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Research paper

Identification of new anti-inflammatory agents based on nitrosporeusine natural products of marine origin

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ABSTRACT

Nitrosporeusines A and B are two recently isolated marine natural products with novel skeleton and exceptional biological profile. Interesting antiviral activity of nitrosporeusines and promising potential in curing various diseases, evident from positive data from various animal models, led us to investigate their anti-inflammatory potential. Accordingly, we planned and synthesized nitrosporeusines A and B in racemic as well as enantiopure forms. The natural product synthesis was followed by preparation of several analogues, and all the synthesized compounds were evaluated for *in vitro* and *in vivo* anti-inflammatory potential. Among them, compounds **25**, **29** and **40** significantly reduced levels of nitric oxide (NO), reactive oxygen species (ROS) and pro-inflammatory cytokines. In addition, these compounds suppressed several pro-inflammatory mediators including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nuclear factor-κB (NF-κB), and thereby can be emerged as potent anti-inflammatory compounds. Furthermore, all possible isomers of lead compound **25** were synthesized, characterized and profiled in same set of assays and found that one of the enantiomer (−)-**25a** was superior among them.

1. Introduction

Inflammation is a biological response to harmful stimuli such as pathogens that cause tissue and cell damage [1]. It is considered as a defensive measure taken by the organism to eliminate noxious stimuli and to begin the healing process. It is classified as either acute or chronic, depending on whether it involves a short response or a prolonged one, respectively [2]. During an inflammatory response, mediators, such as pro-inflammatory cytokines (e.g., interleukin-1β (IL-1β), IL-6, IL-12, and the chemokine IL-8), tumor necrosis factors (e.g., TNF-α and TNF-β), interferons (e.g., IFN-γ), eicosanoids (e.g., prostaglandins and leukotrienes) and vasoactive amines (e.g., histamine) are released [3]. The transcription factor nuclear factor-κB (NF-κB), plays a central role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [4]. In spite of the fact that inflammation is primarily a protective response, the chronic and uncontrolled inflammation becomes detrimental to tissues [5]. The inferences of the chronic inflammation in the pathogenesis of arthritis, cancer, cardiovascular, autoimmune as well as viral infections have made it a serious medical issue [6]. Therefore, research has been directed in recent years to develop safer and potent anti-inflammatory drugs to attenuate the severity of inflammation [6,7]. As the human immune system is a complex process involving many factors and can go awry many times, it is very challenging to develop novel efficient chemical entities for treating inflammation [8].

As a part of our continuous interest [9] in search of biologically active natural molecules with anti-inflammatory activity, we have come across a novel class of compounds with

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benzenecarbothioccyclopenta[*c*]pyrrole-1,3-dione scaffold - nitrosporeusines. Nitrosporeusines A (**1**) and B (**2**) are two new marine natural products having unique skeleton isolated [10] by Lin and co-workers from Arctic Chucki sea (Fig. 1). The structures of both compounds were established through detailed NMR and single crystal X ray studies. Biological evaluation of these compounds revealed that both compounds **1** and **2** have promising potential in treating influenza virus strains A/WSN/33(H1N1) [10]. Following that, *in vivo* studies by Chen and co-workers showed in mouse models that, nitrosporeusine A has exceptional potential in treating wide range of diseases such as rhinitis, oral ulcer, chronic heart failure, acute renal failure and renal fibrosis and all of them were claimed in a series of patents [11] (see Fig. 2 for details). These biological activity results reported for nitrosporeusines, in particular with compound **1** are very impressive, and attracted our attention where we planned for a complete study over this novel scaffold. Due to close association of many microbial and viral infections with inflammation [12], we envisioned to synthesize and study the nitrosporeusines in detail towards its anti-inflammatory potential.

Accordingly, the first total synthesis of nitrosporeusines A & B using a simple chemistry was achieved from this group and reported in a preliminary communication [13]. The developed route is flexible and now we extended it to generate a library of analogues around the natural product skeleton. Full account of design, synthesis and biological evaluation on nitrosporeusine scaffold is discussed in the present paper.

2. Results and discussion

2.1. Chemistry

Nitrosporeusines A and B share a common structural core with difference only in relative stereochemistry between hydroxy group and ring junction.

So, both the natural products are envisioned to be obtained from a common intermediate – maleimycin, which in turn could be obtained from compound **3** (see Scheme 1). This maleimide moiety (**3**) could be prepared from cyclopent-1-ene-1,2-dicarboxylic acid in few synthetic operations. While we were planning for the synthesis, we chose a strategy which would be amenable for synthesis of both natural products, as well as analogue synthesis. We planned to exploit the key maleimide intermediate to make several building blocks for analogues. Our initial aim was to prepare compound **3** in good quantities, so following literature procedures [14a,b], we treated cyclopent-1-ene-1,2-dicarboxylic acid with acetic anhydride/NH_3 which was then subjected to intramolecular condensation using trifluoroacetic anhydride resulting in desired maleimide compound **3** in good yields (Scheme 2). After scaling up the reaction and making sufficient quantities, we first explored the synthesis of nitrosporeusines A and B in racemic forms. Towards that we subjected compound **3** to microwave mediated allylic oxidation using selenium dioxide [15], and after few optimizations we could obtain desired allylic alcohol (**4**) in 68% yield (brsm).

Alternately, the same alcohol **4** was obtained by allylic bromination (NBS/hυ/$CHCl_3$) followed by acetylation and hydrolysis protocol (Scheme 2). Having the required racemic maleimycin in hand we next prepared required acid fragment for Michael addition. Commercially available 4-hydroxybenzoic acid along with solid Lawesson's reagent was dissolved in acetonitrile and irradiated under microwave conditions (100°C/15 min) [16] which gave 4-hydroxybenzothioic *S*-acid (**7**). We then subjected **4** to Michael addition with 4-hydroxybenzothioic *S*-acid (**7**) using water as solvent and obtained both nitrosporeusines A and B in 1:3 diastereomeric ratio (Scheme 2). Both the compounds were racemic, but the diastereomers formed were cleanly separated using silica gel column chromatography, characterized and compared with literature reports [10]. Here, the observed selectivity could possibly be due to strong intermolecular H-bonding between thio-ester carbonyl and hydroxy groups, which directs the incoming nucleophilic addition towards forming intermediate **I** as major compound giving nitrosporeusine B (Scheme 3). The other possibility of addition could result in formation of intermediate **II** resulting in nitrosporeusine A as minor product. Similar H-bonding mediated anti-aza-Michael addition reactions were reported in literature [17] which helped us in proposing this probable mechanism. The successful racemic synthesis of natural products with a simple and efficient route encouraged us to synthesize natural products in enantiopure forms.

The use of enzymatic kinetic resolution as a tool to resolve alcohols is well documented in literature [18]. We subjected alcohol **4** to different conditions in which one of the alcohols would selectively get acetylated and give enantiopure compounds (Scheme 4). Thus after a few attempts with different lipases and conditions (Scheme 4), alcohol **4** was resolved using vinyl acetate in presence of *Amano PS* lipase in dry THF to give acetate (−)-**5** and

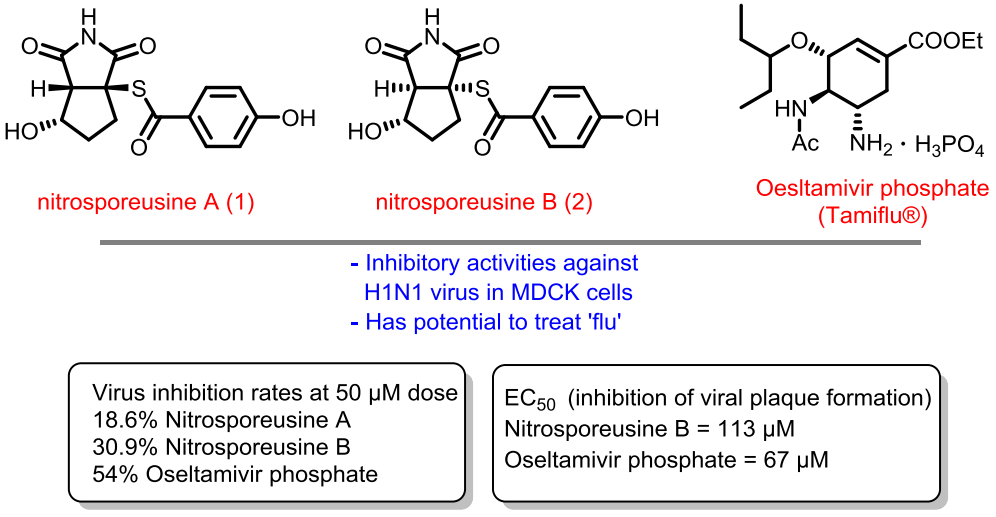

Fig. 1. Natural products nitrosporeusines A (**1**) and B (**2**).