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Biological evaluation of angular disubstituted naphthoimidazoles as anti-inflammatory agents

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ABSTRACT

A series of naphthoimidazoles derivatives (**3a**–**3f**) were tested for potential anti-inflammatory activity on lipopolysaccharide (LPS)-treated macrophages. Naphthoimidazole **3e** exhibited significant inhibitory effects on nitric oxide (NO) production (IC_{50} <10 μM) and decreased the expression of nitric oxide synthase-2 (NOS-2) and cycloxygenase-2 (COX-2) enzymes. It also inhibited the activation of transcription factor NF-κB. Naphthoimidazole **3e** might represent a starting point for the synthesis of new anti-inflammatory naphthoimidazoles derivatives.

Inflammation is one of the first responses of the immune system to harmful stimuli, such as pathogens, physical injury or damaged cells that ultimately lead to the restoration of a normal tissue structure and function. It is characterized by recruitment and accumulation of immune cells in injured sites and by the production of soluble mediators that are essential for controlling the inflammation and tissue repair and include reactive oxygen and nitrogen species, chemokines, lipid mediators and cytokines.[1] Macrophages play a key role in the inflammatory response and serve as an essential interface between innate and adaptive immunity. Overproduction and release of inflammatory mediators, such as nitric oxide (NO), prostaglandins (PGs) and different cytokines, are mediated by the activation and nuclear translocation of inducible transcription factors, such as NF-κB. Pro-inflammatory mediators PGs and NO are generated by the inducible isoforms of cyclooxygenase (COX-2) and NO synthase (NOS-2), respectively.[2–4] Due to its central role in the inflammatory response, NF-κB is involved in many human pathological conditions, including acute and chronic inflammation, and thus constitutes a suitable target for the development of new anti-inflammatory drugs.[5,6]

The naphthoimidazoles constitute a group of compounds with a broad spectrum of biological activities. Naphthoimidazoles derived from the naphthoquinone β-lapachone have displayed trypanocidal activity against *Trypanosoma cruzi*.[7] Some halogenated naphthoimidazole nucleosides have been reported as potent and selective inhibitors of human cytomegalovirus (HCMV) with a novel mode of action[8] and a set of naphtha[1,2-d]imidazoles have presented thrombopoietic activity.[9] Regarding the anti-inflammatory activity, several furan-naphthoimidazoles have exhibited potent inhibitory activity on the LPS-induced PGE_2 production in RAW 264.7 macrophages, by inhibition of mPGES-1 protein expression with no inhibitory effect on COX-2 expression.[10,11]

With these antecedents, we decided to evaluate a set of angular disubstituted naphthoimidazoles (**3a**–**3f**) as anti-inflammatory agents. To investigate the mechanism of action of this class of compounds, we have studied targets relevant to the regulation of the inflammatory response.

Naphthoimidazoles **3a–3e** were synthesized via a domino reaction from 1,4-dimethoxynaphthalen-2-amine (**1**) and imines (obtained from **1** and aromatic aldehydes) in the presence of Sc $(OTf)_3$ (Scheme 1).[12] A plausible pathway for the reaction involves attack of the nucleophilic amine on the imine and further reaction

Abbreviations: COX-2, cycloxygenase-2; LPS, lipopolysaccharide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; mPGES-1, microsomal prostaglandin E2 synthase-1; NO, nitric oxide; NOS-2, nitric oxide synthase; PGE_2, prostaglandin E_2.

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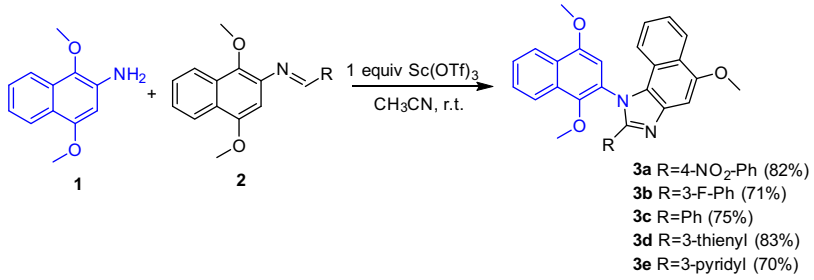
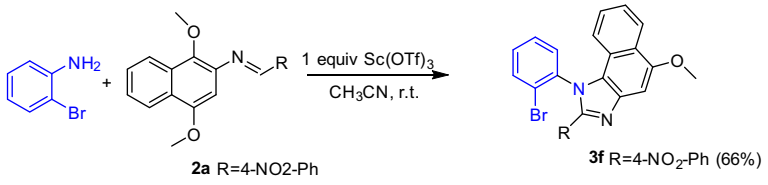


Scheme 1. Synthesis of naphthoimidazoles (**3a**–**3e**).



Scheme 2. Synthesis of naphthoimidazole **3f**.

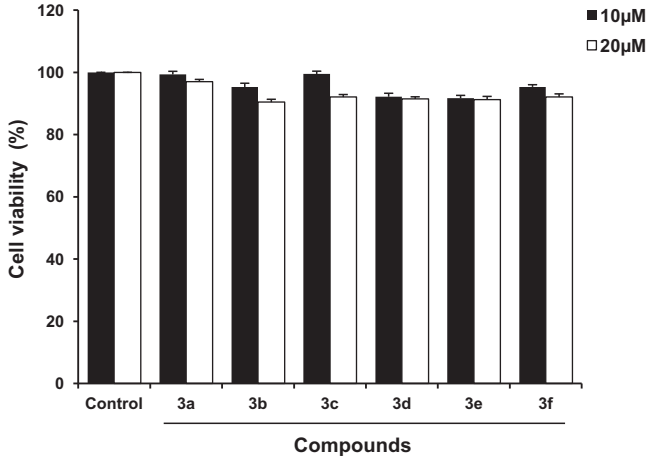


Figure 1. Naphthoimidazoles **3a**–**3f** were not cytotoxic. Macrophages were treated with different concentrations of compounds (10–20 μM) for 24 h. Cell viability was determined by MTT assay, and results are reported as mean ± S.D. of at least three independent experiments ($n = 3$).

of the resulting intermediate by intramolecular cyclization with loss of a methoxy group and subsequent oxidation to yield the final naphthoimidazole.

In the case of the simplified compound **3f**, 2-bromo aniline was used instead of 1,4-dimethoxynaphthalen-2-amine (**1**) and after 14 h the corresponding naphthoimidazol **3f**[13] was obtained in 66% yield (Scheme 2).

Although previous data indicated that these compounds were not cytotoxic in cancer cell lines (HEL, SKBR3, and MCF7),[14] we firstly assayed macrophage viability in the presence of naphthoimidazoles derivatives by the MTT assay.[15] As shown in Figure 1, cell viability was not affected after treatment for 24 h with compounds **3a–3f**.

To evaluate the potential anti-inflammatory activity of naphthoimidazoles **3a–3f**, we tested the ability of these compounds to decrease NO production. In murine macrophage RAW 264.7 cells, LPS induces NOS-2, and then NO production. Therefore, this macrophage cell line provides an excellent model for drug screening and for evaluation of potential inhibitors on the pathway leading to the induction of NOS-2. Treatment of RAW 264.7 macrophages with LPS (1 μg/mL) for 24 h, induced NO production as assessed by

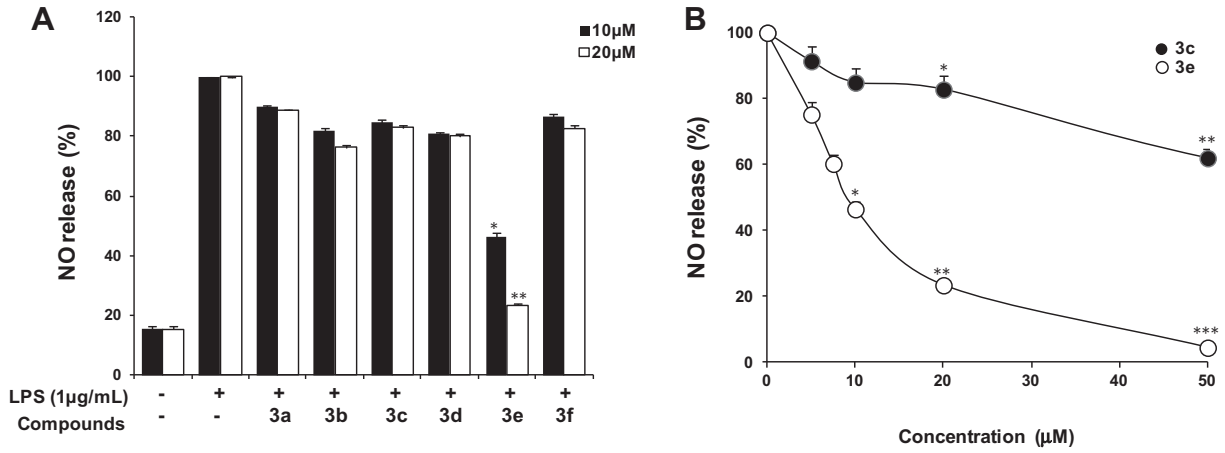


Figure 2. Effects of naphthoimidazoles **3a**–**3f** on nitric oxide (NO) release. (A) RAW 264.7 cells were pretreated with compounds (10 and 20 μM) for 30 min and then stimulated with 1 μg/mL LPS for 20 h. (B) Cells were pretreated with compounds **3c** and **3e** (5–50 μM) for 30 min and then stimulated with 1 μg/ml LPS for 24 h. The accumulation of nitrite in the culture medium was measured with the Griess reagent in both experiments that were carried out in triplicate and the results are the means ± S. D. of three different assays.