Supplemental File

First report of antioxidant 1*H*-benzochromenone from muricid gastropod *Chicoreus ramosus* as dual inhibitors of pro-inflammatory 5-lipoxygenase and carbolytic enzymes

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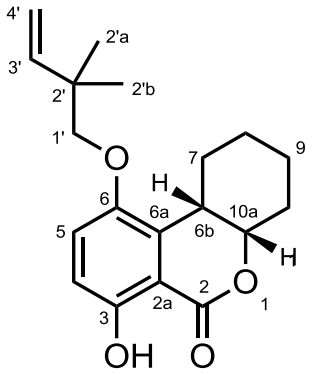
ABSTRACT

Chromene derivatives with manifold structural framework and pharmacological properties were ubiquitous in the mollusks of marine origin. A previously undescribed 1*H*-benzochromenone was isolated through bioassay-guided chromatographic purification of the organic extract of the marine gastropod mollusk *Chicoreus ramosus*. The compound was characterized as 6-(2′,2′-dimethyl)-3′-en-1′-yl-1′-oxy)-3-hydroxy-1*H*-benzo[c]chromene-2(10a*H*)-one based on integrated spectroscopic analysis. The antioxidant studies by employing the stable free radicals reported that the antioxidant activity (IC_{50} 1.4-1.6 mM) was comparable to *α*-tocopherol (IC_{50} 1.4-1.7 mM). The attenuating potential of the studied compound against pro-inflammatory 5-lipoxygenase (IC_{50} 2.12 mM) was significantly greater than that exhibited by anti-inflammatory drug ibuprofen (IC_{50} 4.4 mM), whereas its inhibitory properties against carbolytic *α*-amylase (IC_{50} ~0.72 mM) was comparable with that displayed by acarbose (IC_{50} 0.43 mM). The present study recognized the potential of 1*H*-benzochromenone derivative isolated from *C. ramosus* as important pharmaceutical lead with anti-diabetic and anti-inflammatory potentials to reduce the risk of hyperglycaemia and inflammatory pathologies.

Keywords: Gastropod mollusk, *Chicoreus ramosus*, 1*H*-benzochromenone derivative, pro-inflammatory 5-lipoxygenase, carbolytic enzymes, anti-diabetic and anti-inflammatory

Supplemental Data

Table S1. NMR spectroscopic data of 6-(2′,2′-dimethyl)-3′-en-1′-yl-1′-oxy)-3-hydroxy-1*H*-benzo[c]chromene-2(10a*H*)-one in $CDCl_3$[†]



C. No	^{13}C, type	^{1}H NMR (mult, J in Hz)[‡]	^{1}H-^{1}HCOSY	^{1}H-^{13}C HMBC
1	–	–	–	–
2	171.54, C	-	-	-
2a	112.10, C	-	-	-
3	154.0, C	-	-	-
4	115.89, CH	6.90 (d, 7.1)	H-5	C-2a,3,5,6
5	117.56, CH	7.08 (d, 7.1)	-	C-6, 3, 6a
6	151.0, C	-	-	-
6a	129.70, C	-	-	-
6b	34.39, CH	3.65 (dt,8.1, 2.1)	H-7,10a	C-2a,6a
7	31.80, CH_2	1.86 (dt, 5.0, 1.3)	H-8	C-6a
8	29.60, CH_2	1.47 (m)	H-9	-
9	29.26, CH_2	1.40 (m)	H-10	C-7
10	29.02, CH_2	2.39 (dt, 6.2 ,1.8)	H-10a	-
10a	81.52, CH	4.68 (dt, 8.0, 2.5)	-	C-9, 2, 6a
1′	79.8, CH_2	4.06 (s)	-	C-6, 3′
2′	39.38, C	-	-	-
3′	139.0, CH	5.84 (dd, 16.0, 9.0)	H-4′	-
4′	114.0, CH_2	5.03 (dd, 16.8, 2.2)	-	C-2′,3′
		4.98 (dd, 10.0, 2.1)	-	-
2′a	22.60, CH_3	1.25 (s)	-	C-2′
2′b	22.62, CH_3	1.25 (s)	-	C-3′

[†]NMR spectra recorded using Bruker AVANCE III 500 MHz (AV 500) spectrometers.

[‡]Values in ppm, multiplicity and coupling constants (J = Hz) are indicated in parentheses. Assignments were made with the aid of the ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY experiments.

Table S2. Bioactive potentials of the major column fractions of the crude solvent extract (IC_{50} value) [p]

Column fractions	Antioxidant activity		Anti-inflammatory activity	Antidiabetic activity		Yield (g)
	DPPH scavenging	$ABTS^+$ scavenging	5-LOX attenuating	α-amylase inhibitory	α-glucoxidase inhibitory	
CR_A	$3.40^a \pm 0.05$	$3.35^a \pm 0.03$	$2.42^a \pm 0.05$	$4.03^a \pm 0.01$	$3.99^a \pm 0.02$	9.27
CR_B	$2.45^b \pm 0.03$	$2.58^b \pm 0.01$	$2.98^b \pm 0.02$	$2.81^b \pm 0.01$	$2.03^b \pm 0.05$	5.57
CR_C	$2.25^c \pm 0.01$	$2.33^c \pm 0.03$	$2.20^c \pm 0.03$	$3.96^c \pm 0.02$	$4.09^c \pm 0.03$	6.95
CR_D	$2.58^d \pm 0.04$	$2.44^c \pm 0.01$	$2.71^d \pm 0.02$	$4.88^d \pm 0.01$	$4.50^d \pm 0.01$	2.07
CR_E	$2.32^e \pm 0.05$	$2.55^b \pm 0.03$	$2.53^e \pm 0.03$	$3.20^e \pm 0.03$	$3.90^e \pm 0.02$	5.27
CR_F	$2.08^f \pm 0.02$	$2.25^d \pm 0.01$	$2.44^a \pm 0.01$	$3.71^f \pm 0.02$	$3.88^f \pm 0.01$	4.57
CR_G	**$0.98^g \pm 0.02$**	**$0.96^e \pm 0.01$**	**$1.33^f \pm 0.03$**	**$2.22^g \pm 0.03$**	**$1.90^g \pm 0.02$**	**5.57**
CR_H	$2.20^c \pm 0.03$	$2.34^c \pm 0.03$	$2.55^e \pm 0.03$	$3.78^h \pm 0.01$	$3.98^a \pm 0.02$	7.27
CR_I	$1.09^f \pm 0.02$	$1.53^b \pm 0.03$	$1.78^g \pm 0.01$	$3.98^c \pm 0.02$	$3.71^h \pm 0.02$	0.95
CR_J	$1.03^d \pm 0.05$	$1.33^f \pm 0.03$	$1.20^h \pm 0.03$	$2.90^i \pm 0.02$	$2.78^i \pm 0.01$	1.07
CR_{G-1}	$0.97^a \pm 0.02$	$0.94^a \pm 0.02$	$1.52^a \pm 0.01$	$1.99^a \pm 0.02$	$2.86^a \pm 0.04$	0.08
CR_{G-2}	$0.98^a \pm 0.03$	$0.93^a \pm 0.01$	$1.41^b \pm 0.04$	$1.98^a \pm 0.04$	$1.97^b \pm 0.01$	0.05
CR_{G-3}	$0.92^b \pm 0.02$	$0.94^a \pm 0.04$	$1.35^c \pm 0.02$	$1.59^b \pm 0.02$	$1.57^c \pm 0.02$	0.21
CR_{G-4}	$0.96^a \pm 0.03$	$0.96^a \pm 0.01$	$1.28^d \pm 0.01$	$1.67^c \pm 0.03$	$1.68^d \pm 0.01$	1.6
CR_{G-5}	**$0.76^c \pm 0.02$**	**$0.80^b \pm 0.01$**	**$0.88^e \pm 0.03$**	**$1.09^d \pm 0.01$**	**$1.14^e \pm 0.03$**	**1.08**
CR_{G-6}	$0.90^b \pm 0.01$	$1.22^c \pm 0.02$	$1.09^f \pm 0.02$	$1.64^e \pm 0.01$	$1.58^c \pm 0.02$	1.2
CR_{G-7}	$0.93^b \pm 0.04$	$1.30^d \pm 0.01$	$1.01^g \pm 0.03$	$1.38^f \pm 0.01$	$1.95^b \pm 0.04$	0.64
CR_{G5-1}	$0.68^a \pm 0.03$	$0.67^a \pm 0.01$	$0.78^a \pm 0.03$	$1.07^a \pm 0.01$	$1.09^a \pm 0.01$	0.08
CR_{G5-2}	**$0.43^b \pm 0.01$**	**$0.51^b \pm 0.02$**	**$0.67^b \pm 0.03$**	**$0.23^b \pm 0.01$**	**$0.37^b \pm 0.04$**	**0.045**

[P]The bioactivities were expressed as IC_{50} values (mg/mL).

The samples were analysed in triplicate (n=3) and expressed as mean ± standard deviation. Means followed by different superscripts (a-f) within the same column of the each chromatographic column sub fractions indicated the significant differences ($p<0.05$).

Figure S1 ^{1}H NMR spectrum of 1*H*-benzochromenone derivative (500 MHz, $CDCl_3$)

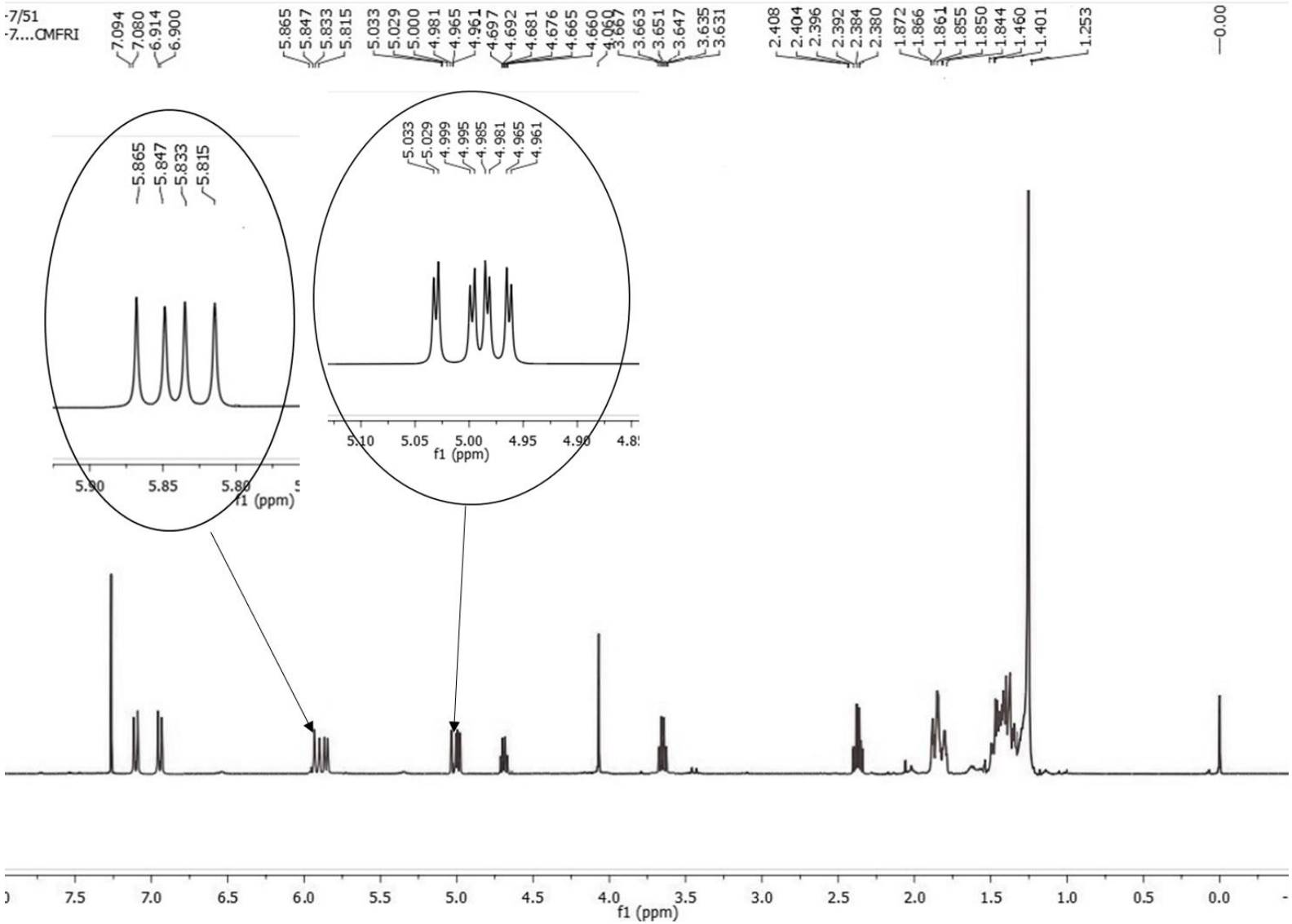


Figure S2 ^{13}C NMR spectrum of 1*H*-benzochromenone derivative (125 MHz, $CDCl_3$)

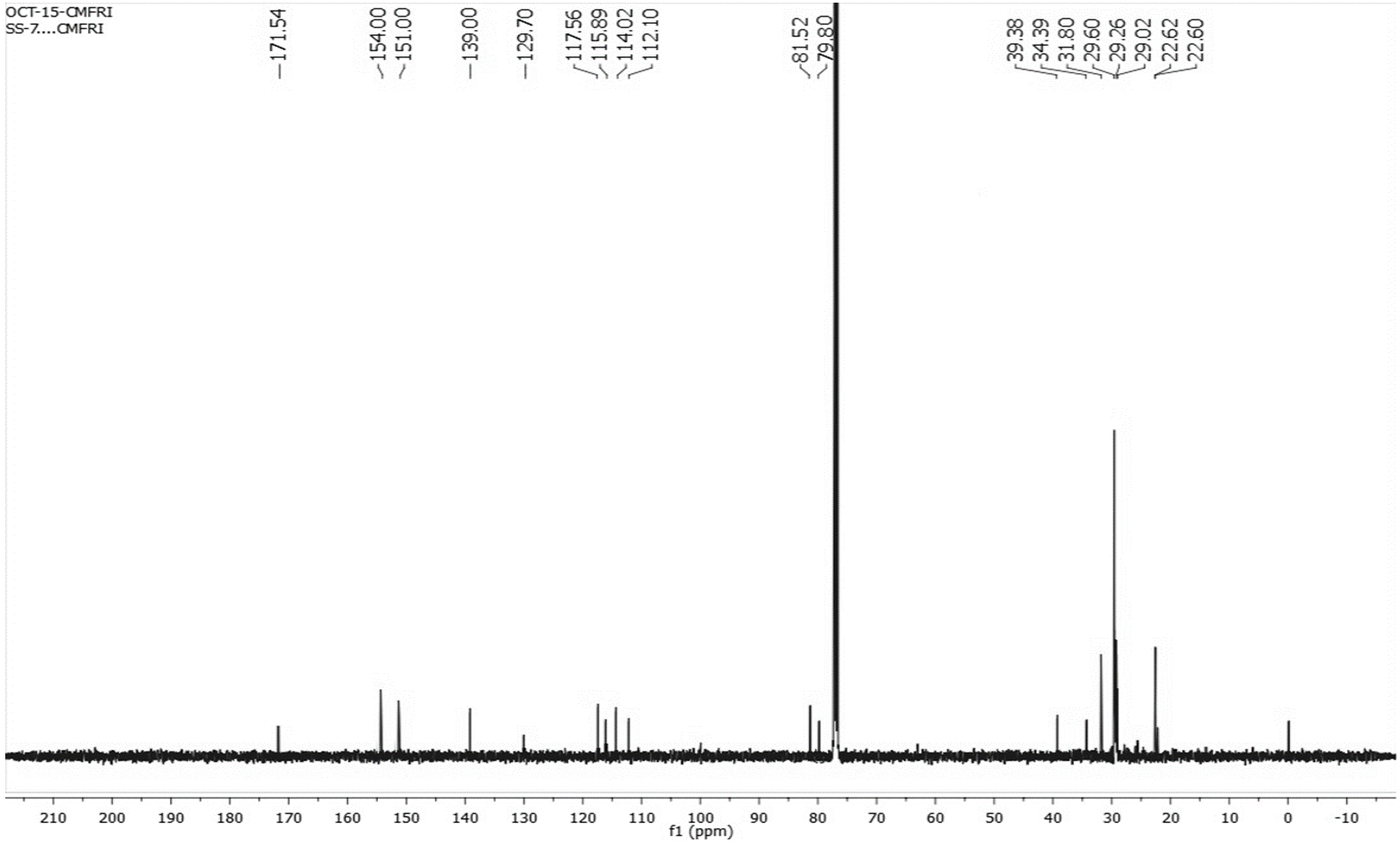


Figure S3 DEPT NMR spectrum of 1*H*-benzochromenone derivative

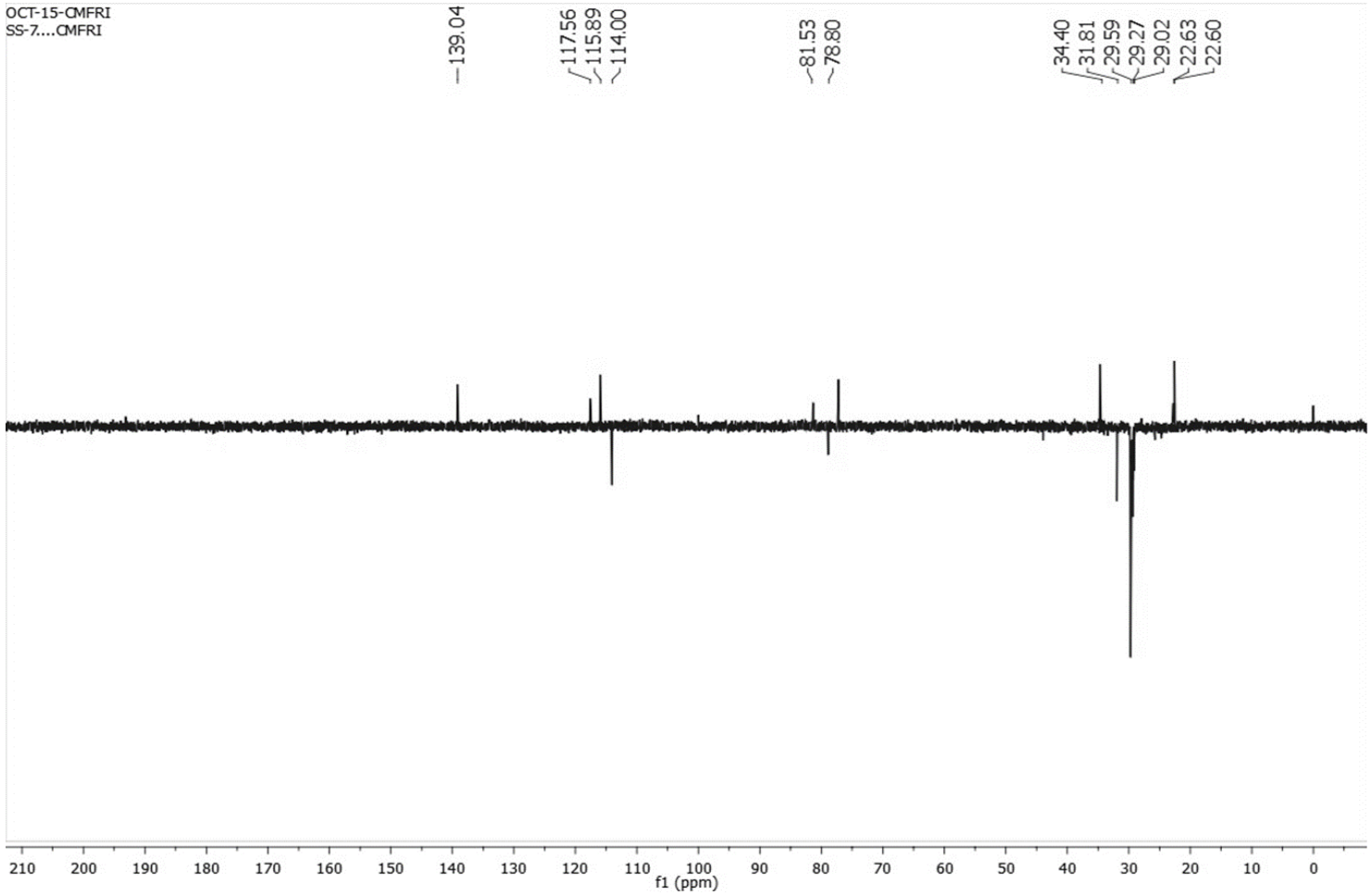
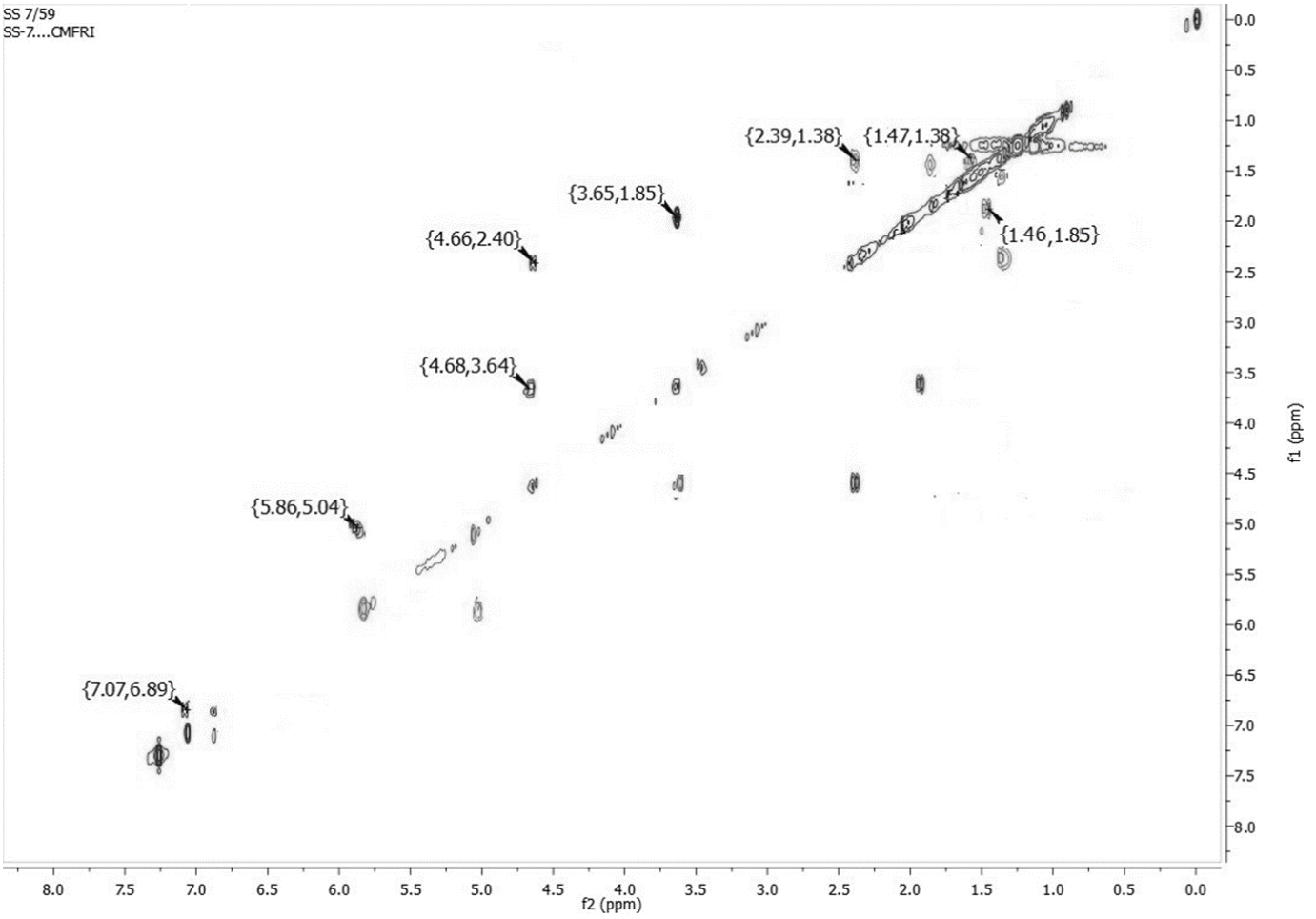


Figure S4 ^{1}H-^{1}H COSY spectrum of 1*H*-benzochromenone derivative



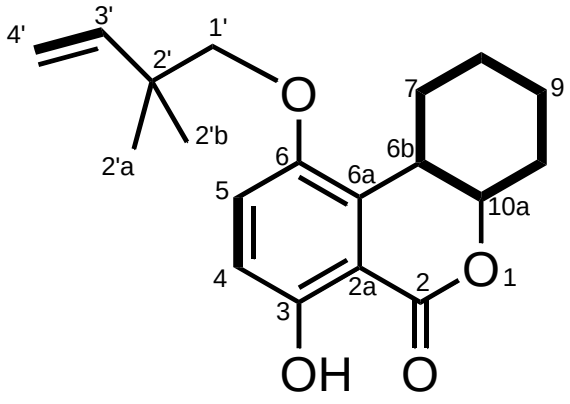


Figure S5 HSQC spectrum of 1*H*-benzochromenone derivative

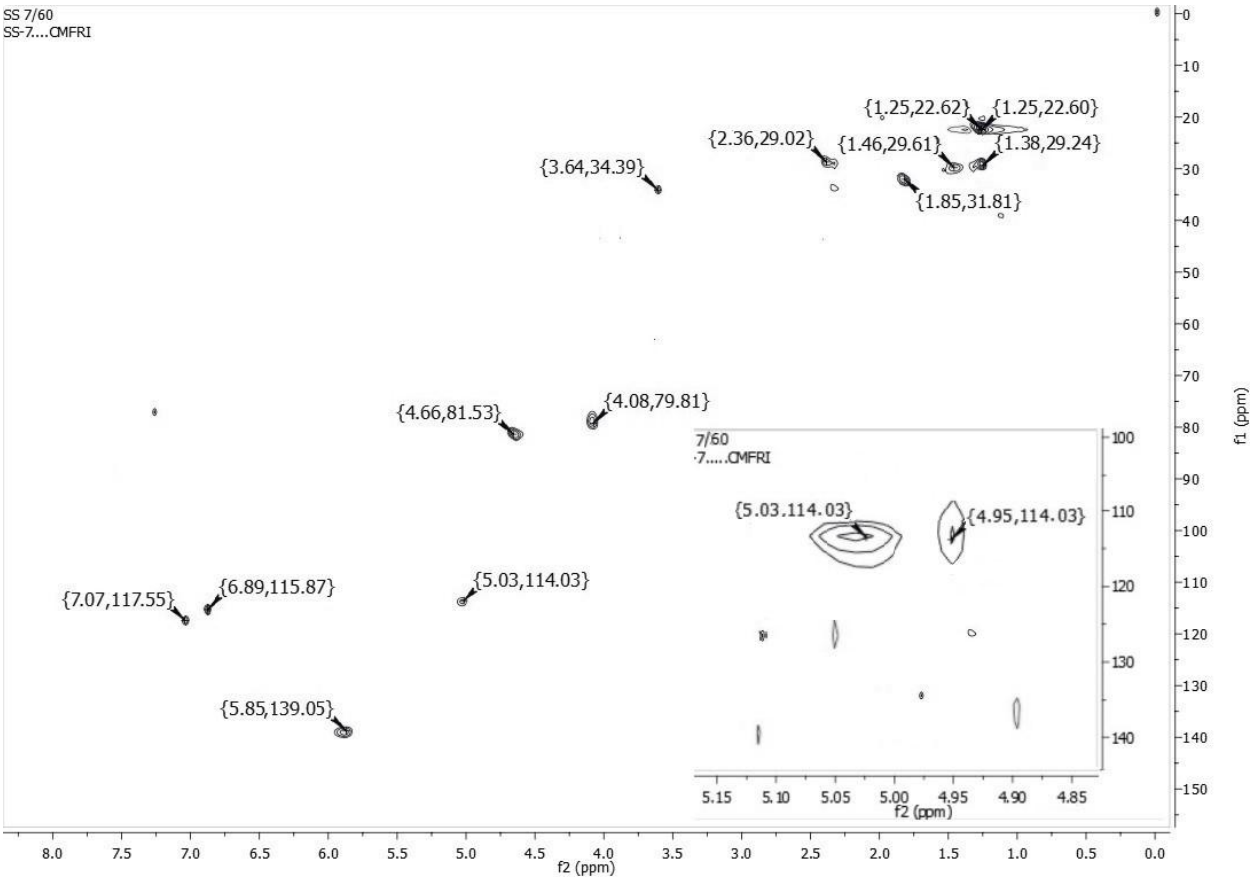
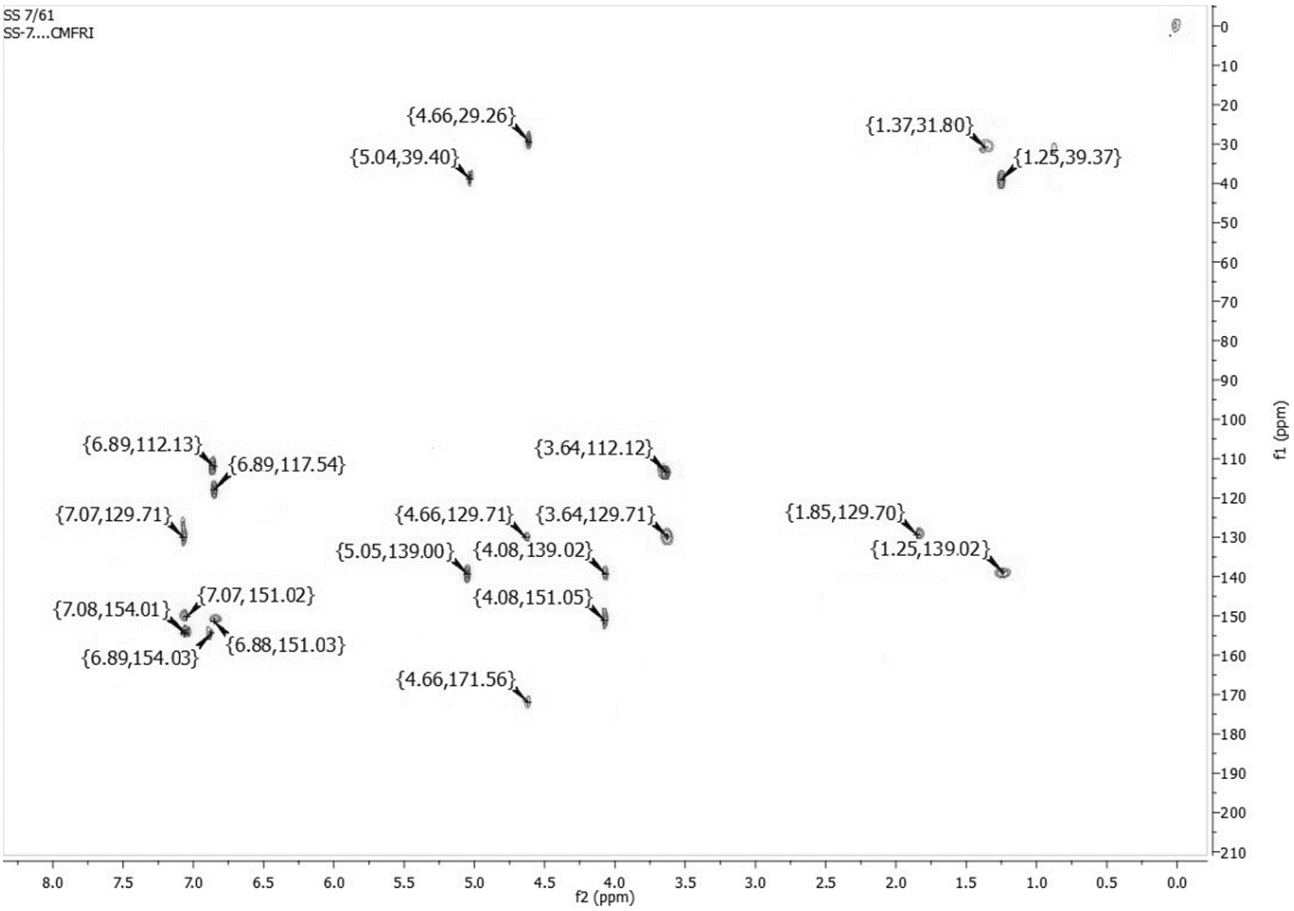


Figure S6 HMBC spectrum of 1*H*-benzochromenone derivative



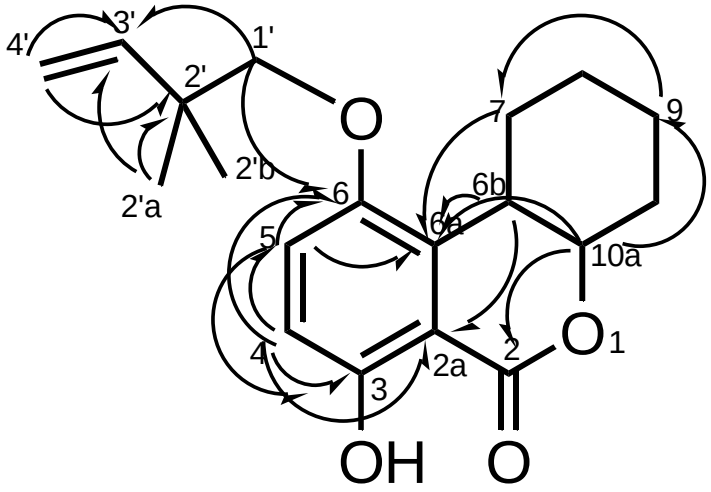
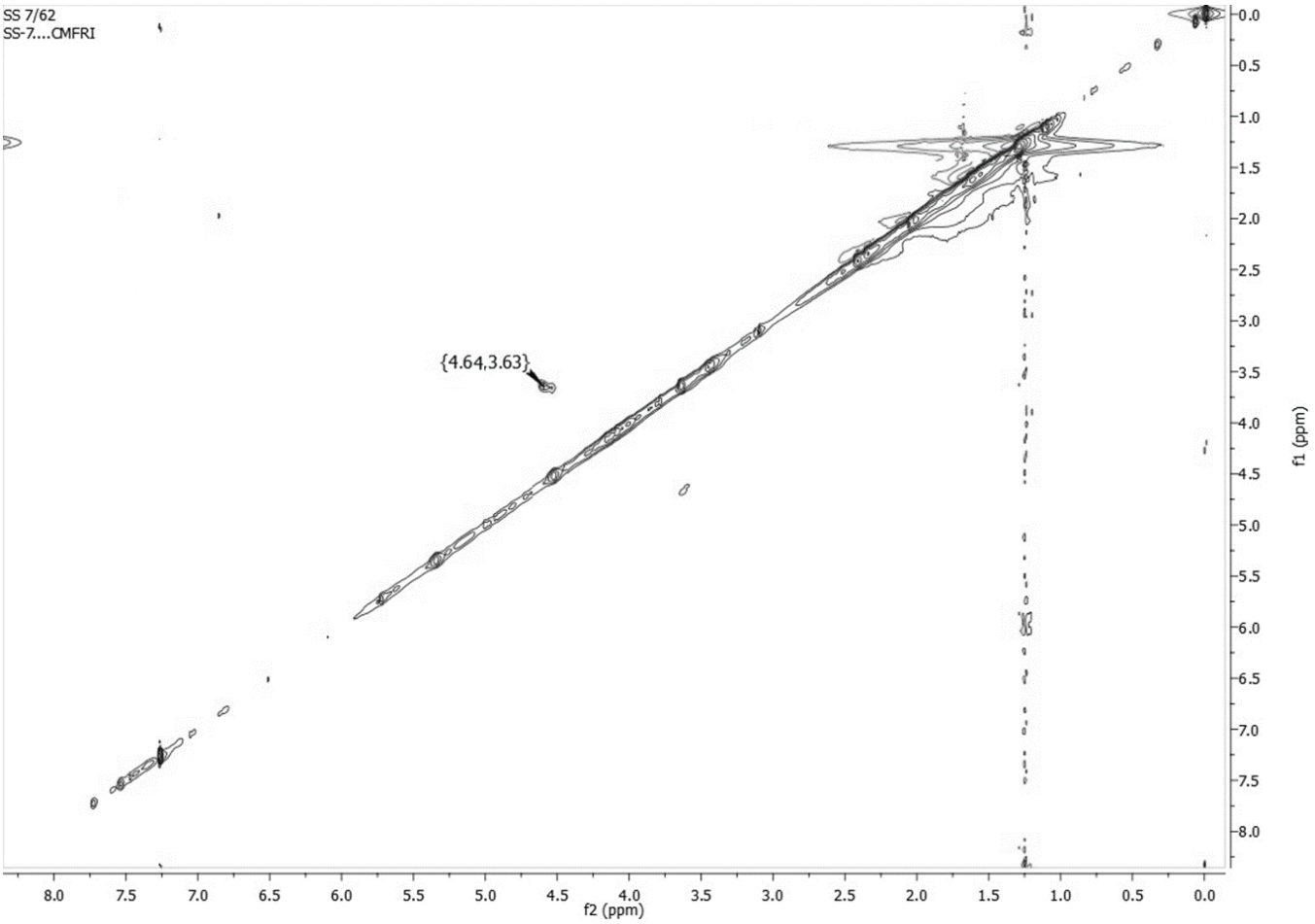


Figure S7 NOESY spectrum of 1*H*-benzochromenone derivative



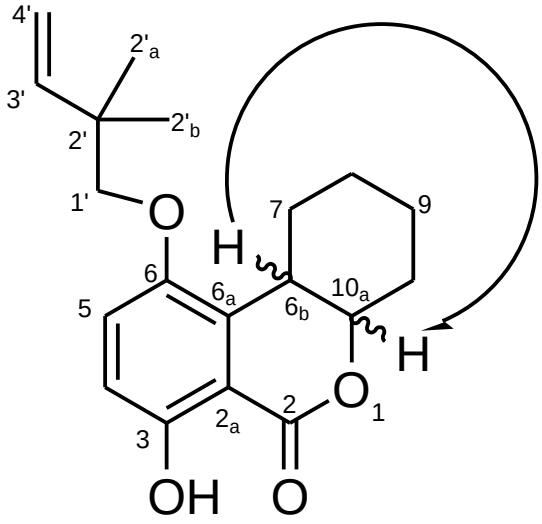


Figure S8 Mass fragmentation scheme of 1*H*-benzochromenone derivative

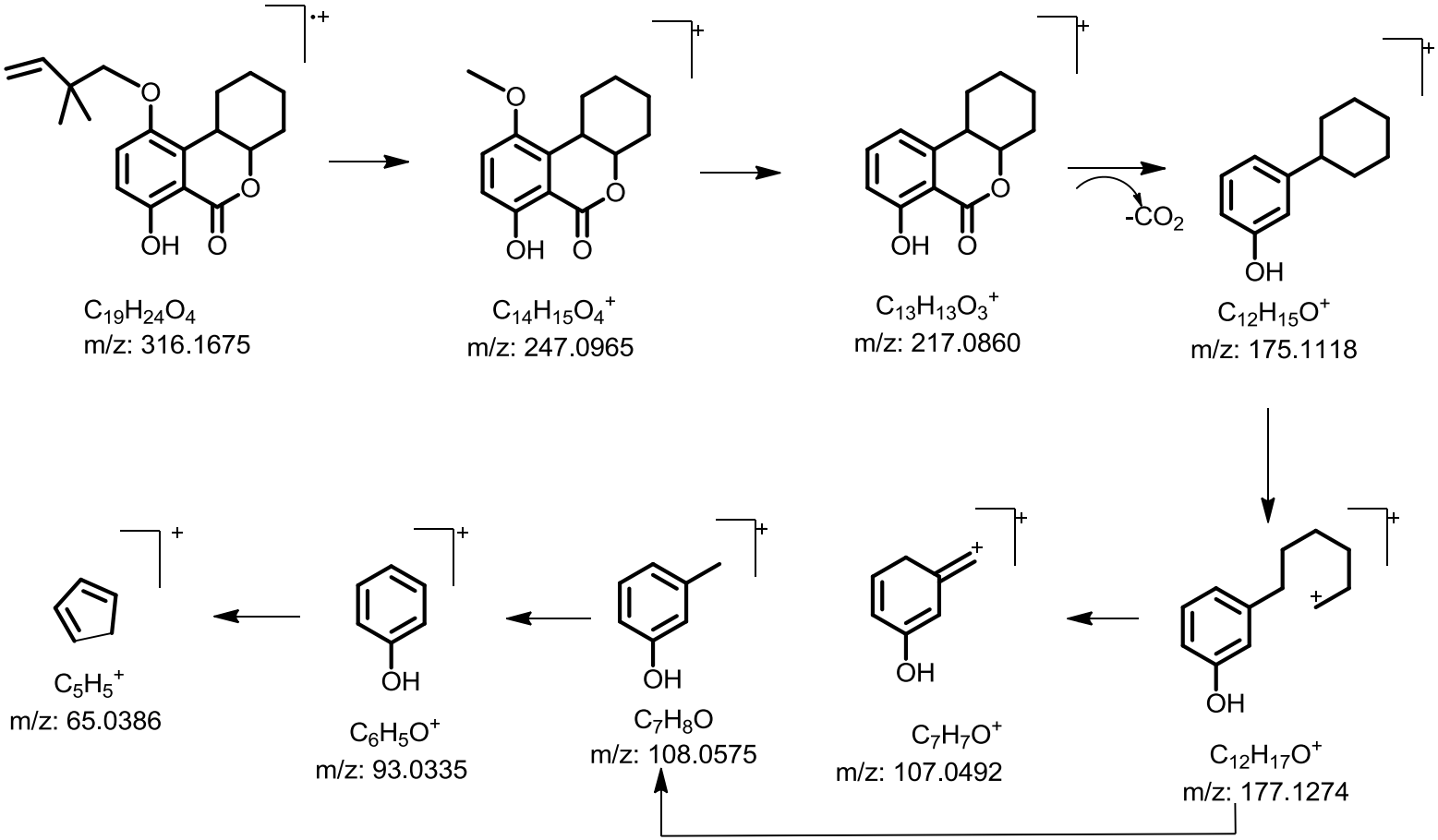


Figure S9 Mass spectrum of 1*H*-benzochromenone derivative

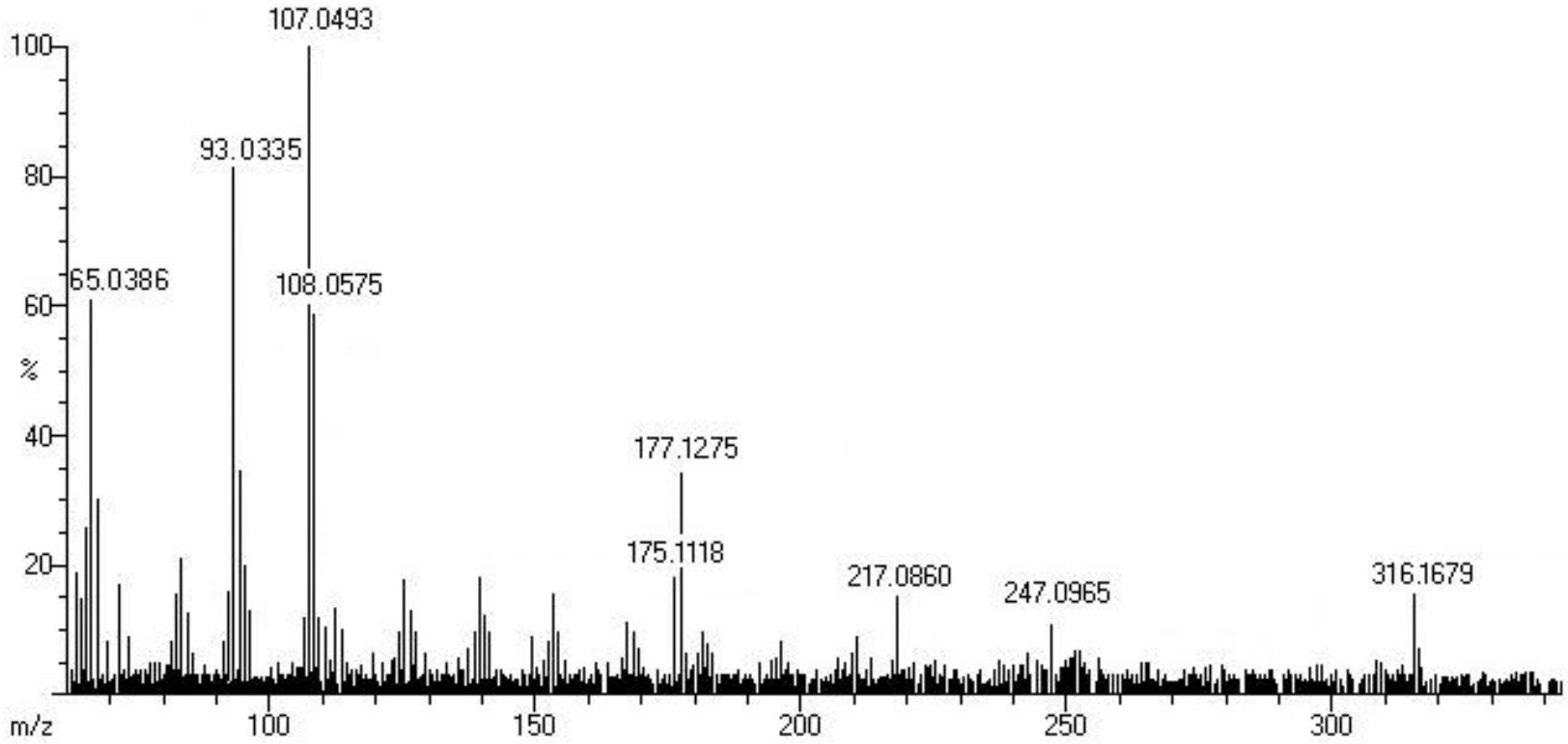


Figure S10 IR spectrum of 1*H*-benzochromenone derivative

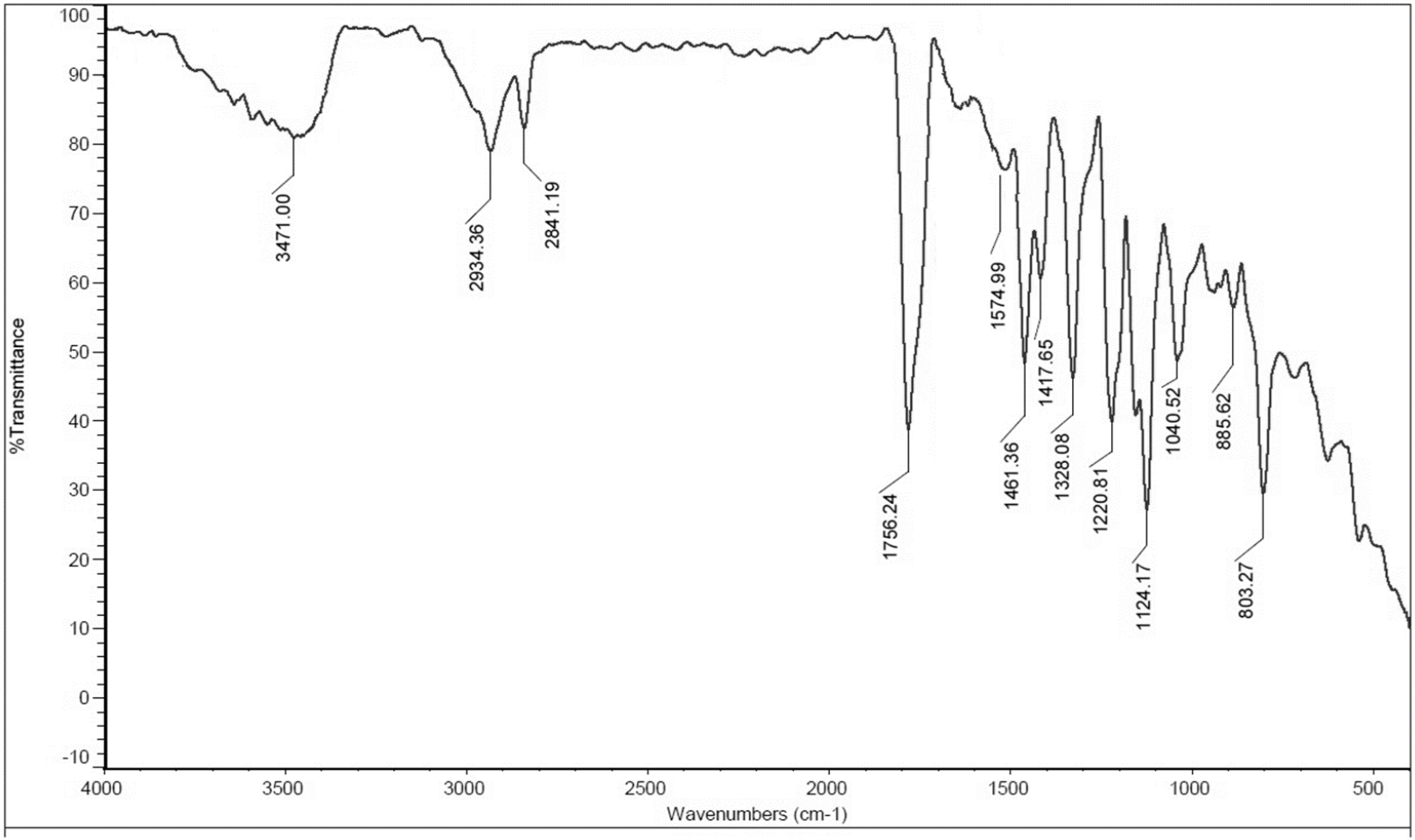


Figure S11 HPLC chromatogram of 1*H*-benzochromenone derivative isolated from muricid gastropod *Chicoreus ramosus*