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Original article

Structure-based design, synthesis and preliminary anti-inflammatory activity of bolinaquinone analogues

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ABSTRACT

As a part of our drug discovery efforts we developed a series of simplified derivatives of bolinaquinone (BLQ), a hydroxyquinone marine metabolite, showing potent anti-inflammatory activity. Thirteen new hydroxyquinone derivatives closely related to BLQ were synthesized and tested on mouse macrophage-like RAW 264.7 cell line in order to investigate their ability to modulate the production of Prostaglandin E_2 (PGE_2). This optimization process led to the identification of three strictly correlated compounds with comparable and higher inhibitory potency than BLQ on PGE_2 production. To evaluate the affinity of BLQ and its analogues for *hs*PLA_2, surface plasmon resonance (SPR) experiments were performed.

1. Introduction

The search for biologically active natural products from marine sources continues to be an important scientific field that offers promising opportunities for the development of new compounds endowed with pharmacological properties.

Natural products with a quinoide system and a decalin-type or related aromatic side chain are characterized by pronounced and several biological properties (Chart 1).

For instance, the marine sesquiterpene quinones such as bolinaquinone (**1**) (**BLQ**) [1], nakijiquinone A–D [2] (**2a**–**d**) and nakijiquinone G-H [3] (**2e**–**f**), ilimaquinone [4] (**3**), avarol [5] (**4**), smenospongine (**5**), smenospongidine (**6**), smenospongiarine [6] (**7**) display antimicrobial, antiviral, anti-inflammatory and cytotoxic activities [7].

Among this class, the marine metabolite bolinaquinone expresses its anti-inflammatory activity by inhibition of secretory phospholipase A_2 (*s*PLA_2) [1].

Phospholipases A_2 (PLA_2) are a class of lipolytic enzymes that catalyze the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids liberating free fatty acids and lysophospholipids [8]. Phospholipases A_2 include several unrelated protein families with common enzymatic activity but with different substrate specificities, cofactor requirements, subcellular localization and cellular functions.

Based on biophysical and biochemical properties more than 15 different forms of PLA_2 enzymes have been identified [8].

For their cellular location PLA_2 enzymes are classified as cytosolic PLA_2 (*c*PLA_2), intracellular PLA_2 (*i*PLA_2) and secretory PLA_2 (*s*PLA_2) [9].

Among the calcium dependent *s*PLA_2, type IIA (*s*PLA_2-IIA) is an isoform first isolated and purified from rheumatoid synovial fluid. Increased plasma levels of this enzyme were found in diseases that involve systemic inflammation such as sepsis, rheumatoid arthritis, and cardiovascular disease [10,11].

Therefore, compounds that can selectively block secretory PLA_2 activity and the assessment of their molecular mechanism of enzyme inactivation are of paramount importance in the field of anti-inflammatory drugs.

Among terpenoidic hydroquinone class, avarol was able to inhibit human recombinant synovial phospholipase A_2 activity with an $IC_{50} = 158$ μM, while quinone derivative avarone failed to show inhibitory activity [12]. BLQ, sharing a hydroxyl-*p*-quinone moiety connected to a trans-decalin terpene unit in a rearranged drimane skeleton is one of the most active metabolites with a selective profile against secretory PLA_2's [1].

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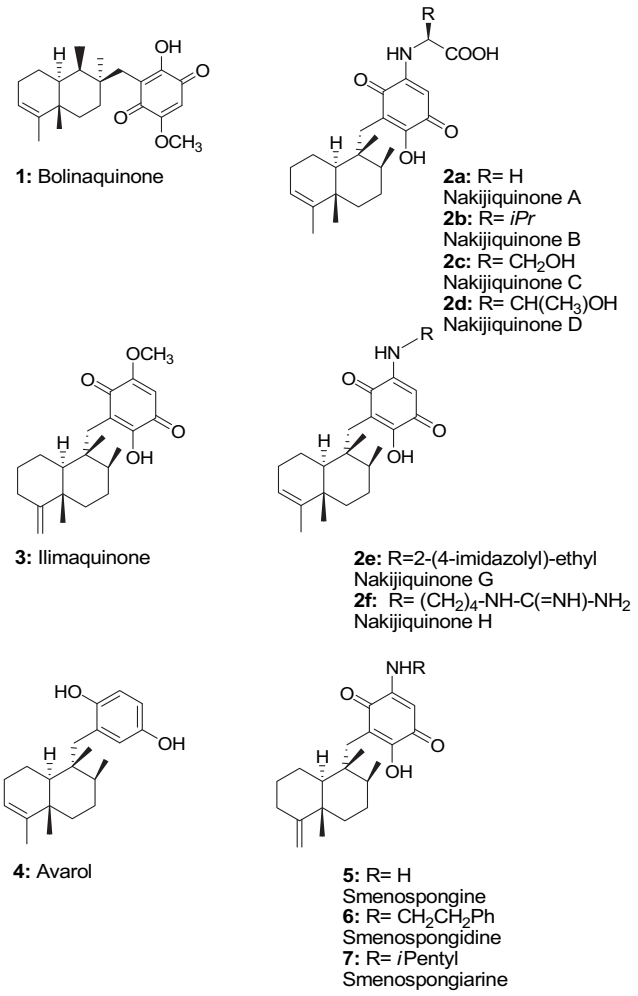

Chart 1. Formulas of natural sesquiterpene quinones and hydroquinones.

BLQ shows the ability to inhibit strongly *hs*PLA_2, bee venom PLA_2 (*bv*PLA_2), with IC_{50} values of 0.2 and 0.1 μM, respectively; remarkably, this product also exerts no effects on cytosolic PLA_2 (*c*PLA_2) [1].

Moreover, bolinaquinone is able to reduce the inflammatory response of adjuvant arthritis by inhibiting PGE_2, NO, and TNF production in paw homogenates without affecting PGE_2 levels in the stomach [13].

In a recent work [14] we have proposed, for the inactivation mechanism of *hs*PLA_2 [15] by BLQ, a key process in the inhibition consisting in the occupation of the active site's hydrophobic pocket by the sesquiterpene unit of the inhibitor.

The molecular basis of the human group IIA secretory phospolipase A_2 inactivation by BLQ is completely due to a non-covalent event involving the 2-hydroxy-1,4-benzoquinone responsible for the chelation with Ca^{2+} ions in the region of binding [16,17], orienting the inhibitor within the binding site.

Mainly, the oxygen atoms of hydroxyl-quinone system are of primary importance for the interaction, namely, both the C-1 carbonyl group and the deprotonated C-2 hydroxyl residue coordinate the calcium ion in a bidentate fashion.

Following our extensive investigation on the PLA_2 inactivation mechanism by BLQ we have developed a series of simplified derivatives related to the natural product.

The general design of novel inhibitors against inflammation-related diseases was guided by the modular composition of this metabolite.

Indeed, on the basis of these premises, we decided to rely on some well-reasoned structural changes of the basic molecule **1**, in the attempt to improve its pharmacological behaviour (Scheme 1).

To generate the chelation of calcium ion responsible of the competitive inhibition process of *hs*PLA_2, we generated focused collection of hydroxyl-benzoquinone analogues. Therefore, the quinone-type building block was not changed entirely but simplified by elimination of methoxy group.

In order to explore the non-covalent interactions with the large hydrophobic surface of the active site, thedrimane moiety of BLQ was replaced with different hydrophobic structures such as *n*-pentyl, benzyl, naphthyl and biphenyl units, also they were shifted from position 3 to 5.

To evaluate the impact of the single linker atom replacement on the overall profile of these molecules, we have substituted the carbon-linked of quinoide derivative with an NH or O bridge.

Optimization of the aromatic region was performed by introduction of different sterical hindrance structures as phenyl, naphthyl, biphenyl, thianthrenyl and dibenzofuranyl moiety directly attached to the quinoide core.

The purpose of the present study is to investigate the anti-inflammatory activity of a series of 2-hydroxy-1,4-benzoquinones, as well as to define the structure–activity relationships (SAR) of this new class. Moreover, preliminary investigations on the compounds synthesized in their potency against *hs*PLA_2 are evaluated.

This report describes the synthesis of new compounds that achieved good potency in the biochemical assay and cell-based system.

2. Chemistry

Synthesis of compounds **8a**–**d** (Scheme 2) was accomplished in three steps.

Commercially available 2,4-dimethoxyphenyl boronic acid (**10**) was subjected to Suzuki coupling with several benzylic halides (**11b**–**d**), to yield derivatives **12b**–**d**. After deprotection, compounds **13a**–**d** were oxidized with Fremy's salt yielding quinones **8a**–**d**. In the case of derivative **8a** we oxidized commercially available *n*-hexylresorcinol.

Compound **8e** was synthesized starting from 2,4,5-trimethoxybenzaldehyde (**14**) that was converted, via oxidation [18], to the phenol derivative **15** which reacted with 2-naphthyl boronic acid providing diaryl ether derivative **16** (Scheme 3).

Treatment of **16** with ammonium cerium nitrate in acetonitrile yielded desired compound.

For derivative **8f**, 2,4-dimethoxy aniline was submitted to Ullmann-type coupling with 2-bromonaphthalene to provide *N*-(2,4-dimethoxyphenyl)-naphthyl-2-amine (**18**). The resulting amine was deprotected and finally oxidized with Fremy's salt to give quinone **8f** (Scheme 4).

For the second set of analogues **9a**–**g**, a different procedure was followed (Scheme 5).

Commercially available 1-iodo-2,4-dimethoxybenzene (**20**) was subjected to Suzuki coupling, under microwave irradiation, with several boronic acids (**21a**–**g**), yielding the corresponding compounds, which were O-demethylated to provide resorcinol derivatives (**23a**–**g**) that were finally oxidized with Fremy's salt yielding quinones.

3. Biological results

3.1. Effects of BLQ and its analogues on LPS-induced PGE_2 production by LPS-stimulated RAW 264.7 cells

To assess the effect of BLQ and compounds **8a**–**f** and **9a**–**g** on LPS-induced PGE_2 production, RAW 264.7 macrophages cells were