



Article

Design, synthesis and in vitro evaluation of novel 8-amino-quinoline combined with natural antioxidant acids

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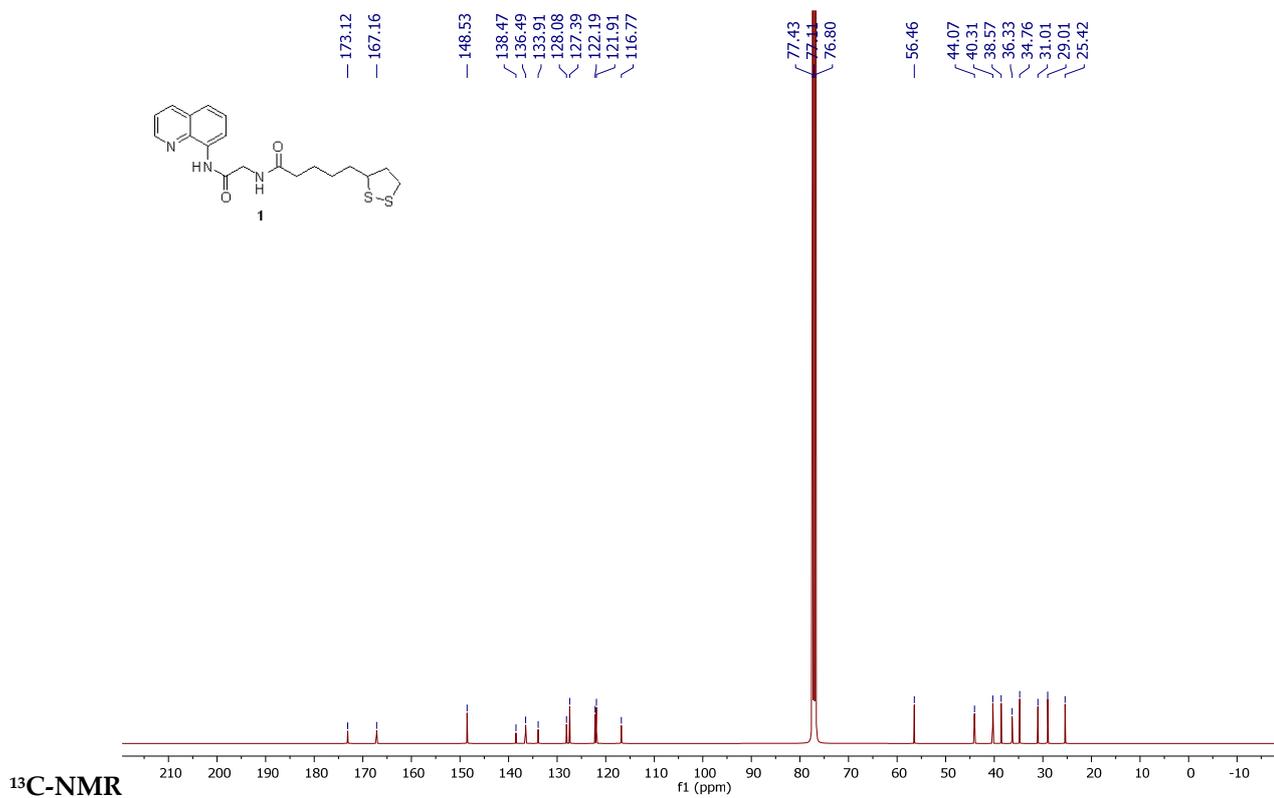
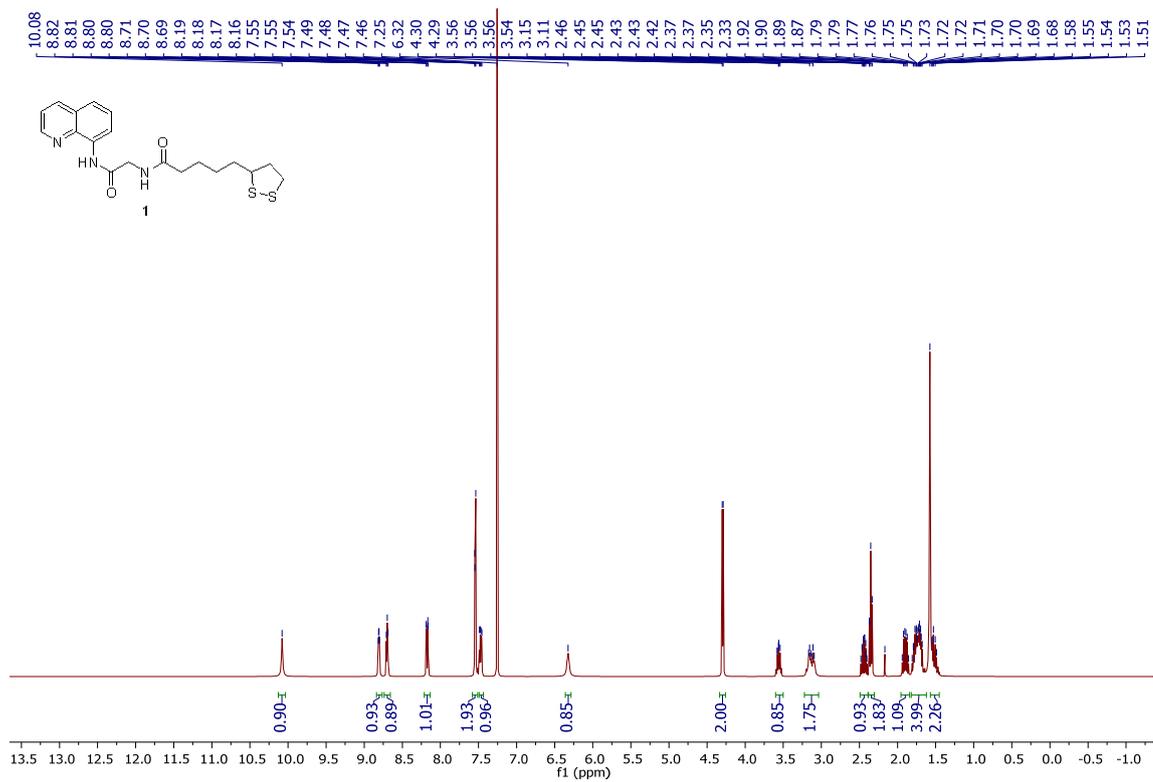
Contents

¹ H-NMR and ¹³ C NMR spectra of 1-8	S1-S9
HPLC Analysis of 1-8.....	S10
Copper chelating study	S18
DPPH assay	S18
Cell viability	S19

1. ¹H-NMR AND ¹³C-NMR SPECTRA OF FINAL PRODUCTS 1-8

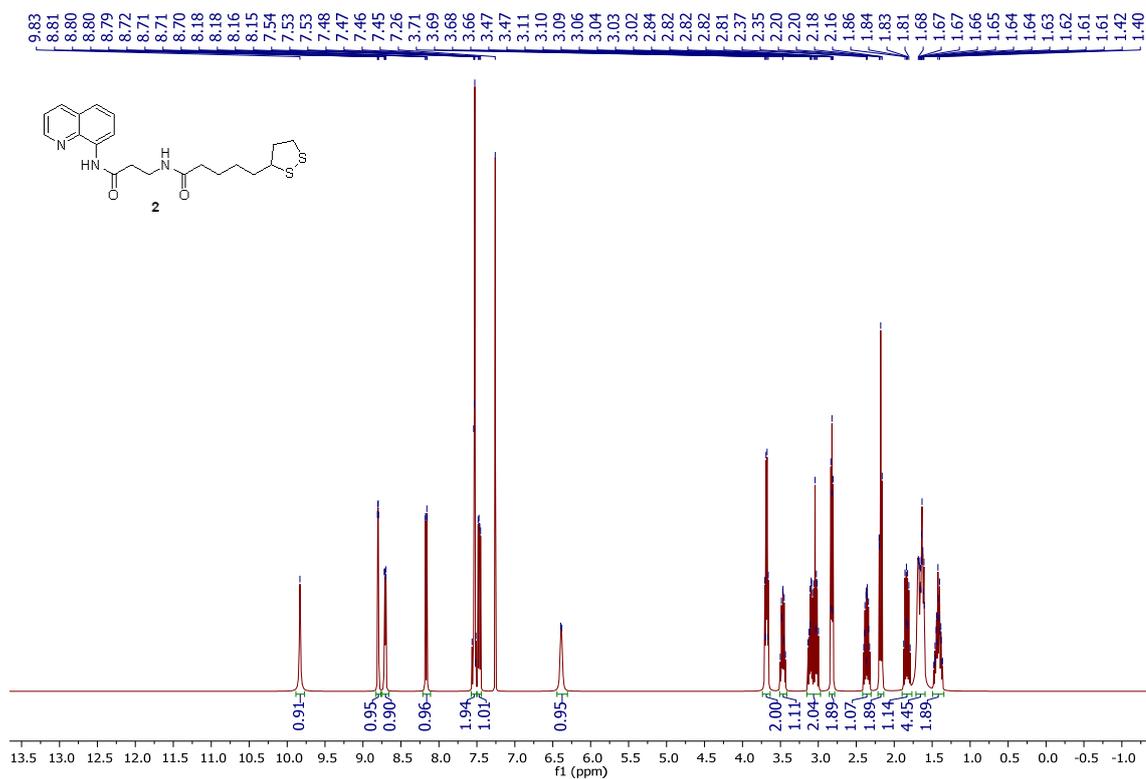
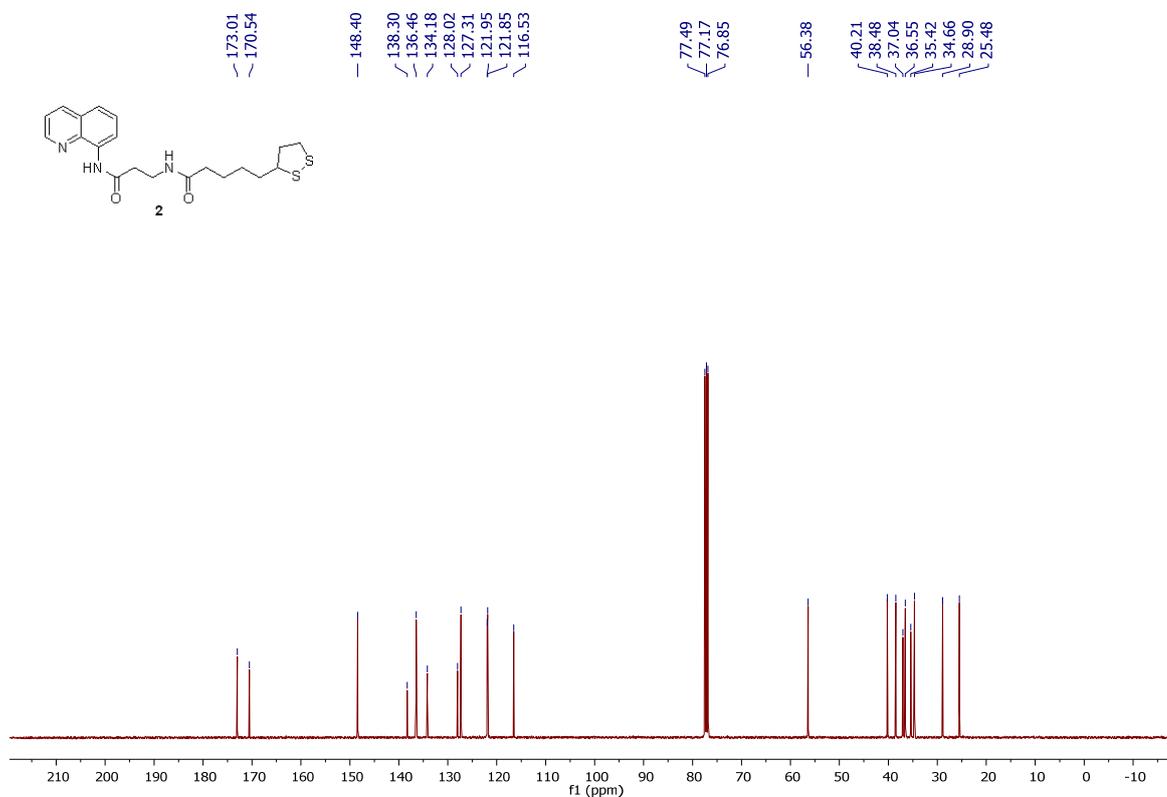
5-(1,2-dithiolan-3-yl)-N-(2-oxo-2-(quinolin-8-ylamino)ethyl)pentanamide (1)

¹H-NMR



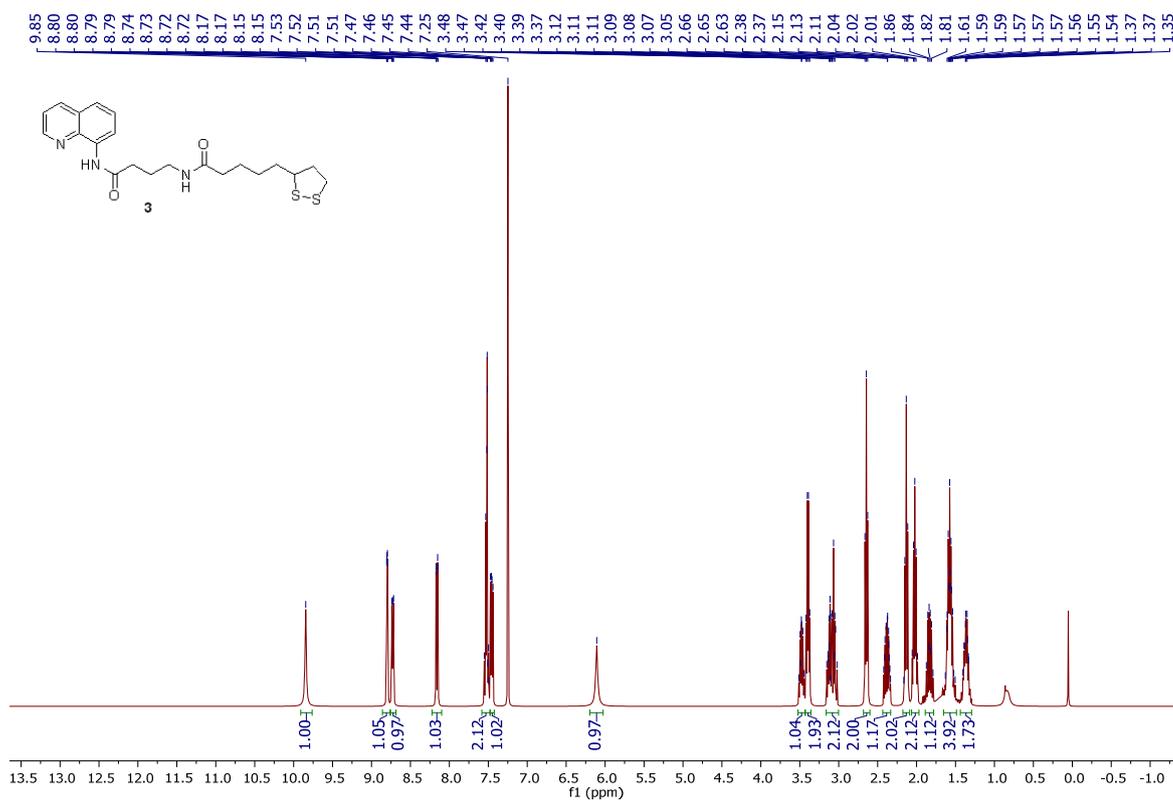
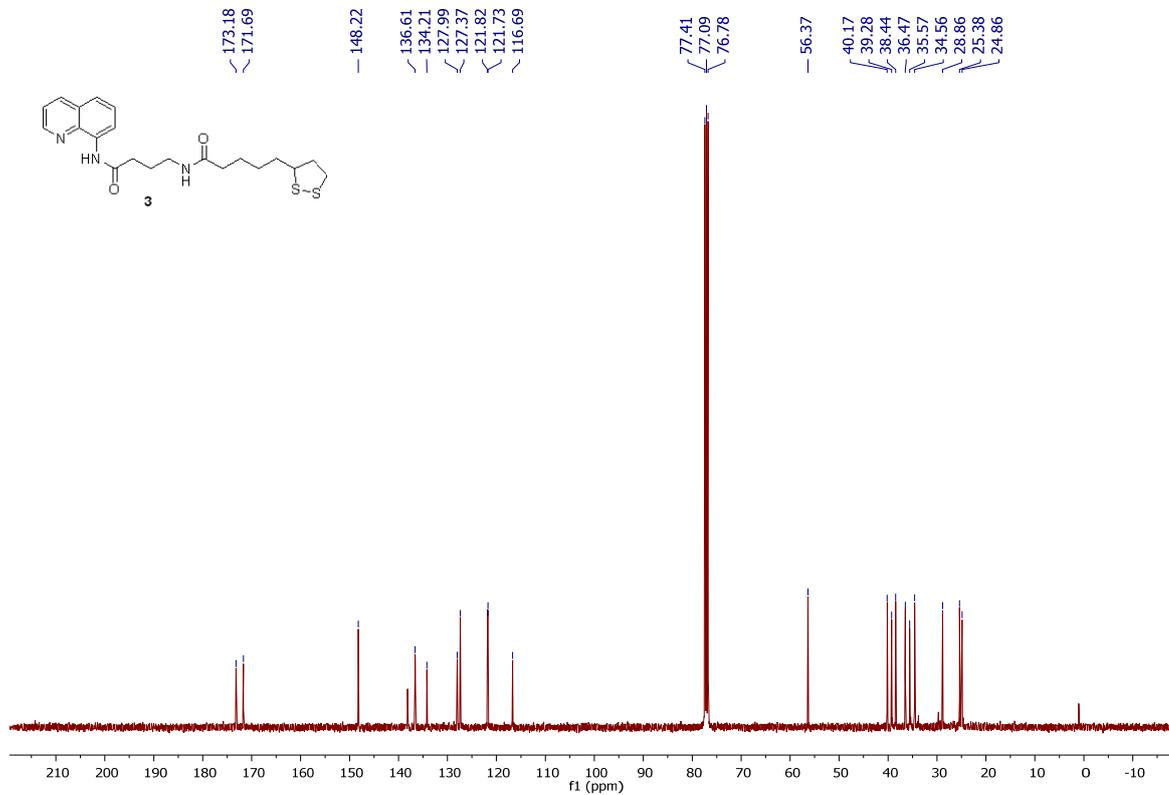
5-(1,2-dithiolan-3-yl)-N-(3-oxo-3-(quinolin-8-ylamino)propyl)pentanamide (2)

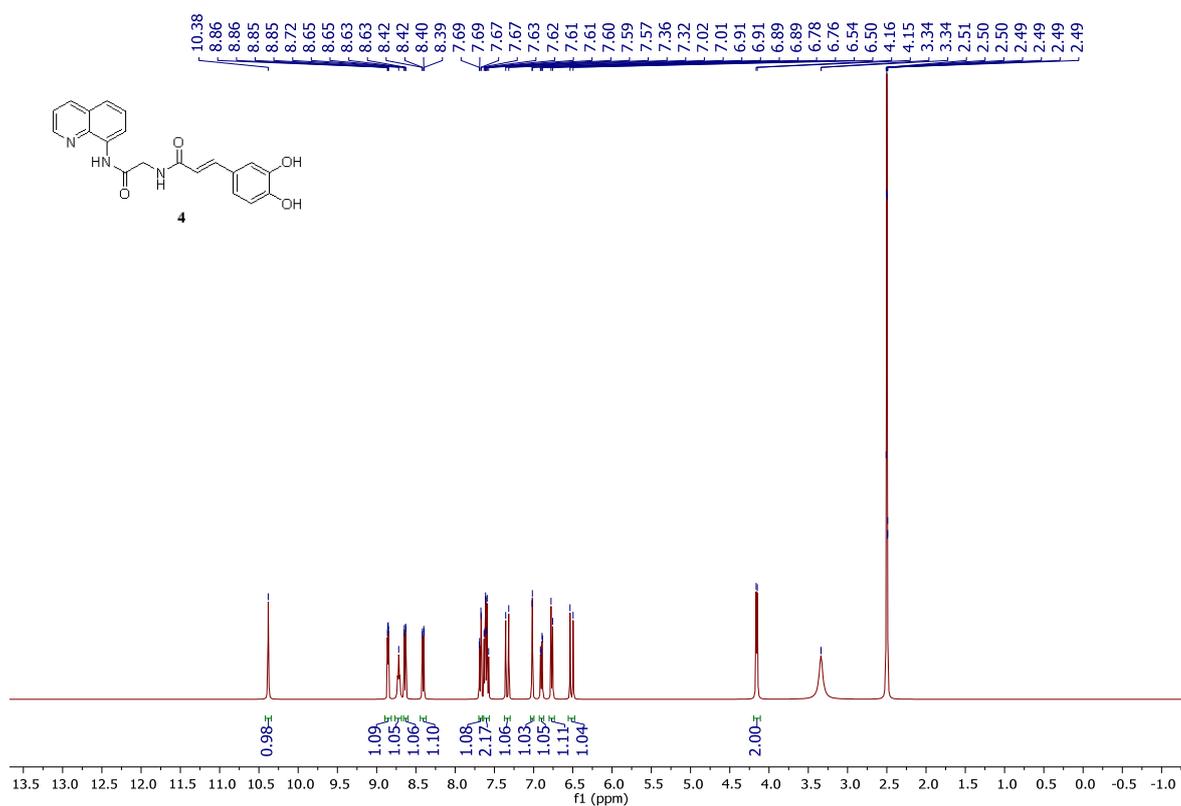
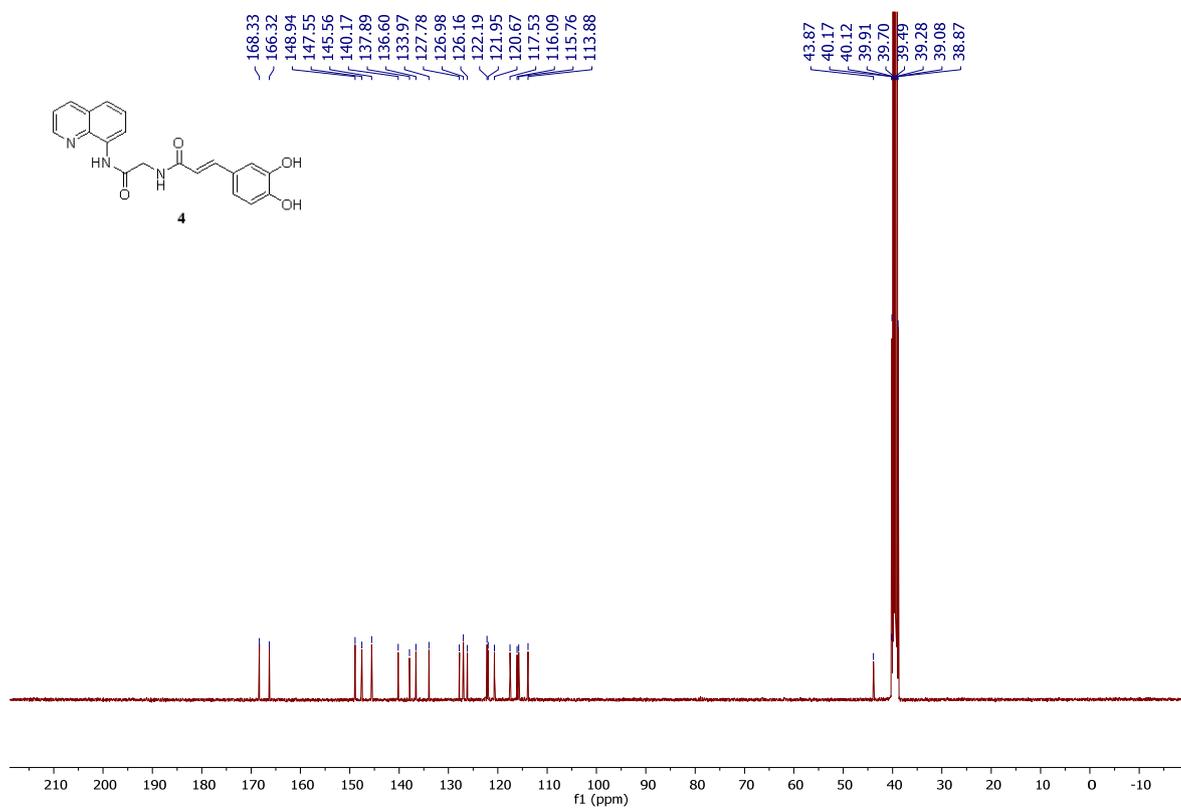
¹H-NMR

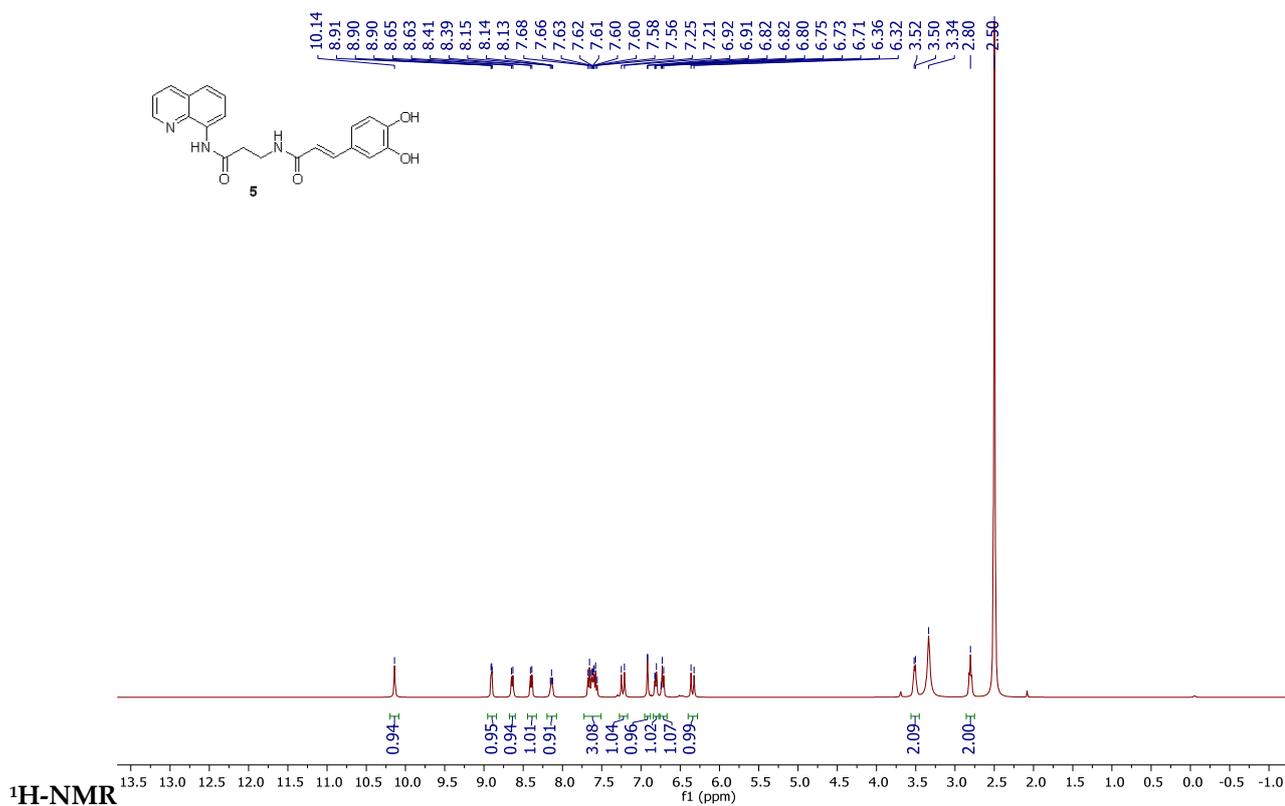
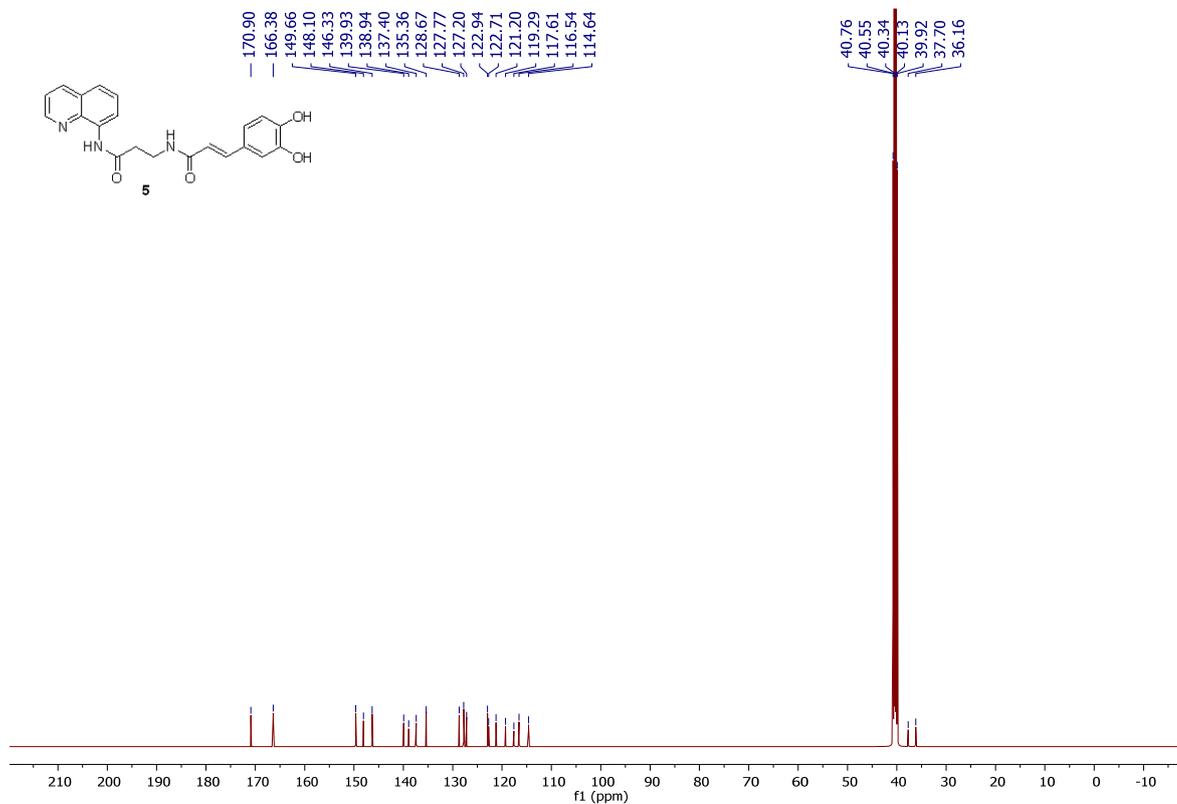
¹³C-NMR

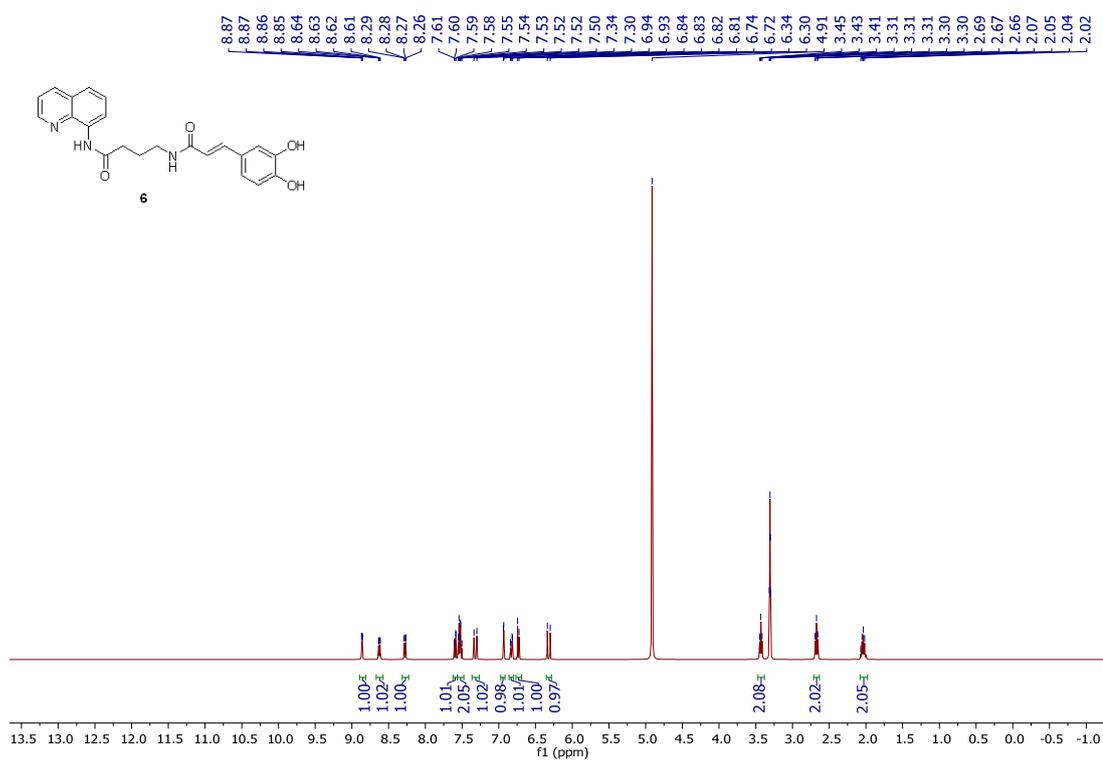
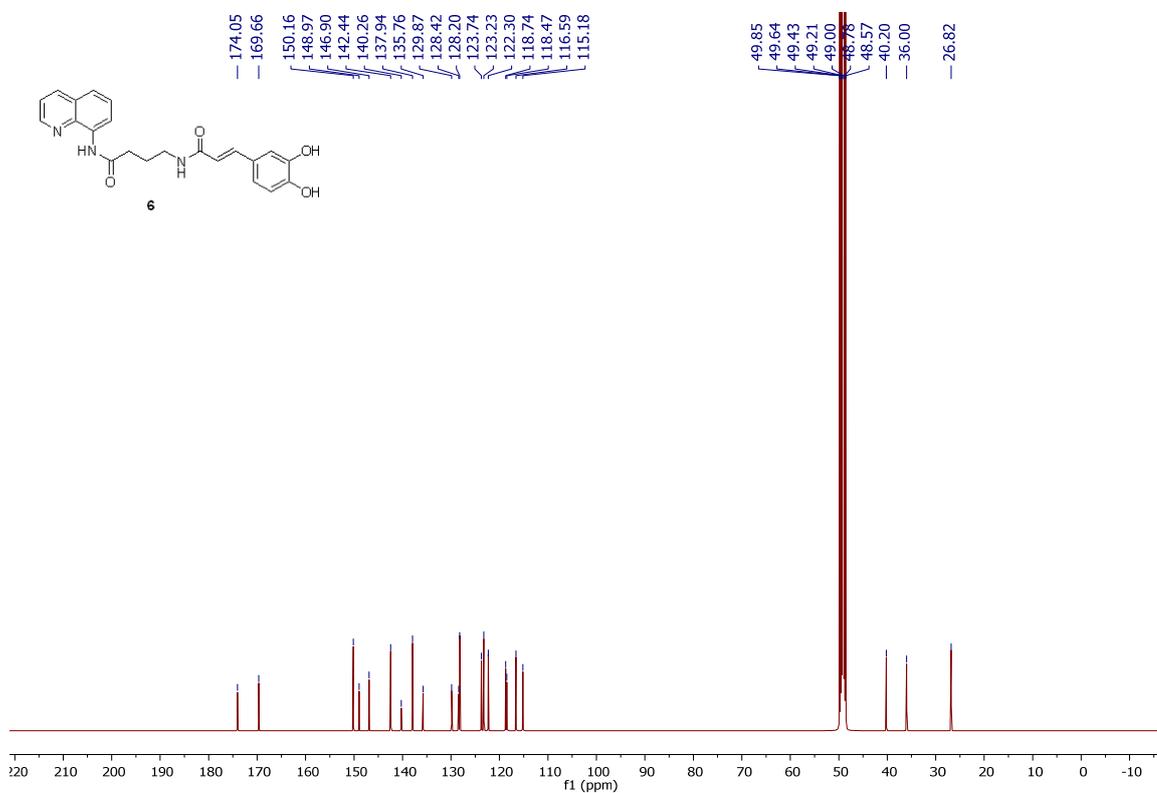
5-(1,2-dithiolan-3-yl)-N-(4-oxo-4-(quinolin-8-ylamino)butyl)pentanamide (3)

¹H-NMR

**¹³C-NMR****(E)-3-(3,4-dihydroxyphenyl)-N-(2-oxo-2-(quinolin-8-ylamino)ethyl)acrylamide (4)****¹H-NMR**

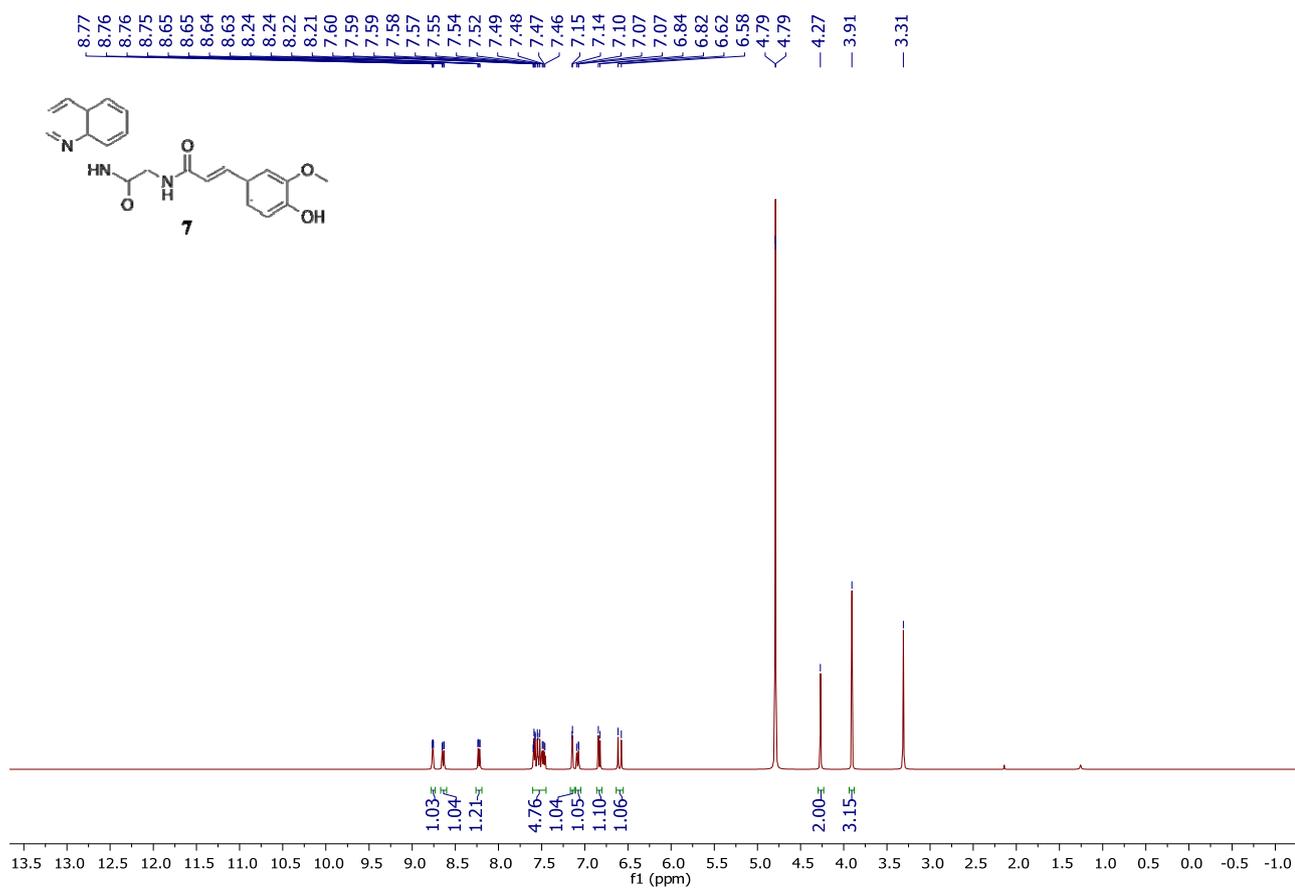
**¹³C-NMR****(E)-3-(3,4-dihydroxyphenyl)-N-(3-oxo-3-(quinolin-8-ylamino)propyl)acrylamide (5)**

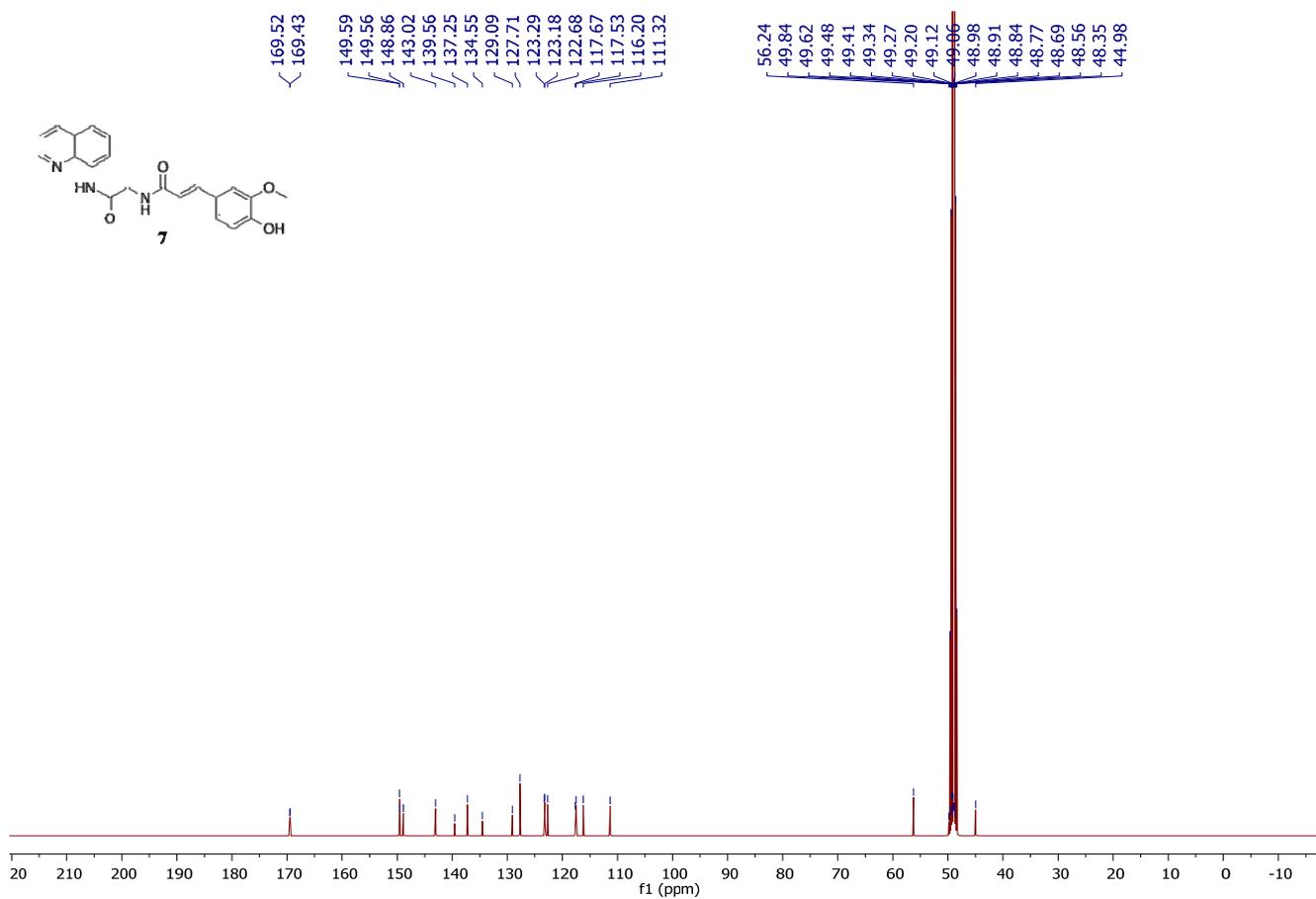
**¹³C-NMR****(E)-4-(3-(3,4-dihydroxyphenyl)acrylamido)-N-(quinolin-8-yl)butanamide (6)****¹H-NMR**

**¹³C-NMR**

(E)-3-(4-hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-8-ylamino)ethyl)acrylamide (7)

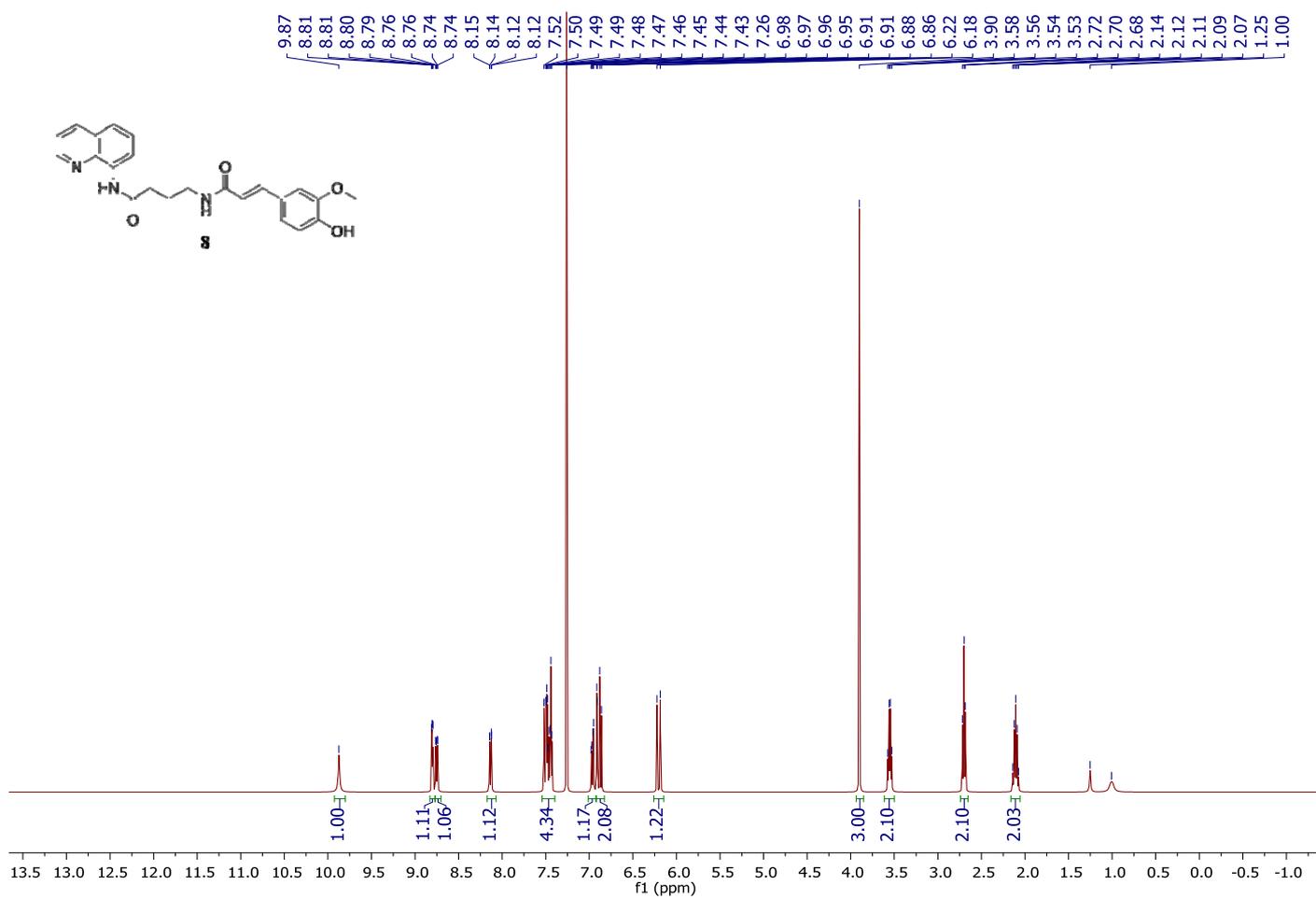
¹H-NMR



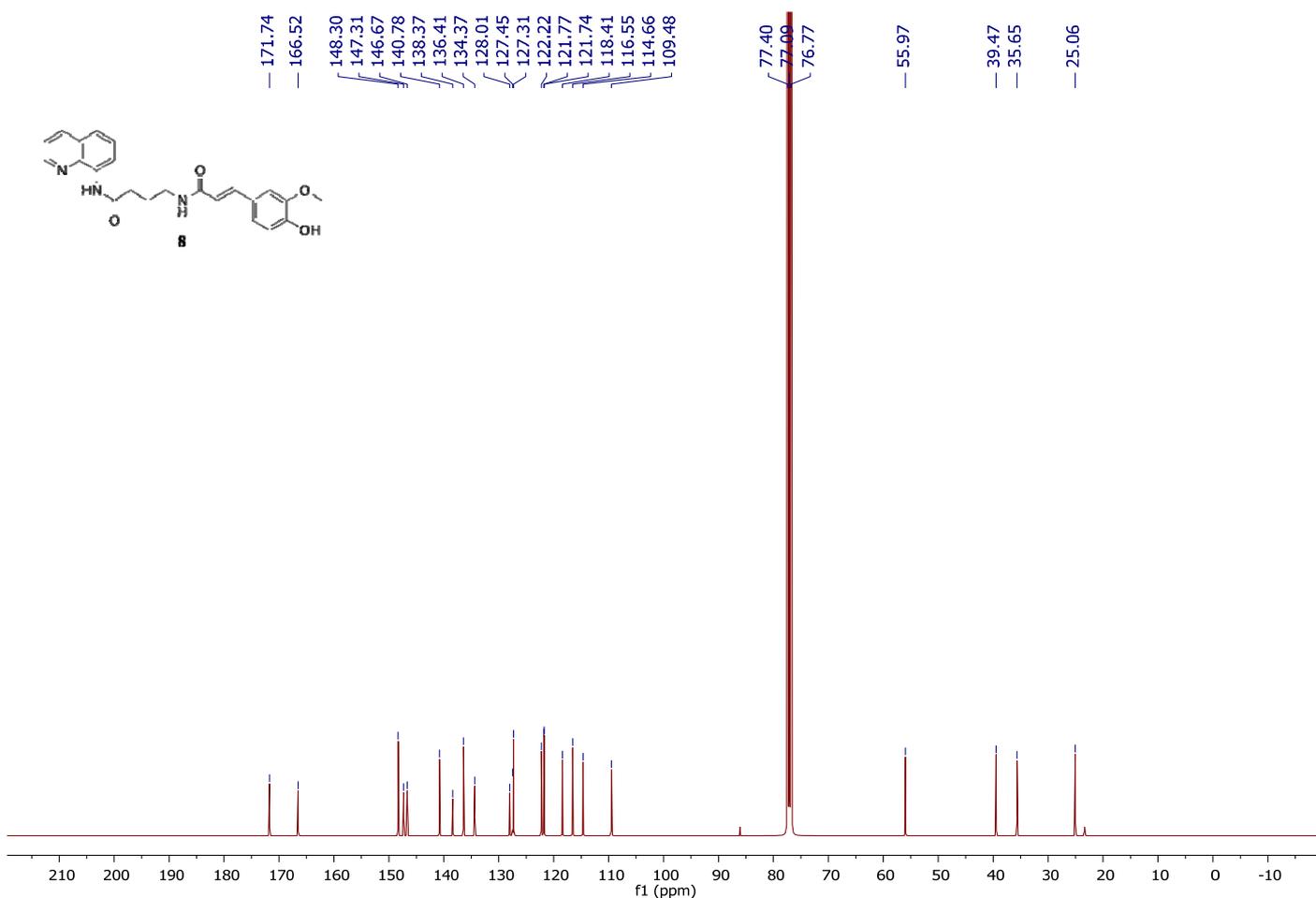


(E)-4-(3-(4-hydroxy-3-methoxyphenyl)acrylamido)-N-(quinolin-8-yl)butanamide (8)

¹³C-NMR



¹³C-NMR

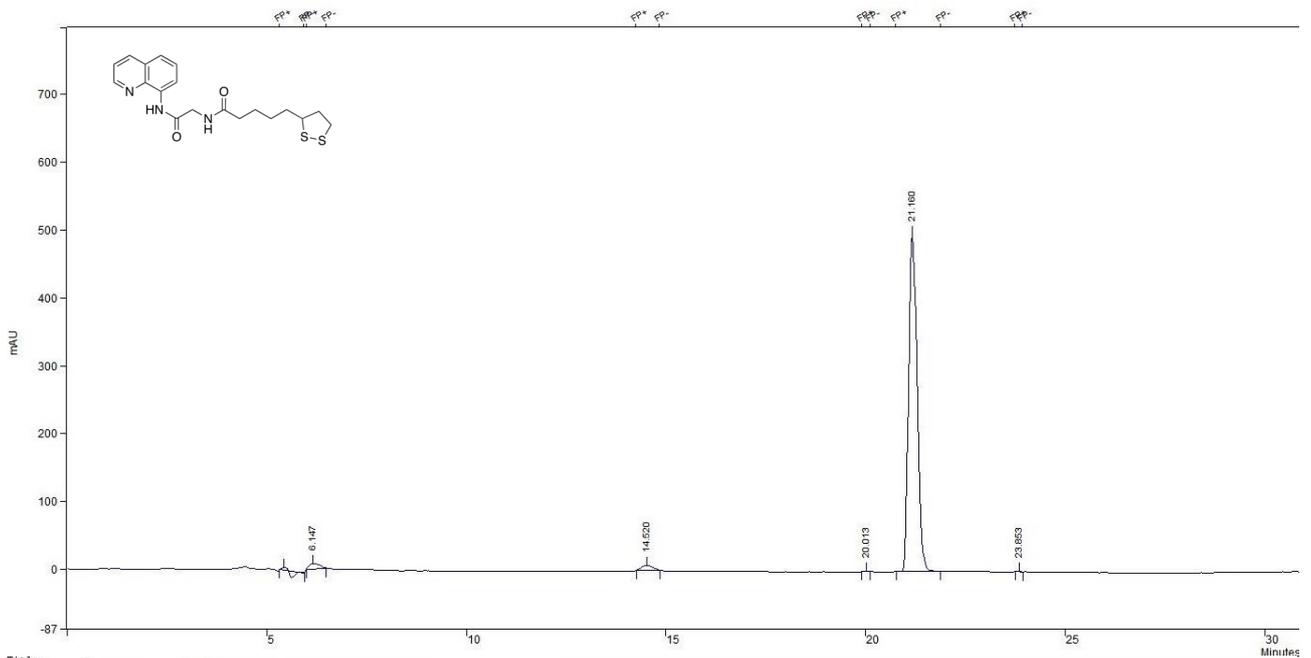


2. HPLC analysis

HPLC purity determination was performed on a Varian Pro Star 330PDA detector, a ternary HPLC pump Varian 9012 and a Rheodyne injector with 20 μ l loop. A RP column ThermoScientific TM Hypersil TM C18 ODS (5 μ m, 250 \times 4.6 mmID) HPLC was used for all analysis (detection at 240 nm). Stock solutions of compounds 1-8 were prepared in a mixture of MeOH/ACN (2:3) and stored at 4°C. As the mobile phase, acetonitrile was used as eluent (A) and eluent (B) was water, with the elution gradient varying according to the method depicted in the table. HPLC analysis confirmed the $\geq 95\%$ purity of all compounds 1-8.

Time (minutes)	Flow rate (mL/min)	%ACN (A)	%H ₂ O (B)
Initial	0.60	20	80
20 min	0.60	80	20
30 min	0.60	20	80

Compound 1



Title :
 Run File : c:\documents and settings\varian\desktop\bacci\compound 1 h acn.run
 Method File : c:\star\data\bacci\si58 h acn 1-12 c-2.mth
 Sample ID : si58 h acn 1-12 c

Injection Date: 11/05/2022 12.53 Calculation Date: 11/05/2022 13.49

Operator :
 Workstation: HPLC
 Instrument : Varian Star #1
 Channel : 2 = 240.00 nm

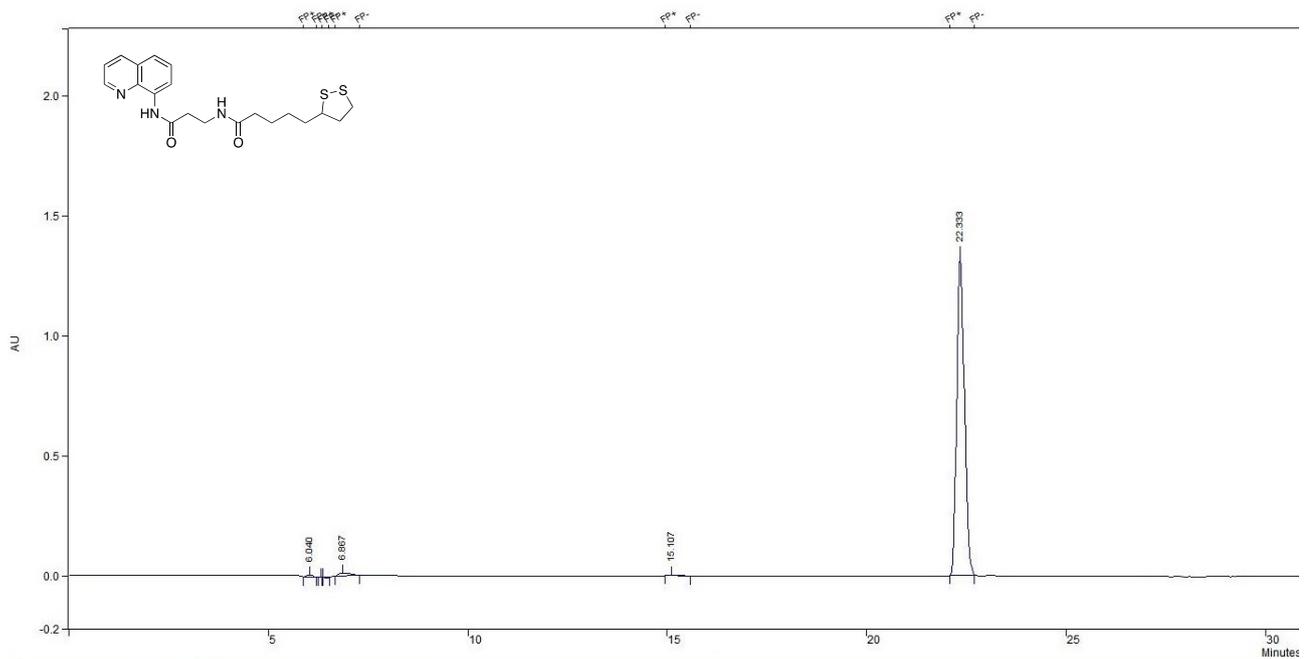
Detector Type: 330 UV-Vis. PDA
 Bus Address : 71
 Sample Rate : 0.63 Hz
 Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01938-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result (%)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 Code (sec)	Status Codes
1		2.0315	6.147	0.000	901499	BB	21.3	
2		1.8085	14.520	0.000	718510	BB	19.5	
3		0.0545	20.013	0.000	21515	BB	8.6	
4		96.0749	21.160	0.000	37905088	BB	14.2	
5		0.0306	23.853	0.000	12080	BB	6.1	
Totals:		100.0000		0.000	39453692			

Compound 2



Title :
 Run File : c:\documents and settings\varian\desktop\bacci\compound 2 h acn.run
 Method File : c:\star\data\bacci\sl 56 h acn 1-12 c-2.mth
 Sample ID : SI 64 H ACN 1-12

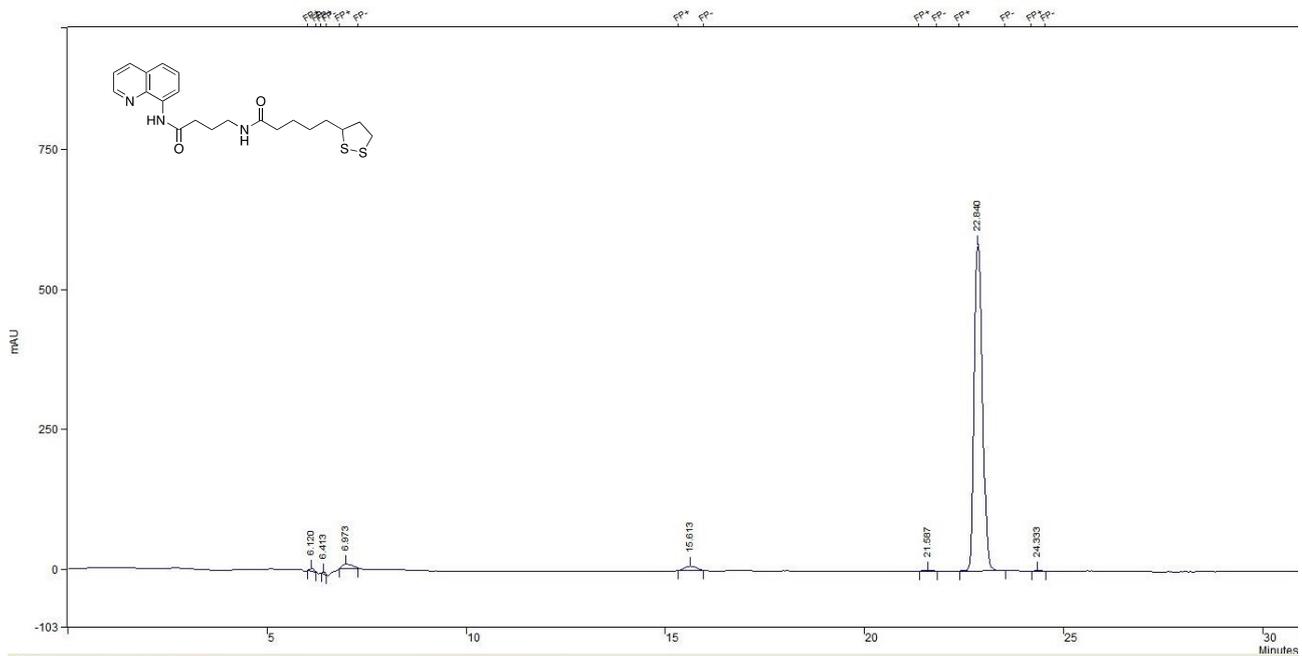
Injection Date: 09/05/2022 16.52 Calculation Date: 09/05/2022 17.58

Operator : Workstation: HPLC Detector Type: 330 UV-Vis. PDA
 Bus Address : 71
 Instrument : Varian Star #1 Sample Rate : 0.63 Hz
 Channel : 2 = 240.00 nm Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01938-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result (t)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 (sec)	Status Codes
1		0.5170	6.040	0.000	498785	BB	10.1	
2		1.1710	6.867	0.000	1107053	BB	23.3	
3		0.3042	15.107	0.000	287635	BB	13.2	
4		99.0078	22.333	0.000	92657848	BB	12.9	
Totals:			100.0000	0.000	94541321			



Title :
 Run File : c:\documents and settings\varian\desktop\bacci\compound 3 h acn.run
 Method File : c:\star\data\bacci\si 56 h acn 1-12 c-2.mth
 Sample ID : SIS6 H ACN H 1-12 C

Injection Date: 09/05/2022 13.59 Calculation Date: 13/05/2022 10.54

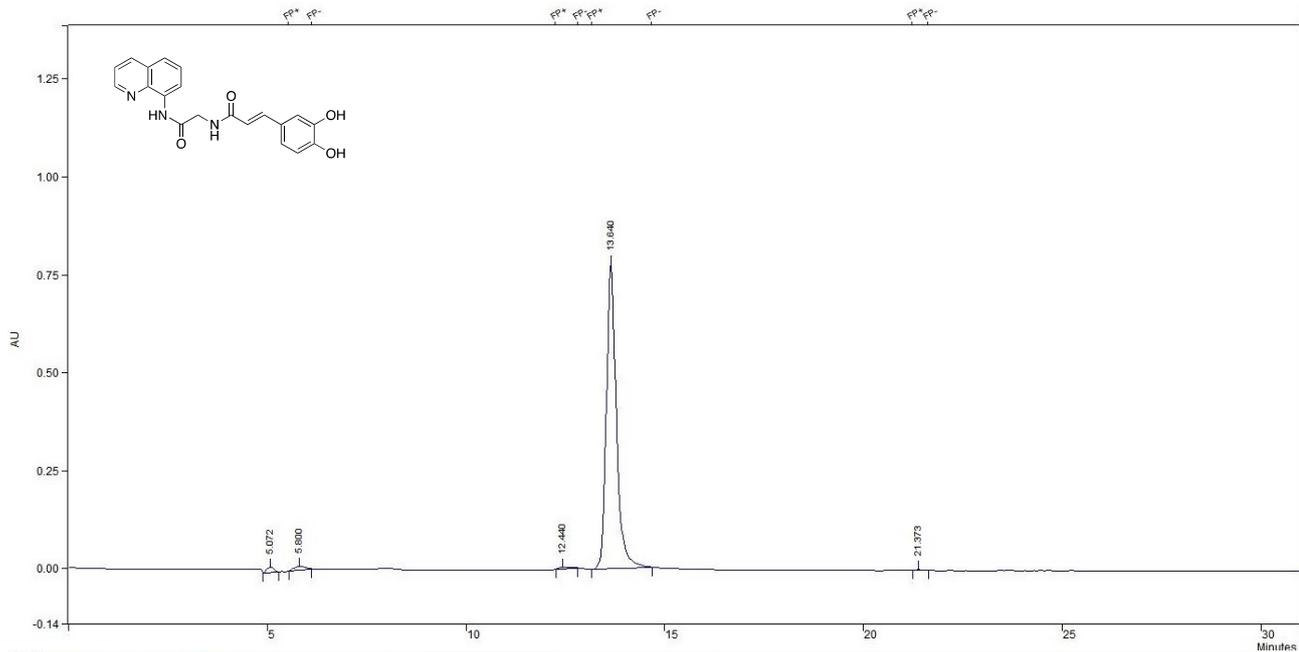
Operator :
 Workstation: HPLC
 Instrument : Varian Star #1
 Channel : 2 = 240.00 nm
 Detector Type: 330 UV-Vis. PDA
 Bus Address : 71
 Sample Rate : 0.63 Hz
 Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01938-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result (%)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 (sec)	Status Codes
1		0.4992	6.120	0.000	226791	BB	7.3	
2		0.1659	6.413	0.000	75357	BB	3.4	
3		1.4702	6.973	0.000	667949	BB	18.6	
4		1.7996	15.613	0.000	817613	BB	21.3	
5		0.2441	21.587	0.000	110917	BB	15.8	
6		95.7174	22.840	0.000	43457596	BB	13.8	
7		0.1036	24.333	0.000	47080	BB	13.3	
Totals:		100.0000		0.000	46433303			

Compound 4



Title :
 Run File : c:\documents and settings\varian\desktop\baccai\compound 4 h acn.run
 Method File : c:\star\data\baccai\si 27 h acn-2.mth
 Sample ID : SI 27 H ACN

Injection Date: 06/05/2022 10.18 Calculation Date: 11/05/2022 15.32

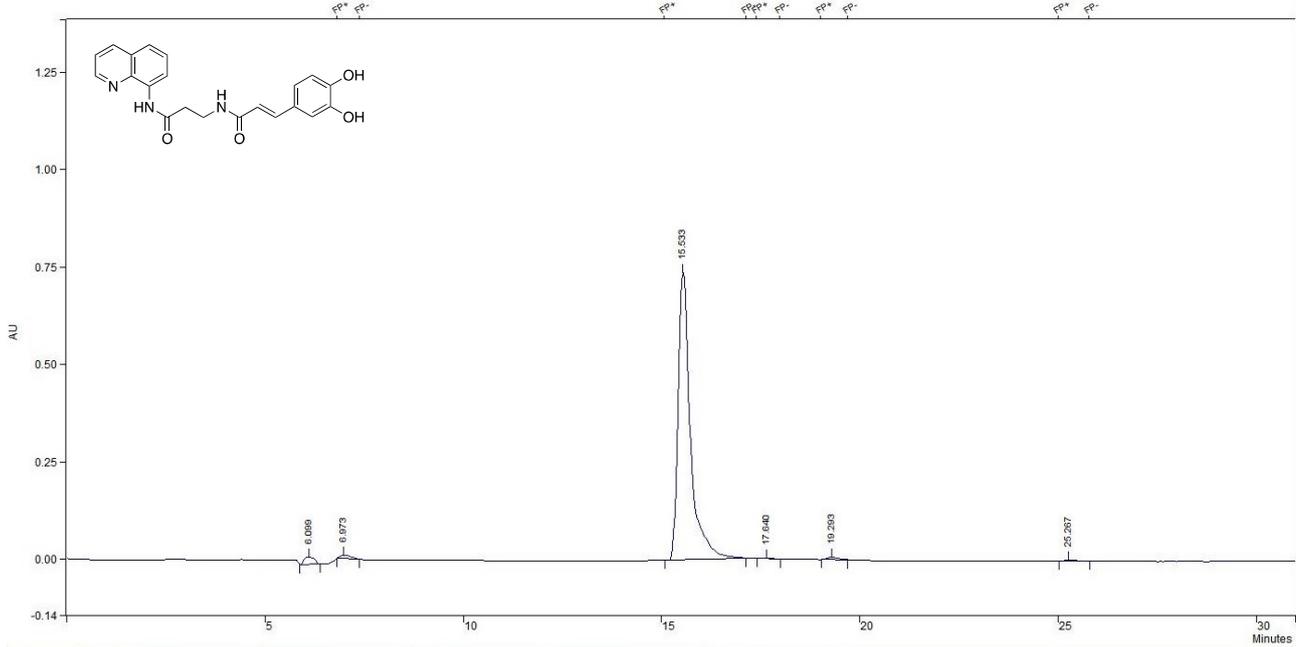
Operator :
 Workstation: HPLC Detector Type: 830 UV-Vis. PDA
 Bus Address : 71
 Instrument : Varian Star #1 Sample Rate : 0.63 Hz
 Channel : 2 = 240.00 nm Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01998-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 Code (sec)	Status Codes
1		1.0883	5.072	0.000	791303	BB	13.1	
2		1.2115	5.800	0.000	880839	BB	26.2	
3		0.5502	12.440	0.000	400043	BB	30.3	
4		97.0028	13.640	0.000	70528120	BB	15.2	
5		0.1471	21.373	0.000	106976	BB	10.4	
Totals:			99.9999	0.000	72707281			

Compound 5



Title :
 Run File : c:\documents and settings\varian\desktop\bacci\compound 5 h acn.run
 Method File : c:\star\data\bacci\si 63 h acn 1-6-2.mth
 Sample ID : SI 63 H ACN 1-6

Injection Date: 06/05/2022 15.08 Calculation Date: 13/05/2022 10.50

Operator :
 Workstation: HPLC
 Instrument : Varian Star #1
 Channel : 2 = 240.00 nm

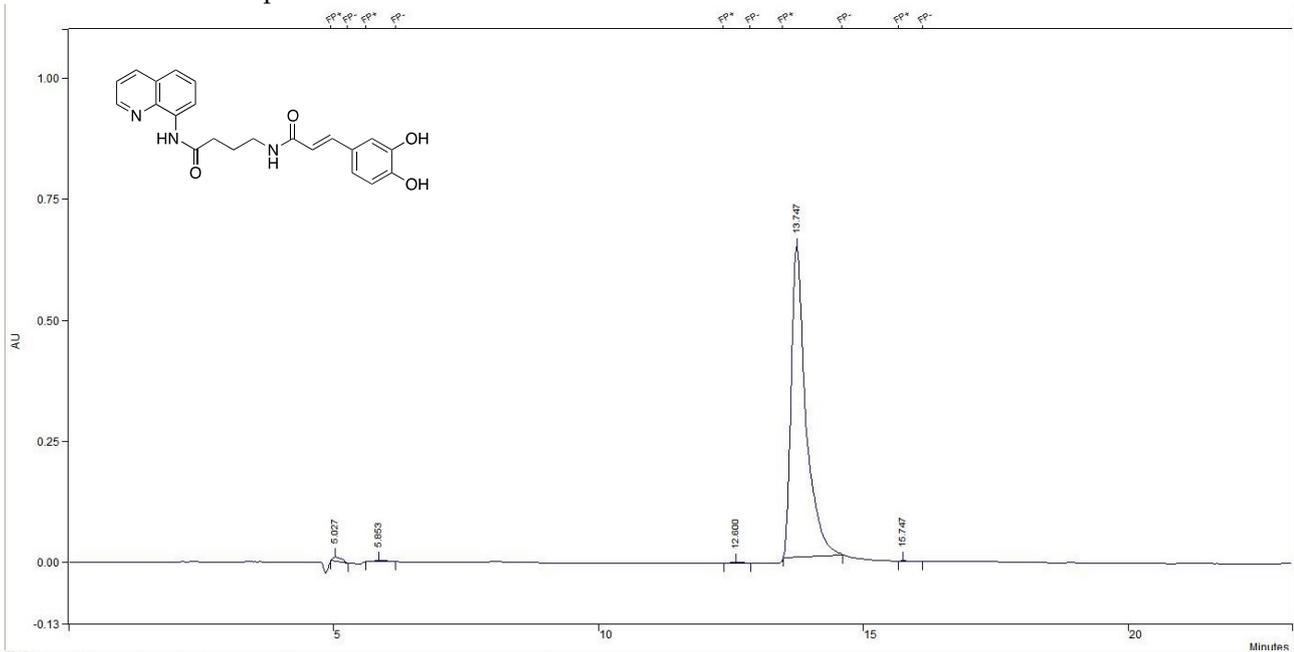
Detector Type: 330 UV-Vis. FDA
 Bus Address : 71
 Sample Rate : 0.63 Hz
 Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01938-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result (%)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 (sec)	Status Codes
1		1.9285	6.099	0.000	1654871	BB	19.0	
2		0.9576	6.973	0.000	821741	BB	17.6	
3		95.9189	15.533	0.000	82310720	BB	17.8	
4		0.2148	17.640	0.000	184284	BB	12.7	
5		0.6672	19.293	0.000	572517	BB	17.8	
6		0.3131	25.267	0.000	269664	BB	20.5	
Totals:		100.0001		0.000	85812797			

Compound 6



Title :
 Run File : c:\documents and settings\varian\Desktop\bacci\compound 6 h acn .run
 Method File : c:\star\data\bacci\si 24 h acn -2.mth
 Sample ID : sSI 24 H ACN

Injection Date: 05/05/2022 16.38 Calculation Date: 13/05/2022 10.49

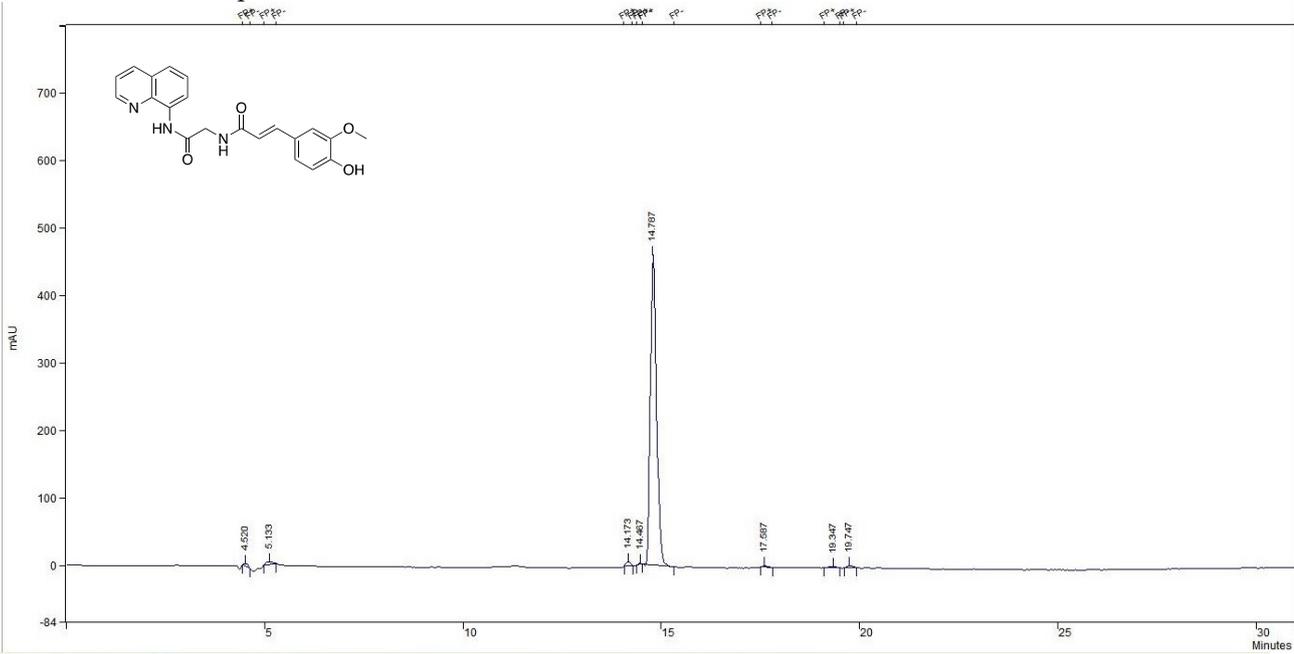
Operator :
 Workstation: HPLC
 Instrument : Varian Star #1
 Channel : 2 = 240.00 nm
 Detector Type: 330 UV-Vis. PDA
 Bus Address : 71
 Sample Rate : 0.63 Hz
 Run Time : 23.120 min

** LC Workstation Version 6.41 ** 01998-61c0-aa4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 (sec)	Status Codes
1		0.7476	5.027	0.000	482938	BB	9.2	
2		0.4164	5.853	0.000	268990	BB	15.7	
3		0.1795	12.600	0.000	116946	BB	19.8	
4		98.5946	13.747	0.000	63687904	BB	16.1	
5		0.6619	15.747	0.000	39989	BB	4.4	
Totals:		100.0000		0.000	64595767			

Compound 7



Title :
 Run File : c:\documents and settings\baccii\desktop\baccii\compound 7 h acn.run
 Method File : c:\star\data\baccii\si 37 h acn 1-6 at2-2.mth
 Sample ID : si 37 h acn 1-6 at2

Injection Date: 12/05/2022 16.58 Calculation Date: 12/05/2022 18.53

Operator :
 Workstation: HPLC
 Instrument : Varian Star #1
 Channel : 2 = 240.00 nm
 Detector Type: 330 UV-Vis. PDA
 Bus Address : 71
 Sample Rate : 0.63 Hz
 Run Time : 31.013 min

** LC Workstation Version 6.41 ** 01938-61c0-ea4-04b0 **

Run Mode : Analysis
 Peak Measurement: Peak Area
 Calculation Type: Percent

Peak No.	Peak Name	Result (%)	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	1/2 (sec)	Status Codes
1		0.6667	4.520	0.000	175246	BB	6.1	
2		0.8573	5.133	0.000	225365	BB	15.9	
3		0.6451	14.173	0.000	169566	BB	7.8	
4		0.1353	14.467	0.000	45174	BB	5.9	
5		96.1359	14.787	0.000	25271500	BB	9.9	
6		0.3115	17.587	0.000	81891	BB	7.9	
7		0.4977	19.347	0.000	190838	BB	14.0	
8		0.7025	19.747	0.000	184674	BB	10.3	
Totals:			100.0000	0.0000	26287254			

3. Copper chelating study

Standard solutions of compounds 1-8 were prepared in 100% DMSO and diluted in absolute ethanol to reach the concentration of 20 μM (DMSO final concentration < 0.1%). A 4X stock solution of CuCl_2 was prepared at concentration of 800 μM in absolute ethanol. 100 μL of this solution was transfected in a 96-well plate and diluted to obtain progressive final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0 μM . Then, 100 μL of each compound solutions were added in every well obtaining a final concentration of compounds of 10 μM (duplicated). After 30 minutes of incubation in a dark room, the absorption spectrum was recorded at room temperature with UV-vis spectrophotometer PerkinElmer EnSpire 2300. Absorption spectra were collected detecting values at different wavelengths (230-500 nm) and normalized with control (ethanol solution) and then analyzed the absorbance values in function of wavelength using SpectraGryph 1.2.

4. DPPH assay

In this method, the scavenging activity of the colored radical by synthesized compounds is followed by spectrophotometry. Standard solutions of compounds 1-8 were prepared in 100% DMSO and diluted in absolute methanol to reach the concentration of 400 μM (DMSO final concentration < 0.1%). 75 μL of each compound solution were properly diluted to 200 and 100 μM and then added with 75 μL of 1mM methanolic solution of DPPH (final DPPH concentration 500 μM , final concentrations of compounds 200, 100 and 50 μM , duplicated). The well was incubated in a dark room, at room temperature for 45 minutes and the absorbance values was measured at 531 nm with PerkinElmer Enspire 2300 multiplate reader. Instead, for detect IC_{50} of selected compounds, in a 96-well plate 300 μM methanolic solutions of compounds (75 μL) were properly diluted to 150, 75, 37.5, 18.8, 9.4, 4.7 and 0 μM (duplicated). Then, in each well were added 75 μL of a 1 mM methanolic solutions of DPPH (final well concentration 500 nM). The well was incubated in a dark room, at room temperature for 45 minutes and the absorbance values was measured at 531 nm with PerkinElmer Enspire 2300 multiplate reader. All the values were then mediated, normalized with control (only methanol) and the scavenging activity percentage calculated with the formula:

$$\text{scavenging capacity \%} = 100 * \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{compound}}}{\text{Abs}_{\text{DPPH}}}$$

5. Cell viability

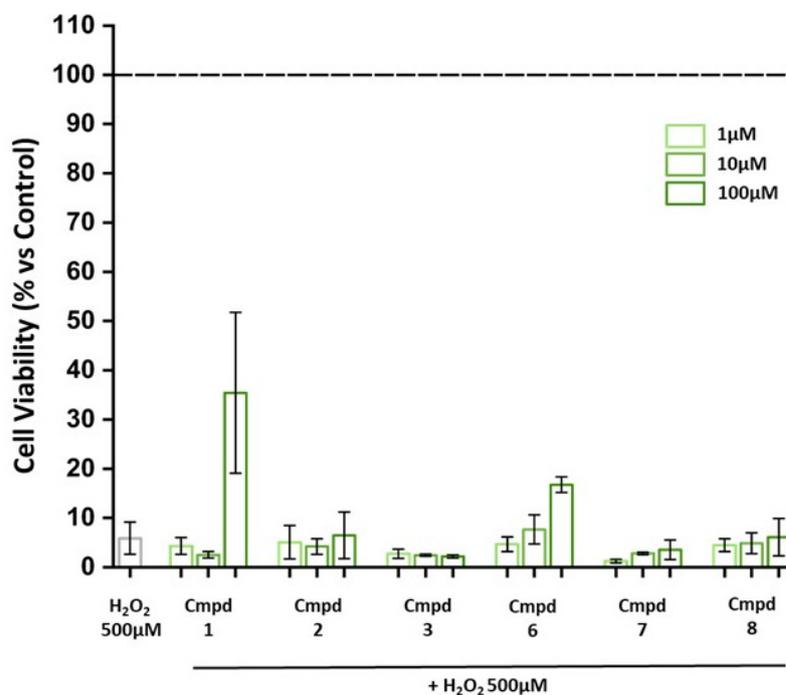


Figure S1. Cell viability was analysed by CellTiter 96 Aqueous – One solution Reagent. Cells were pre-treated for 24 h with compounds at various concentration (1-10-100µM) and then exposed for 3 h with H₂O₂ 500µM. The dashed line indicates the reference value of Ctrl: control group - no compounds or H₂O₂ exposure. Values in the graph indicate % viability as the mean ± SE obtained from a n=3 of independent experiments