Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 81 (1) S1–S5 (2016) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

A novel compound isolated from *Sclerochloa dura* has anti-inflammatory effects

SYED MAJID BUKHARI[1,2]*, ASTRID JULLUMSTRØ FEUERHERM[3]**, HANNA MAJA TUNSET[3], STIAN MOE ISAKSEN[1], MARI SÆTHER[3], THOR HÅKON KRANE THVEDT[1], SUSANA VILLA GONZALEZ[1], RUDOLF SCHMID[1], ANDERS BRUNSVIK[4], ERIK FUGLSETH[5], BOJAN ZLATKOVIĆ[6], BERIT JOHANSEN[3] and NEBOJŠA SIMIĆ[1]

[1]Department of Chemistry, Norwegian University of Sciences and Technology, 7491 Trondheim, Norway, [2]Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad-22060, KPK, Pakistan, [3]Department of Biology, Norwegian University of Sciences and Technology, 7491 Trondheim, Norway, [4]SINTEF Materials and Chemistry, Department of Mass Spectrometry, Sem Sælands vei 2a, 7465 Trondheim, Norway, [5]Technology transfer office, Norwegian University of Sciences and Technology, 7491 Trondheim, Norway and [6]Department of Biology, University of Niš, 18000 Niš, Serbia

J. Serb. Chem. Soc. 81 (1) (2016) 1–12

ISOLATION AND IDENTIFICATION OF BIOACTIVE FRACTIONS OF *S. dura*

The isolation was realized by liquid chromatography using a multistage fractionation approach. The crude extract was subjected to prefractionation by MPLC using a double injection with total injected weight of 85.0 g per injection. The parameters used for the MPLC are given in Table S-I. Nine prefractions (A-I) were collected starting at time 10 min. After the start, fractions were collected at 8 min intervals. The obtained prefractions were tested on human fibroblastlike synoviocyte cell line SW982 to determine any effect on PLA_2 activity by measuring the release of AA. Three very active prefractions were found that were subsequently fractionated into 259 fractions by 3 respective separations on preparative HPLC. The parameters used for preparative HPLC separation are given in Table S-II. A total of 80 fractions from prefraction E, 90 fractions from prefraction F and 89 fractions from prefraction G were obtained. The injected amounts of prefractions E, F and G were 2.3638, 0.7078 and 1.0822 g respectively, at a flow rate of 109 mL min^{-1}. All fractionations were based on time, 18 to 20 s per fraction. All the obtained fractions were tested for inhibition of PLA_2 activity by measuring the release of AA. Based on the similarity of chroma-

*,** Corresponding authors. E-mail: (*) majid_bukhari@hotmail.com;
(**) astrid.j.feuerherm@bio.ntnu.no

tograms and bioactivity, some the fractions of prefraction F were pooled into groups, *e.g.*, F40–F44 as one group and F70–F74 as another. These groups were subjected to semi-preparative HPLC separations. The parameters used for semi-preparative separation are given in Table S-III. Five subfractions from group F40–F44 (injected amount, 34.3 mg and flow rate, 5 mL min^{-1}), 7 subfractions from group F70–74 (injected amount, 39.6 mg and flow rate, 5 mL min^{-1}) and 2 subfractions from group G50–G51 (injected amount, 45.0 mg and flow rate, 15 mL min^{-1}) were obtained. All subfractions collections were based on time – 18–20 s per subfraction. Based on TLC, fractions G50–G51 of prefraction G were pooled together and subjected to semi-preparative HPLC separation.

TABLE S-I. Method used for medium pressure liquid chromatography; individual gradient

Time, min	Flow rate, mL min^{-1}	Water content %	Methanol content %	2-Propanol content %
0.0	100	100	0	0
5.0	100	100	0	0
5.1	130	100	0	0
10.0	130	100	0	0
10.1	100	100	0	0
18.0	100	100	0	0
51.0	100	30	70	0
61.0	100	10	90	0
61.1	150	0	100	0
66.0	150	0	100	0
66.1	30	0	0	100
70.0	30	0	0	100

TABLE S-II. Method used for preparative high pressure liquid chromatography

Gradient	Time, min	Water content %	Methanol/acetonitrile 1:1 content %
Fraction E	0.0	85	15
	57.7	58	42
	58.0	0	100
	63.0	0	100
Fraction F	0.0	77	23
	57.7	43	57
	58.0	0	100
	63.0	0	100
Fraction G	0.0	45	55
	57.7	9	91
	58.0	0	100
	63.0	0	100

TABLE S-III. Method used for semi-preparative high pressure liquid chromatography

Gradient	Time, min	Water content %	Methanol/acetonitrile 1:1 content %
Group F40–F44	0.0	100	0
	57.7	94	6
	58.0	0	100
	63.0	0	100
Group F70–F74	0.0	94	6
	57.7	86	14
	58.0	0	100
	63.0	0	100
Group G50–G51	0.0	37	63
	25.0	20	80
	25.1	0	100
	30.0	0	100

TABLE S-IV. Assignments of ^{1}H- and ^{13}C-NMR resonances of compounds **1–6** (**1**–**5**: CD_3OD; **6**: DMSO); δ / ppm

C	**1**		**2**		**3**	
	C	H	C	H	C	H
1	100.3	4.77, *d*, (3.7 Hz)	–	–	–	–
2	73.9	3.41, *dd*, (3.7 & 9.6 Hz)	166.0	–	165.9	–
3	75.3	3.65, *m*	103.6	6.53, *s*	106.0	6.77, *m*
4	75.1	3.08, *dd*, (8.8 & 9.6 Hz)	183.7	–	184.0	–
5	70.0	4.10, *m*	162.2	–	163.1	–
6	54.4	2.92, *dd*, (9.3 & 14.3 Hz); 3.36, *m*	109.8	–	101.1	6.50, *s*
7	–	–	167.8	–	164.8	–
8	–	–	96.0	6.42, *s*	96.1	6.87, *s*
9	–	–	159.1	–	158.7	–
10	–	–	104.5	–	107.1	–
1′	71.0	3.36, *m* 4.05, *m*	75.5	4.90 (overlap by solvent)	101.7	5.08, *d*, (6.6 Hz)
2′	72.7	3.89, *m*	72.6	4.24, *t*, (9.2 Hz)	77.8	3.51, *m*
3′	64.4	3.59, *m*	80.4	3.48, *m*	74.7	3.51, *m*
4′	–	–	71.9	3.48, *m*	71.2	3.40, *m*
5′	–	–	82.7	3.42, *m*	78.4	3.55, *m*
6′	–	–	63.0	3.74, *dd*, (5.3 & 12.2 Hz); 3.87, *dd*, (2.2 & 12.2 Hz)	62.2	3.68, *m*
1″	176.2	–	123.1	–	127.5	–
2″	34.9	2.31, *t*, (7.5 Hz)	129.5	7.80, *d*, (8.8 Hz)	105.1	7.23, *s*
3″	26.2	1.60, *m*	117.0	6.90, *d*, (8.8 Hz)	154.8	–

TABLE S-IV. Continued

C	**1**		**2**		**3**	
	C	H	C	H	C	H
4″	30.3	1.33, *m*	163.3	–	140.6	–
5″	30.4[a]	1.33, *m*	117.0	6.90, *d*, (8.8 Hz)	154.8	–
6″	30.5[a]	1.33, *m*	129.5	7.80, *d*, (8.8 Hz)	105.1	7.23, *s*
7″	30.6[a]	1.33, *m*	–	–	87.4	4.46, *m*
12″	129.3[c]	5.34, *m*	–	–	–	–
13″	129.4[c]	5.34, *m*	–	–	–	–
14″	26.5	2.81, *m*	–	–	–	–
15″	128.9[b]	5.31, *m*	–	–	–	–
16″	132.9	5.37, *m*	–	–	–	–
17″	21.6	2.09, *m*	–	–	–	–
18″	14.8	0.98, *t*, (7.5 Hz)	–	–	–	–
1‴	–	–	–	–	133.4	–
2‴	–	–	–	–	111.5	7.00, *m*
3‴	–	–	–	–	148.6	–
4‴	–	–	–	–	146.8	–
5‴	–	–	–	–	115.6	6.73, *m*
6‴	–	–	–	–	120.5	6.81, *m*
			OMe			
3″, 5″	–	–			57.0	3.92, *s*
3‴					56.5	3.83, *s*

C	**4**		**5**		**6**	
	C	H	C	H	C	H
1	170.1	–	66.3	4.17, *m* 4.26, *m*	–	–
2	125.2	5.81, *s*	71.1	3.88, *m*	164.5	–
3	202.1		63.9	3.59, *m*	102.1	6.94, *s*
4	48.2	1.96, *m*, *α* 2.47, *d*, (17.2 Hz), *β*	–	–	181.6	–
5	37.1	–	–	–	161.0	–
6	52.2	1.99, *m*, *β*	–	–	99.3	6.43, *d*, (1.8 Hz)
7	27.0	1.50, *m* 1.97, *m*	–	–	162.8	–
8	37.5	1.62, *m* 1.68, *m*	–	–	95.1	6.89, *d*, (1.8Hz)
9	75.6	3.88, *m*	–	–	156.8	–
10	20.0	1.18, *d*, (6.2 Hz)	–	–	105.2	–
11	29.2	1.01, *s*, *β*	–	–		
12	27.6	1.09, *s*, *α*	–	–		
13	25.1	2.05, *s*	–	–		

TABLE S–IV. Continued

C	**4**		**5**		**6**	
	C	H	C	H	C	H
1′	102.3	4.32, *d*, (7.8 Hz)	126.1	–	100.1	5.04, *d*, (7.5 Hz)
2′	75.3	3.14, *dd*, (7.8 & 9.2 Hz)	111.4	7.20, *m*	73.1	3.27, *m*
5′	78.0	3.25, *m*	116.5	6.81, *m*	77.3	3.45, *m*
6′	63.0	3.65, *m* 3.86, *m*	123.9	7.08, *m*	60.6	3.46, *m* 3.72, *m*
1″	–	–	146.8	7.66, *m*	123.6[c]	–
2″	–	–	115.0	6.39, *m*	104.9	7.30
3″	–	–	–	–	148.8	–
4″	–	–	–	–	135.8[d]	–
5″	–	–	–	–	148.8	–
6″	–	–	–	–	104.9	7.30
			OMe			
3′			56.3	3.90, *s*		
3″, 5″					56.2	3.84, *s*

[a,b]Assignments marked with letter “a” and “b” are interchangeable; [c,d]tentative assignments