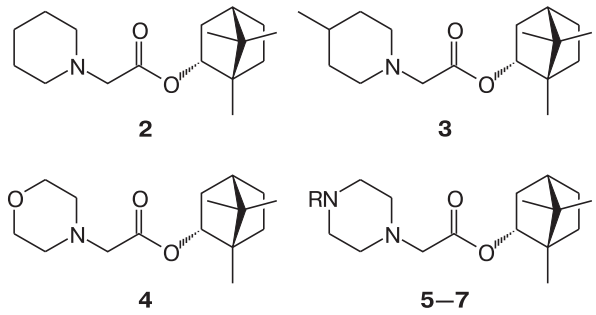
and Genetics, Siberian Branch of Russian Academy of Sciences (Novosibirsk) and kept under standard conditions with free access to food and water. The animals were randomized by weight and divided into groups of six animals. All procedures with animals were carried out in compliance with European convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986).

Indomethacin-induced ulcer model was applied according to the published procedure.[11] The analyzed compounds, which are the (−)-borneol esters containing piperidine (**2**), 4-methylpiperidine (**3**), morpholine (**4**), piperazine (**5**), *N*-ethylpiperazine (**6**) and 4-ethoxycarbonyl piperazine (**7**) moieties, were given intragastrically after an overnight fast at a dose of 100 mg kg^{-1} as a solution in 0.05% aqueous Tween 80. Omeprazole (Akrikhin, Russian Federation) was used as a reference drug at a dose of 100 mg kg^{-1}, and Famotidine (Hemofarm, Russian Federation) at a dose of 20 mg kg^{-1}. The controls were given aqueous Tween 80 intragastrically. One hour after the intragastric administration of the examined compounds, Indomethacin was given to the animals *per os* at a dose of 25 mg kg^{-1}. One day after the administration of Indomethacin, the animals were euthanized using diethyl ether. The removed stomachs were opened along the lesser curvature, and the number of lesions was determined.



Antiulcerogenic activity of the examined compounds was determined according to the method developed by Pauls. To this purpose, the Pauls' index (PI) was calculated for each experimental group using the equation

$$PI = (A \times B)/100, \quad (1)$$

where A is the number of ulcers per animal in the group, and B is the percentage of the animals with ulcers.

Antiulcer index (AI) was calculated as follows

$$AI = PI_{control}/PI_{exp}. \quad (2)$$

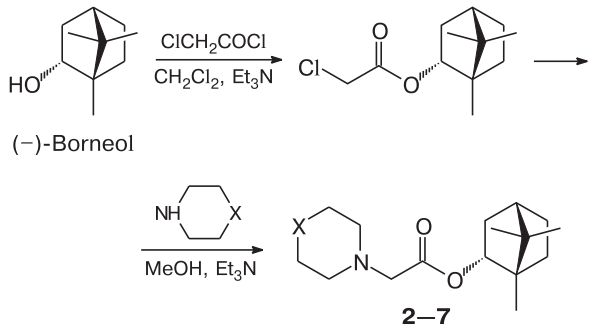
The compounds with antiulcer index greater than or equal to 2 were regarded as antiulcerogenic compounds.[12]

Ethanol-induced ulcer was modeled as described earlier.[13] Compound **5** was given to the animals intragastrically after an overnight fast at a dose of 100 mg kg^{-1} in 0.05% aqueous Tween 80. Omeprazole (Akrikhin, Russian Federation) at a dose of 100 mg kg^{-1} and Famotidine (Hemofarm, Russian Federation) at a dose of 20 mg kg^{-1} was used as reference drug standards. The controls were given aqueous Tween 80 *per os*. One hour later, 96% aqueous ethanol was administered to the animals orally at a dose of 0.5 mL per 100 g live weight (5 mL kg^{-1}). One hour after this procedure, the animals were euthanized using diethyl ether. The stomachs were removed, opened along the greater curvature, flattened and photographed with Canon Powershot G9. The area of lesions was evaluated using AxioVision 4.9.1 software. Statistical analysis was applied to the obtained data using a Statistica 6.0 software package, and means and the standard error of mean were determined. Student's t-test was used as a significance criterion. The results were considered statistically significant at $p < 0.05$.

Results and Discussion

With a view to the development of novel drugs with a significant antiulcerogenic activity, we synthesized N-heterocyclic esters of borneol **2—7** (Scheme 1). As a starting compound, natural (−)-borneol was obtained from the verdure extraction residue wastes using a special procedure.[14] Compounds **2—7** were obtained as we described in our early paper[15] by reaction of N-heterocycles with borneol chloroacetate. In the cited article, physico-chemical characteristics of these compounds are presented.

Scheme 1



Peptic ulcer disease is caused by the action of multiple exogenous and endogenous factors, such as infection, gastric acid, dietary and autoimmune factors. These factors affect different parts and functions of the stomach, and hence, the agents used for the treatment of this pathological state are characterized with different types of action. Basic groups of medicines administered against peptic ulcer are antibiotics, antisecretory drugs (proton pump inhibitors, histamine H_2-receptors, etc.), antacids, immunotherapeutic and anti-inflammatory drugs, medicines with regenerative and reparative effect. Therefore, estimation of antiulcer activity of new candidates has to be done using several experimental models simulating different types of action on the gastric mucosa. For example, Indomethacin, as a non-steroidal anti-inflammatory drug, affects the formation of prostaglandins, which stimulate gastric mucus production, induces apoptosis of gastric cells and acts as a weak acid.[16] Ethanol, which demon-

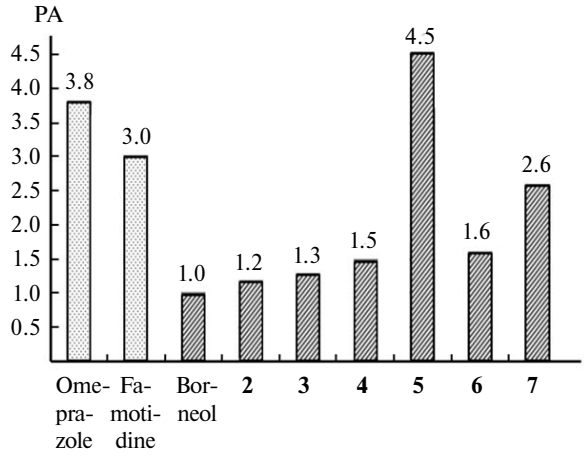


Fig. 1. Antiulcerogenic activity of the studied borneol derivatives determined using indomethacin-induced ulcer model.

strates both hydrophilic and lipophilic properties, destroys gastric mucous layer and induces oxidative stress and gastric cell necrosis.[13]

The results of the study of antiulcerogenic activity of the compounds against indomethacin-induced ulcer (Fig. 1) show that compounds **5** and **7** with PA indexes 4.5 and 2.6, respectively, demonstrate considerable antiulcerogenic activity at a dose of 100 mg kg^{-1}. Furthermore, the AI index of compound **5** exceeded these found for Omeprazole (AI = 3.8) and Famotidine (AI = 3.0). Thus, it was concluded that prophylactic treatment with agent **5**, which comprises a piperazine moiety, is able to considerably reduce the number of ulcerative lesions caused by the action of Indomethacin.

Antiulcerogenic activity of compound **5** was also examined using the ethanol-induced ulcer model. The results of the experiment demonstrate that this compound substantially decreases the area of erosive lesions of the gastric mucosa of the animals in comparison to the control group,

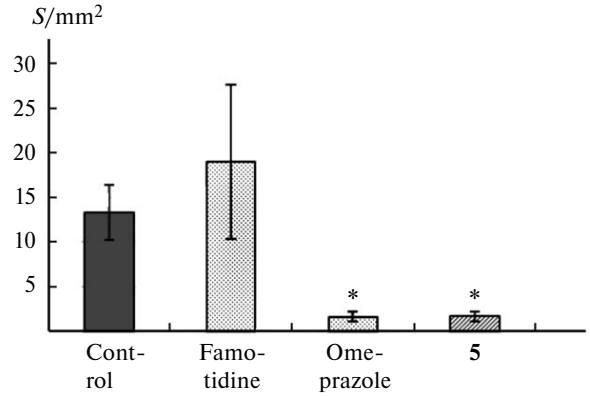


Fig. 2. Area of lesions (*S*) of the stomach mucosa determined using an ethanol-induced ulcer model. Statistically significant difference from control is marked with an asterisk ($p < 0.05$).

and its efficiency is similar to that of Omeprazole (Fig. 2). Therefore, we have shown that agent **5** exerts a substantial antiulcerogenic action against concentrated ethanol if administered prophylactically at a dose of 100 mg kg^{-1}.

In consistence with the previously published data[17] Famotidine at a dose of 20 mg kg^{-1} did not exhibit any protective effect on ethanol-induced ulcer model. Famotidine is commonly used for the treatment of pathological conditions of gastrointestinal tract and belongs to the third generation of H_2-antagonists. This medication blocks histamine H_2 receptors, which are located on the cells of the stomach, and, in particular, on vascular endothelial cell membranes. Histamine is a native activator of acid secretion, and it stimulates the local blood circulation. Upon the action of 96% aqueous ethanol, histamine receptors of the stomach are blocked, and hence, the effect of histamine on the blood vessels is abolished, thus promoting ischemia, which results in decreasing the resistance of the mucosa to the action of the ulcerogen. This result demonstrates the difference in the mode of action of medicinal substances and provides the insight into the mechanism of action of newly synthesized compounds. On the basis of the described experiments we can conclude, that antiulcerogenic action of the studied compound **5** does not involve blocking of histamine H_2 receptors.

In conclusion, the results obtained in this study showed that two compounds (**5** and **7**) among six investigated borneol derivatives demonstrated antiulcerogenic activity on indomethacin-induced ulcer model, and one of these compounds (agent **5**) was found to exert considerable gastroprotective effect on ethanol-induced ulcer model, which was similar to that of Omeprazole. The data obtained in the experiment using the ethanol-induced ulcer model also suggested that the mechanism of action of compound **5** does not involve blocking of H_2 receptors. Based on the characteristics of the experimental model and the mechanism of action of Omeprazole, we can conclude that compound **5** possesses antioxidant activity or suppresses the hydrochloric acid production by inhibition of proton pump. In conclusion, this borneol derivative containing piperazine moiety is a promising compound for the further investigation as an antiulcerogenic agent.

References

1. N. F. Salakhutdinov, K. P. Volcho, O. I. Yarovaya, *Pure Appl. Chem.*, 2017, **89**, 1105.
2. P. R. C. Corrêa, R. R. S. Miranda, L. P. Duarte, G. D. F. Silva, S. A. Vieira Filho, A. A. Okuma, F. Carazza, J. A. Morgado-Díaz, P. Pinge-Filho, L. M. Yamauchi, C. V. Nakamura, S. F. Yamada-Ogatta, *Pathog. Glob. Health*, 2012, **106**, 107.
3. A. Kononova, A. Sokolova, S. Cheresiz, O. Yarovaya, R. Nikitina, A. Chepurnov, A. G. Pokrovsky, N. Salakhutdonov, *Med. Chem. Commun.*, 2017, DOI: 10.1039/C7MD00424A.

4. M. Šala, A. M. De Palma, H. Hrebabecky, R. Nencka, M. Dracínský, P. Leyssen, J. Neyts, A. Holy, *Bioorg. Med. Chem.*, 2010, **18**, 4374.
5. R. M. C. Vasconcelos, F. C. Leite, J. A. Leite, S. R. Mascarenhas, L. C. Rodrigues, M. R. Piuvezam, *Immunopharmacol. Immunotoxicol.*, 2012, **34**, 1028.
6. I. Gurbuz, E. Yesilada, B. Demirci, E. Sezik, F. Demirci, K. H. C. Baser, *J. Ethnopharmacol.*, 2013, **148**, 332.
7. C. Takayama, F. Meira de-Faria, A. C. A. de Almeida, R. J. Dunder, L. P. Manzo, E. A. R. Socca, L. M. Batista, M. J. Salvador, A. R. M. Souza-Brito, A. Luiz-Ferreira, *Asian Pac. J. Trop. Biomed.*, 2016, **6**, 677.
8. B. Yu, M. Ruan, X. Dong, Y. Yu, H. Cheng, *J. Ethnopharmacol.*, 2013, **150**, 1096.
9. X. Xu, J. Li, S. Han, C. Tao, L. Fang, Y. Sun, J. Zhu, Z. Liang, F. Li, *Eur. J. Pharm. Sci.*, 2016, **88**, 178.
10. A. S. Sokolova, O. I. Yarovaya, A. A. Shtro, M. S. Borisova, E. A. Morozova, T. G. Tolstikova, V. V. Zarubaev, N. F. Salakhutdinov, *Chem. Heterocycl. Compd.*, 2017, **53**, 371.
11. M. G. Derelanko, G. T. Long, *Dig. Dis. Sci.*, 1980. **25**, 830.
12. F. Pauls, A. M. Wick, E. M. MacKey, *Gastroenterology*, 1947, **8**, 774.
13. M. B. Adinortey, C. Ansah, I. Galyuon, A. Nyarko, *Ulcers*, 2013, **2013**, Article ID 796405 DOI: 10.1155/2013/796405.
14. Pat. RF 2464035, *Buyll. Izobret.*[*Invention Bull.*], 2012, No. 29 (in Russian).
15. A. S. Sokolova, O. I. Yarovaya, M. D. Semenova, A. A. Shtro, I. R. Orshanskaya, V. V. Zarubaev, N. F. Salakhutdinov, *Med. Chem. Commun.*, 2017, **8**, 960.
16. H. Matsui, O. Shimokawa, T. Kaneko, Y. Nagano, K. Rai, I. Hyodo, *J. Clin. Biochem. Nutr.*, 2011, **48**, 107.
17. A. Dembiński, Z. Warzecha, P. Ceranowicz, T. Brzozowski, M. Dembiński, S. J. Konturek, W. W. Pawlik, *Eur. J. Pharmacol.*, 2005, **508**, 211.

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