

**Isolation, Characterization, Genome Annotation,
and Evaluation of Hyaluronidase Inhibitory
Activity in Secondary Metabolites of *Brevibacillus*
sp. JNUCC 41: A Comprehensive Analysis
through Molecular Docking and Molecular
Dynamics Simulation**

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Supplementary materials

Table S1. COG functional gene classification of the genome	2
Table S2. A list of genetic determinants of CAZy detected in the chromosome of <i>Brevibacillus</i> sp. JNUCC 41	3
Table S3. A list of genetic determinants of virulence (VFs) detected in the chromosome of <i>Brevibacillus</i> sp. JNUCC 41	5
Table S4. ADMET properties of compounds.....	6
Table S5. Drug-likeness properties of compounds.....	7
Figure S1. ¹ H NMR of compound 1	8
Figure S2. ¹³ C NMR of compound 1.....	9
Figure S3. ¹ H NMR of compound 2	10
Figure S4. ¹³ C NMR of compound 2	11
Figure S5. ¹ H NMR of compound 3	12
Figure S6. ¹³ C NMR of compound 3.....	13
Figure S7. ¹ H NMR of compound 4.....	14
Figure S8. ¹³ C NMR of compound 4.....	15
Figure S9. ¹ H NMR of compound 5	16
Figure S10. ¹³ C NMR of compound 5.....	17

Table S1. COG functional gene classification of the genome

COG_class	Description	Number
J	Translation, ribosomal structure and biogenesis	290
A	RNA processing and modification	0
K	Transcription	408
L	Replication, recombination and repair	152
B	Chromatin structure and dynamics	0
D	Cell cycle control, cell division, chromosome partitioning	236
Y	Nuclear structure	0
V	Defense mechanisms	116
T	Signal transduction mechanisms	291
M	Cell wall/membrane/envelope biogenesis	256
N	Cell motility	47
Z	Cytoskeleton	4
W	Extracellular structures	3
U	Intracellular trafficking, secretion, and vesicular transport	41
O	Posttranslational modification, protein turnover, chaperones	188
X	Mobilome: prophages, transposons	31
C	Energy production and conversion	250
G	Carbohydrate transport and metabolism	309
E	Amino acid transport and metabolism	513
F	Nucleotide transport and metabolism	142
H	Coenzyme transport and metabolism	273
I	Lipid transport and metabolism	274
P	Inorganic ion transport and metabolism	247
Q	Secondary metabolites biosynthesis, transport and catabolism	118
R	General function prediction only	397
S	Function unknown	220

Table S2. A list of genetic determinants of CAZy detected in the chromosome of *Brevibacillus* sp. JNUCC 41

Gene ID	dbCAN_sub	Gene ID	dbCAN_sub
QOS87884.1	GT2(7-176)	QOS90431.1	AA4(13-229)
QOS87908.1	AA4(3-228)	QOS90512.1	GT51(99-282)
QOS88030.1	GT51(62-236)	QOS90731.1	CE4(42-145)
QOS88137.1	CBM34(6-124)+GH13_20(173-469)	QOS91004.1	GH18(242-517)
QOS88138.1	GH13_45(65-358)	QOS91021.1	GT1(180-390)
QOS88139.1	GH13_31(28-372)	QOS91054.1	GT2(7-97)
QOS88211.1	CE1(15-237)	QOS91071.1	GT4(189-353)
QOS88245.1	GH1(2-466)	QOS91177.1	GH18(154-452)
QOS88275.1	CE3(31-225)	QOS91197.1	GT4(1033-1177)
QOS88374.1	CE4(82-204)	QOS91204.1	GH73(959-1089)
QOS88571.1	CE4(125-249)	QOS91214.1	AA1(98-488)
QOS88624.1	GH4(6-185)	QOS91215.1	GH8(65-417)
QOS88730.1	GH25(7-177)	QOS91235.1	GT28(202-349)
QOS88860.1	GH13_29(25-369)	QOS91252.1	CE4(269-389)
QOS88980.1	CE14(8-126)	QOS91284.1	GT4(257-413)
QOS89144.1	GH170(1-333)	QOS91377.1	GH18(131-408)
QOS89145.1	GH3(116-351)	QOS91399.1	GT51(60-236)
QOS89148.1	GH109(1-152)	QOS91471.1	GH13_31(28-376)
QOS89360.1	AA4(6-242)	QOS91582.1	GT4(184-326)

QOS89377.1	AA4(4-227)	QOS91583. 1	GT26(59-227)
QOS89427.1	GH13_14(192-506)	QOS91586. 1	GT4(203-354)
QOS89451.1	GT4(168-325)	QOS91590. 1	GT2(38-162)
QOS89455.1	GT4(165-320)	QOS91684. 1	GT51(62-238)
QOS89460.1	GT4(168-326)	QOS91706. 1	CE14(8-126)
QOS89474.1	GT5(2-471)	QOS91766. 1	GH13_5(31-371)
QOS89476.1	CBM48(33-116)+GH13_9(185-485)	QOS91945. 1	CE4(51-175)
QOS89477.1	GH15(17-318)	QOS91995. 1	CE9(11-383)
QOS89598.1	GT27(61-340)	QOS92067. 1	GT51(84-264)
QOS89599.1	GT2(5-160)	QOS92210. 1	GH179(19-334)
QOS89600.1	GT4(406-567)	QOS92211. 1	GH179(19-342)
QOS89602.1	GT2(5-173)+GT2(248-408)	QOS92258. 1	CBM50(29-70)
QOS89608.1	GH18(118-405)	QOS92305. 1	CE4(56-180)
QOS89692.1	CE4(42-155)	QOS92331. 1	AA7(21-250)
QOS89707.1	GT28(191-339)	QOS92425. 1	AA6(2-169)
QOS89754.1	CE3(53-256)	QOS92473. 1	GT4(256-362)
QOS89782.1	CE4(78-197)	QOS92609. 1	CE4(34-143)
QOS89808.1	GH170(3-358)	QOS92619. 1	GH3(115-338)
QOS89809.1	GH4(8-188)	QOS92655. 1	CE4(117-243)
QOS89888.1	GT51(83-257)	QOS92740. 1	GH23(105-206)
QOS89916.1	GT4(194-343)	QOS92746. 1	GH179(18-206)

QOS89917.1	CE14(10-114)	QOS92800.1	CE4(37-160)
QOS90205.1	CE4(42-157)	QOS92805.1	AA6(5-195)
QOS90283.1	GT4(201-355)	QOS92828.1	GH171(51-408)
QOS90285.1	GT4(194-345)	QOS92853.1	GH14(24-415)+CBM20(435-520)

Table S3. A list of genetic determinants of virulence (VFs) detected in the chromosome of *Brevibacillus* sp. JNUCC 41

VFclass	Virulence factors		JNUCC 41 (Prediction)
Adherence	Fibronectin-binding protein (Listeria) (fbpA)		QOS88467.1
	LPS O-antigen (P. aeruginosa) (Pseudomonas)		QOS92532.1
	Streptococcal lipoprotein rotamase A (Streptococcus) (slrA)		QOS91396.1
Enzyme	Serine-threonine phosphatase (Listeria) (stp)		QOS88478.1
Immune evasion	Hyaluronic acid (HA) capsule (hasC)		QOS89457.1
	Polyglutamic acid capsule	capA	QOS90628.1
		capB	QOS90626.1
		capC	QOS90627.1
		capD	QOS89054.1; QOS90629.1; QOS92890.1
		capE	QOS92939.1
	Polysaccharide capsule		QOS87883.1; QOS89453.1; QOS89456.1; QOS90270.1; QOS90281.1; QOS90284.1; QOS90288.1; QOS90290.1; QOS90291.1; QOS91196.1; QOS91283.1; QOS92531.1
Regulation	LOS (Campylobacter)		QOS91024.1
	Carbon storage regulator A (Legionella) (csrA)		QOS91567.1
	CheA/CheY (Listeria) (cheY)		QOS88534.1
	LisR/LisK (Listeria) (lisR)		QOS91489.1

Secretion system	T6SS-II(Klebsiella) (clpB)	QOS88168.1
	TTSS (yyc-yop) secretion apparatus (Yersinia) (yycN)	QOS88524.1
Toxin	Hemolysin III (hlyIII)	QOS89682.1
	Cytolysin (Enterococcus) (cylR2)	QOS90796.1
Antiphagocytosis	Capsule (Enterococcus)	QOS88554.1
	Capsule (Klebsiella)	QOS92091.1
Iron uptake	ABC transporter (Corynebacterium) (fagC)	QOS87867.1
	Achromobactin biosynthesis and transport (Pseudomonas) (cbrD)	QOS88087.1
	Periplasmic binding protein-dependent ABC transport systems (Vibrio) (vctC)	QOS91480.1
	Pyoverdine (Pseudomonas) (pvdH)	QOS91765.1
Motility and export apparatus	Flagella (Campylobacter) (fliP)	QOS88536.1
Nutritional factor	Allantoin utilization (Klebsiella)	QOS87959.1
Other adhesion-related proteins	PDH-B (Mycoplasma) (pdhB)	QOS88033.1
Stress adaptation	Catalase (Neisseria) (katA)	QOS91358.1
Surface protein anchoring	Lipoprotein-specific signal peptidase II (Listeria) (lspA)	QOS88455.1

Table S4. ADMET properties of compounds

ADMET properties	Methyl indole-3-acetate	EGCG
Absorption		
Caco-2 permeability (cm/s)	-4.459	-6.717
P-gp inhibitor	No	Yes
P-gp substrate	No	Yes
Human intestinal absorption	94.739%	47.395%
Distribution		
Plasma protein binding	56.817%	88.242%
Volume distribution (L/kg)	0.976	0.485

Blood–brain barrier	Yes	No
Metabolism		
CYP1A2 inhibitor	Yes	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	Yes
Elimination		
Clearance rate (mL/min/kg)	10.460	14.450
T _{1/2} (h)	0.898	0.496
Toxicity		
Hepatotoxicity	No	No
Ames toxicity	No	No
Skin sensitization	No	No
hERG inhibition	No	Yes
LD ₅₀ of acute toxicity (mol/kg)	2.314	2.522

LD₅₀: lethal dose;
CYP: cytochrome
p450; T_{1/2}: time
required for plasma
concentration of a
drug to decrease by
50%; hERG: human

Ether-a-go-go-Related Gene.

Table S5. Drug-likeness properties of compounds

Drug-likeness properties	Methyl indole-3-acetate	EGCG
MW	189.08	458.38
HBA	3	11
HBD	1	8
MR	54.16	112.06
RB	3	4
TPSA (Å ²)	42.090	225.060
Log <i>p</i>	2.200	1.893
Lipinski's Rule	Yes	No
Ghose Filter	Yes	Yes
Veber Filter	Yes	No
Egan Filter	Yes	No

MW: molecular weight; HBA: num. H-Bond acceptors; HBD: num. H-Bond donors; RB: num. rotatable bonds; MR: molar refractivity.

Methyl indole-3-acetate

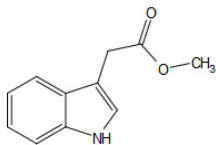


Figure S2. ^{13}C NMR of compound 1

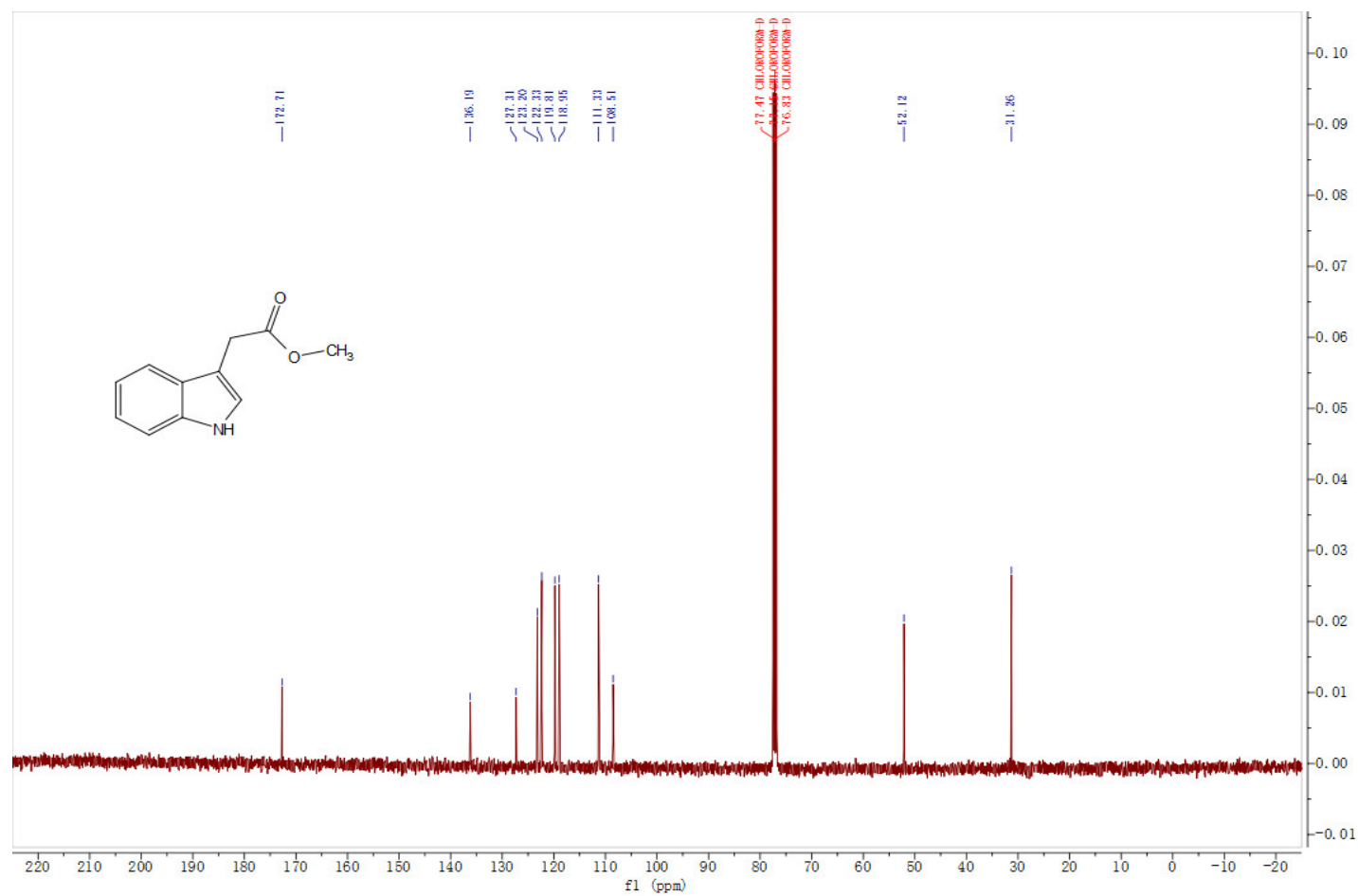


Figure S3. ^1H NMR of compound 2

Dibutyl phthalate

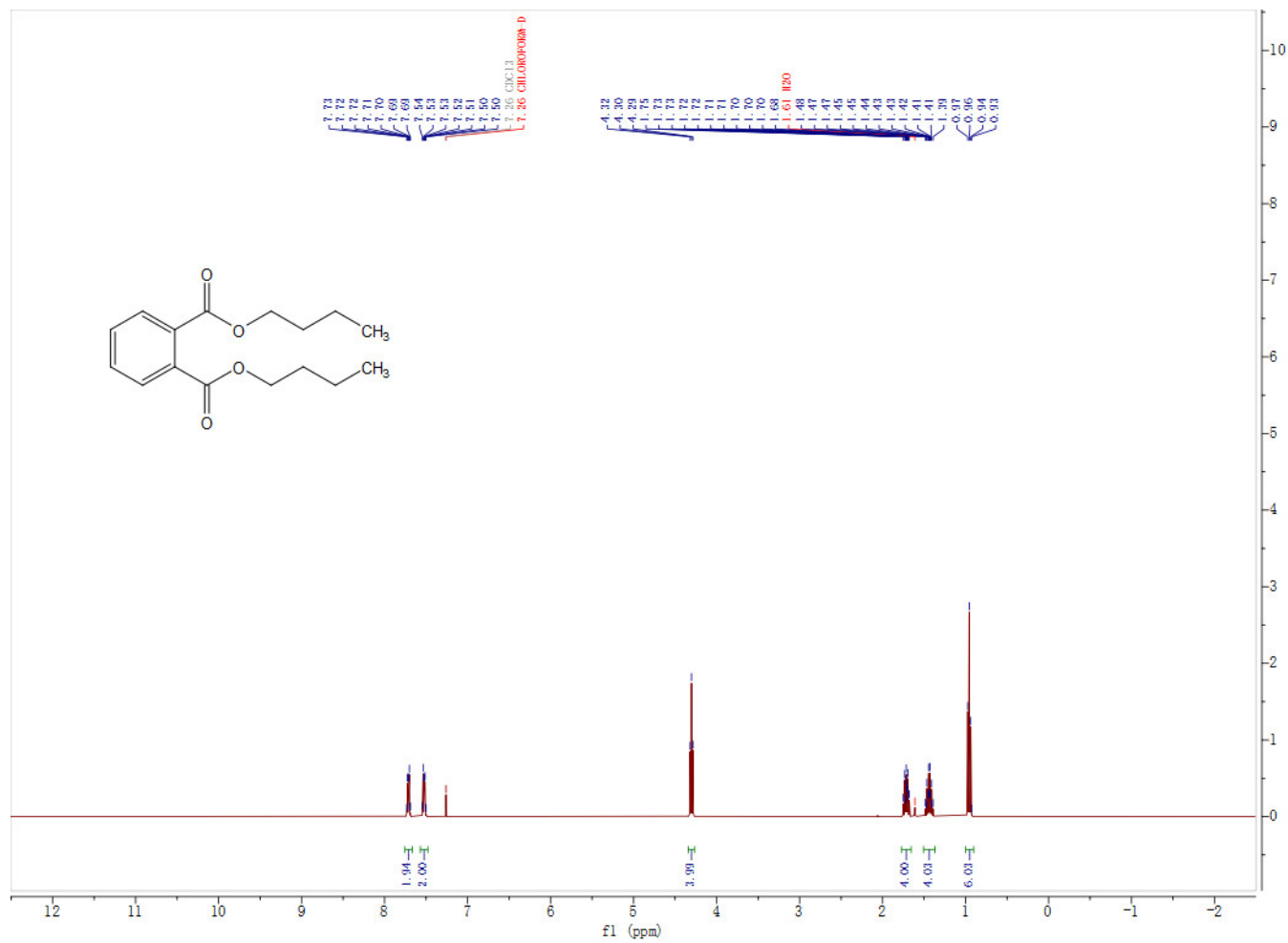
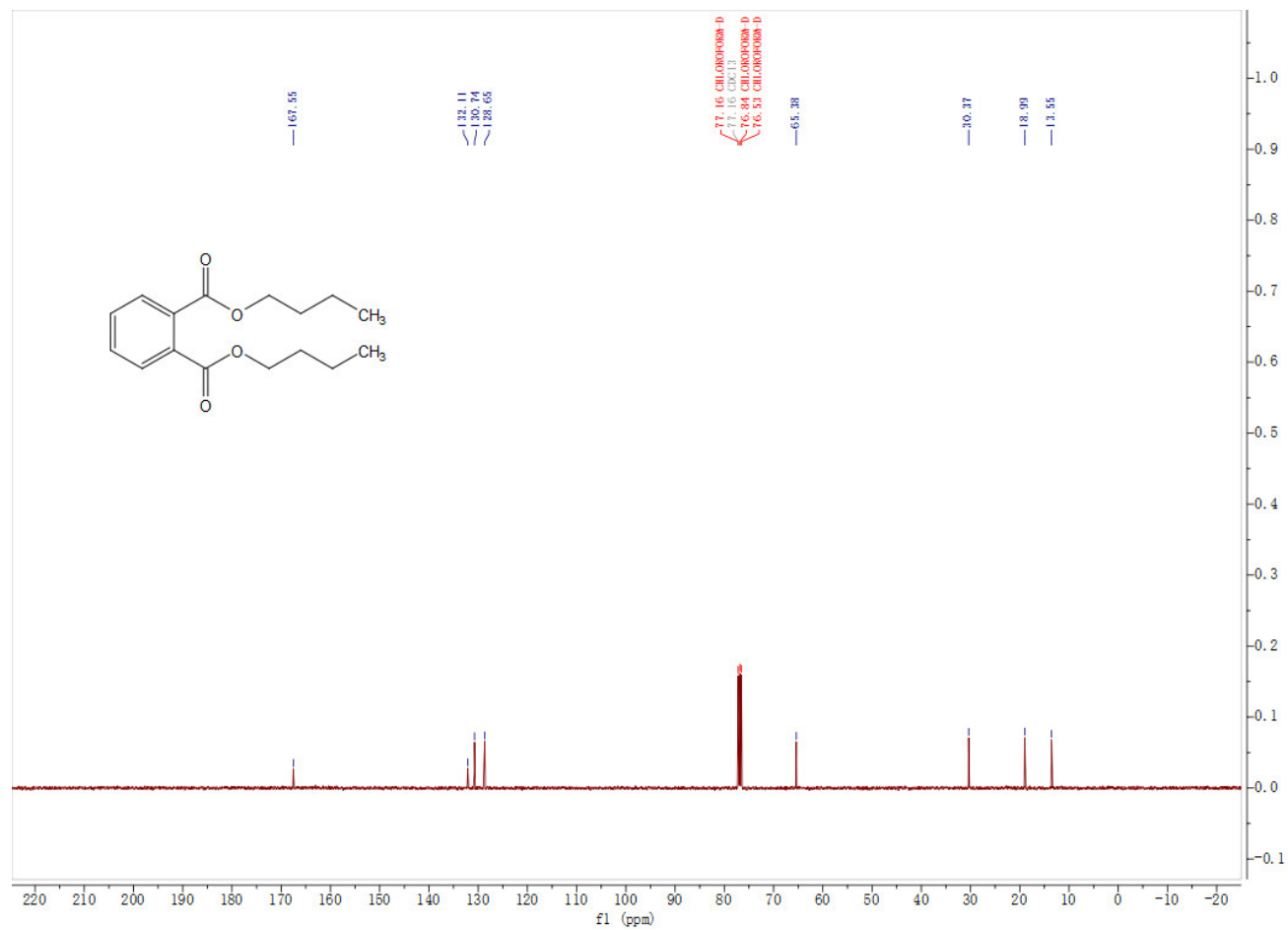


Figure S4. ^{13}C NMR of compound 2



Daidzein

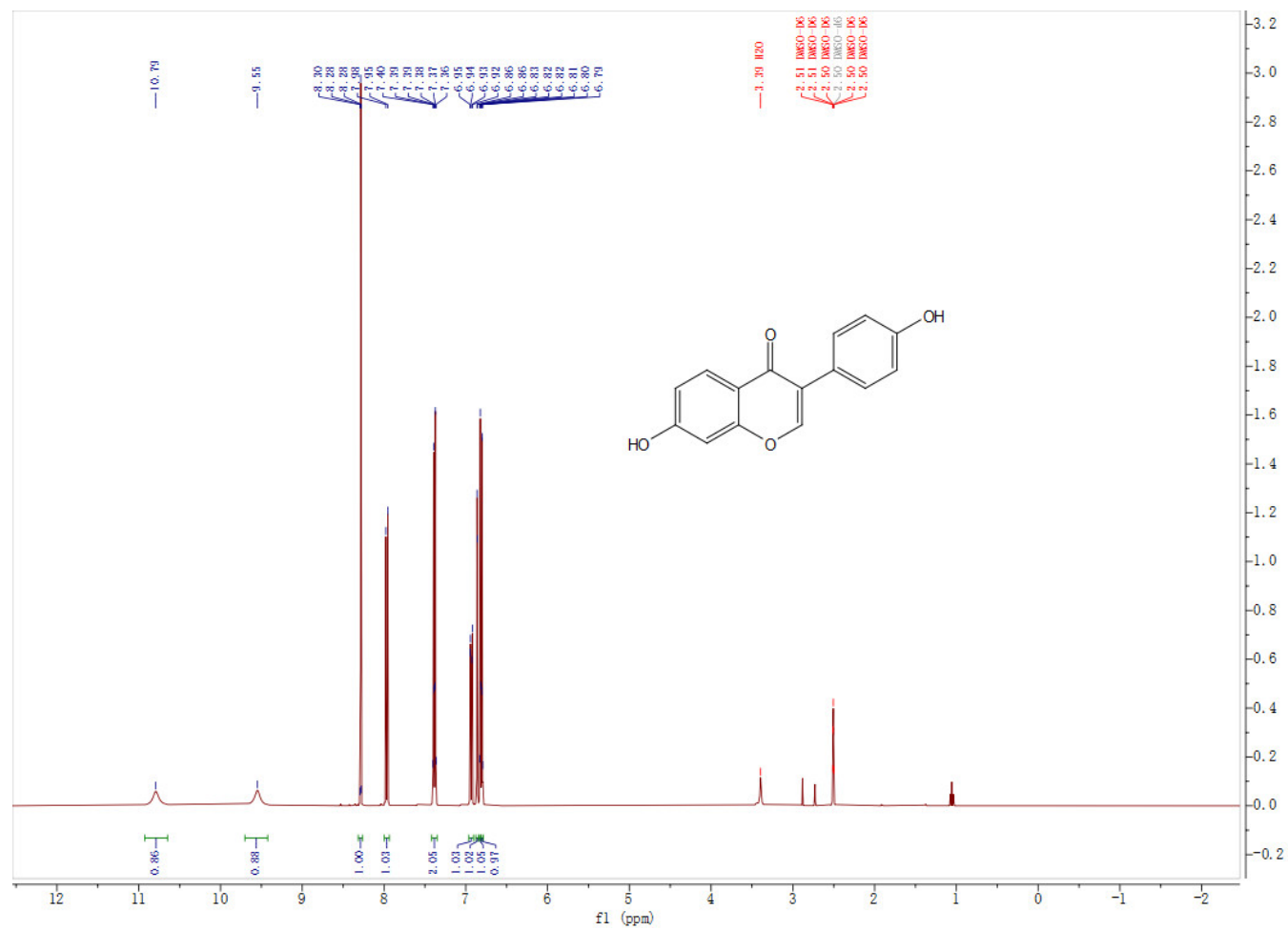


Figure S6. ^{13}C NMR of compound 3

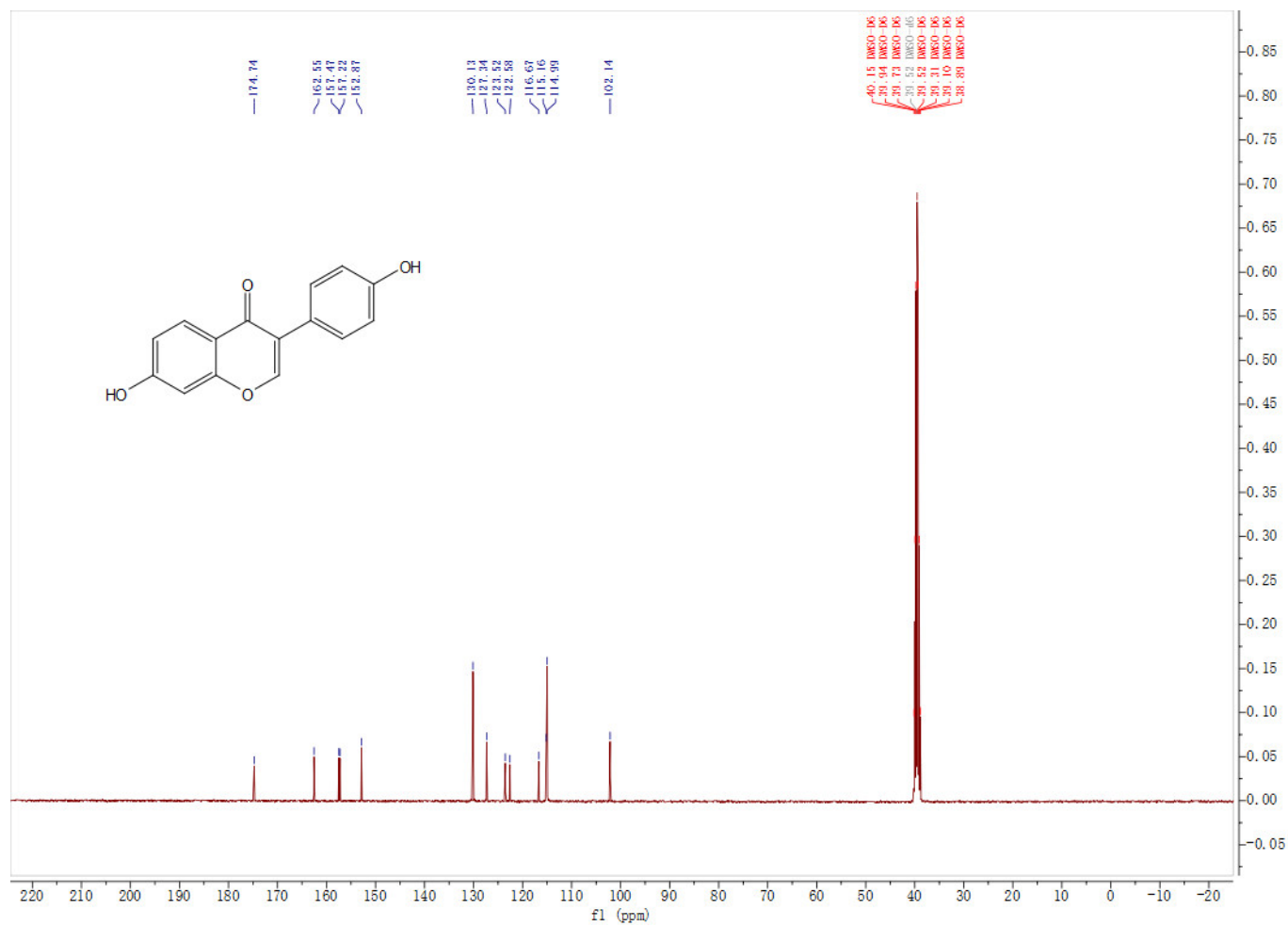


Figure S7. ^1H NMR of compound 4

Maculosin

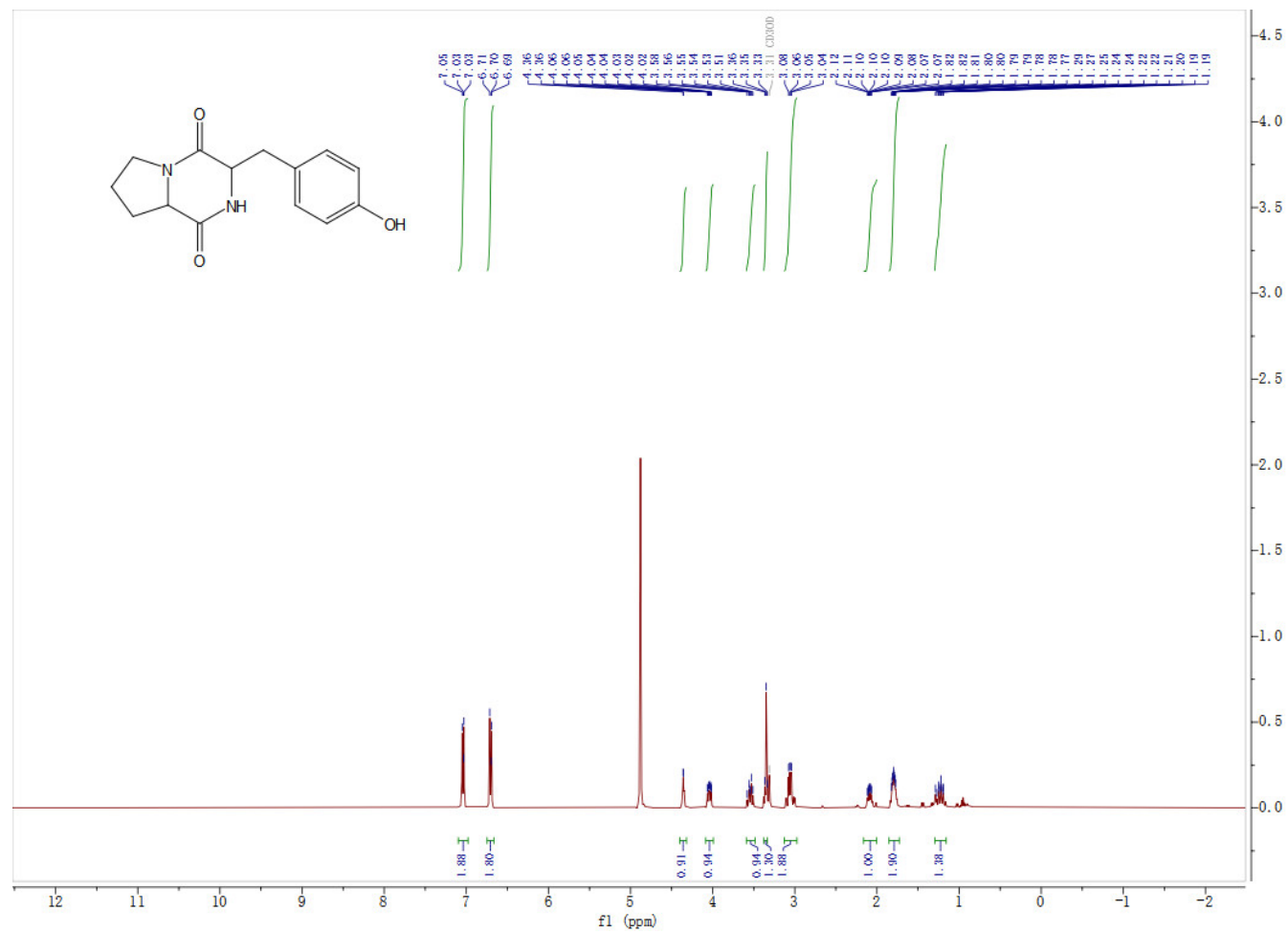
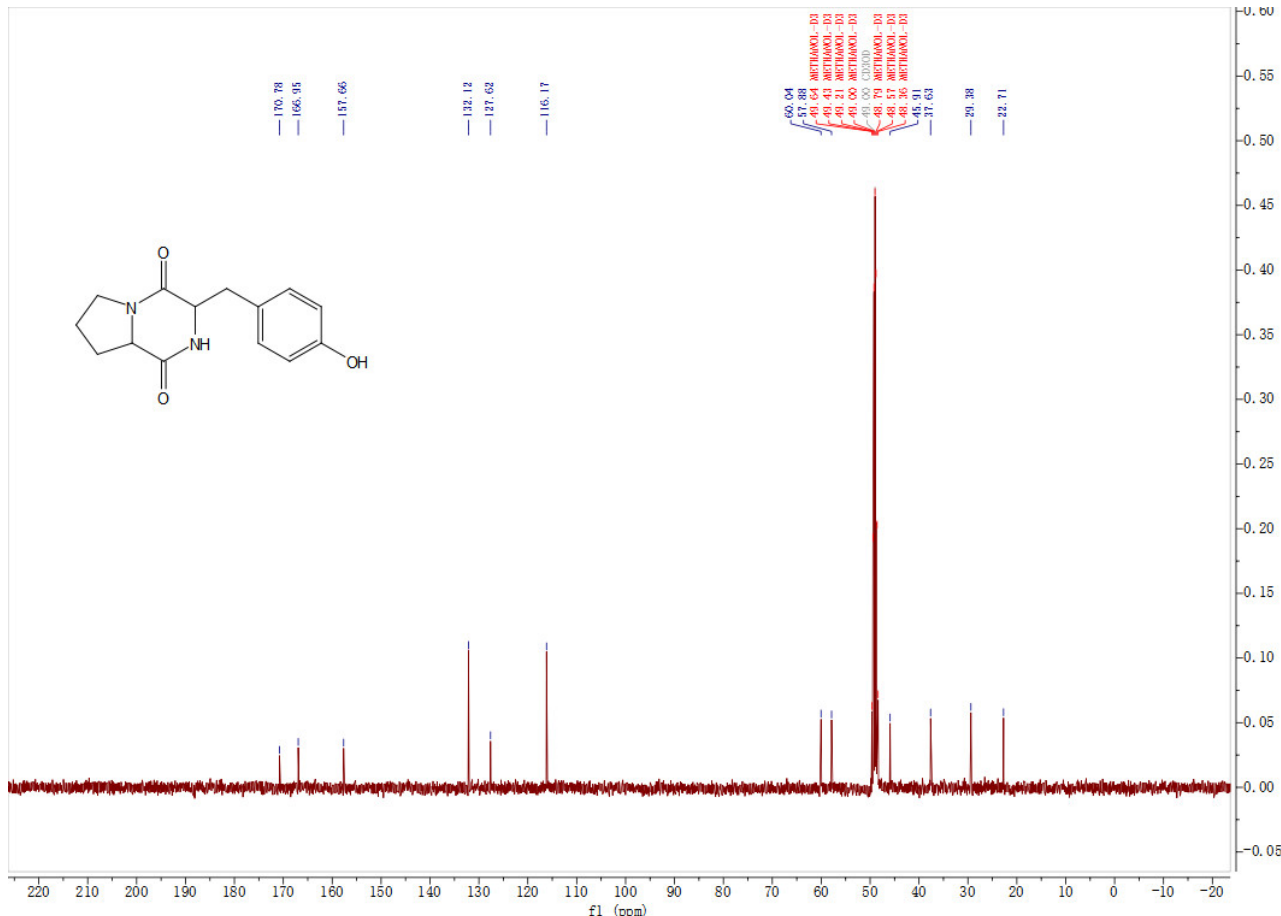


Figure S8. ^{13}C NMR of compound 4



N-Acetyl-L-tryptophan



Figure S10. ^{13}C NMR of compound 5

