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

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Supporting Information

Experimental Procedures

General. NMR spectra were recorded on a Varian Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. ^{1}H and ^{13}C were referenced to the peak solvent of DMSO-d_6 at δ_H 2.49 and δ_C 39.5. Standard parameters were used for 1D and 2D NMR spectra which included ^{1}H, ^{13}C, DEPT, gradient COSY, HMQC, HMBC, CIGAR and ROESY. UV spectra were recorded on a GBC 916 UV-Visible spectrometer and IR spectra were recorded on a Perkin Elmer 1725X FTIR spectrometer. Optical rotation was measured on a Jasco P-1020 polarimeter. Davisil® C_{18} powder (30-40 μm) was used for MPLC column packing. A YMC ODS-Aqueous HPLC column (5μm, 10 x 150 mm) connected to a Waters 600 pump, 717 Autosampler, 996 Photodiode Array Detector and Fraction Collector were used for semi-preparative chromatography separations. HRESIMS were measured on a Bruker BioAPEX 47e mass spectrometer. LRESIMS mass spectra were measured on a Fisons Single Quadrupole VG Platform II, using negative electrospray ionization mode.

Animal Material. The sponge sample *Neopetrosia exigua* was collected by SCUBA diving at Lady Musgrave Is., Australia, and the voucher sample G314093 is lodged at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried sponge material (2.0 g) was ground and exhaustively extracted inline through a C_{18} MPLC column using gradient elution from 100% H_2O to 100% MeOH. The medium-polarity active fractions were pooled and submitted to HPLC fractionation on an aqueous ODS column with gradient elution from MeOH/H_2O 5:95 to MeOH/H_2O 30:70. The active fractions were pooled and re-submitted to HPLC (aqueous ODS) using isocratic elution of MeOH/H_2O 32:68 with 0.2 M NaCl. The fraction eluting at 7.5 min contained exiguaquinol (**2**).

Biological Protocol. MurI activity was determined using an assay based on NADH fluorescence. The following procedure was used for the HST of crude extracts. The 100-μL assay was performed in 140 mM Tris HCl buffer (pH 8.2) containing 5 mM dithiothreitol (DTT), 150 μM NADH, 5 mM *D*-SOS, 2.6 units of lactic dehydrogenase (LDH), 90 mM sodium sulfate, 1.5% v/v glycerol, 1.0 mM EDTA, 3.0 μg bovine serum albumin (BSA), 0.03% PEG-8000, and 120 nM *H. pylori* MurI. Each assay contained 2 μL of DMSO. The extract in 2 μL of DMSO and enzyme in 80 μL of assay buffer (Tris, DTT, NADH, BSA, EDTA and PEG-800) were pre-incubated for 15 min, and then 15 μL of *D*-SOS solution was added. A first fluorescence measurement was taken within 15 min and a final measurement was taken after 120 min during which approximately 90% of the NADH was consumed. The fluorescence measurements were made with an excitation wavelength of 340 nm and an emission wavelength of 460 nm, using a Wallac 1420 Multilabel Counter.

The following modified assay was used for the testing of pure compounds. All final reagent concentrations were maintained, but the addition of reagents and the number of fluorescent readings was varied. Assay buffer (65 μL) containing DTT, BSA, EDTA, PEG-8000, and MurI were added to 2 μL of extract in DMSO, and after a brief shake, the fluorescence was recorded. NADH (15 μL) in the above buffer was then added and after shaking, the fluorescence was again recorded. The change in fluorescence (ΔF460) was used as an "in well" control value. A further 15 μL of the substrate solution (*D*-SOS, sodium sulfate, glycerol and LDH, pH 8.0) was then added and after shaking a third reading was made. The plate was then left at room temperature for 120 min before a final F460 was recorded. Activity calculations made use of the ΔF460 due to the reaction (reading 3 minus reading 4) corrected by the ΔF460 due to the added NADH (reading 2 minus reading 1). In this way fluorescence differences that occurred due to quench phenomena caused for

example by inherent absorption of compound **1** chromophores, could be taken into account on a per well basis.

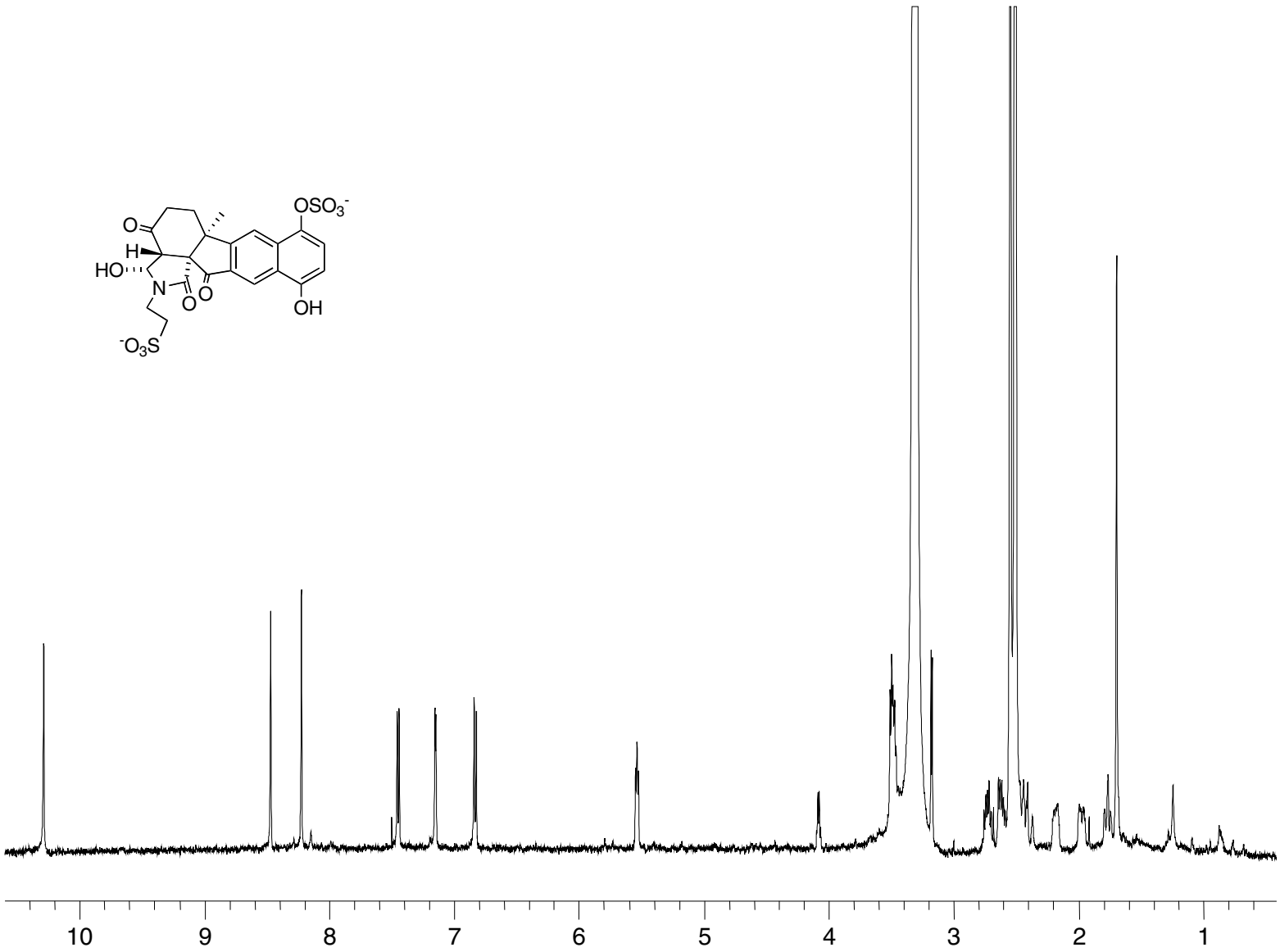
Exiguaquinol (2): orange powder (2 mg, 0.1% dry wt.): $[\alpha]^{26}_{D}$ –12° (c 0.14, MeOH); UV (MeOH) λ_{max} (logε) 272.0 nm (3.6), 395.7 nm (2.6); IR ν_{max} (NaCl cell) 3448, 1685, 1627, 1343, 1206, 1048 cm^{-1}; (-) LRESIMS *m/z* 276.7 (100%) $[C_{22}H_{21}NO_{12}S_2\text{-}2H^+]^{-2}$, *m/z* 455.1 (3%) $[C_{22}H_{21}NO_{12}S_2\text{-}H_2O\text{-}SO_3^-\text{-}2H^+]^{-1}$, *m/z* 535.9 (11%) $[C_{22}H_{21}NO_{12}S_2\text{-}H_2O\text{-}H^+]^{-1}$, m/z 553.9 (22%) $[C_{22}H_{21}NO_{12}S_2\text{-}H^+]^{-1}$, *m/z* 575.9 (15%) $[C_{22}H_{21}NO_{12}S_2\text{-}2H^+\text{+}Na^+]^{-1}$; (-) HRESIMS *m/z* 276.5189 $[C_{22}H_{21}NO_{12}S_2\text{-}2H^+]^{-2}$ (calc. 276.5180).

NMR Data

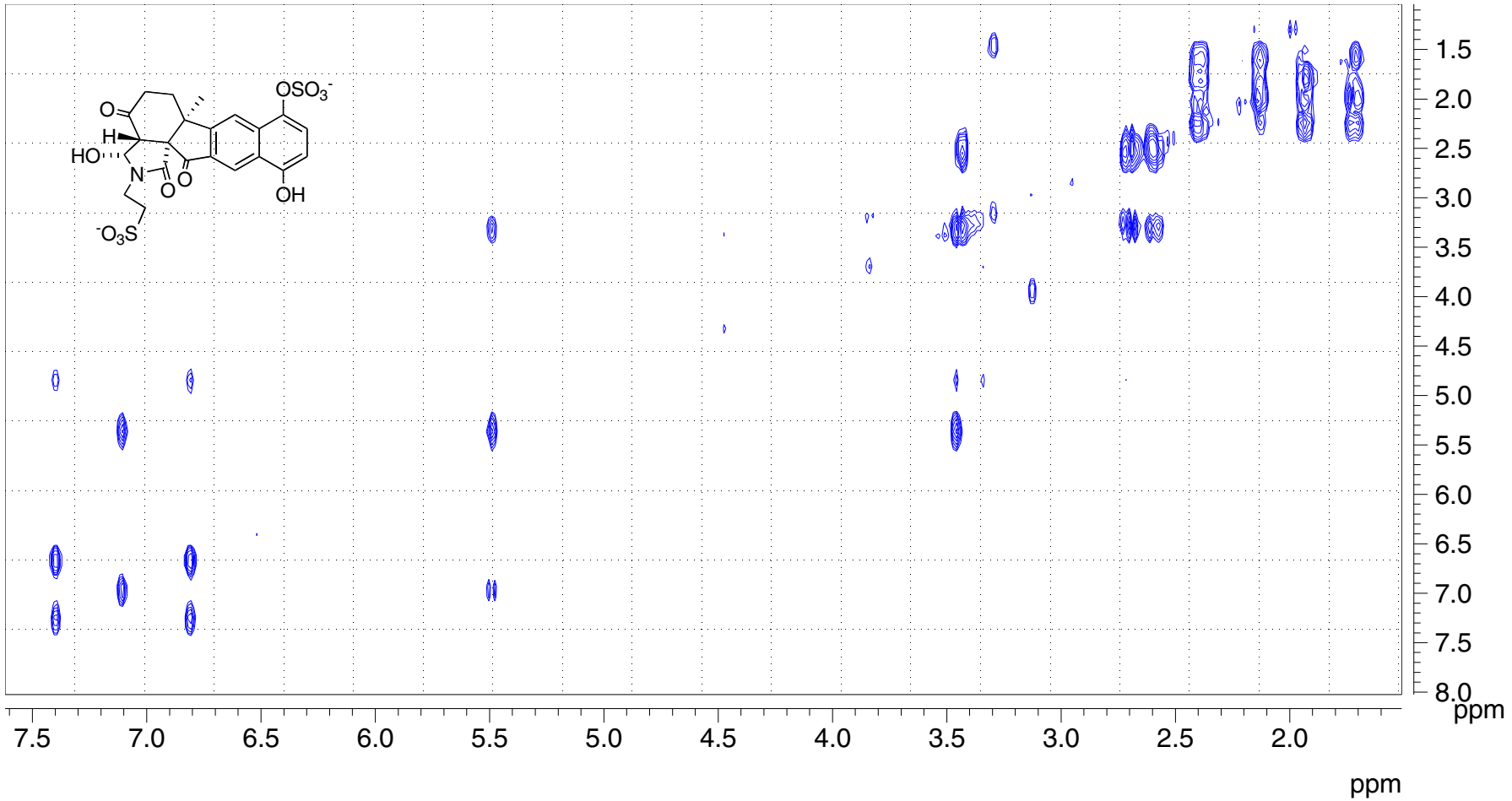
NMR data for exiguaquinol in DMSO-d_6[a]

pos.	^{13}C NMR	^{1}H NMR	$^{2}J_{CH}$ and $^{3}J_{CH}$	COSY	ROESY[b]
1	170.8	-	-	-	-
2	80.9	5.51 (dd, *J*=3.8, 6.6 Hz)	C1, C8	H3, 2-OH	H3, H21, H22β, H22α
3	50.6	3.47 (m)	C1, C2, C4, C7, C8, C9	H2	H5
4	206.7	-	-	-	-
5β	37.3	1.95 (m)	C4, C7	H5α, H6β, H6α	H5α, H6β
5α		2.42 (dt, *J*=3.2, 14 Hz)		H5β, H6β, H6α	H5β, H6α, H20
6β	38.5	1.74 (dt, *J*=3.2, 14 Hz)	C4	H5β, H5α, H6α	H5β, H6α, H18
6α		2.15 (m)		H5β, H5α, H6β	H5α, H6β, H18, H20
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	13-OH, H15	13-OH
15	121.2	7.42 (d, *J*=8.0 Hz)	C13, C16, C17	H14	-
16	141.5	-	-	-	-
17	132.7	-	-	-	-
18	116.8	8.20 (s)	C7, C10, C12, C16	-	H6β, H6α, H20
19	154.1	-	-	-	-
20	20.0	1.67 (s)	C6, C7, C8, C19	-	2-OH, H5α, H6α, H18
21	37.6	3.45 (m)	C1, C2, C22	H22β	H2, 2-OH, H22β, H22α
22β	49.4	2.60 (m)	C21	H21, H22α	2-OH, H2, H21, H22α
22α		2.71 (dd, *J*=7.8, 13.2 Hz)	C21	H21, H22β	H2, H21, H22β
2-OH	-	7.13 (d, *J*=3.8 Hz)	C2, C3	H2	H5α, H20, H21, H22β
13-OH	-	10.34 (s)	C12, C13	H14	H14

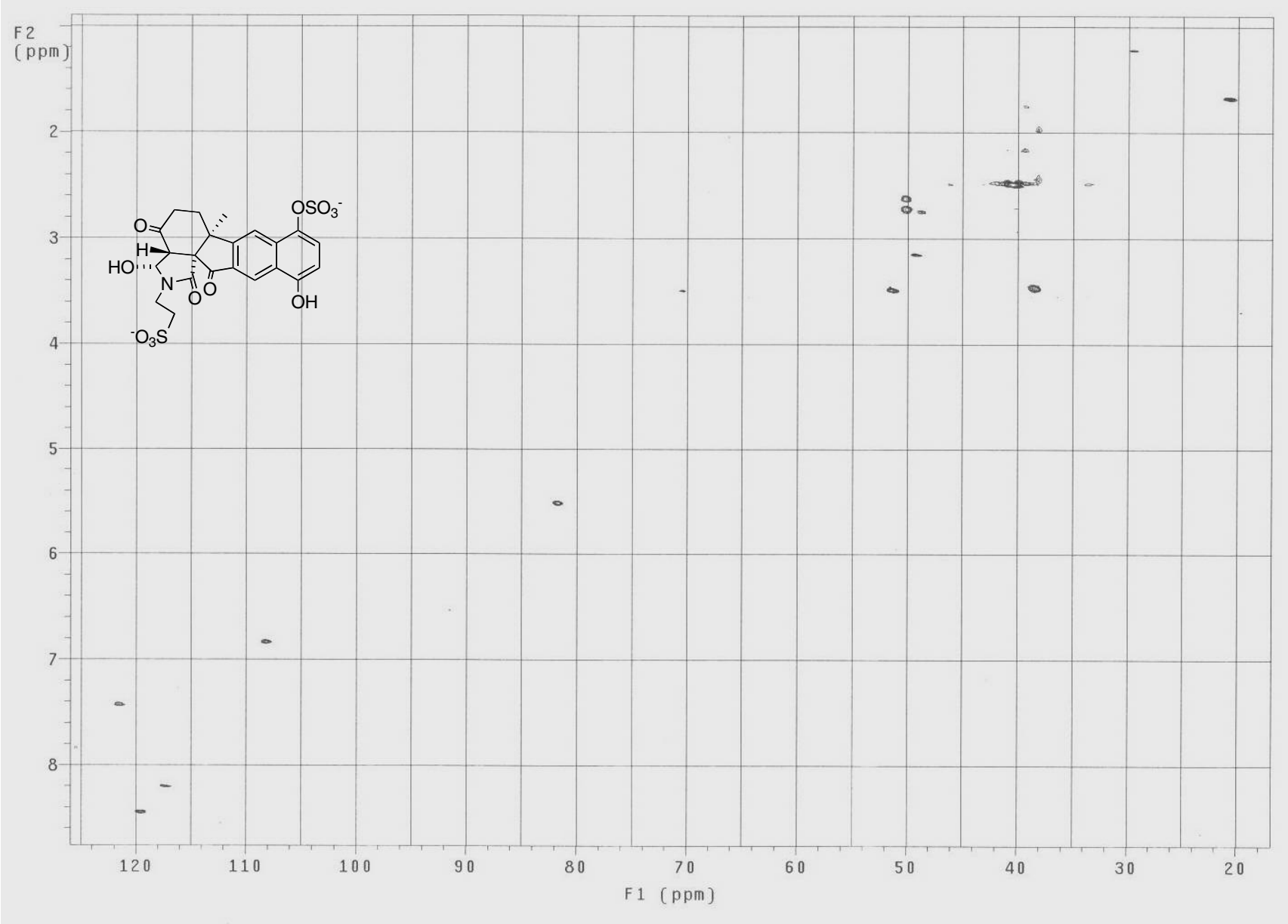
[a] ^{1}H NMR at 600 MHz referenced to residual DMSO solvent (δ_H 2.49) and ^{13}C NMR at 150 MHz referenced to DMSO (δ_C 39.5). [b]ROESY experiment was acquired using a mixing time of 500 ms.



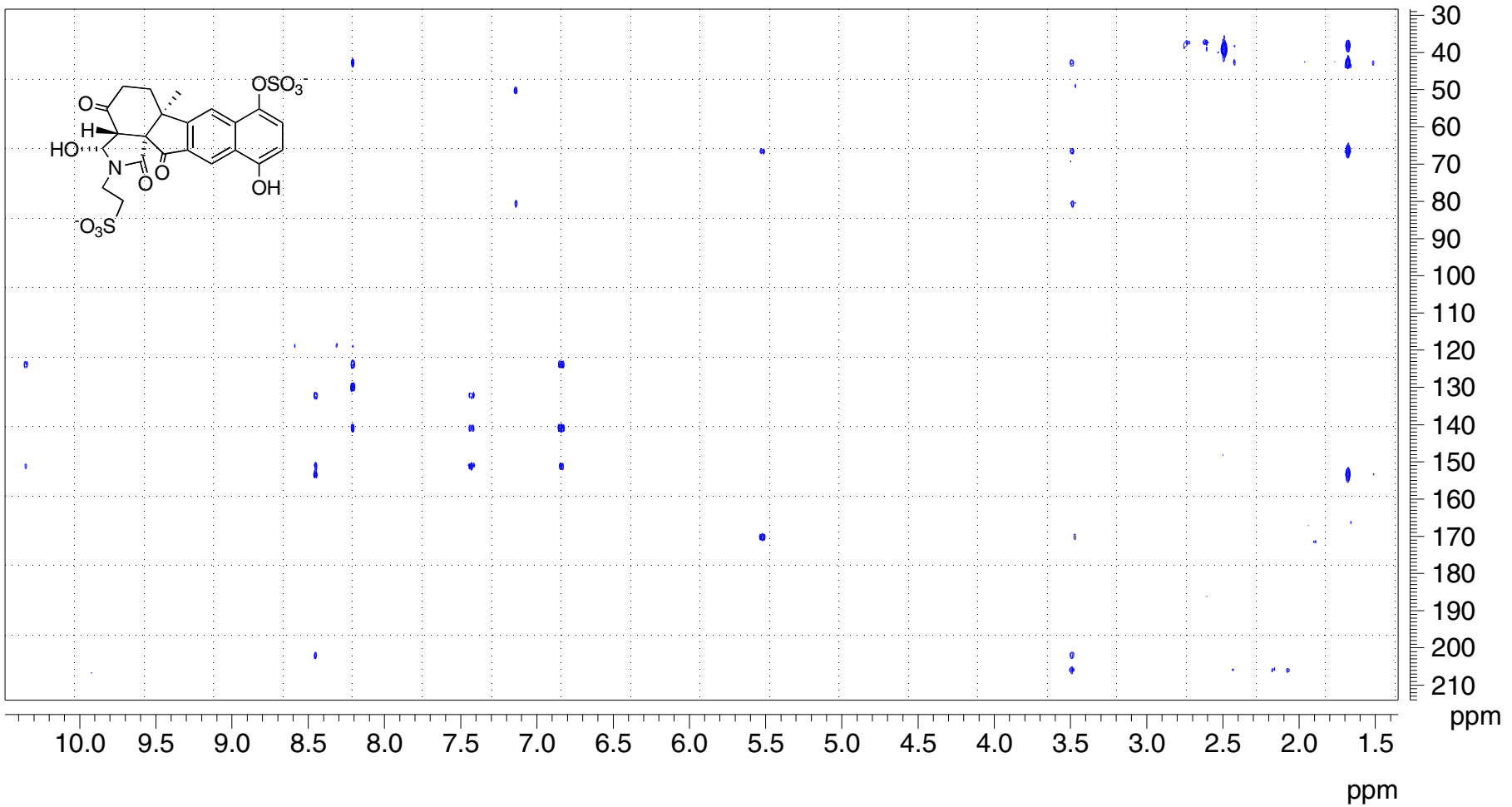
^{1}H NMR spectrum of exiguaquinol (2) in DMSO-*d6*



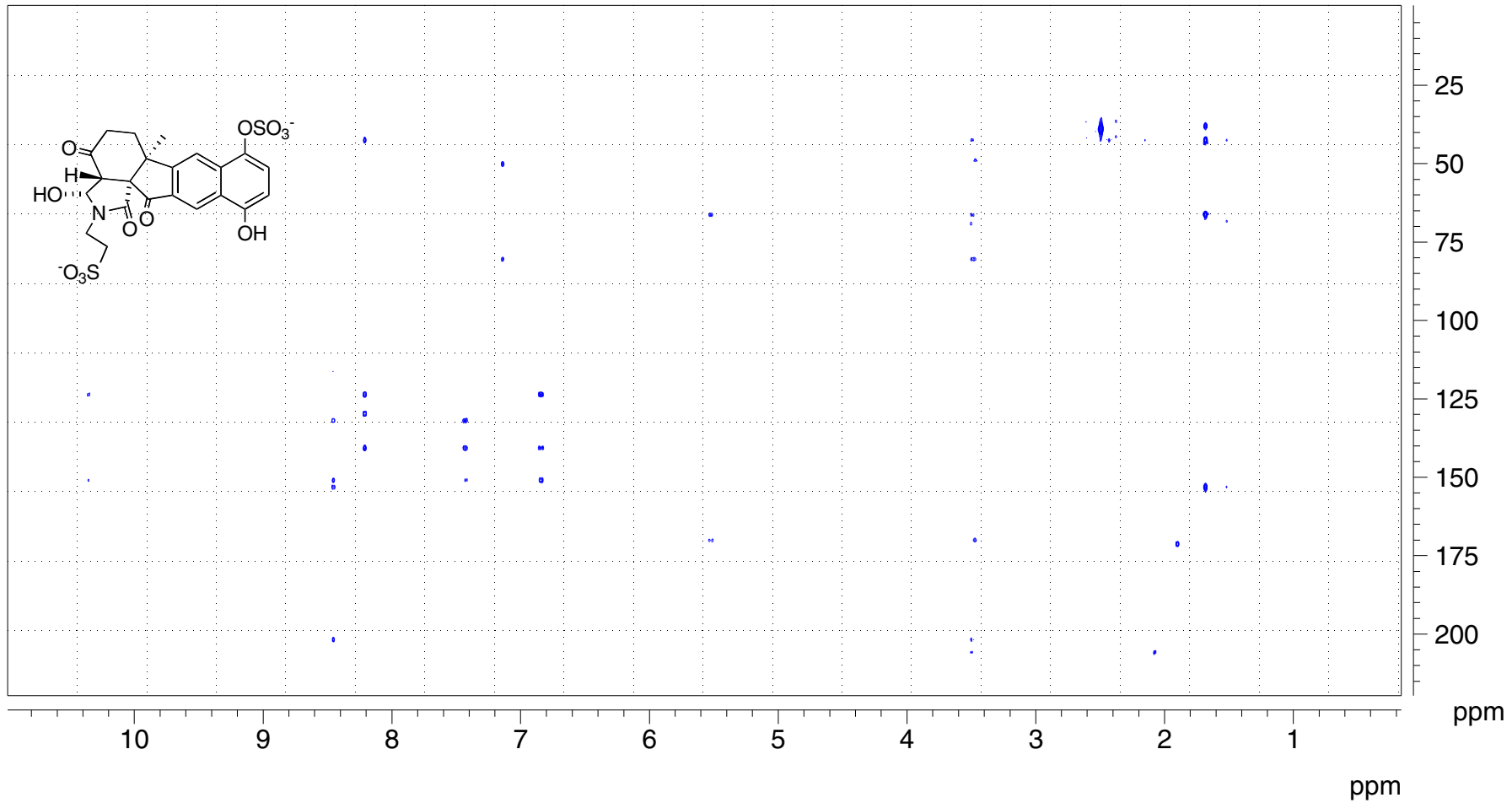
COSY spectrum of exiguaquinol (2) in DMSO-*d6*



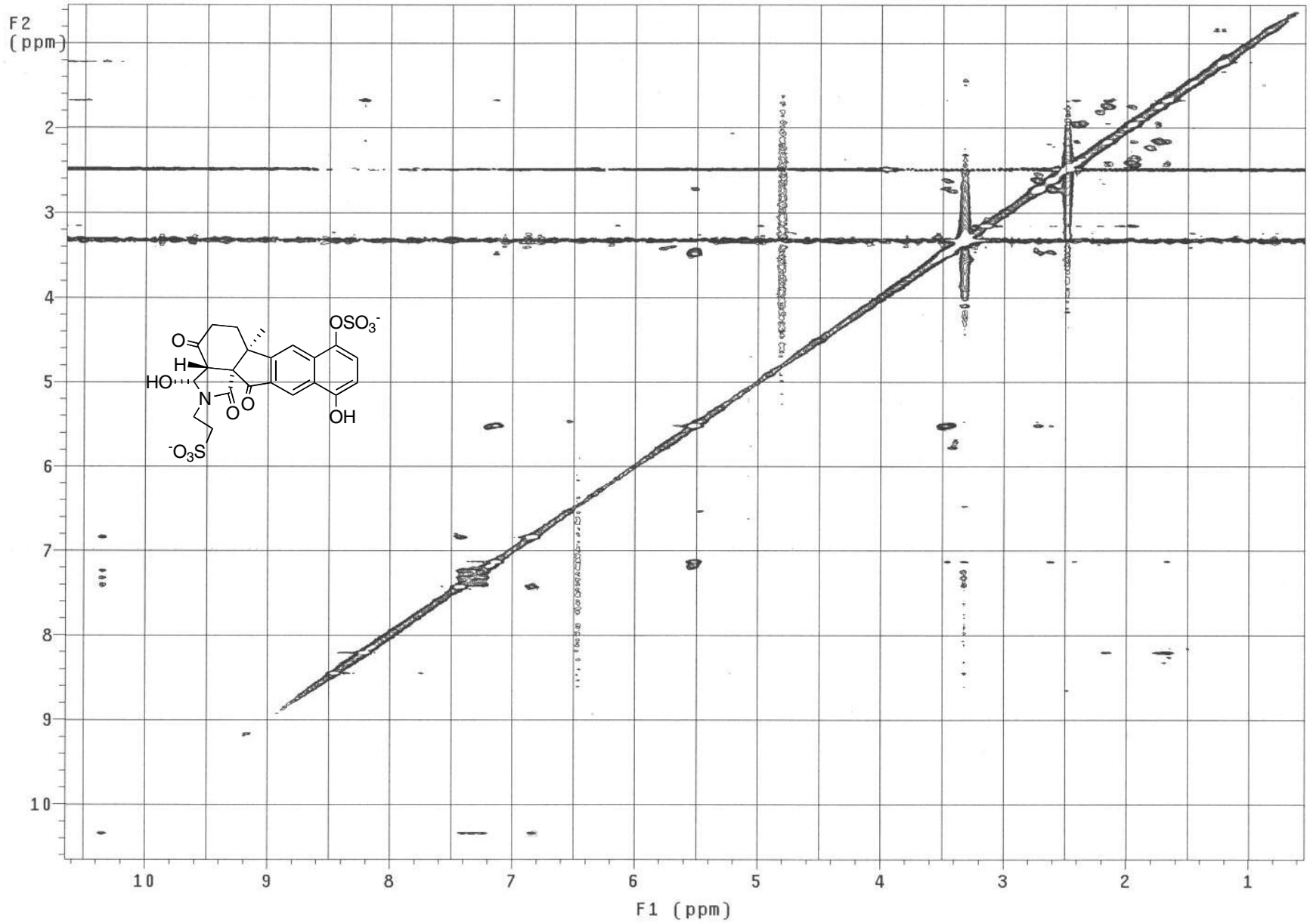
Phase sensitive HSQC spectrum of exiguaquinol (2) in DMSO-*d6*



HMBC spectrum of exiguaquinol (2) in DMSO-*d6*



CIGAR spectrum of exiguaquinol (2) in DMSO-*d6*



ROESY spectrum of exiguaquinol (2) in DMSO-*d6*

Molecular Modelling

Docking was done using the program GOLD[1-4] (Genetic Optimisation of Ligand Docking) version 3.1 and standard default settings to produce 50 solutions for each experiment. The protein structure of *H. pylori* MurI complexed with D-glutamate and compound **1** were prepared by extracting the ligand, removing all waters, lone pairs and dummy atoms, and adding hydrogens. The exiguaquinol (**2**) was prepared by checking atom types and bond types followed by a minimisation using the MMFF94s force field, MMFF94 charges, conjugate gradient optimisation method, and termination at a gradient of 0.05 kcal/(mol*A) without any initial optimisation. Solutions for hydrogen bonding analysis were selected on the basis of GoldScore fitness function.[1, 2] Hydrogen bond interactions were analysed using a previously reported method.[5]

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