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Exiguaquinol: A Novel Pentacyclic Hydroquinone from *Neopetrosia exigua* that Inhibits *Helicobacter pylori* MurI

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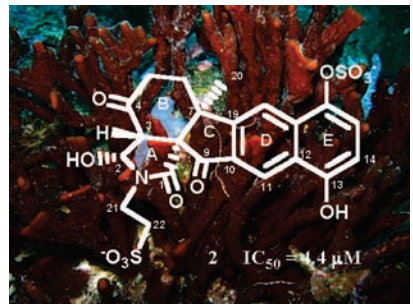
ABSTRACT



Bioassay-guided fractionation of the methanol extract of the Australian sponge *Neopetrosia exigua* led to the isolation of exiguaquinol (2), a new pentacyclic hydroquinone that inhibited *Helicobacter pylori* glutamate racemase (MurI) with an IC_{50} of 4.4 μM. Its structure and relative configuration were assigned on the basis of spectroscopic data. Exiguaquinol (2), bearing a novel pentacyclic ring skeleton, is the first natural product to show inhibition of *H. pylori* MurI. Its protein−ligand modeling is also discussed.

In the search for new and effective antibiotics, pharmaceutical companies have targeted cell wall components specific to human pathogens. One of these targets is MurI, a glutamate racemase essential for the survival of some pathogenic eubacteria.[1] MurI catalyzes the interconversion of L and D-glutamate, providing D-glutamate for incorporation into the growing peptidoglycan chain that forms the cell walls. Glutamate racemases of several pathogenic bacteria, including *Helicobacter pylori* have been recently characterized and their regulation mechanisms described.[2] A series of benzodiazepine-derived compounds, such as compound **1**, have recently been reported as inhibitors of *H. pylori* MurI.[2,3] X-ray and NMR structural studies have revealed that these compounds are allosteric inhibitors.[2]

We employed an NADH fluorescence high-throughput screening assay to detect inhibition of *H. pylori* MurI. Forty thousand extracts derived from plants and marine organisms collected in Queensland were tested, and the methanol extract from the sponge *Neopetrosia exigua* (formerly *Xestospongia exigua*) was targeted for further evaluation since it inhibited the enzyme at 0.25 mg dry wt/mL. Bioassay-guided purifica-

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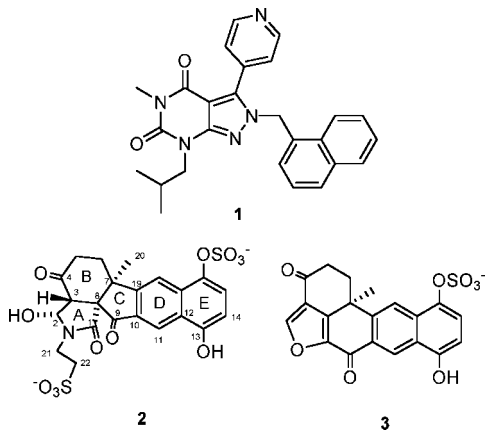
tion led to a novel pentacyclic hydroquinone, exiguaquinol (**2**), being isolated as the bioactive component.



1

2 **3**

Quinone and hydroquinone compounds with varying degrees of oxidation and unsaturation have been previously isolated from *Neopetrosia exigua*[4] *Xestospongia* sp.,[5] *X. sapra*,[6,7] *X. cf. carbonaria*,[8] and *Adocia* sp.[9] with a range of biological activities reported from antimicrobial to protein tyrosine kinase inhibition.

In this report, we describe the isolation, structure elucidation, biological activity, and protein−ligand modeling of exiguaquinol (**2**). Exiguaquinol (**2**) is the first natural product that shows inhibitory activity against *H. pylori* MurI.

Exiguaquinol (**2**) was isolated as an orange optically active powder. Negative HRESIMS did not yield a well-resolved $[M - H^+]^-$ ion. A peak at *m*/*z* 276.5189, however, indicated a doubly charged ion $[M - 2H^+]^{2-}$, where M is consistent with the formula $C_{22}H_{21}NO_{12}S_2$.

The infrared absorption bands at 3448 and 1685 cm^{-1} suggested hydroxyl and ketone functionalities, and the bands at 1206 and 1048 cm^{-1} suggested a sulfonate group.

The ^1H NMR spectrum of **2** (Table 1) showed one quaternary methyl group at δ_H 1.67 and several resonances between δ_H 1.74 and 3.47, integrating to nine protons. The downfield region showed three one-proton singlets, three one-proton doublets, and a one-proton doublet of doublets at δ_H 5.51. The ^{13}C NMR spectrum (Table 1) contained the resonance of 22 carbons: 1 methyl group (δ_C 20.0), 3 carbonyl groups (two ketones δ_C 202.7 and 206.7; one amide δ_C 170.8), 10 unsaturated and 8 aliphatic carbons, 1 bearing oxygen (δ_C 80.9).

Three of the 13 units of unsaturation implied by the molecular formula were accounted for by three carbonyl groups. The presence of two aromatic rings was supported by carbon NMR data, and three additional rings accounted for the remaining three units of unsaturation. This information combined with mass spectroscopy data suggested that exiguaquinol (**2**) bore resemblance to hydroquinone compounds such as halenaquinol sulfate **3**.[5]

Table 1. NMR Data for Exiguaquinol (**2**) in DMSO-d_6[a]

no.	δ_C	δ_H	$^2J_{CH}$ and $^3J_{CH}$
1	170.8	-	-
2	80.9	5.51 (dd, J = 3.8, 6.6 Hz)	C1, C8
3	50.6	3.47 (m)	C1, C2, C4, C7,C8,C9
4	206.7	-	-
5β	37.3	1.95 (m)	
5α		2.42 (dt, J = 3.2, 14 Hz)	C4, C7
6β	38.5	1.74 (dt, J = 3.2, 14 Hz)	
6α		2.15 (m)	C4
7	43.1	-	-
8	67.0	-	-
9	202.7	-	-
10	130.6	-	-
11	119.2	8.44 (s)	C9,C13,C17,C19
12	124.4	-	-
13	151.7	-	-
14	107.9	6.83 (d, J = 8.0 Hz)	C12,C13, C16
15	121.2	7.42 (d, J = 8.0 Hz)	C13,C16, C17
16	141.5	-	-
17	132.7	-	-
18	116.8	8.20 (s)	C7,C10,C12,C16
19	154.1	-	-
20	20.0	1.67 (s)	C6, C7, C8,C19
21	37.6	3.45 (m)	C1, C2, C22
22	49.4	2.60 (m)	C21
		2.71 (dd, J = 7.8, 13.2 Hz)	C21
2-OH	-	7.13 (d, J = 3.8 Hz)	C2, C3
13-OH	-	10.34 (s)	C12, C13

[a] ^1H NMR at 600 MHz referenced to residual DMSO solvent (δ_H 2.49) and ^{13}C NMR at 150 MHz referenced to DMSO (δ_C 39.5).

A series of HMBC correlations (Table 1) from the four aromatic protons H-11, H-14, H-15, and H-18 (δ_H 8.44, 6.83, 7.42, and 8.20, respectively) allowed a naphthalene moiety to be assigned (C-10 to C-19). HMBC correlations from the hydroxyl group at δ_H 10.34 to carbons C-12 and C-13 (δ_C 124.4 and 151.7) and a COSY correlation to proton H-14 gave further support to the aromatic moiety, which has a sulfate group attached to the quaternary carbon at δ_C 141.5 (C-16) and the hydroxyl group attached to C-13. This was consistent with the reported data for compound **3** (C-13 at δ_C 151.4 and C-16 at δ_C 141.1). The presence of one sulfate and one hydroxyl group in the aromatic ring E implied that the second sulfur-containing moiety was attached to another region in the molecule.

The naphthalene moiety was attached to ring C based on correlations from H-11 (δ_H 8.44) to C-9 and C-19 and from H-18 (δ_H 8.20) to C-7 and C-10. The bridgehead proton H-3 (δ_H 3.47) showed HMBC correlations to C-1, C-2, C-4, C-8, and C-9, which assisted in the assignment of the tricyclic system A−B−C. COSY correlations from H-2 at δ_H 5.51 to H-3 and to the hydroxyl group 2-OH at δ_H 7.13 also supported this assignment. Other HMBC correlations were observed from the methyl group H-20 to carbons C-6, C-7, C-8, and C-19.

A series of HMBC and CIGAR[10] correlations from H-21 (δ_H 3.45) to C-1 and C-2 indicated the presence of an ethylene side chain attached to ring A. This side chain, a

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taurine moiety, contained a sulfonate group attached to C-22 (δ_C 49.4) and a tertiary nitrogen, which was attached to C-21 (δ_C 37.6) and formed part of ring A.

Further confirmation for the structure of exiguaquinol (**2**) was obtained by analysis of the negative LRESIMS spectrum. The presence of an alcohol group was indicated by loss of water yielding the ion peak at *m*/*z* 535.9. Further fragmentation of this ion could have led to loss of a sulfonate group (*m*/*z* 455.1). The loss of sulfate and oxidation of the hydroquinone to a quinone in the aromatic ring E could also account for the *m*/*z* 455.1 peak.

ROESY experiments were used to define the relative configuration of the four stereogenic centers. Correlations from the hydroxyl group at C-2 to methyl H-20 and methylene H-5α and from the methyl H-20 to methylenes H-5α and H-6α established that all these groups were on the α face of the molecule. H-2 and H-3 were on the opposite *β* face of the molecule as these protons had strong correlation to each other.

Exiguaquinol (**2**) contains a novel pentacyclic ring system, and although no detectable amount of halenaquinol sulfate (**3**) was found in the sponge crude extract, it is possible that **2** could be derived from **3**. Oxidation of the furan ring followed by hydrolyses and nucleophilic addition of taurine to ring C would lead to subsequent ring C contraction, stabilized by the α,*β*-unsaturated ketone in ring B, forming a more stable amide bond in ring A (Figure 1). Examples of

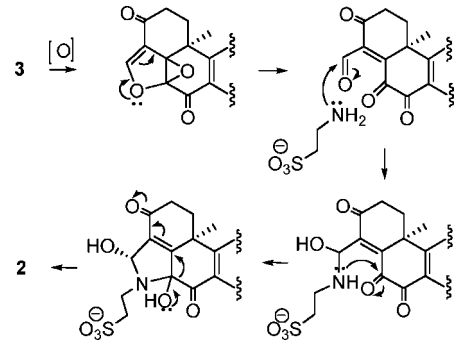

Figure 1. Proposed biogenesis of exiguaquinol (**2**) from **3**.

taurine-substituted quinones have been previously reported from *Adocia* sp.[9]

Due to the scarcity of material, it was not possible to determine the absolute configuration of exiguaquinol (**2**) directly; however, this could be inferred from halenaquinol sulfate (**3**) whose absolute configuration has been determined by CD spectroscopy.[11] From the biosynthetic perspective, the *S* absolute configuration at C-7 in compound **3** could be assumed to be the same for C-7 in **2**. The absolute configuration of exiguaquinol's (**2**) additional three stereogenic centers at C-2, C-3, and C-8 could then be assumed as *R*, *S*, and *S*, respectively, from their relative configuration.

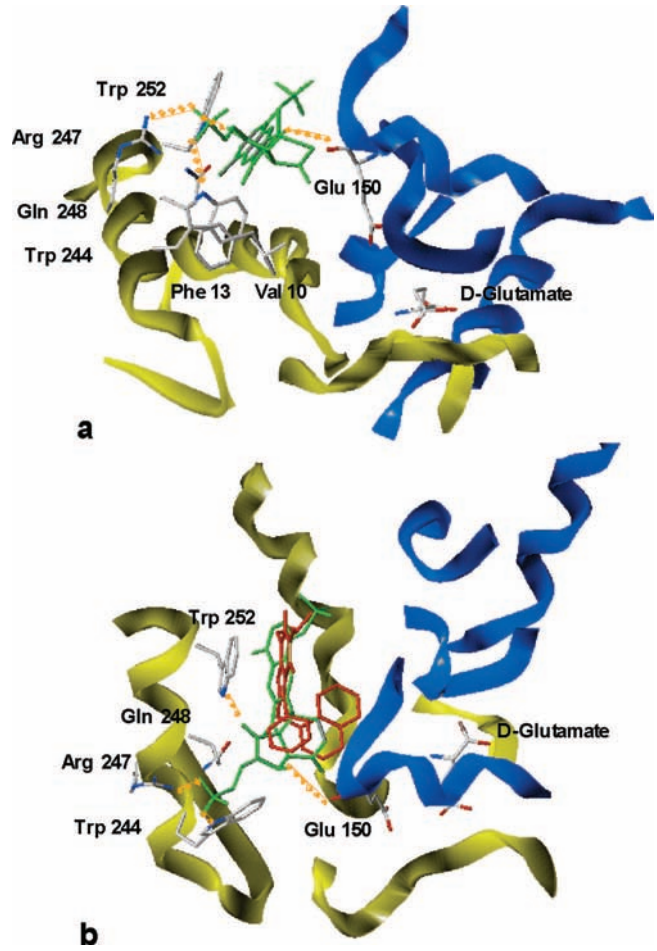

Figure 2. *H. pylori* MurI enzyme−inhibitor−substrate complex. (a) Exiguaquinol (**2**) (green) in inhibitor-binding site with stabilizing hydrogen bonds; (b) overlay top view of compounds **1** (red) and **2** in inhibitor-binding site.

Exiguaquinol (**2**) showed an IC_{50} value of 4.4 μM for the inhibition of *Helicobacter pylori* MurI in the D-SOS (D-serine-O-sulfate) assay described here and did not significantly quench the NADH fluorescence. A subsequent assay utilizing natural D-glutamate instead of D-SOS as the substrate revealed weak inhibition of MurI at 361 μM. Differences in binding to MurI enzyme by the two substrates may explain the differences in inhibitory activity observed for **2**.

The basis for this hypothesis is that key interactions are formed between the *γ*-carboxylate of the substrate and Tyr39−Gly40 main chain atoms (A-domain) and hydrophobic interactions with the D-glutamate side chain and Val146 (B-domain) resulting in the A- and B-domains being tightly packed at the domain interface. It is likely that the larger sulfate group in D-SOS affects these interactions and leads to a more open conformation, thereby allowing inhibitors to bind in the presence of D-SOS that would be excluded by

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the tighter conformation induced by the D-glutamate. Confirmation of this hypothesis could be obtained when the crystal structure of the enzyme−D-SOS complex becomes available.

Docking of exiguaquinol (**2**) into the crystal structure of *Helicobacter pylori*MurI enzyme−substrate (D-glutamate) complex, where **1** was removed, was performed using GOLD program.[12] The majority of the docked solutions adopted the conformation shown in Figure 2. Exiguaquinol (**2**) docked into the same allosteric site as **1** and achieved hydrogen bonds with the nitrogen of the side chains of Trp252, Arg247, and Trp244 and with the oxygen of the backbone of Glu150 (Figure 2a). Overlay of the docked exiguaquinol (**2**) with the crystal structure of **1** showed that the π-stacking interaction with Trp252 was conserved (Figure 2b), that **1** does not have any hydrogen bonding interactions with the enzyme, and that the pocket occupied by the naphthyl moiety of **1** (red) is not occupied by the docked **2**(green).

Exiguaquinol (**2**) is the first natural product to show inhibition of *H. pylori* MurI. Structure modification of **2** such as the introduction of a hydrophobic group to fill the vacant pocket could lead to a more potent compound.

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Supporting Information Available: Detailed description of experimental procedures, IR, UV, MS data, and 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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