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# Analgesic and anti-inflammatory properties of synthesized imidazopyrinidyl-chalcones: Relationship activity and structure

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## Abstract

**Background**: The effective management of pain in clinic is still challenging practitioner because of the many side effects associated with the use of current drugs, which can even affect life quality of the patients. Chalcones are described as compounds that have various pharmacological activities such as antioxidants, anti-inflammatories, anticancer, including antifungals and antibacterials. The objective of this study was to evaluate the analgesic and anti-inflammatory properties of two (2) synthesized imidazopyridinyl-chalcones.

**Materials and Methods**: Imidazopyridinyl-chalcones tested V1 and V2, different by the substituent, type hydroxyl group for V1 and diethylamine for V2, were synthesized by the Department of Organic and Therapeutic Chemistry of Pharmaceutical and Biological Sciences (Côte d'Ivoire). The analgesic and anti-inflammatory activities were performed in mice and rats respectively by acetic acid-induced writhes test according to the method described by Koster *et al* and formalin-induced irritation test performed by Dubuisson *et al*.

**Results**: V1 and V2 showed inhibition of contortions induced by acid acetic 1%, with greater analgesic effect for V2 at lower doses, while the opposite was observed for V1. At concentration of 3.125 mg/kg b. wt., V2 was around 77.78% and V1 reach this percentage around 72.22% at 50 mg/kg b. wt., whereas that of paracetamol 100 mg/kg b. wt., used as a reference was about 48%. The anti-inflammatory effect of V2 (43.51%) was also higher compared to V1 (34.85%) at 3.125 mg/kg b. wt., but when doses increases at 12.25 mg/kg b. wt., the effect was non-significantly different to that of ketoprofen (69.98%) at 10 mg/kg b. wt., and range 48.57% and 47.73% respectively for V2 and V1.

**Conclusion:** Imidazopyridinyl-chalcones is a good model for the development of new molecules and it would appear that the presence of electron donor group like diethylamine is better than hydroxyl to push up analgesic and/or anti-inflammatory activities.

**Keywords:** Chalcones, pain, inflammation.

## 1. Introduction

Pain is a complex symptom almost constant in pathologies for which the care does not require any delay [1]. Various types of medications are offered clinically to control pain [2], especially opioids and non-steroidal anti-inflammatory drugs; however these products only relieve 50% of pain in approximately 30% of patients [3]. In addition, many side effects are reported that can significantly affect the health and quality life of patients. [4]

Faced with these findings, the development of new drugs effective against pain, interfering little or not on physiological functions, remains a major challenge of medicinal chemistry. [5]

Chalcones are widely describe in literature, as compounds that have various pharmacological activities such as anticancer [6], anti-inflammatory [**7**], including antioxidants. [8]

A previous work undertaken in Pharmacology Laboratory (Training and Research Unit of Pharmaceutical and Biological Sciences), found that, two imidazopyridinyl-chalcones showed an antioxidant and non-cytotoxic properties on healthy human cells (HUVEC). These compounds were retained for further investigation, because good antioxidant and non-cytotoxic substances are potential candidates for analgesic and anti-inflammatory drugs research. [9]

The objective of the current study was to assess the pharmacological properties, such as analgesic and anti-inflammatory activities of 2 imidazopyridinyl-chalcones (V1, V2), so that to propose them as potential candidate drug against moderate pain and inflammatory manifestations.

## 2. Materials and methods

### 2.1 Samples under study

Imidazopyridinyl-chalcones (V1, V2), were synthesized by the Laboratory of Therapeutic Chemistry of Training and Research Unit of Pharmaceutical and Biological Sciences (Côte d'Ivoire). V1 powder is yellow whereas V2 is orange, and those compounds are soluble in carboxymethylcellulose (CMC), and insoluble in water and the usual organic solvents, excepted dimethylsulfoxide (DMSO).

### 2.2 Chemicals

Acetic acid (Cooper, France), Carboxymethyl cellulose (CMC, Prolabo®, Belgium); paracetamol (Doliprane® packet, Aventis Pharma, France); formalin (Sharlau, Germany); ketoprofen (Sigma-Aldrich, France)

### 2.3 Experimental Animals

Healthy adult Wistar albino rats (*Rattus norvegicus*), weighting between 175 ± 25g, and female Swiss albino mice weighing between 26 ± 4 g, were used for the study. In the pet shop, inside Training and Research Unit of Pharmaceutical and Biological Sciences, Felix Houphouet-Boigny University, Ivory Coast, animals were kept in large spacious, hygienic plastic cages during the course of the experimental period on standard environmental temperature of 26±1°C and relative humidity 50±5% with 12h light-dark cycle. The rats were fed with FACI® (Food Manufacturing in Ivory Coast) pellets and drank tap water.

### 2.4 Pharmacological tests

#### 2.4.1 Analgesic activity experiments: Acetic acid-induced writhes test

The acetic acid-induced writhes test was carried out according to **Koster and *al.***, 1959, method with slight modification. [10]

Intraperitoneal injection of acetic acid 1% in the mouse causes a pain syndrome which is reflected by abdominal contortions or "cramping" characterized by contractions of dorso-abdominal region muscles, associated with stretching of hind legs. Analgesic, anti-inflammatory and myorelaxant substances protect animals against these painful syndromes.

✓ **Operating mode of writhing test**

Animals fasted for 4 hours on experiment day were divided into 7 homogeneous weighing groups of six mice. The volume administered by gavage to each animal was function of body weight at a rate of 10 mL / kg b. wt.:

- Group 1 as control, constituted with animals that received only CMC 2% suspension
- Group 2 as reference, received paracetamol at a rate of 100 mg / kg b.w.
- The other test groups (3 to 7) received different doses (from 100 to 1.625 mg / kg b. wt.) of samples V1 and V2

The protocol was led in this way:

- Performing a sample concentration range (from 10 to 0.1625 mg/mL), by successive dilutions to half (1/2) in CMC 2% suspension
- T0: Taking up substances to animals and waiting thirty minutes
- T30: Applying the pain syndrome by intraperitoneal injection of acid acetic 1%, and waiting 5 minutes
- T35-55: Counting the crampings done by the animals over a period of twenty minutes.

Cramps inhibition percentage was estimated according to the following formula:

$$\text{Pain inhibition (\%)} = \frac{\text{Number of writhes in control group} - \text{Number of writhes in treated group}}{\text{Number of writhes in control group}} \times 100$$

### 2.5 Anti-inflammatory activity experiments: formalin-induced irritation test

Evaluation was conducted using the method described by Dubuisson *et al* [11] with some modifications [12]. The injection of phlogogenic substance (formalin) under aponeurosis plantar of the rat hind paw, leads appearance of a painful inflammatory syndrome which rises in two phases:

- A neurogenic phase (phase 1) ranging from 0 to 5 minutes after the application of the stimulus. This step corresponds to a central stimulation of the pain.
- An inflammatory phase (phase 2) ranging from 15 to 30 minutes after the application of the stimulus.

Preventive administration of an anti-inflammatory substance inhibits inflammatory phenomena during the second phase of observation. Opioids inhibit both phases.

✓ **Operating mode of formalin test:**

Animals fasted for 16 hours before the experiment had free access to water. Rats were divided into 9 homogeneous weighing groups of six rats. The volume administered by gavage to each animal was function of body weight at 10 ml / kg b. wt.:

- Group 1 as control, constituted with animals that received only CMC 2% suspension
- Group 2 as reference, received ketoprofen at 10 mg / kg b. wt.
- The other test lots (3 to 9) received three doses (with best analgesic effects) of the compounds V1 and V2

The protocol was led in this way:

- Preparing a solution of formalin at 2.5% by homogenizing it with a physiological saline solution (NaCl 0.9%)

Before the experiment, familiarising the rats with the environment, putting them in a transparent Plexiglas box (20cm x 20cm x 30cm) for 30 min (the box is equipped on three sides with a mirror inclined at 45° on the ground, which allows to observe the behaviour of rats)

- T0: Taking up substances to animals and waiting thirty minutes
- T30: Applying inflammatory pain syndrome by injection of 50 µL of formalin into aponeurosis plantar of rat hind paw, and put it in the observation box
- T30-35: Recording in seconds the duration of paw licking during first 5 minutes (neurogenic phase) and waiting 10 minutes
- T45-60: Recording in seconds the duration of paw licking for 15 minutes (inflammatory phase)

Inflammation percentage inhibition of was calculated according to precedent formula used for writhing test.

$$\textbf{Inflammatory inhibition (\%)} = \frac{\text{Licking time in control group} - \text{Licking time in treated group}}{\text{Licking time in control group}} \times 100$$

## 2.6 Ethical Approval

The experimental procedures were conducted after the approval of the Ethical Guidelines of the University Committee on Animal Resources in Ivory Coast. All procedures were in strict accordance with the guidelines for Care and Use of Laboratory Animals and the statements of the European Union regarding the handling of experimental animals (86/609/EEC). [13]

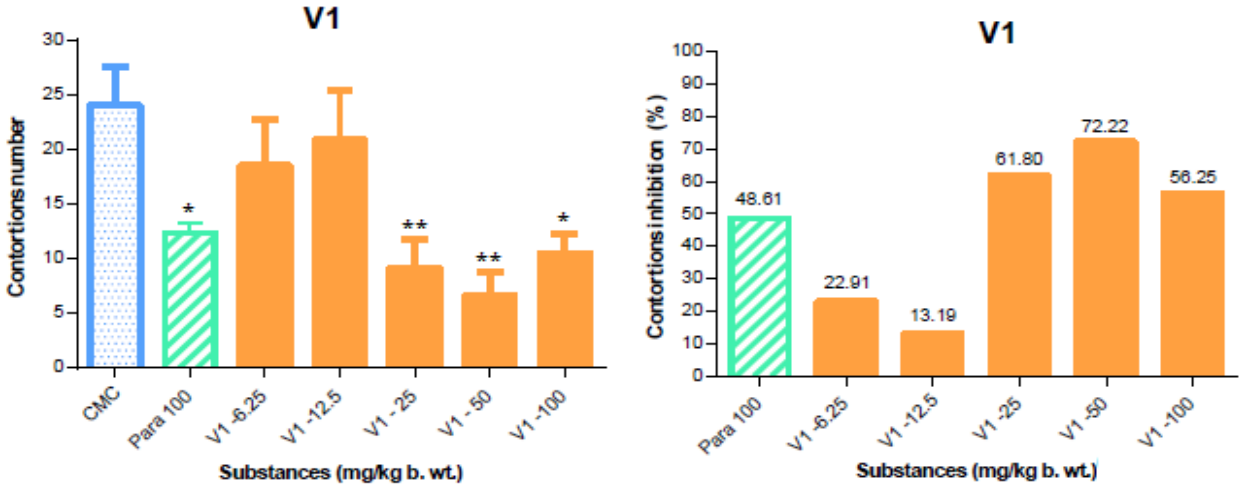
## 2.7 Statistical Method

The data were analysed using Graph Pad Prism® 7.0 software. The Dunnett's test compared mean values ± SD (standard deviation) by analysis of variance (ANOVA) at risk $\alpha = 0.05$.

The codification for statistically significant difference was: *: $p \geq 0.01$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$

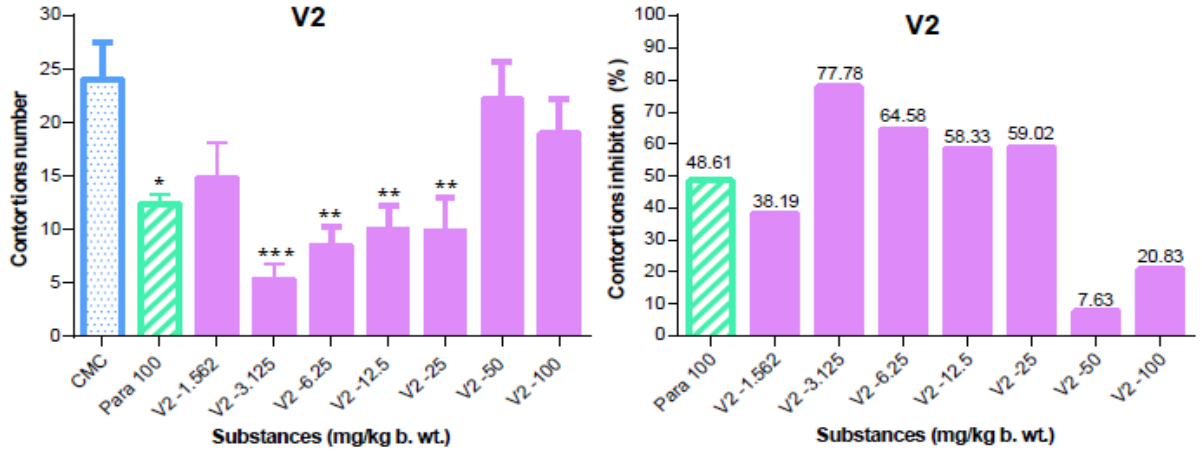
# 3. Results

**Figure 1: V1 effects against pain syndrome**



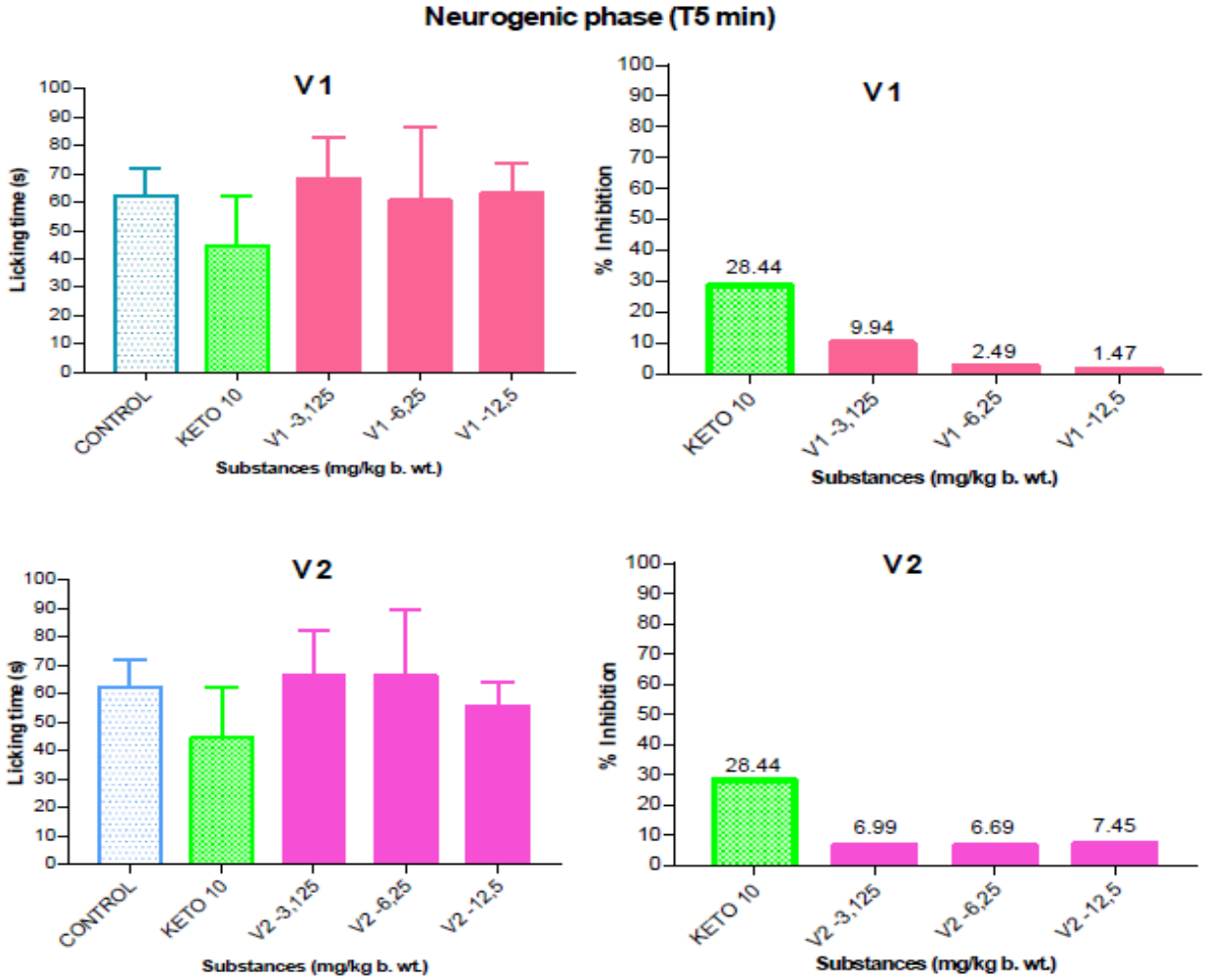

*Dunnett's test at risk α = 0.05%. Values expressed on average ± SD (standard deviation)*
**: p ≥ 0.01: Significant difference compared to Control (CMC): para 100 (p = 0.0494); V3-100 mg/kg (p = 0.0177)*
***: 0.001 ≤ p ≤ 0.01: V3-25 mg/kg (p = 0.0080); V3-50 mg/kg (p = 0.0016)*

**Figure 2: V2 effect against pain syndrome**



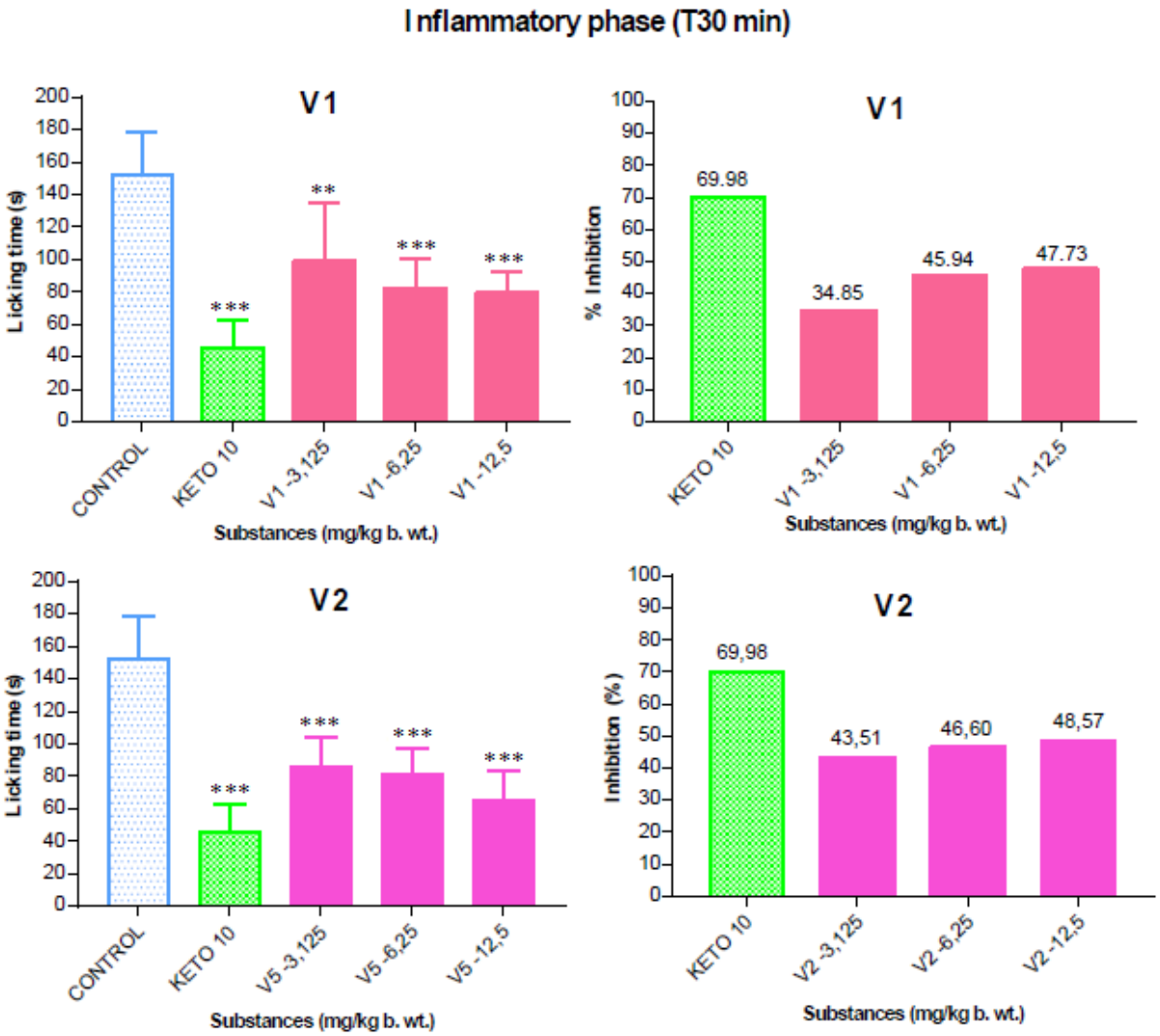

*Dunnett's test at risk α = 0.05%. Values expressed on average ± SD (standard deviation)*
**: p ≥ 0.01: Significant difference compared to Control (CMC): para 100 (p = 0.0494)*
***: 0.001 ≤ p ≤ 0.01: V5-6.25 mg/kg (p = 0.0016); V5-12.5 mg/kg (p = 0.0051); V5-25 mg/kg (p = 0.0045)*
****: p ≤ 0.001: V5-3.125 mg/kg (p = 0.0001)*

**Figure 3: V1 and V2 effect on the neurogenic phase**




*Dunnett's test at risk α = 0.05%. Values expressed on average ± SD (standard deviation).*
*No Significant difference compared to Control: V1-3.125 mg/kg (p = 0. 0659); V1-6.25 mg/kg (p =0.2967); V1-12.5 mg/kg (p = 0.1917); V2-3.125 mg/kg (p = 0.0761); V2-6.25 mg/kg (p =0.0793); V2-12.5 mg/kg (p = 0.5624)*

**Figure 4: V1 and V2 effect on the inflammatory phase**




*Dunnett's test at risk α = 0.05%. Values expressed on average ± SD (standard deviation).*
***: 0.001 ≤ p ≤ 0.01: Significant difference compared to Control: V1-3.125 mg/kg (p = 0.0050)*
****: p ≤ 0.001: V1-6.25 mg/kg (p = 0.0003); V1-12.5 mg/kg (p = 0.0002); V2-3.125 mg/kg (p = 0.0003); V2-6.25 mg/kg (p = 0.0003); V2-12.5 mg/kg (p = 0.0002)*

## 4. Discussion

The writhing test is a nociception model used to screen analgesic agents with an opioid mechanism action or not. [14,15]

The doses of V1 (25, 50, 100 mg/kg b. wt.) and V2 (3.125, 6.25, 12.50; mg/kg b. wt.) significantly inhibited the pain induced by the injection of acetic acid 1% compare to control. The effects of V2 (58.33 to 77.78%) which appear at the lower doses, were greater than that of V1 (56.25 to 61.80%), and similar or slightly superior to that of paracetamol 100 mg/kg b. wt., also names acetaminophen, used as a reference and which pain inhibition percentage was about 48%.

Therefore, V1 and V2 could have inhibited the release of many chemical mediators, such as histamine, bradykinin and serotonin, which are involved in nociceptors sensitization at the origin of the onset of pain syndrome [16,17], protecting animals from abnormal motor coordination.

It makes sense to evoke the strong probability that V1 and V2 would act as paracetamol which peripheral well-known action results from the inhibition of algogenic mediator's release [18]. They could also act in central nervous system performing like agonist of transient receptor potential channel (TRP) including TRPA1, which activation results in depolarization by calcium influx [19,20], thus reducing the neuronal excitability and the release of algogenic neurotransmitters in the postsynaptic nerve fibers.

The formalin-induced paw irritation test is widely used to evaluate analgesic and anti-inflammatory properties of compounds. The administration of formalin in the rat's

paw causes direct stimulation of nociceptors (neurogenic phase: phase 1) leading to the release of chemical pain mediators such as substance P, bradykinin, histamine, serotonin, [21], which secondarily activate the production of prostaglandins at the origin of inflammation (inflammatory phase: phase 2). [22]

Opioid drugs, such as narcotic analgesics [23], inhibit both phases, whereas non-steroidal anti-inflammatory drugs such as ketoprofen inhibit only the late phase. During the neurogenic phase, best analgesic doses of V1 or V2 did not significantly inhibit pain syndrome. In the second phase, at 3.125 mg/kg b. wt., V2 (43.51%) anti-inflammatory effect was higher compared to V1 (34.85%), and this effect was non-significantly different to that of ketoprofen (69.98%) at 10 mg/kg b. wt., when doses scale up at 12.5 mg/kg b. wt., for V2 (48.57%) and V1 (47.73%).

The introduction of diethylamine at position 4 (V2), made possible to obtain compounds with analgesic and/or anti-inflammatory activities greater than hydroxyl group at position 2 (V1). The mechanism of these compounds could be exerted by blocking the expression of cyclooxygenases, in particular COX2, and decreasing the release of cytokines and proinflammatory molecules such as IL-1β, IL-6, and TNF-α. [24, 25]

## 5. Conclusion

Imidazopyridinyl chalcones seem to be adequate models for the development of new molecules with analgesic and / or anti-inflammatory activities. The intensity of the activity would be better when there are electron-donating substituents of diethylamine profile than the hydroxyl type. These compounds are helpful to design new future potential agent against moderate pain and inflammatory manifestations.

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