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Original article

3-Hydroxy-piperidinyl-*N*-benzyl-acyl-arylhydrazone derivatives reduce neuropathic pain and increase thermal threshold mediated by opioid system

Nathália M. dos Santos[a], Nayara C. Pereira[b], Angélica P.S. de Albuquerque[b], Flávia P. Dias Viegas[a], Clarice Veloso[c], Fabiana C. Vilela[d], Alexandre Giusti-Paiva[d], Marcelo L. da Silva[b], Josie R.T. da Silva[b], Claudio Viegas Jr.[a,*]

[a] PeQuiM - Laboratory of Research in Medicinal Chemistry, Institute of Chemistry, Federal University of Alfenas, 37133-840, Alfenas, MG, Brazil
[b] Physiotherapy Department, Federal University of Alfenas, 37133-840, Alfenas, MG, Brazil
[c] Faculty of Pharmaceutical Sciences, Federal University of Amazonas, 69067-005, Manaus, AM, Brazil
[d] Department of Physiological Sciences, Federal University of Alfenas, 37133-840, Alfenas, MG, Brazil

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ABSTRACT

Here in, we report the preparation and evaluation of four 3-hydroxy-piperidine-*N*-benzyl-aryl-acylhydrazone derivatives (**6a–d**) for their potential antinociceptive activity. In the tail flick test, compounds **6a** and **6d** exhibited a significant increase in the latency time of the animals, in comparison to the control group. These two compounds also showed a significant increase in the nociceptive threshold from 1 to 6 h after treatment in the CCI neuropathic pain model. In both cases, the antinociceptive activity was blocked by naloxone, suggesting an opioid mechanism of action, but without sedative or motor coordination effects.

1. Introduction

Pain is a multidimensional experience that involves sensory and emotional aspects, which usually have a protective function, but under pathological response can significantly affect the quality of life [1]. Painful conditions are present in the life of millions of people worldwide, which are affected by a number of varied diseases such as cancer, diabetes, inflammatory illnesses in general, among others [2]. Although there has been a breakthrough in medicine for the treatment of severe diseases, there is still a great deficiency in the therapy of pain resulting from these pathologies. Thus, pain is considered a global health problem and entails countless social and economic consequences [3].

The most common way of treating pain is by using drugs. In general, non-steroidal anti-inflammatory drugs, opioids, antidepressants, anticonvulsants and local anesthetics are used as first choice medicines, but other therapeutical classes could be also used as adjuvants [4]. However, all of them have limitations in the therapeutics of some types of pain, such as low efficiency, low potency and a great variety of side effects, such as respiratory depression, gastrointestinal and hepatotoxicity, as well as dependence caused by the use of opioids [5]. In this context, the search for new therapeutic alternatives, including the discovery and development of effective and safe new drugs, continues to be of paramount importance.

In a previous work (data not published), our group synthesized a series of 3-hydroxy-piperidinyl-*N*-benzyl-acyl-arylhydrazone derivatives designed as multifunctional candidates of drug prototypes with acetylcholinesterase inhibition and anti-inflammatory properties designed by molecular hybridization of the structure of donepezil and acyl- and arylhydrazone derivatives from the literature. During the pharmacological evaluation *in vivo*, four compounds showed unexpected and interesting antinociceptive effect (PCT/BR2016/050010). Thus, aiming a more detailed study of their potential central antinociceptive effects, compounds **6a–d** (Scheme Scheme 1) were selected for evaluation on different pain models in rats such as neuropathic pain induced by chronic constriction injury (CCI) and mechanical allodynia in the electronic von Frey test. In addition, thermal threshold was also evaluated in the tail flick test.

2. Results

2.1. Chemistry

The synthetic route for the preparation of the target compounds is shown in Scheme Scheme 1. Commercial methyl 4-formylbenzoate (**1**) was used as starting material and submitted to a reductive amination reaction with *rac*-3-hydroxy-piperidine (**2**) in the presence of $NaBH_3CN$ and anhydrous zinc chloride, leading to the corresponding *N*-benzyl-piperidine ester **3**. In a second step, compound **3** was reacted with

* Corresponding author at: PeQuiM - Laboratory of Research in Medicinal Chemistry, Institute of Chemistry, Federal University of Alfenas, 37133-840, Alfenas, MG, Brazil.
E-mail address: cvjviegas@gmail.com (C. Viegas Jr.).

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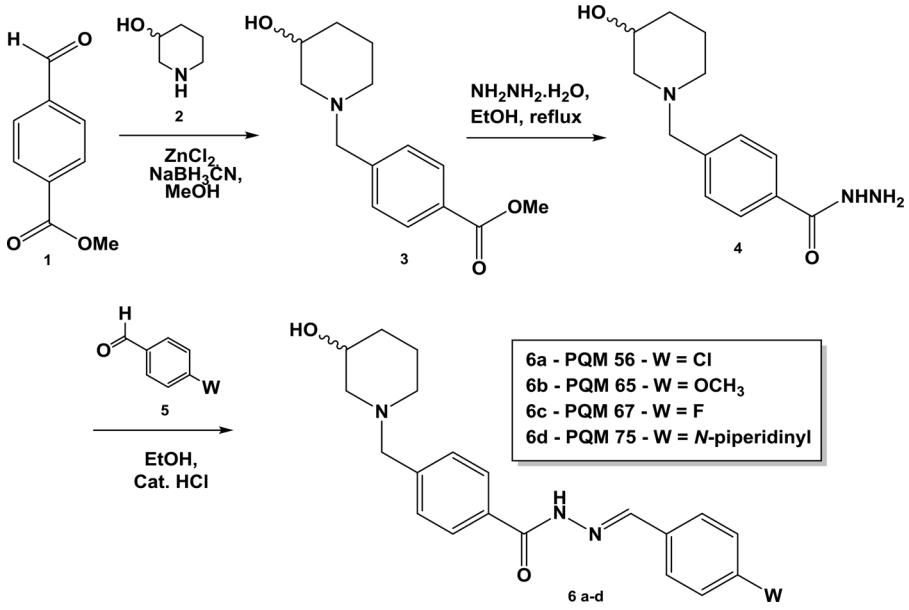

Scheme 1. Synthetic route for the preparation of the target compounds PQM-56 (**6a**), PQM-65 (**6b**), PQM-67 (**6c**) e PQM-75 (**6d**).

hydrazine monohydrate, generating the hydrazide **4** as the key intermediate of the divergent synthetic approach. A sequence of acid-catalyzed coupling reactions of **4** with a series of adequately functionalized benzaldehydes (**5**) led to the desired compounds **6a–d**. The structures of all these compounds were confirmed by IR, 1H and 13C NMR and HRMS experiments.

2.2. Pharmacological evaluation

2.2.1. Formalin test in mice

Fig. 1 shows the licking time in the first and second phases of the formalin test. According to our data, it can be seen that treatment with all compounds or with morphine, except for indomethacin, reduced the licking time when compared to the vehicle group in the first phase ($F(10.77) = 14.57$, $p < 0.001$; Fig. 1A). Regarding the second phase, we can observe that standard drugs and compounds **6a–c**, but not compound **6d**, reduced the licking time when compared to the vehicle group ($F(10.77) = 12.15$, $p < 0.001$; Fig. 1B). In view of these results, and according to the availability of synthesis, compounds **6a–d** were chosen for further evaluation of their potential effect in neuropathic pain and thermal threshold.

2.2.2. Electronic von frey test in rats

Fig. 2 demonstrates the withdrawal threshold of animals submitted or not to CCI surgery and the area under the curves (AUC) versus time for paw withdrawal thresholds in the von Frey test. According to the results for the animals submitted to sham surgery (control group), it can be observed that the surgery procedure did not alter the withdrawal threshold at the times analyzed by pre-treatments and treatments in comparison to the Sham veh + sal group. In the Sham morp + sal group, there was an increase ($p < 0.05$) in the withdrawal threshold at T0-6 times, differently of the Sham veh + sal group (time factor: $F(6.35) = 4.08$, $p < 0.001$; pretreatment factor: $F(11.385) = 56.17$, $p < 0.001$; interaction: $F(66.385) = 11.06$, $p < 0.001$; Fig. 2A). Regarding the animals submitted to CCI surgery, it can be observed that pre-treatment with compounds PQM-56 (**6a**) or PQM-75 (**6d**) or with morphine, followed by treatment with saline, raised the withdrawal threshold when compared to the veh + sal group ($p < 0.05$). There was no difference among the other groups, demonstrating that the treatment with naloxane blocked the effect of these compounds (time factor: $F(6.35) = 1174$, $p < 0.001$; pretreatment factor: $F(11.385) = 595.4$, $p < 0.001$; interaction: $F(66.385) = 82.14$, $p < 0.001$; Fig. 2B). These effects can be evidenced in Fig. 2C and D, which demonstrate AUC versus time for paw withdrawal thresholds. According to the Fig. 2C, it can be observed that only morphine exerted effect on sham animals ($p < 0.001$), when compared to veh + sal group ($F(11.60) = 34.55$). The effects of compounds PQM-56 (**6a**) or PQM-75 (**6d**) or morphine ($p < 0.001$) were observed in animals submitted to CCI surgery (Fig. 2D), in comparison to the veh + sal group. In addition, these effects were reversed when animals were treated with naloxane ($p < 0.001$), when compared to their respective controls (morp + salt, PQM-56 and PQM-75; $F(11.60) = 110.12$).

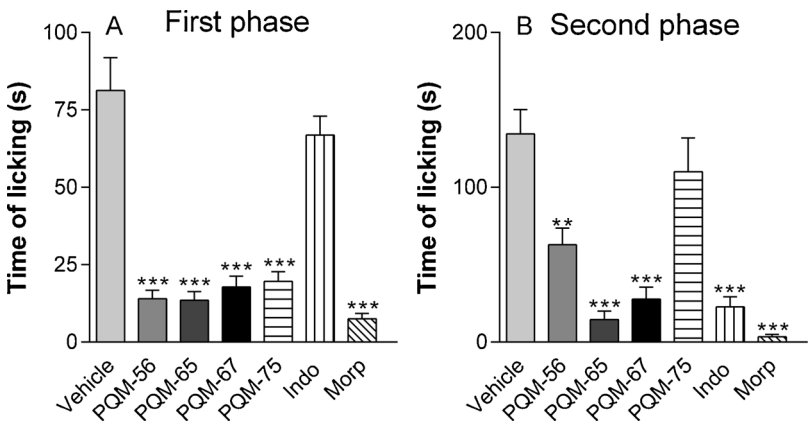

Fig. 1. Effects of 3-hydroxy-piperidinyl-*N*-benzyl-acyl-arylhydrazone derivatives PQM-56, PQM-65, PQM-67 and PQM-75 (**6a–d**) given by oral route on the licking induced by formalin in mice. Animals were pretreated orally with vehicle (1 ml/kg), derivatives compounds (100 µmol/kg) indomethacin (Indo; 100 µmol/kg) or morphine (Morp; 39 µmol/kg; i.p.) prior to formalin. The total time spent licking the hind paw was measured in the first (panel A) and second (panel B) phases after intraplantar injection of formalin. Each column represents the mean with the S.E.M. for eight mice in each group. The asterisks denote the significance levels when compared with the control group: *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$.