European Journal of Pharmacology 791 (2016) 195–204

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Design, synthesis and pharmacological evaluation of new anti-inflammatory compounds

Amanda F. Cidade [a], Patrícia A. Vasconcelos [b], Daiany P.B. Silva [b], Iziara F. Florentino [b], Géssica A. Vasconcelos [c], Boniek G. Vaz [c], Elson A. Costa [b,*], Luciano M. Lião [d], Ricardo Menegatti [a]

[a] Faculty of Pharmacy, Laboratory of Medicinal Pharmaceutical Chemistry, Federal University of Goiás, Goiânia, GO, Brazil
[b] Institute of Biological Sciences, Department of Pharmacology, Federal University of Goiás, Campus Samambaia, Goiânia, GO, Brazil
[c] Chemistry Institute, Laboratory of Chromatography and Mass Spectrometry, Federal University of Goiás, Goiânia, GO, Brazil
[d] Chemistry Institute, Federal University of Goias, Campus Samambaia, Goiânia, GO, Brazil

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
12 August 2016
Accepted 29 August 2016
Available online 30 August 2016

Keywords:
Pyrazole compounds
LQFM-146
LQFM-147, LQFM-148
Inflammation

ABSTRACT

Inflammatory diseases and pain are among the main problems that significantly influence the lifestyle of millions of people and existing therapies are not always effective and can cause several adverse effects. In this context, the molecular modifications or synthesis of compounds continue being the best strategies for the identification of new compounds for the treatment of pain and inflammation. The aim of this study was to evaluate the analgesic and anti-inflammatory activities of new analogues of pyrazole compounds containing subunits N-phenyl-1-*H*-pirazoles and 1,3,4-oxadiazole-2(3*H*)-thione, LQFM-146, LQFM-147 and LQFM-148. In the acetic acid-induced abdominal writhing test, treatments with LQFM-146, LQFM-147 or LQFM-148 at doses 89, 178 and 356 μmol/kg p.o. reduced the abdominal writhing in a dose-dependent manner. In the formalin test, these compounds at dose 178 μmol/kg p.o. reduced the licking time only in inflammatory phase of this test, suggesting an antinociceptive effect dependent of the anti-inflammatory effect. The treatment with the three compounds in intermediate dose (178 μmol/kg p.o.) reduced the edema at all tested time points in the carrageenan-induced paw edema test and reduced polymorphonuclears cell migration, activity myeloperoxidase and TNF-α levels in the carrageenan-induced pleurisy test. Our date suggest that the new compounds LQFM-146, LQFM-147 and LQFM-148 possess satisfactory anti-inflammatory and antinociceptive effects that involves the reduction of pro-inflammatory cytokines and inhibition of the myeloperoxidase enzyme.

1. Introduction

Inflammation is the response of the organism to an external challenge or tissue injury, giving birth to immunological processes of repair and healing. However, prolonged inflammation ceases to be a beneficial event and contributes to the pathogenesis of many diseases, such as, rheumatoid arthritis and osteoarthritis, are characterized by accumulation of inflammatory cells in the joints and leads to joint damage (Gilroy et al., 2004).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammation symptoms and signs, and exert their effects by inhibition of the synthesis of cyclooxygenase (COX), key enzymes in the production prostaglandins, biosynthesized from arachidonic acid. There are two main types of cyclooxygenases enzymes (COX-1 and COX-2). COX-1 is constitutively and responsible for, among others, by a cytoprotective activity, while COX-2 is induced during the inflammatory process, pain and fever (Rainsford, 2007; Jahn et al., 2008; Rao and Knaus, 2008).

The first line of clinical treatment for the inflammatory disorders is NSAIDs via COX pathway. Due the gastrointestinal bleeding and toxicity associated with NSAIDs long term use does the investigation of new analgesic and anti-inflammatory agents a major challenge. Multiple-ligand drugs using hybridization techniques can act on a single or multiple targets with synergistic action and minimize toxicity or adverse reactions (Abdellatif et al., 2009; Hernández et al., 2012; Shenvi et al., 2015).

COX-2 inhibitors are marketed as a new generation of NSAIDs, their mechanism of action exhibit less gastrointestinal toxicity than traditional NSAIDs, but studies have shown that continuous use causes cardiovascular effects and induced asthma frames.

* Correspondence to: Laboratório de Farmacologia de Produtos Naturais e Sintéticos, Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, CP 131, CEP 74001-970, Goiânia, GO, Brazil.
E-mail address: xico@ufg.br (E.A. Costa).

http://dx.doi.org/10.1016/j.ejphar.2016.08.033
0014-2999/

Then, the attention has been focused another acid arachidonic metabolic pathway, the lipoxygenases (LOX) pathway, since it possesses important action in the production and maintenance of inflammation. In this context, dual inhibitors on the routes of COX-2 and 5-LOX have shown significant anti-inflammatory activity supported by tests "in vitro" and "in vivo" with a reduction of adverse action (Leval et al., 2002; Burnett and Levy, 2012; Hwang et al., 2013).

Synthetic approaches based on chemical structure of NSAIDs are being conducted in order to improve its safety profile. Thus structural changes in drug flufenamic acid led to the derived (2), which not only kept his inhibiting the synthesis of prostaglandins (COX), but also featured 5-LOX inhibitor activity (Boschelli et al., 1993; Julemont et al., 2004). This derivative has in its structure the nucleus 1,3,4-oxadiazole-2(3*H*)-thione, widely described in the literature due to the anti-inflammatory activities, giving for derived low gastric toxicity (Oliveira et al., 2012; Khalilullah et al., 2012).

Sub-units *N*-phenyl-1-*H*-pirazoles are described by their anti-inflammatory and analgesic activities, these come gained prominence after the development of the Celecoxibe (1) how COX-2 selective inhibitor (Bansal et al., 2014; Küçükgüzel; Şenkardeş, 2015). In this context, our group has published studies on new pyrazole compounds, 5-(1-(3-fluorophenyl)-1-*H*-pyrazol-4-yl)-2*H*-tetrazole (1), shown analgesic activity involving the peripheral opioid receptors and activation of the NO/cGMP/K(ATP) pathway (Florentino et al., 2015a). In addition, we also found that 5-(1-(3-fluorophenyl)-1-*H*-pyrazol-4-yl)-2*H*-tetrazole (1) possess anti-inflammatory activity involving the reduction leukocyte migration, the TNF-α and IL-1β levels and decreased of the myeloperoxidase activity. Furthermore, was observed that the inhibition of nitric oxide synthase promoted by L-NAME reduced the anti-inflammatory effect of this compound, shown that the nitric oxide is important to this effect (Florentino et al., 2015b).

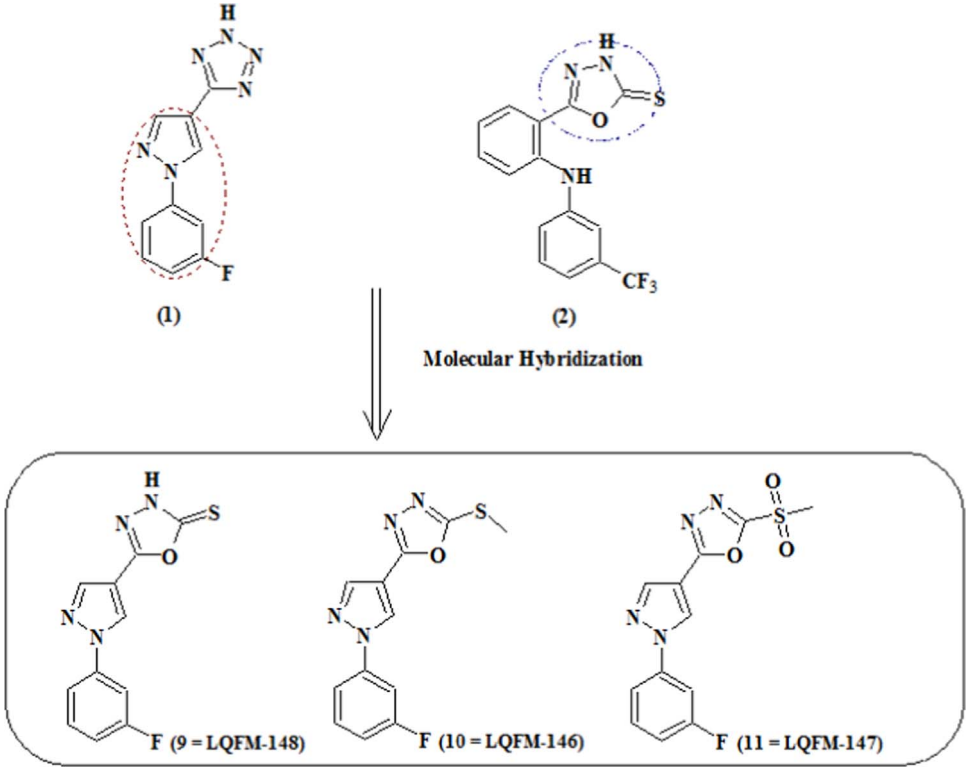
Thus, stimulated by perspective in obtaining compounds with anti-inflammatory activity satisfactory, we describe the synthesis and evaluation of analgesic and anti-inflammatory activities of new analogues containing subunits N-phenyl-1-*H*-pirazoles and 1,3,4-oxadiazole-2(3*H*)-thione, with potential for clinical development.

In the scope of a research program aimed at drug development for treatment of anti-inflammatory disease, we describe in the present study the synthesis and biological evaluation of new heterocyclic compounds (**9–11**). The compounds (**9–11**) were designed through strategy of hybridization molecular from 5-(1-(3-fluorophenyl)-1-*H*-pyrazol-4-yl)-2*H*-tetrazole (**1**) and derivative (**2**) (Scheme 1). The synthetic route of title compounds 9–11 is shown in Scheme 2.

2. Materials and methods

2.1. General

Reactions were monitored by TLC using commercially available precoated plates (Whatman 60 F254 silica) and developed plates were examined under UV light (254 and 365 nm). ^{1}H and ^{13}C NMR spectra were recorded in the indicated solvent on Bruker Avance III 500 MHz spectrometer. Chemical shifts are quoted in parts per million downfield from TMS and the coupling constants are in Hertz. Infrared spectra were recorded on a Perkin-Elmer Spectrum Bx-II FT-IR System spectrophotometer instrument as films on KBr discs. Melting points were performed using a Marte melting point apparatus, and the results were uncorrected. All assignments of the signals of ^{1}H and ^{13}C NMR spectra are consistent with the chemical structures of the products described. The organic solutions were dried over anhydrous sodium sulfate and organic solvents were removed under reduced pressure in a rotary evaporator. Mass spectra (MS) were obtained with a Q-Exactive (Thermo Scientific, Bremen, Alamanha). The sample preparation for mass spectrometry analysis consisted of diluting 1 mg of each sample in 1 ml of methanol. To perform the analysis in positive



Scheme 1. Structural design of the derivate 9 (LQFM-148), 10 (LQFM-146) and 11 (LQFM-147).