ABSTRACT

The treatment of chronic pain, e.g. neuropathic pain with currently available analgesics is not fully effective. Moreover, these drugs possess numerous side effects and have the potential to lead dependence. The search for safe and non-addictive analgesics with a new mechanism of action is one of the challenges of modern pharmacotherapy. TRPA1 channel antagonists that blocking the pain peripherally are considered one of the promising strategy for the treatment of neuropathic pain.

The aim of the present thesis was to design and synthesize novel amide and hydrazide derivatives of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione and 1*H*-benzimidazole, and to determine their antagonistic activity against the TRPA1 channel *in vitro*. Compounds with this mechanism of action may be effective in the treatment of chronic pain, including neuropathic pain and inflammatory conditions.

A library of 135 compounds belonging to the amide and hydrazide derivatives of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione **7**-**26** (series Ia) and **123**-**134** (series Ib) and 8-amino- and 8-alkoxy-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione **51**-**78** (series IIa), **93**-**119** (series IIIa), **136**-**139** (series IIb) and **141**-**143** (series IIIb), as well as 1*H*-benzimidazole **150**-**170** (series IVa) and **181**-**192** (series IVb) and 2-piperydyno-1*H*-benzimidazole **174**-**177** (series Va) and **194**-**197** (series Vb) was designed by structural modification of HC-030031 - the model TRPA1 channel antagonist and drug-likeness analysis *in silico*.

The designed new compounds were obtained by multi-step procedure, using classical chemical methods, and microwave-assisted synthesis. In the first step, intermediate esters were obtained, followed by acids and hydrazides. In the final step, the condensation reaction with amines or aldehydes took place, respectively leading to the final *N*-phenylamides (series Ia-Va) and *N*'-benzylidenohydrazides (series Ib-Vb). The identity of the new final products and intermediates was confirmed by spectral (^{1}H NMR) and for the compounds **93**, **94**, **97**, **98**, **101**, **102**, **105**-**107**, **109**-**117** by elemental analysis. Molecular weights were verified by UPLC/MS. The purity of the obtained compounds was determined using the UPLC/MS technique.

For the all 135 novel compounds, the antagonistic activity of the TRPA1 channel was determined using a fluorometric calcium imaging assay and HEK-293 cells stably expressing human TRPA1 channel. The biological tests *in vitro* allowed to select from the series of tested compounds the potent TRPA1 channel antagonists, with better antagonistic activity than that of HC-030031.

Analysis of the structure-activity relationship (SAR) in the search groups of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione and 1*H*-benzimidazole derivatives allowed to determine the influence of structural elements on the antagonistic activity of the TRPA1 channel. Among others, the length of the aliphatic chain and its branching, the type of substituent in the amide and hydrazide moieties, introduction of an additional amino or alkoxy substituent, and replacement of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione with a 1*H*-benzimidazole system were assessed.

For representative novel TRPA1 channel antagonists from the group of amide (compounds **94** and **102**; series IIIa) and hydrazide (compounds **142** and **143**; series IIIb) derivatives of 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, a potential binding mode to the TRPA1 ion channel was determined using molecular modeling. The obtained results allowed to draw conclusions regarding the structural requirements for this class of antagonists, ensuring optimal binding with this biological target.

Based on the results of *in vitro* tests, the most promising compounds **94** and **102**, with stronger antagonistic activity than the HC-030031 were selected for *in vivo* pharmacological study in order to confirm the analgesic

and anti-inflammatory properties. In the second (inflammatory) phase of the formalin test, compounds **94** and **102** showed stronger analgesic activity than that of HC-030031. Compounds **94** and **102** revealed antiallodynic properties in oxaliplatin-induced neuropathic pain model. In the von Frey test, the analgesic activity of the tested compounds was comparable or weaker than that of pregabalin, used as a positive control, while in the cold plate test it was higher than that of pregabalin. Both evaluated compounds also showed more potent anti-inflammatory properties than HC-030031 in the carrageenan-induced edema model. Compound **102** possessed comparable activity to ketoprofen (positive control), the effect of compound **94** was weaker than reference drug.

The findings of this study confirmed that the presented novel TRPA1 channel antagonists are promising for the treatment of chronic pain, including neuropathic pain and inflammatory conditions.