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Original research article

Analgesic and anti-inflammatory activity of 7-substituted purine-2,6-diones

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ABSTRACT

Background: In an effort to develop new analgesic and anti-inflammatory agents, we determined a series of 7-substituted purine-2,6-diones.
Methods: The obtained compounds (**1**–**6**) were evaluated pharmacologically in four *in vivo* models: the writhing syndrome, the formalin tests, the carrageenan-induced edema model and the zymosan-induced peritonitis. The influence of the investigated compounds on the phosphodiesterase (PDE) and PDE4B activity was also determined. In addition, determination of the antioxidant activity was determined by the FRAP assay.
Results: A majority of the tested compounds showed a significant analgesic and anti-inflammatory activity. The strongest analgesic and anti-inflammatory effect was observed for **1** and **2**. The active compound **1** was more efficient than theophylline in inhibiting the PDE and more efficient than rolipram in inhibiting the PDE4B activity. The tested compounds did not show significant antioxidant properties.
Conclusion: Active compounds (**1**–**6**) inhibited the PDE activity, while compound **1** significantly inhibited the PDE4B activity, what may suggest that this mechanism may be involved in their analgesic/anti-inflammatory properties.

Introduction

Inflammatory process is regulated by endogenous antioxidant mechanisms, involving glutathione, catalase (CAT), superoxide dismutase (SOD) and also by other exogenous substances possessing antioxidant properties [1,2]. The mechanism of tissue damage due to inflammatory processes has been partly linked to the release of reactive oxygen species (ROS) from activated neutrophils and macrophages. ROS support and spread inflammation by the stimulation of cytokine production (IL-1, TNF-α, INF-γ) which increase further neutrophil and macrophage influx. Thus, free radicals are indispensable mediators in inducing and maintaining inflammation [3]. In addition, phosphodiesterases (PDE), especially isozymes 4B, play a key role in inflammation. PDE4 isoenzymes are the predominant cAMP-degrading isozymes in most, if not all, immune and inflammatory cells, including T cells, B cells, eosinophils, neutrophils, monocytes, and macrophages [4]. By increasing cAMP levels, PDE4 inhibitors show a broad spectrum of anti-inflammatory effects in almost all inflammatory cells. Many PDE4 inhibitors have been evaluated in clinical trials for treatment of various inflammatory conditions [4].

PDE4 inhibitors have considerable efficacy, but they also have adverse effects, such as nausea and emesis which limit their dosing and subsequently their immunomodulatory activity. Thus, the development of PDE4 inhibitors with improved therapeutic indexes has been a major focus of pharmaceutical research searching for the treatment of chronic inflammatory diseases. The side effects of the PDE4 inhibitors are probably a result of their nonselectivity at all four PDE4 subtypes, and thus generation of new PDE4 inhibitors with subtype selectivity may provide clinical benefits by maintaining therapeutic efficacy while decreasing the side effects [4].

Theophylline has been used to treat airway diseases for more than 80 years [5]. The anti-inflammatory effects of theophylline have been previously described, and the proposed mechanisms of

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action vary from phosphodiesterase inhibition to mediator inhibition, increased apoptosis or inhibition of nuclear factor-κB (NF-κB) [6]. More recently it has been shown to have anti-inflammatory effects in asthma and chronic obstructive pulmonary disease (COPD) at lower concentrations [6]. In patients with COPD oxidative stress reduces histone deacetylase (HDAC) activity. The molecular mechanism of the anti-inflammatory effect of theophylline implicate the inhibition of PDE4 and histone deacetylase-2 activation, resulting in switching off the activated inflammatory genes [6–9]. In addition theophylline inhibits free oxygen radical production [9]. Furthermore, theophylline may inhibit the production of various inflammatory mediators. It is believed that the therapeutic effect of theophylline is based on its anti-inflammatory effect, as well as its bronchodilator activity [6,10]. Some studies suggest that theophylline has anti-inflammatory activity *in vivo* be influencing the glucocorticoid (GC)–GC receptor system and reducing TNF-α concentration [6,10].

Theophylline has a narrow therapeutic index; as a result, toxicity can be a significant problem with its chronic use. These disadvantages together with the fact that theophylline is nonselective for most of PDEs expressed in body cells, prompted us to modify its structure.

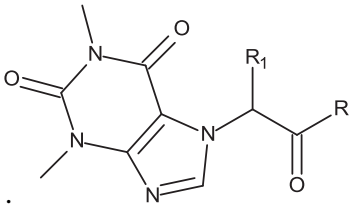
The aim of the present study was to evaluate analgesic and anti-inflammatory activity of six compounds: 7-theophylline acetic and propionic acids and their esters and amides. In addition, in order to clarify the mechanism of action of the active compounds, we determined their antioxidant action and the effect on phosphodiesterase, especially its 4B isoenzyme.

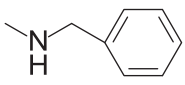
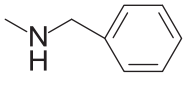
Materials and methods

Chemistry

Chemical structures of the evaluated 7-substituted purine-2,6-diones **1**–**6** are presented in Table 1. The previously reported 2-(1,3-dimethyl-2,6-dioxo-purinyl-7-yl) acetic acid (**1**) and its ethyl ester (**5**) and benzylamide (**3**) derivatives were synthesized according to the published procedure [1–3]. The 2-(1,3-dimethyl-2,6-dioxo-purinyl-7-yl) propanoic acid (**2**) and its ethyl ester (**6**) and benzylamide (**4**) derivatives were resynthesized by previously described methods [11–13].

Table 1
Chemical structures of the evaluated 7-substituted purine-2,6-diones **1**–**6**.



Compounds	*R*	R_1
1	–OH	–H
2	–OH	$-CH_3$
3		–H
4		$-CH_3$
5	$-OC_2H_5$	–H
6	$-OC_2H_5$	$-CH_3$

Pharmacology

Animals

The *in vivo* experiments were carried out on male albino Swiss mice weighing 18–26 g. The animals were housed in constant temperature facilities exposed to 12:12 light–dark cycle and maintained on a standard pellet diet and tap water given *ad libitum.* Control and experimental groups consisted of 6–8 animals each. The investigated compounds were administered intraperitoneally (*ip*) in the form of a suspension in 0.5% methylcellulose. Control animals received the equivalent volume of solvent.

All procedures were conducted according to the guidelines of ICLAS (International Council on Laboratory Animals Science) and were approved by The Local Ethics Committee of the Jagiellonian University in Krakow (agreement no. 47/2014).

Statistical analysis

The data are expressed as the mean ± SEM (standard error of the mean). Differences between vehicle control and treatment groups were tested using one- and two-way ANOVA followed by Duncan's multiple comparison test. When there were only two groups, a two-tailed Student's *t*-test was used. The difference of means was statistically significant if $p < 0.05$.

The writhing syndrome test

Mice were treated with 0.25 ml of 0.02% phenylbenzoquinone solution 30 min after *ip* administration of the investigated compound or the vehicle. Then, the mice were placed individually in glass beakers and 5 min were allowed to elapse. After that period each animal was observed for 10 min and the number of characteristic writhes was counted. The analgesic effect of the tested substances was determined by a decrease in the number of writhes observed [14]. The ED_{50} values and their confidence limits were estimated by the method of Litchfield and Wilcoxon [15].

The formalin test

The mice were pretreated with the test compound or the vehicle and were allowed to acclimate in Plexiglas observation chambers (20 × 30 × 15 cm) for 30 min before the test. Then, 20 μl of a 5% formalin solution was injected intraplantarly into the right hind paw using a 26-gauge needle. Immediately after formalin injection, the animals were placed individually into glass beakers and were observed during the next 30 min. Time (in seconds) spent on licking or biting of the injected paw in selected intervals, 0–5, 15–20, 20–25, and 25–30 min, was measured in each experimental group and was an indicator of nociceptive behavior [16]. The ED_{50} values and their confidence limits were estimated by the method of Litchfield and Wilcoxon [15].

Rota-Rod test

Animals were placed on a 1-inch diameter knurled plastic rod rotating at 24 rpm. Non-toxic (normal) mice can remain on a rod rotating at this speed almost indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as the number of animals exhibiting toxicity/number of animals tested. Animals are considered toxic if they fail this test on three successive attempts.

Carrageenan-induced edema model

Mice were divided into four groups, one of them being the control. In order to produce inflammation, 0.1 ml of 1% carrageenan