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Synthesis and evaluation of the anti-nociceptive and anti-inflammatory activity of 4-aminoquinoline derivatives

Raquel de Meneses Santos [a], Pooline Rocha Barros [a], Janaína Heberle Bortoluzzi [a], Mario Roberto Meneghetti [a,*], Yolanda Karla Cupertino da Silva [b], Amanda Evelyn da Silva [b], Mariana da Silva Santos [b], Magna Suzana Alexandre-Moreira [b,*]

[a] GCaR—Grupo de Catálise e Reatividade Química, Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, AL, Brazil
[b] LaFI—Laboratório de Farmacologia e Imunidade, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, Maceió, AL, Brazil

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ABSTRACT

In this paper, we describe the synthesis and pharmacological evaluation of a series of 4-aminoquinolines. The compounds were characterised and tested in models of pain and inflammation, using the writhing test with acetic acid, formalin test, peritonitis test by zymosan and arthritis test with Freund's adjuvant complete assay. The results revealed that all of the 4-aminoquinolines that were prepared promoted anti-nociceptive activity as well as acute and chronic anti-inflammatory effects, with marked activity in the derivates labelled with **BAQ** and **7-CF$_3$-MAQ**. After 7 days of treatment, **7-CF$_3$-MAQ** did not induce significant hepatotoxicity, gastrotoxicity or nephrotoxicity.

1. Introduction

Inflammation is a biological reaction to disrupted tissue homeostasis. At its basic level, it is a tissue-destroying process that involves the recruitment of blood-derived products, such as plasma proteins, fluid and leukocytes, into perturbed tissue. This migration is facilitated by alterations in the local vasculature that lead to vasodilatation, increased vascular permeability and increased blood flow.[1] When not regulated properly, excessive inflammation can have devastating effects, resulting in excessive collateral damage and pathology.[2] Thus, several drugs are used to treat inflammation, including chloroquine, a well known anti-malarial drug that is also classified as a modifying agent for rheumatic disease because of its immunosuppressive potential. This context prompted us to search and test 4-aminoquinoline derivatives (analogous molecules to chloroquine) in order to evaluate their potential as anti-nociceptive and anti-inflammatory agents. For that study, we synthesised and tested in models of pain and inflammation four 4-aminoquinoline compounds labelled **BAQ**, **7-Cl-MAQ**, **7-CF3-MAQ** and **2-CF3-MAQ** (Fig. 1).

Anti-rheumatic disease-modifying drugs (ARDMDs) are a group of drugs with the potential to decrease and prevent joint damage and preserve the functionality of the joints. Synthetic ARDMDs include chloroquine, hydroxychloroquine, sulfasalazine, methotrexate and cyclosporine.[3] Chloroquine and its analogues are used to treat diseases other than malaria, including various chronic diseases such as systemic lupus erythematosus, discoid lupus and sarcoidosis, among others.[4] For that reason, we develop and test aminoquinoline derivatives as drug prototypes against rheumatism.

2. Results and discussion

2.1. Synthesis of the compounds

All aminoquinoline derivatives were prepared in good yields, using the same strategy developed elsewhere (Fig. 1), with slight modifications.[5] The mechanisms of all of the synthetic reactions operate via a S_NAr route. All compounds were isolated as white or slightly yellow solids and were fully characterised by NMR, MS, and IR spectroscopy.

2.2. Spectroscopic characterisation

The ^1H and ^{13}C NMR spectra as well the infrared spectra of **7-Cl-MAQ** and **BAQ** were the same as those already published

* Corresponding authors. Tel.: +55 82 3214 1744; fax: +55 82 3214 1384 (M.R.M.); tel.: +55 82 3214 1684; fax: +55 82 3214 1528 (M.S.A.-M.).
E-mail addresses: mrm@qui.ufal.br (M.R. Meneghetti), suzana.magna@gmail.com (M.S. Alexandre-Moreira).

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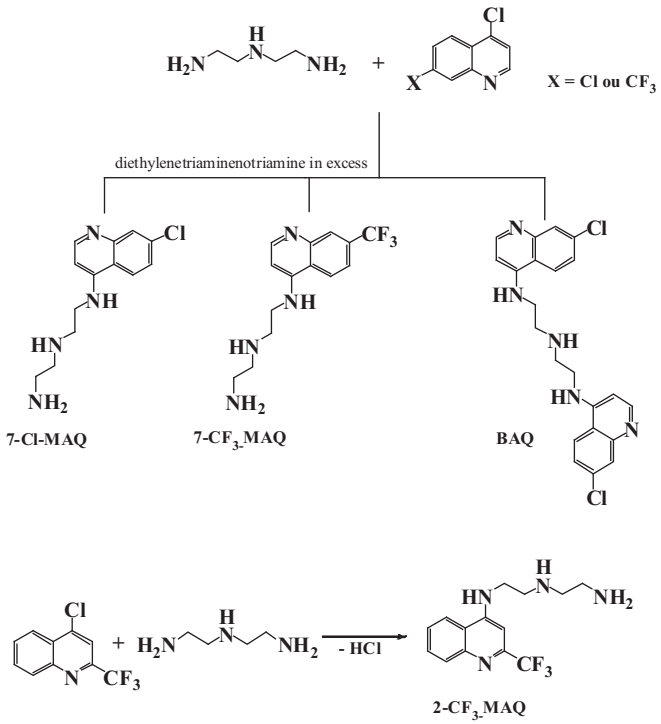

Figure 1. Design of the 4-aminoquinoline derivatives.

elsewhere.[5] For **2-CF3-MAQ**, the ^{1}H NMR spectrum displayed five signals (two triplets, two doublets and one singlet) for the aromatic hydrogen atoms that were observed between 8.16 and 6.79 ppm, as well as an additional four triplets related to the methylenic groups between 3.53 and 2.73 ppm. The hydrogen signals of the amino groups were either not observed or had integration values lower than those predicted, owing to fast H/D exchange with the deuterated solvent. In the case of the ^{13}C NMR spectrum of this compound, it was possible to identify the signal related to the CF_3 group (124.8 ppm), the nine signals related to the aromatic carbons (between 156.7 and 97.1 ppm) as well as the four signals associated with the methylenic carbons (between 54.8 and 44.2 ppm). In the infrared spectrum of **2-CF3-MAQ**, typical absorption bands for this kind of chemical structure were observed. Similarly, **7-CF3-MAQ** presents a ^{1}H NMR spectrum with five signals related to the aromatic hydrogen atoms (four doublets and one singlet) as well as the four signals associated with the methylenic groups; the expected 14 signals in the ^{13}C NMR spectrum were also observed. In addition, the ^{13}C NMR spectrum of **7-CF3-MAQ** shows the signals related to the CF_3 group (124.1 ppm), the nine aromatic carbons (between 155.7 and 97.8 ppm) and the four peaks associated with the methylenic carbons (between 54.1 and 43.9 ppm). In the infrared spectrum of **7-CF3-MAQ**, typical absorption bands for this kind of chemical structure were observed.

2.3. Biological assays

The present study was undertaken to evaluate the anti-nociceptive and anti-inflammatory effects of 4-aminoquinolines derivatives on four classical models: the acetic-acid-induced writhing test, the formalin test, peritonitis by zymosan and arthritis assessment by the complete Freund's adjuvant (CFA) assay in rats.

The acetic-acid-induced writhing test is a classical model that is widely used to screen prototype agents with analgesic activity when neurogenic and/or inflammatory pain are involved.[6] The intra-peritoneal (ip) administration of the 4-aminoquinoline derivatives (**BAQ**, **7-Cl-MAQ**, **7-CF3-MAQ** and **2-CF3-MAQ**) at concentrations of 100, 30, 3 and 0.3 μmol/kg (Table 1) caused a significant reduction in the number of writhing episodes induced by acetic acid compared to the ID_{50} of the control groups: **BAQ** (11.5 ± 3.7 μmol/kg, 97,2%), **7-Cl-MAQ** (39.4 ± 13.4 μmol/kg, 86.3%), **7-CF3-MAQ** (15.3 ± 4.2 μmol/kg, 96.7%) and **2-CF3-MAQ** (26.6 ± 6.4 μmol/kg, 95.1%). **BAQ**, **7-CF3-MAQ** and **2-CF3-MAQ** cause a reduction in the number of writhes with high potency and efficacy, which are higher than the standard drug, dipyrone (36.8 ± 6.7 μmol/kg, 74.8%). The **BAQ** and **7-CF3-MAQ**, derivatives presented minors ID_{50} and anti-nociceptive effects above 96%. It seems that the presence of a CF_3 group as a substituent at the quinoline ring and the addition of a second quinoline portion promoted an increase in anti-nociceptive activity compared to other compounds. These data suggest that these aminoquinolines derivatives have, indeed, a significant anti-nociceptive potential and can modulate levels of inflammatory mediators.

In order to confirm the anti-nociceptive effect of the aminoquinoline derivatives, the formalin test was also carried out. As shown in Figure 2, all derivatives were able to reduce, statistically and significantly, the time took to lick the paw of animals in the neurogenic and inflammatory phase of the formalin test, with a marked reduction in response for the **7-CF3-MAQ** derivate (89.6%) in the second phase. The standard drug (indomethacin) inhibited 50.8% of paw licking. The formalin test has an advantage over other tests frequently used, since it involves a biphasic response with an early and a late phase, for neurogenic and inflammatory pain, respectively, and agents can be screened for activity in these two models of pain.[7] There was no apparents increases in activity among the derivatives with different substituents.

To confirm the anti-inflammatory effect suggested by the formalin test, the test of peritonitis induced by zymosan was also performed. The zymosan is an insoluble polysaccharide component of the wall of yeast *Saccharomyces cerevisiae* cell. It causes a reproducible inflammatory reaction and remains the standard irritant for examining acute inflammation and anti-inflammatory drugs.[8] Inflammation induced by zymosan develops immediately following subcutaneous injections, caused by the combined action of prostaglandins, bradykinin, histamine, tachykinins and reactive oxygen species. Neutrophils readily migrate to sites of inflammation.[9]

The results obtained with zymosan-induced peritonitis test are summarised in Figure 3. The data revealed that, at the dose tested, the treatment with **BAQ** and **7-CF3-MAQ** (100 μmol/kg, ip) caused a significant reduction in the leukocyte migration into the peritoneal cavity in comparison to the control group. **BAQ** inhibited cell migration by 66.5%, whereas **7-CF3-MAQ** inhibited 59.1%. Indomethacin (10 μmol/kg, ip), the standard drug, inhibited 75.8% of the inflammatory response. The 4-aminoquinoline derivatives were able to inhibit cell migration in the zymosan-induced peritonitis model, suggesting that these compounds display anti-inflammatory activity.

Various drugs may reduce cell migration assay in zymosan, including: the glucocorticoids, leukotriene antagonists[10] and

Table 1
The ID_{50} of 4-aminoquinoline derivates (100, 30, 3 and 0.3 μmol/kg) and dipyrone (100, 30, 3 and 0.3 μmol/kg) on the 0.6% acetic-acid-induced abdominal constrictions in mice over a period of 25 min

Compound	ID_{50} (μmol/kg ± SEM)	E_{max} (% ± SEM)
7-Cl-MAQ	39.4 ± 13.4	86.4 ± 7.6
7-CF3-MAQ	15.3 ± 4.2	96.7 ± 5.4
2-CF3-MAQ	26.6 ± 6.4	95.1 ± 7.4
BAQ	11.5 ± 3.7	97.3 ± 2.3
Dipyrone	36.8 ± 6.7	74.8 ± 3.8