The effect of benzilic acid derivatives on the pain threshold using the “hot plate” test

The search for new highly effective analgesics is a topical issue of modern pharmacology since drugs currently used in clinical practice do not meet the requirements of efficiency and safety.

**Aim.** To study of the effect of new derivatives of benzilic acid on the pain threshold using the “hot plate” test.

**Materials and methods.** The objects of the study were new derivatives of benzilic acid. Determination of the pain threshold was carried out by the “hot plate” test. The substances studied were introduced orally as aqueous solutions in the dose of 12 mg/kg. The latent periods of the first licking of the paw sole and the first jump were recorded during the study.

**Results.** Six- and seven-membered thienolactames did not show the analgesic activity, and acid benzilic amides yielded far exceeded their activity. Hence, formation of cyclic products led to a decrease of the activity. Introduction of the chlorine atom into molecules also led to the decrease of activity. At the same time, the structural modification of the amide group in thienopyrroles was effective. In the experiment the most active substance was KMS-49 containing the dimethylacetyl group. Another active compound was a derivative of KMS-284 belonging to other chemical group of compounds under study – benzilic acid amides.

**Conclusions.** The most active compounds under research appeared to be KMS-49 and KMS-284 after their introduction the latent period of the first licking of the paw sole increased by 2.2 and 1.6 times, respectively; and the latent period of the first jump – by 2.8 and 2.5 times, respectively, compared to the control group at the significance level p ≤ 0.05.

**Key words:** benzilic acid derivatives; analgesic activity; “hot plate” test

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Mатериалы и методы. Объектами исследования стали новые производные бензиловой кислоты. Определение порога болевой чувствительности проводили с помощью теста «горячая пластина». Исследуемые вещества вводили перорально в виде водных растворов в дозе 12 мг/кг. В ходе исследования регистрировали латентное время первого облизывания подошвы и латентное время первого прыжка.

Результаты и их обсуждение. Шести- и семичленные тиенолактамы не проявили анальгетической активности, исходные амиды бензиловой кислоты значительно превышали их по активности, следовательно, образование циклических продуктов приводит к уменьшению активности. Введение в молекулы атома хлора также приводило к уменьшению активности. В то же время эффективной оказалась структурная модификация амидной группировки в составе тиепирролов. В эксперименте самой активной оказалась субстанция KMS-49, которая содержит в своем составе диметилацетильную группу. Другим активным соединением оказался производное KMS-284, которое относится к иной химической группе исследуемых соединений — амидам бензиловой кислоты.

Выводы. Наиболее активными из исследованных веществ оказались KMS-49 и KMS-284, при введении которых увеличивался латентный период первого облизывания лапы в 2,2 и 1,6 раза, соответственно; и латентный период первого прыжка в 2,8 и 2,5 раза, соответственно, по сравнению с группой контроля при уровне значимости р ≤ 0,05.

Ключевые слова: производные бензиловой кислоты; анальгетическая активность; тест «горячая пластина»

Determination of the body pain threshold is an essential part of biomedical, physiological and psychophysiological studies. Indicators of the pain threshold depend on the immune status, emotional state, stress factors and the degree of adaptation to it. Determination of the pain threshold is important for the study of efficacy of drugs with the analgesic action.

The search for new highly effective analgesics is a topical issue of modern pharmacology since drugs currently used in clinical practice do not meet the requirements of efficiency and safety. Narcotic analgesics are characterized by a strong analgesic effect, providing the possibility of their use for injuries and diseases accompanied by pain. However, they also affect the central nervous system: the first use cause euphoria, and further there is addiction and dependence [1, 2].

To determine the pain threshold the studies on laboratory animals are conducted using the “hot plate” test. This test is based on behavioral reactions controlled by supraspinal structures in response to the painful effect. After placing the animals on a hot surface when reaching the pain threshold there are motor anxiety reactions: withdrawal of paws, paw sole licking, jumping characterized by the most complex organization of reflex involving cortical and subcortical brain structures [3].

The aim of our work was to study the effect of new derivatives of benzilic acid on the pain threshold using the "hot plate" test.

Materials and methods
The objects of the study were new derivatives of benzilic acid synthesized at the Department of Organic Chemistry of National University of Pharmacy. The substances studied were introduced orally as aqueous solutions in the dose of 12 mg/kg. The control animals received the corresponding volume of saline. Animals were kept in the vivarium under standard conditions on a regular diet and with free access to water.

Determination of the pain threshold was carried out using the “hot plate” test. Laboratory animals were placed in chambers with the size of 200 × 200 × 300 mm, which floor was heated to the temperature of 54-55 °C. The latent time of the first licking of the paw sole and latent time of the first jump were recorded during the study [3, 4].

The statistical analysis of the results was performed using the parametric Student t-test. The difference was considered to be significant at p < 0.05.

Results and discussion
The results of the effect of the benzyl acid derivatives studied on the pain threshold in laboratory animals are shown in Tab.

According to the results concerning the effect of the benzyl acid derivatives studied on physical endurance of the experimental animals introduction of KMS-303, KMS-258, KMS-230 and KMS-71 led to a decrease of the latent period of the first licking of the paw sole with significant changes observed in the groups of animals.

<table>
<thead>
<tr>
<th>Substance</th>
<th>The latent period of the first licking of the paw sole, sec</th>
<th>The latent period of the first jump, sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.8 ± 1.3</td>
<td>15.8 ± 2.4</td>
</tr>
<tr>
<td>KMS-49</td>
<td>21.4 ± 2.3*</td>
<td>43.8 ± 2.6*</td>
</tr>
<tr>
<td>KMS-69</td>
<td>12.8 ± 1.9*</td>
<td>30.4 ± 1.1*</td>
</tr>
<tr>
<td>KMS-228</td>
<td>13.6 ± 1.1*</td>
<td>29.0 ± 1.6*</td>
</tr>
<tr>
<td>KMS-230</td>
<td>8.4 ± 1.1</td>
<td>22.8 ± 1.9*</td>
</tr>
<tr>
<td>KMS-71</td>
<td>8.2 ± 1.3</td>
<td>17.2 ± 2.6</td>
</tr>
<tr>
<td>KMS-284</td>
<td>16.0 ± 1.6*</td>
<td>39.8 ± 1.5*</td>
</tr>
<tr>
<td>KMS-303</td>
<td>6.6 ± 1.1*</td>
<td>13.2 ± 2.6</td>
</tr>
<tr>
<td>KMS-258</td>
<td>4.6 ± 1.5*</td>
<td>13.2 ± 1.6</td>
</tr>
<tr>
<td>KMS-68</td>
<td>12.8 ± 2.6*</td>
<td>22.6 ± 1.7*</td>
</tr>
<tr>
<td>KMS-73</td>
<td>14.0 ± 2.9*</td>
<td>18.8 ± 2.2</td>
</tr>
<tr>
<td>KMS-229</td>
<td>10.0 ± 0.7</td>
<td>21.2 ± 1.3*</td>
</tr>
<tr>
<td>KMS-282</td>
<td>11.2 ± 1.9</td>
<td>17.4 ± 1.1*</td>
</tr>
</tbody>
</table>

Note: * – p ≤ 0.05 (vs. control animals).
injected with KMS 303 and KMS-258 compared to the group of control animals (Fig. 1). After introduction of KMS-229 KMS-282 the pain threshold remained at the same level compared to the control group. A significant increase of physical endurance revealing in the extension of the latent period of the first licking of the paw sole was observed in the groups of animals after introduction of KMS-69, KMS-68, KMS-228, KMS-73, KMS-284 and KMS-49. The most active substance was KMS-49, which introduction led to an increase of the pain threshold in the experimental mice by 2.2 times compared to the control group of animals.

The second parameter studied was the latent period of the first jump (Fig. 2). Similarly, as in the previous experiment, introduction of KMS-303 and KMS-258 resulted in a decrease of the latent period of the first jump compared to the group of control animals, and introduction of KMS-71, KMS-282 and KMS-73 did not significantly affect this parameter. The rest of the substances studied increased the latent period of the first jump. Notably that the most active substances were KMS-49 and KMS-284.

In the experiment several compounds – benzilic acid amides (KMS-228, KMS-230, KMS-282, KMS-283, KMS-284), products of its cyclization: KMS-229, KMS-258, KMS-303 and six- and seven-membered thienolactames and five-membered thienolactames with the functionalized amide function (KMS-49, KMS-68, KMS-69, KMS-71, KMS-73) were studied. Six- and seven-membered thienolactames did not show the analgesic activity (at the level of the control group), and acid benzilic amides yielded far exceeded their activity. Hence, formation of cyclic products led to a decrease of the activity. Introduction of the chlorine atom into molecules also led to the decrease of activity (such compounds in pairs: KMS-228 – KMS-230 and KMS-229 – KMS-258). At the same time, the structural modification of the amide group in thienopyrroles was effective. In the experiment

\[ \text{Fig. 1. The effect of the substances studied on the latent period of the first licking of the paw sole (* – p ≤ 0.05 vs. control animals)} \]

\[ \text{Fig. 2. The effect of the substances studied on the latent period of the first jump (* – p ≤ 0.05 vs. control animals)} \]
The most active substance was KMS-49 containing the dimethylacetyl group. Probably in the body this group is able to transform into the aldehyde or carboxyl group effectively binding to the active sites of enzymes. Another active compound was a derivative of KMS-284 belonging to other chemical group of compounds under study – benzilic acid amides. In the molecule of this compound there is 3-(5-methoxyindolil), which is a known pharmacophore with the pronounced effects on the CNS. Therefore, further search for effective substances with the central analgesic effect among derivatives of benzilic acid and functionalized derivatives of 6-oxo-4,4-diphenyl-5,6-dihydro-4H-thieno[3,4-c]pyrrole-1-carboxylic acid is promising.

CONCLUSIONS
The most active compounds under research appeared to be KMS-49 and KMS-284 after their introduction the latent period of the first licking of the paw sole increased by 2.2 and 1.6 times, respectively; and the latent period of the first jump – by 2.8 and 2.5 times, respectively, compared to the control group at the significance level $p \leq 0.05$.

Conflict of Interests: authors have no conflict of interests to declare.

REFERENCES