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SUPPLEMENTARY MATERIAL

Kumbicins A–D: bis-indolyl benzenoids and benzoquinones from an Australian soil fungus, *Aspergillus kumbius*

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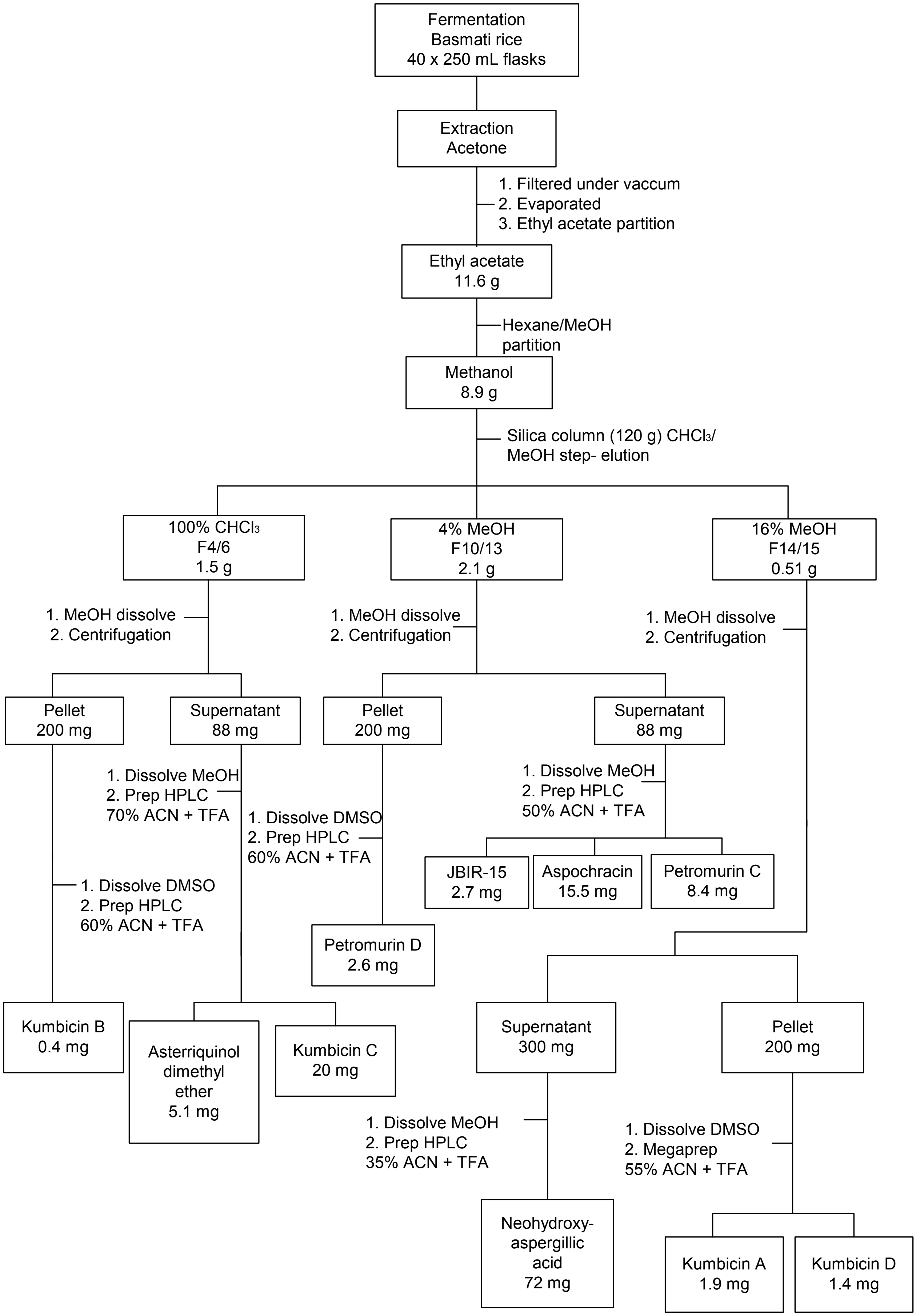


Figure S1. Fractionation scheme for *Aspergillus kumbius*

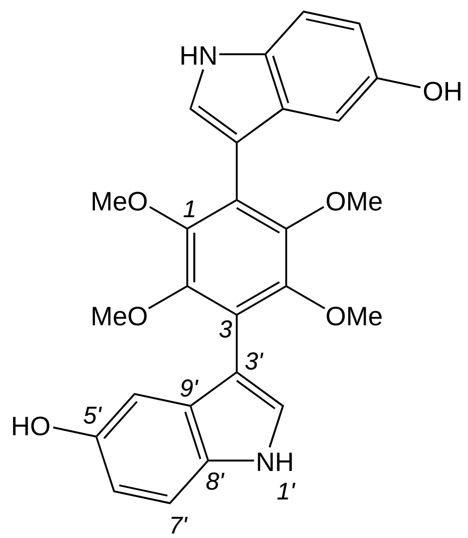


Table S1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kumbicin A (**1**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
2		147.6			
3		122.2			
2-OMe	3.41, s	60.3	2		2′, 4′
1′	10.96, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.33, d (2.4)	125.5	3, 3′, 8′, 9′	1′	2-OMe
3′		106.2			
4′	6.71, d (2.3)	104.2	3′, 5′, 6′, 8′		2-OMe
5′		150.4			
6′	6.61, dd (8.6, 2.3)	111.1	4′, 5′, 8′	7′	
7′	7.20, d (8.6)	111.6	5′, 9′	6′	1′
8′		130.4			
9′		128.0			
5′-OH	8.56, s				

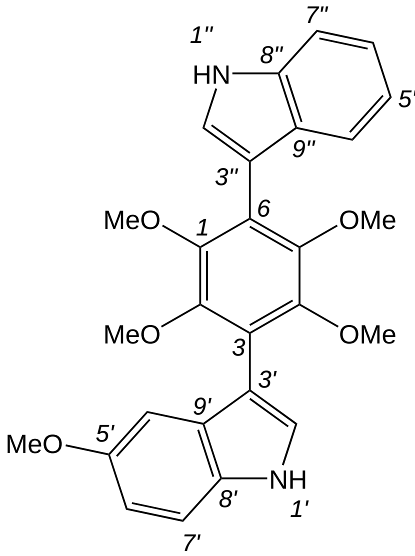


Table S2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kumbicin B (**2**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1/5		147.60[a]			
2/4		147.59[a]			
3		122.0			
6		122.2			
1/5-OMe	3.42, s	60.25[b]	1/5		2″, 4″
2/4-OMe	3.44, s	60.28[b]	2/4		2′, 4′
1′	11.13, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.41, d (2.4)	126.0	3′, 8′, 9′	1′	2/4-OMe
3′		106.7			
4′	6.86, d (2.4)	102.0	3′, 5′, 6′, 8′		2/4-OMe, 5′-OMe
5′		153.1			
6′	6.76, dd (8.7, 2.4)	110.9	4′, 5′, 8′	7′	5′-OMe
7′	7.31, d (8.7)	111.9	5′, 9′	6′	1′
8′		131.0			
9′		127.4			
5′-OMe	3.71, s	55.2	5′		4′, 6′
1″	11.27, d (2.4)		2″, 3″, 8″, 9″	2″	7″
2″	7.45, d (2.4)	125.2	3″, 8″, 9″	1″	1/5-OMe
3″		106.9			
4″	7.41, d (8.0)	120.3	3″, 6″, 8″	5″	1/5-OMe
5″	6.99, d (8.0, 7.6)	118.7	7″, 9″	4″, 6″	
6″	7.10, dd (8.2, 7.6)	120.8	4″, 8″	5″, 7″	
7″	7.43, d (8.2)	111.4	5″, 9″	6″	1″
8″		135.9			
9″		127.0			

[a–b] Assignments interchangeable

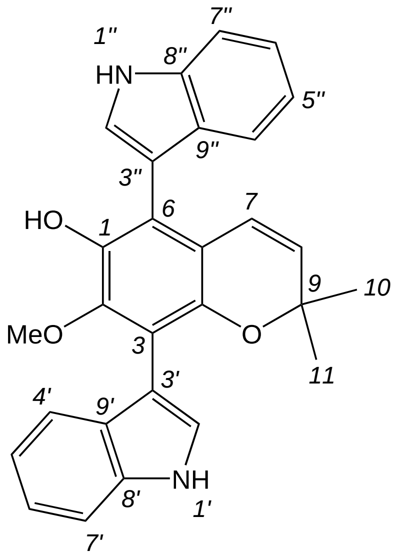


Table S3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kumbicin C (**3**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1		141.7			
2		146.2			
3		115.6			
4		143.6			
5		116.4			
6		118.1			
7	6.07, d (9.1)	121.9	4, 5, 6, 9	8	
8	5.48, d (9.1)	128.4	5, 9, 10, 11	7	10, 11
9		74.2			
10	1.31, s	26.9	8, 9, 11		8, 2′, 4′
11	1.27, s	27.3	8, 9, 10		8, 2′, 4′
1-OH	7.89, s		1, 2, 6		2-OMe
2-OMe	3.22, s	59.6	2		2′, 1-OH
1′	11.19, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.40, d (2.4)	125.5	3, 3′, 8′, 9′	1′	10, 11
3′		107.0			
4′	7.45, d (8.0)	120.6	3′, 6′, 8′	5′	10, 11
5′	6.99, dd (8.0, 7.6)	118.7	7′, 9′	4′, 6′	
6′	7.09, dd (8.1, 7.6)	120.6	4′, 8′	5′, 7′	
7′	7.43, d (8.1)	111.5	5′, 9′	6′	1′
8′		135.8			
9′		127.0			
1″	11.28, d (2.4)		2″, 3″, 8″, 9″	2″	7″
2″	7.36, d (2.4)	126.0	6, 3″, 8″, 9″	1″	
3″		108.7			
4″	7.25, d (8.0)	119.9	3″, 6″, 8″	5″	
5″	6.98, dd (8.0, 7.6)	118.3	7″, 9″	4″, 6″	
6″	7.10, dd (8.1, 7.6)	120.8	4″, 8″	5″, 7″	
7″	7.41, d (8.1)	111.3	5″, 9″	6″	1″
8″		135.9			
9″		127.5			

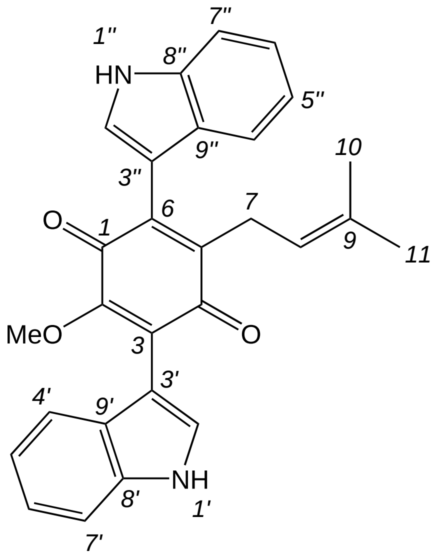


Table S4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kumbicin D (**4**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1		182.9			
2		153.4			
3		124.4			
4		187.3			
5		142.3			
6		136.3			
7	3.19, d (6.9)	27.5	4, 5, 6, 9	8	10
8	5.01, tm (6.9, 1.3)	121.4	5, 10, 11	7	11
9		132.4			
10	1.25, s	17.5	8, 9, 11		7, 11
11	1.54, s	25.4	8, 9, 10		8, 10
2-OMe	3.73, s	60.0	2		4′
1′	11.60, br s			2′	7′
2′	7.58, s	128.9	3, 3′, 8′, 9′	1′	
3′		104.5			
4′	7.37, d (8.0)	120.8	3′, 6′, 8′	5′	2-OMe
5′	7.04, dd (8.0, 7.6)	119.4	7′, 9′	4′, 6′	
6′	7.14, dd (8.2, 7.6)	121.3	4′, 8′	5′, 7′	
7′	7.45, d (8.2)	111.8	5′, 9′	6′	1′
8′		135.8			
9′		126.6			
1″	11.54, br s			2″	7″
2″	7.43, s	127.3	6, 3″, 8″, 9″	1″	
3″		107.0			
4″	7.33, d (8.0)	119.9	3″, 6″, 8″	5″	
5″	7.03, dd (8.0, 7.6)	119.3	7″, 9″	4″, 6″	
6″	7.13, dd (8.2, 7.6)	121.3	4″, 8″	5″, 7″	
7″	7.44, d (8.2)	111.7	5″, 9″	6″	1″
8″		135.9			
9″		126.7			

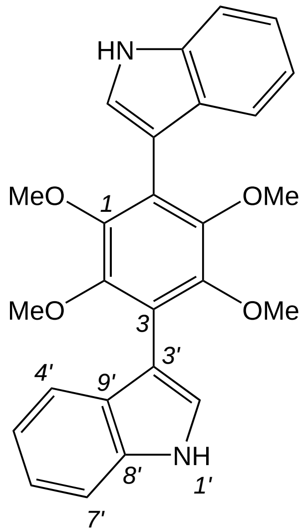


Table S5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for asterriquinol D dimethyl ether (**5**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
2		147.6			
3		122.1			
2-OMe	3.42, s	60.3	2		2′, 4′
1′	11.28, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.46, d (2.4)	125.2	3, 3′, 8′, 9′	1′	2-OMe
3′		106.9			
4′	7.40, d (8.0)	120.2	3′, 6′, 8′	5′	2-OMe
5′	7.00, dd (8.0, 7.6)	118.7	7′, 9′	4′, 6′	
6′	7.10, dd (8.1, 7.6)	120.8	4′, 8′	5′, 7′	
7′	7.43, d (8.1)	111.4	5′, 9′	6′	1′
8′		135.9			
9′		127.1			

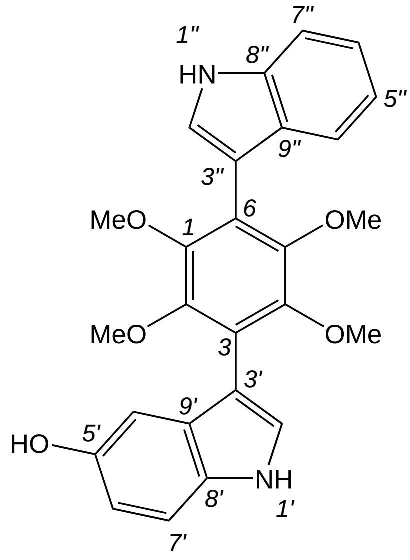


Table S6. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for petromurin C (**6**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1/5		147.60[a]			
2/4		147.59[a]			
3		122.5			
6		121.9			
1/5-OMe	3.42, s	60.25[b]	1/5		2″, 4″
2/4-OMe	3.42, s	60.31[b]	2/4		2′, 4′
1′	10.97, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.34, d (2.4)	125.5	3, 3′, 8′, 9′	1′	2/4-OMe
3′		106.1			
4′	6.73, d (2.4)	104.2	3′, 5′, 6′, 8′		2/4-OMe, 5′-OH
5′		150.4			
6′	6.61, dd (8.6, 2.4)	111.1	4′, 5′, 8′	7′	5′-OH
7′	7.20, d (8.6)	111.6	5′, 9′	6′	1′
8′		130.4			
9′		127.9			
5′-OH	8.56, s		4′, 5′, 6′		4′, 6′
1″	11.27, d (2.4)		2″, 3″, 8″, 9″		7″
2″	7.45, d (2.4)	125.2	6, 3″, 8″, 9″		1/5-OMe
3″		107.0			
4″	7.39, d (8.0)	120.2	3″, 6″, 8″	5″	1/5-OMe
5″	7.00, d (8.0, 7.6)	118.7	7″, 9″	4″, 6″	
6″	7.10, dd (8.2, 7.6)	120.8	4″, 8″	5″, 7″	
7″	7.43, d (8.2)	111.4	5″, 9″	6″	1″
8″		135.9			
9″		127.1			

[a–b] Assignments interchangeable

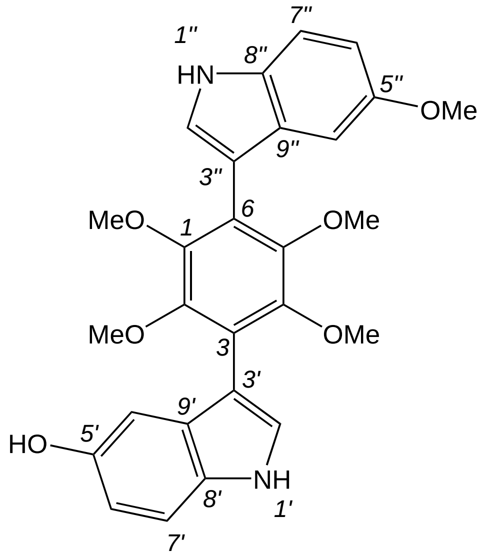


Table S7. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for petromurin D (**7**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1/5		147.60[a]			
2/4		147.55[a]			
3		122.4			
6		121.9			
1/5-OMe	3.44, s	60.3	1/5		2″, 4″
2/4-OMe	3.42, s	60.2	2/4		2′, 4′
1′	10.96, d (2.4)		2′, 3′, 8′, 9′	2′	7′
2′	7.34, d (2.4)	125.6	3, 3′, 8′, 9′	1′	2/4-OMe
3′		106.1			
4′	6.73, d (2.4)	104.3	3′, 5′, 6′, 8′		2/4-OMe, 5′-OH
5′		150.4			
6′	6.61, dd (8.6, 2.4)	111.1	4′, 5′, 8′	7′	5′-OH
7′	7.20, d (8.6)	111.6	5′, 9′	6′	1′
8′		130.4			
9′		127.9			
5′-OH	8.55, s				4′, 6′
1″	11.13, d (2.4)		2″, 3″, 8″, 9″	2″	7″
2″	7.41, d (2.4)	125.9	6, 3″, 8″, 9″	1″	2/4-OMe
3″		106.8			
4″	6.85, d (2.4)	102.0	3″, 5″, 6″, 8″		2/4-OMe, 5″-OMe
5″		153.1			
6″	6.76, dd (8.7, 2.4)	110.9	4″, 5″, 8″	7″	5″-OMe
7″	7.31, d (8.7)	111.9	5″, 9″	6″	1″
8″		131.0			
9″		127.4			
5″-OMe	3.71, s	55.2	5″		4″, 6″

[a] Assignments interchangeable

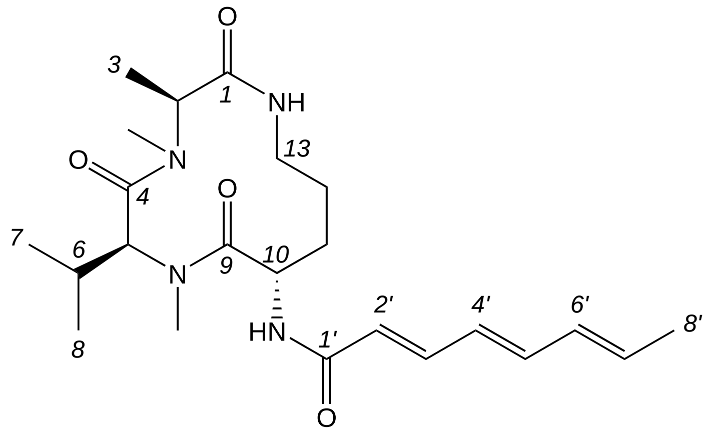


Table S8. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for aspochracin (**8**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1		170.6			
2	4.49, q (7.1)	54.4	1, 2-NMe, 3, 4	3	5, 13-NH
2-NMe	2.84, s	29.7	2, 4		3
3	1.37, d (7.1)	16.3	1, 2	2	2-NMe, 13-NH
4		169.1			
5	4.96, d (10.4)	57.5	2-NMe, 4,6,7,8,9	6	2, 7, 8
5-NMe	2.82, s	29.6	5, 9		8, 10
6	2.21, m	26.4	4, 5, 7, 8	5, 7, 8	
7	0.79, d (6.4)	19.8	5, 6, 8	6	5, 8
8	0.62, d (6.8)	17.7	5, 6, 7	6	5, 7, 5-NMe
9		171.8			
10	4.70, ddd (7.8, 7.8, 2.0)	49.4	9, 11, 12, 1′	10-NH, 11a/b	5-NMe, 12b
10-NH	8.12, d (7.8)		10, 11, 1′	10	2′
11a	1.94, m	28.2	10, 12, 13	10, 11b, 12a/b	13-NH
11b	1.65, m		10, 12, 13	10, 11a, 12b	13-NH
12a	1.62, m	22.8	10, 11, 13	11a, 12b, 13a/b	
12b	1.45, m		10, 11, 13	11a/b, 12a, 13a/b	10
13a	3.02, m	39.0	1, 11, 12	12a/b, 13b, 13-NH	
13b	2.84, m		1, 11, 12	12a/b, 13a, 13-NH	
13-NH	7.48, dd (6.0, 6.0)		1, 13	13a/b	2, 3, 11a/b
1′		164.3			
2′	6.14, d (15.0)	122.3	1′, 3′, 4′	3′	10-NH
3′	7.00, dd (15.0, 11.3)	139.5	1′, 2′, 4′, 5′	2′, 4′	5′
4′	6.22, dd (14.9, 11.3)	128.1	2′, 3′, 5′, 6′	3′, 5′	
5′	6.54, dd (14.9, 10.8)	139.0	3′, 4′, 6′, 7′	4′, 6′	3′, 7′
6′	6.18, dd (15.0, 10.8)	131.5	4′, 5′, 7′, 8′	5′, 7′	8′
7′	5.89, dt (15.0, 6.9)	133.4	5′, 6′, 8′	6′, 8′	5′
8′	1.76, d (6.9)	18.3	6′, 7′	7′	6′

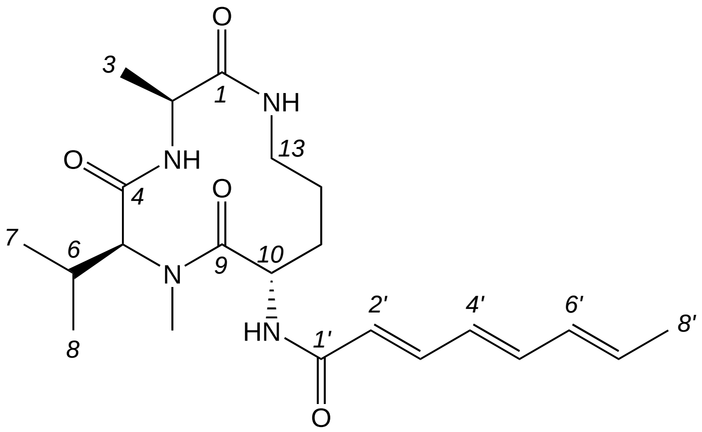


Table S9. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for JBIR-15 (**9**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1		171.9			
2	4.04, dq (9.3, 7.4)	50.8	1, 3, 4	3, 2-NH	5, 13-NH
2-NH	7.64, d (9.3)		2, 3, 4, 5	2	3
3	1.22, d (7.4)	18.7	1, 2	2	2-NH, 13-NH
4		170.0			
5	4.79, d (10.6)	57.0	4, 6, 7, 8, 9	6	2, 7, 8
5-NMe	2.86, s	29.7	5, 9		7, 8, 10
6	2.16, m	25.9	4, 5, 7, 8	5, 7, 8	
7	0.79, d (6.4)	19.6	5, 6, 8	6	5, 8, 5-NMe
8	0.62, d (6.8)	17.7	5, 6, 7	6	5, 7, 5-NMe
9		172.0			
10	4.71, ddd (7.8, 7.8, 2.0)	49.3	9, 11, 12, 1′	10-NH, 11a/b	5-NMe, 12b
10-NH	8.10, d (7.8)		10, 1′	10	2′
11a	1.94, m	28.1	10, 12, 13	10, 11b, 12a/b	13-NH
11b	1.67, m		10, 12, 13	10, 11a, 12b	13-NH
12a	1.66, m	22.6	10, 11, 13	11a, 12b, 13a/b	
12b	1.43, m		10, 11, 13	11a/b, 12a, 13a/b	10
13a	3.02, m	39.1	1, 11, 12	12a/b, 13b, 13-NH	
13b	2.83, m		1, 11, 12	12a/b, 13a, 13-NH	
13-NH	7.57, dd (6.0, 6.0)		1, 13	13a/b	2, 3, 11a/b
1′		164.3			
2′	6.14, d (15.0)	122.3	1′, 3′, 4′	3′	10-NH
3′	7.00, dd (15.0, 11.3)	139.5	1′, 2′, 4′, 5′	2′, 4′	5′
4′	6.22, dd (14.9, 11.3)	128.1	2′, 3′, 5′, 6′	3′, 5′	
5′	6.54, dd (14.9, 10.8)	139.0	3′, 4′, 6′, 7′	4′, 6′	3′, 7′
6′	6.18, dd (15.0, 10.8)	131.5	4′, 5′, 7′, 8′	5′, 7′	8′
7′	5.89, dt (15.0, 6.9)	133.4	5′, 6′, 8′	6′, 8′	5′
8′	1.76, d (6.9)	18.3	6′, 7′	7′	6′

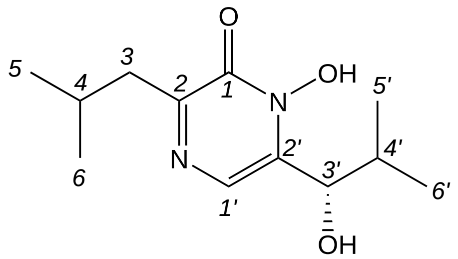


Table S10. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for neohydroxyaspergillic acid (**10**) in DMSO-d_6

Pos.	δ_H, mult (J in Hz)	δ_C	HMBC	COSY	ROESY
1		151.9			
2		154.4			
3a	2.59, dd (14.0, 6.9)	41.5	1, 2, 4, 5, 6	3b, 4	5, 6
3b	2.53, dd (14.0, 7.0)		1, 2, 4, 5, 6	3a, 4	5, 6
4	2.12, m	26.2	2, 3, 5, 6	3a/b, 5, 6	
5	0.87, d (6.4)	22.4	3, 4, 6	4	3a/b
6	0.87, d (6.4)	22.4	3, 4, 5	4	3a/b
1′	7.25, s	119.8	2, 2′, 3′		3′, 4′, 5′, 6′
2′		141.0			
3′	4.63, br s	69.6	1′, 2′, 4′, 5′, 6′	4′	1′
4′	2.02, m	31.5	3′, 5′, 6′	3′, 5′, 6′	1′
5′	0.92, d (6.5)	19.5	3′, 4′, 6′	4′	1′
6′	0.77, d (6.4)	15.8	3′, 4′, 5′	4′	1′
OH	5.54, br s				

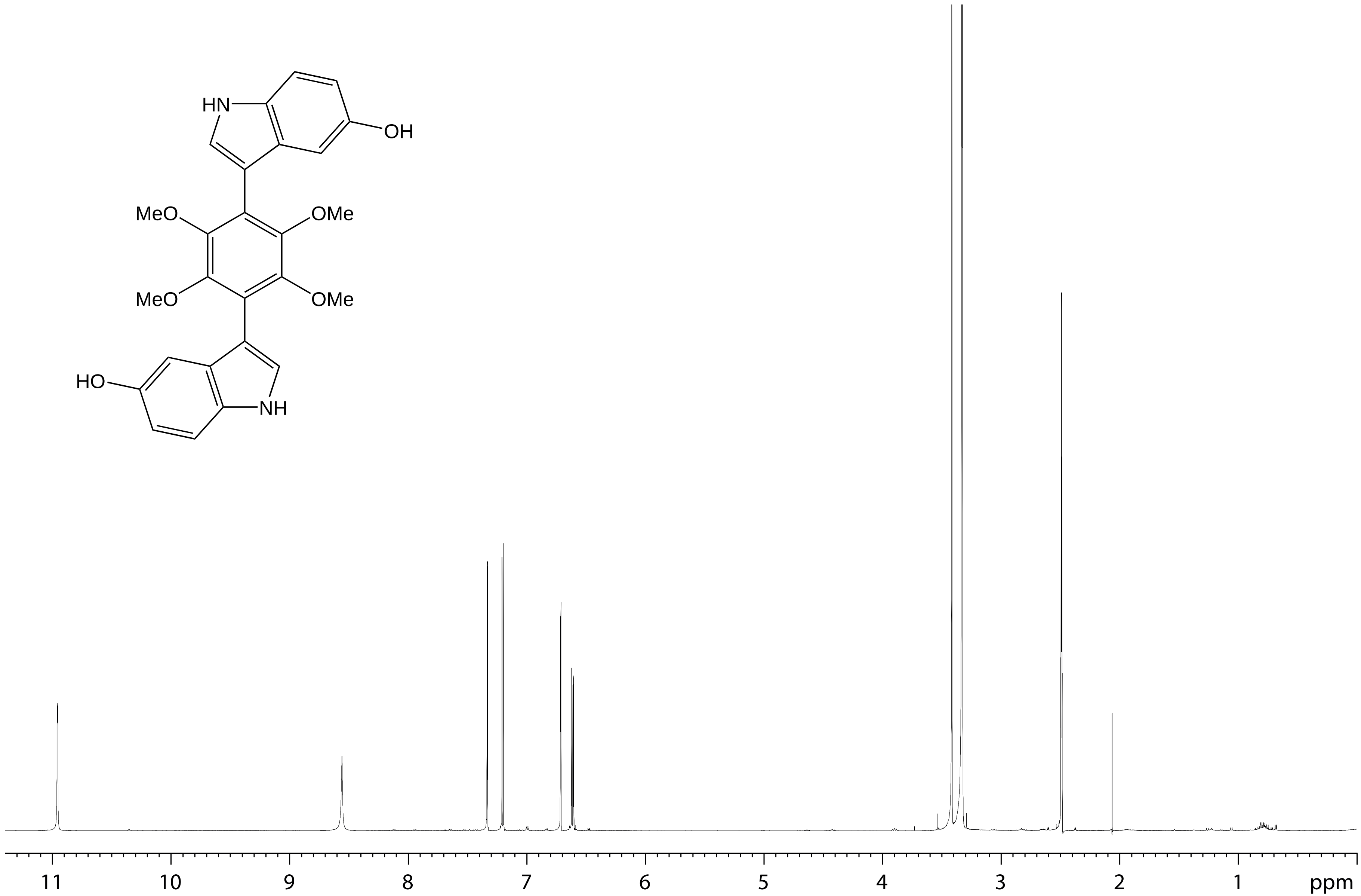


Figure S2. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of kumbicin A (**1**)

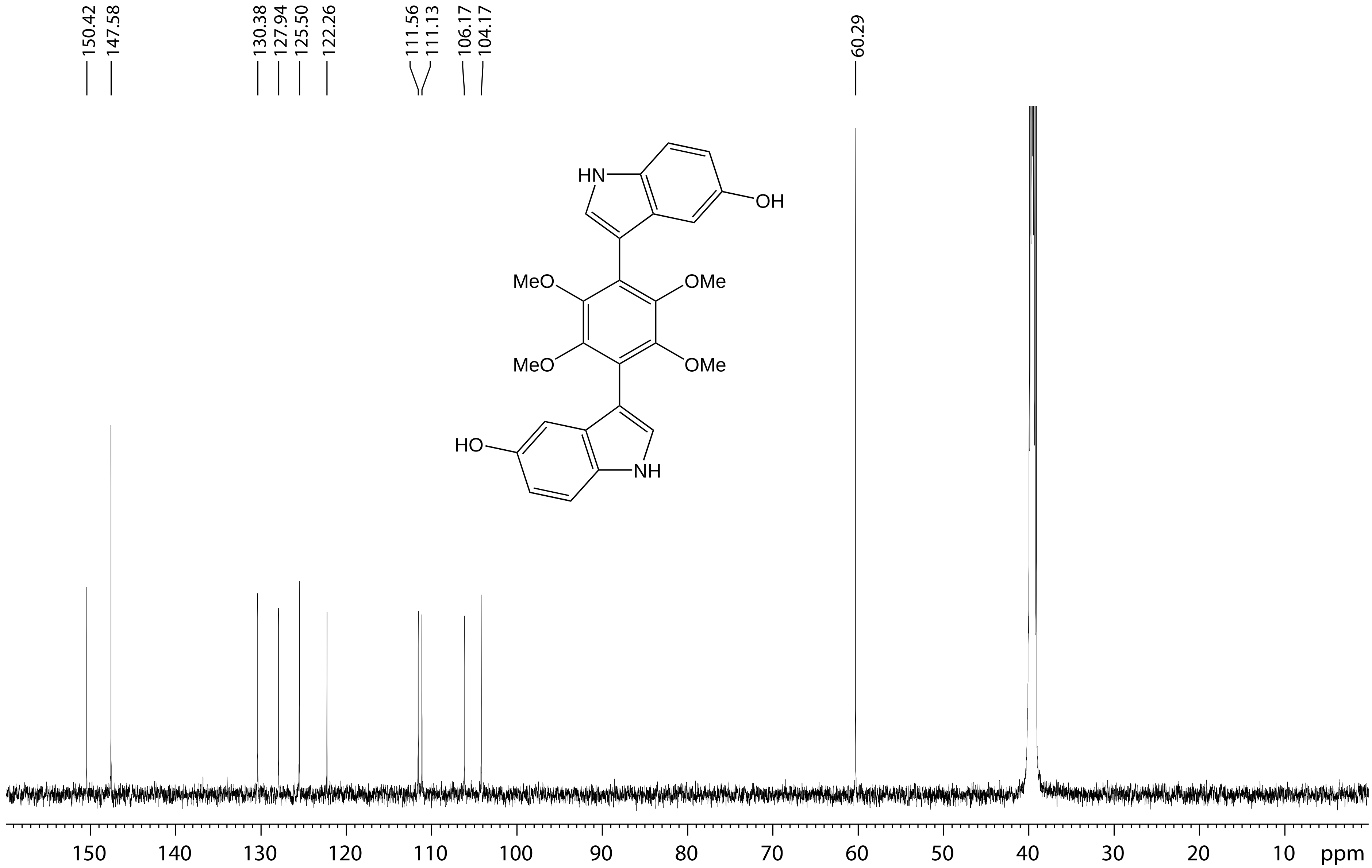


Figure S3. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of kumbicin A (**1**)

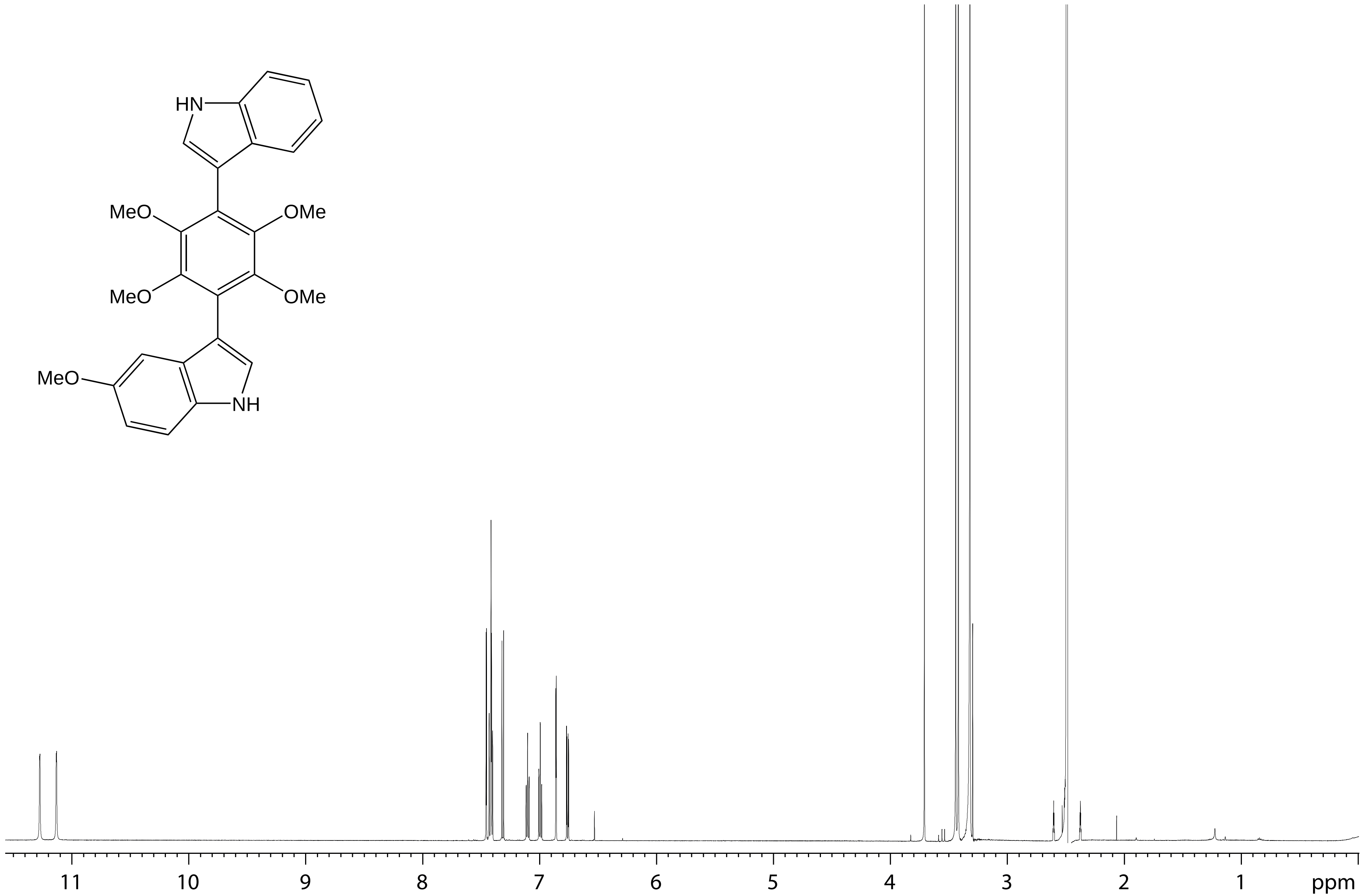


Figure S4. [1]H NMR spectrum (600 MHz, DMSO-d_6) of kumbicin B (**2**)

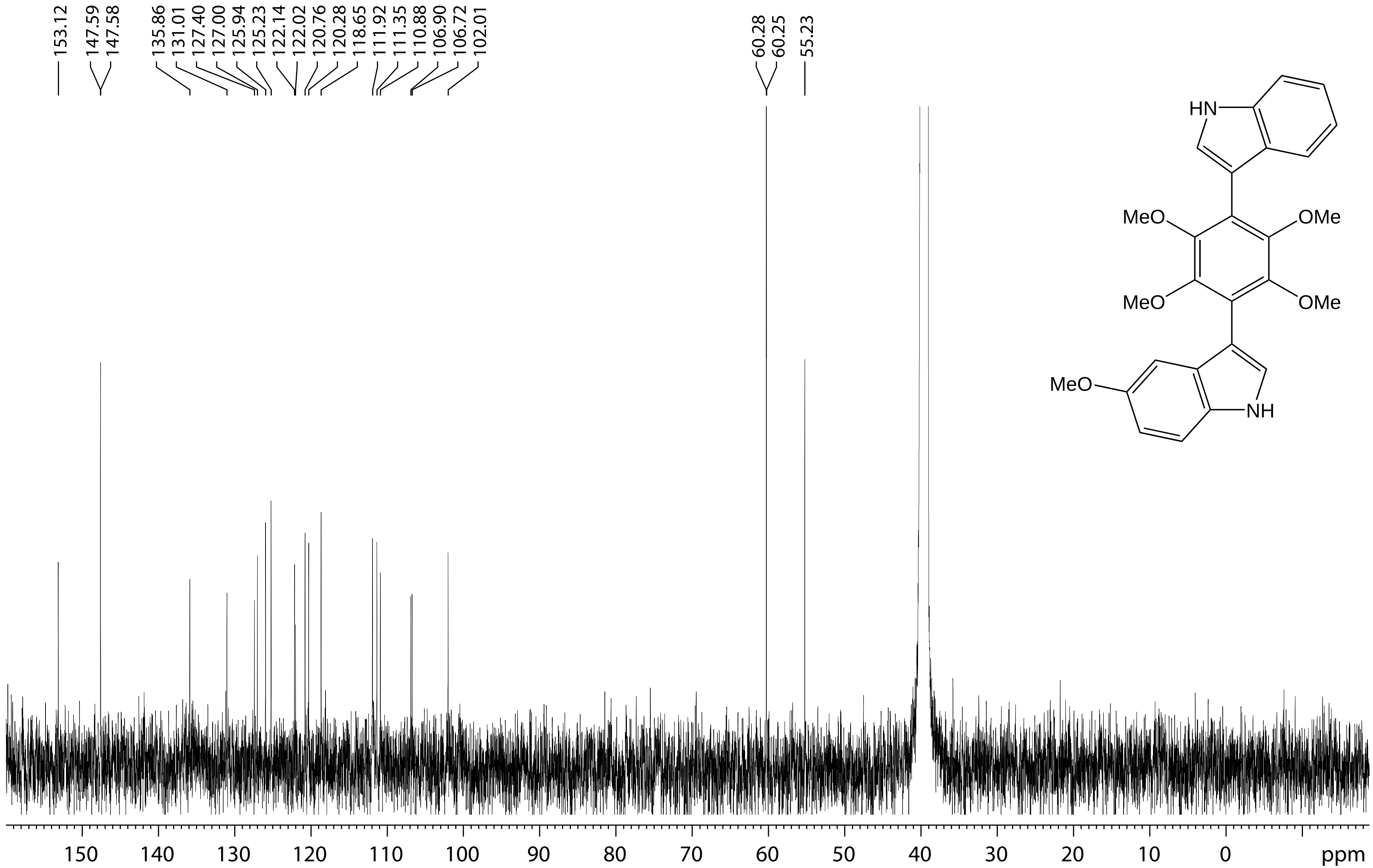


Figure S5. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of kumbicin B (**2**)

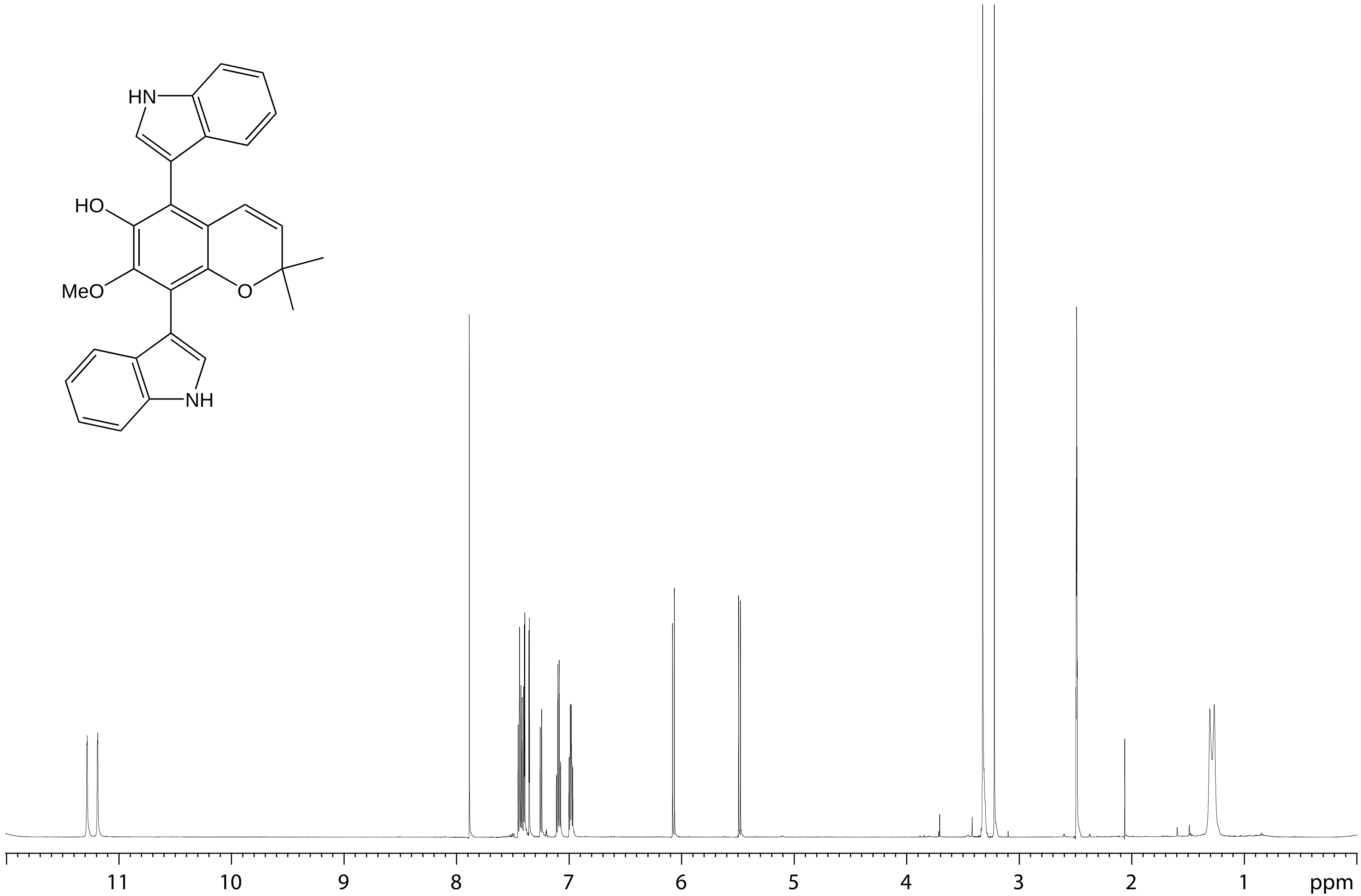


Figure S6. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of kumbicin C (**3**)

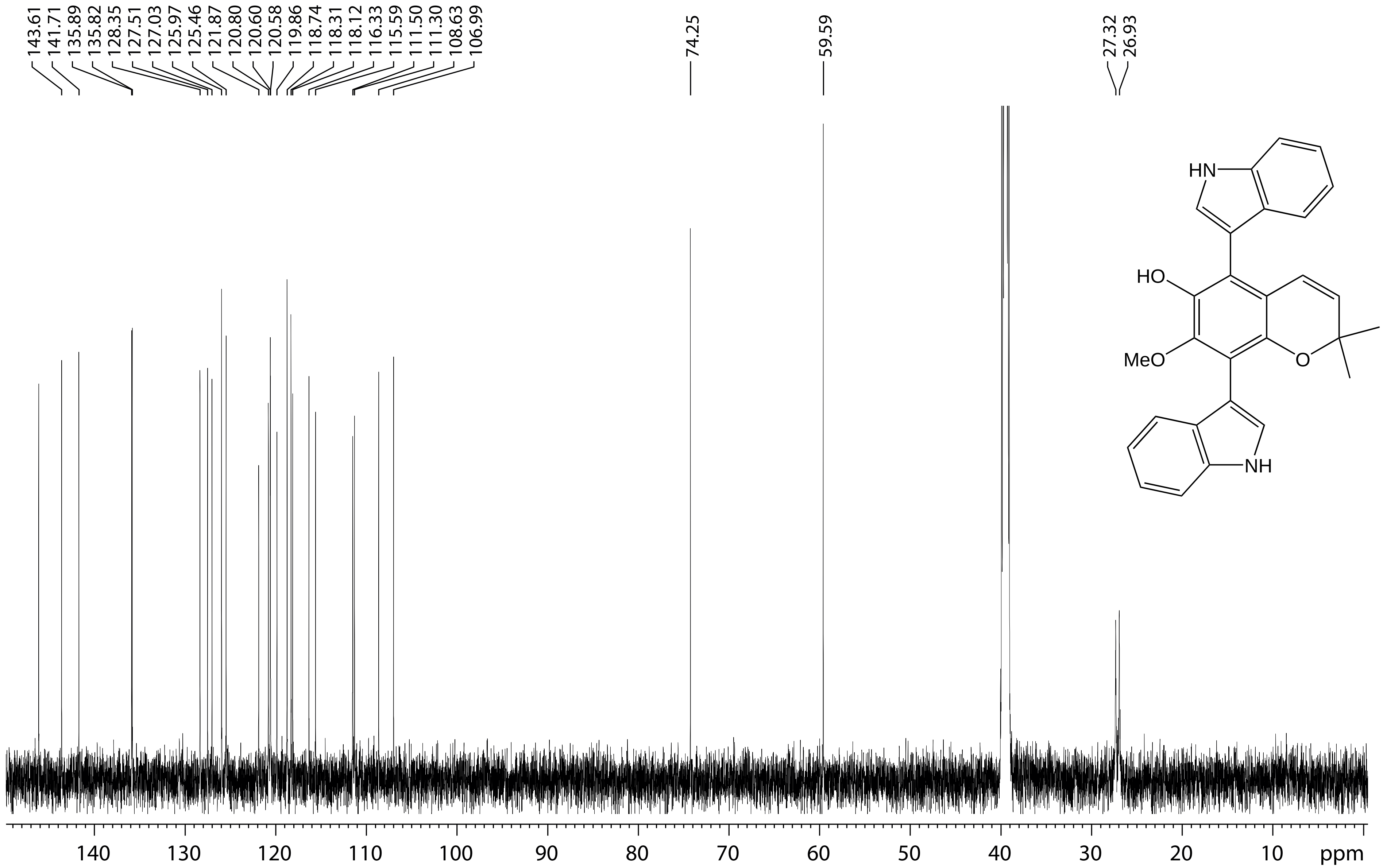


Figure S7. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of kumbicin C (**3**)

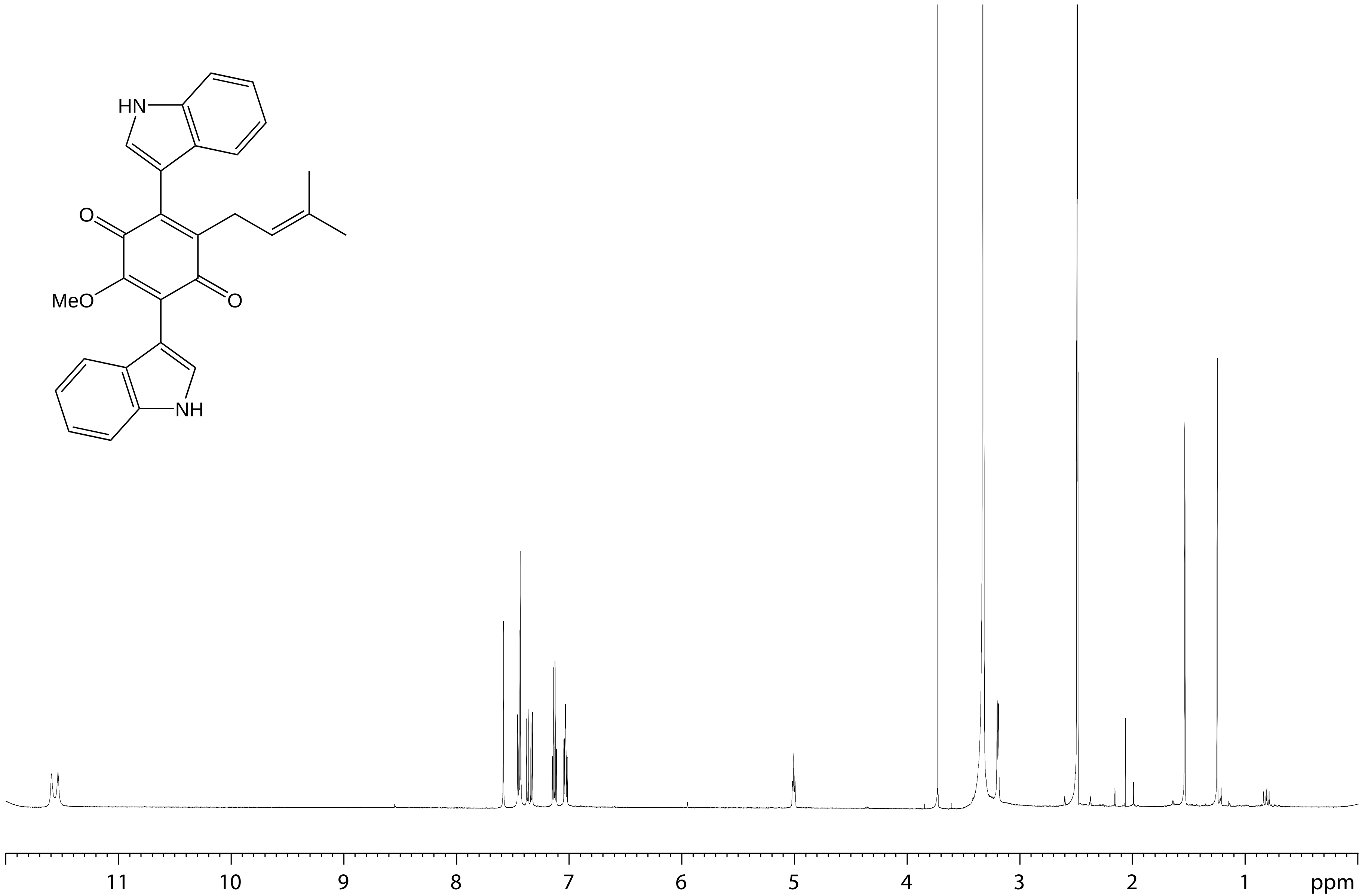


Figure S8. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of kumbicin D (**4**)

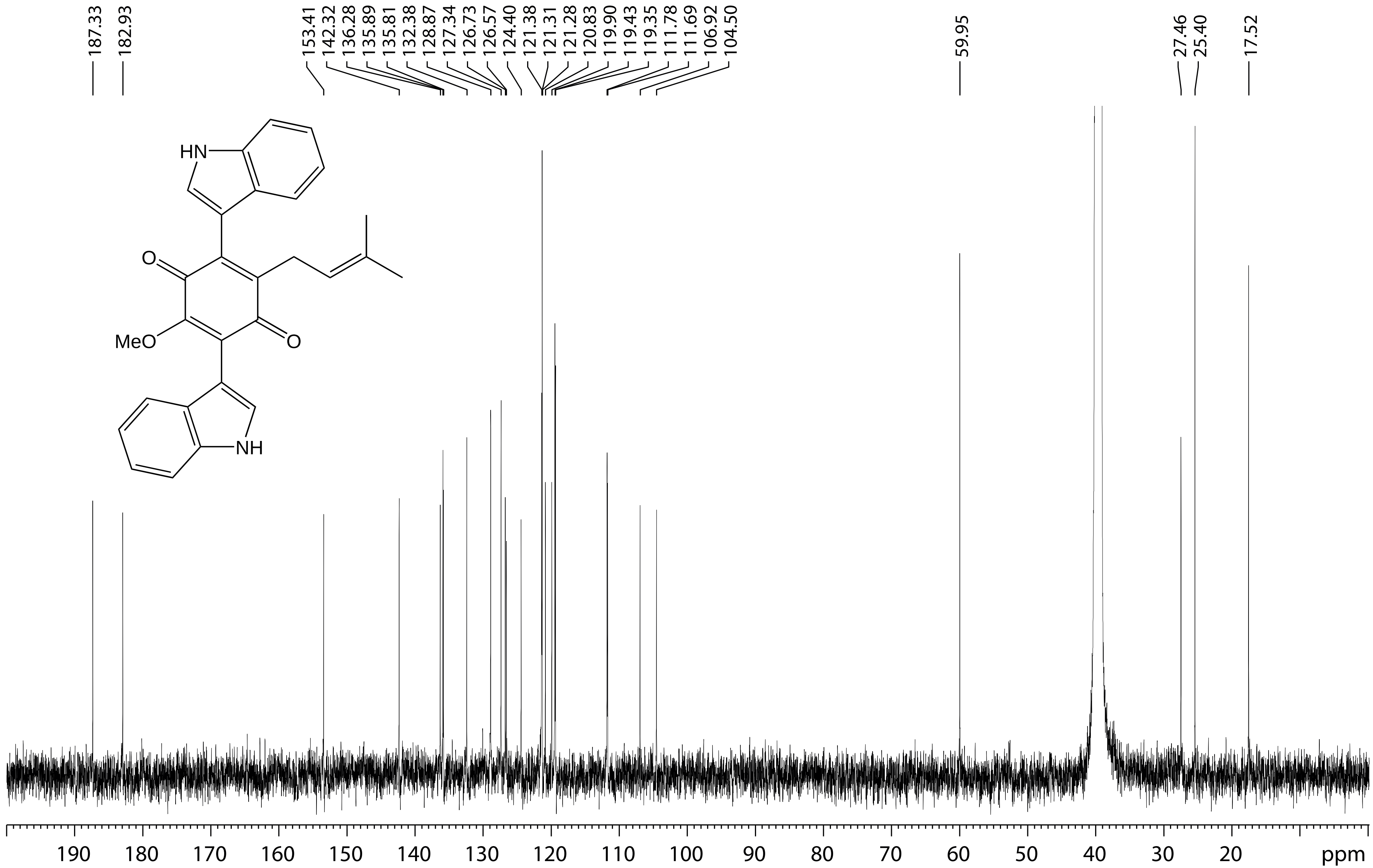


Figure S9. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of kumbicin D (**4**)

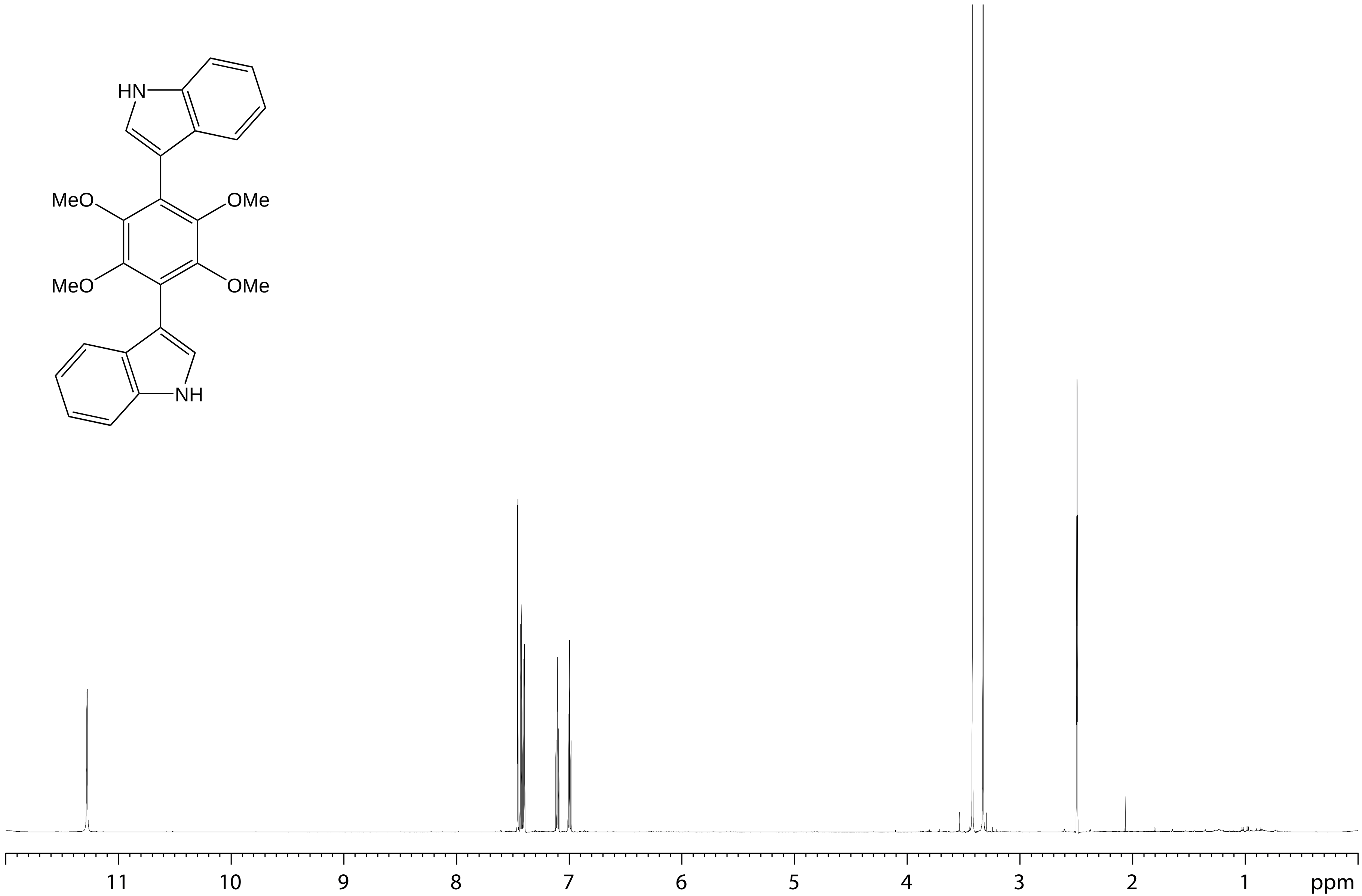


Figure S10. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of asterriquinol D dimethyl ether (**5**)

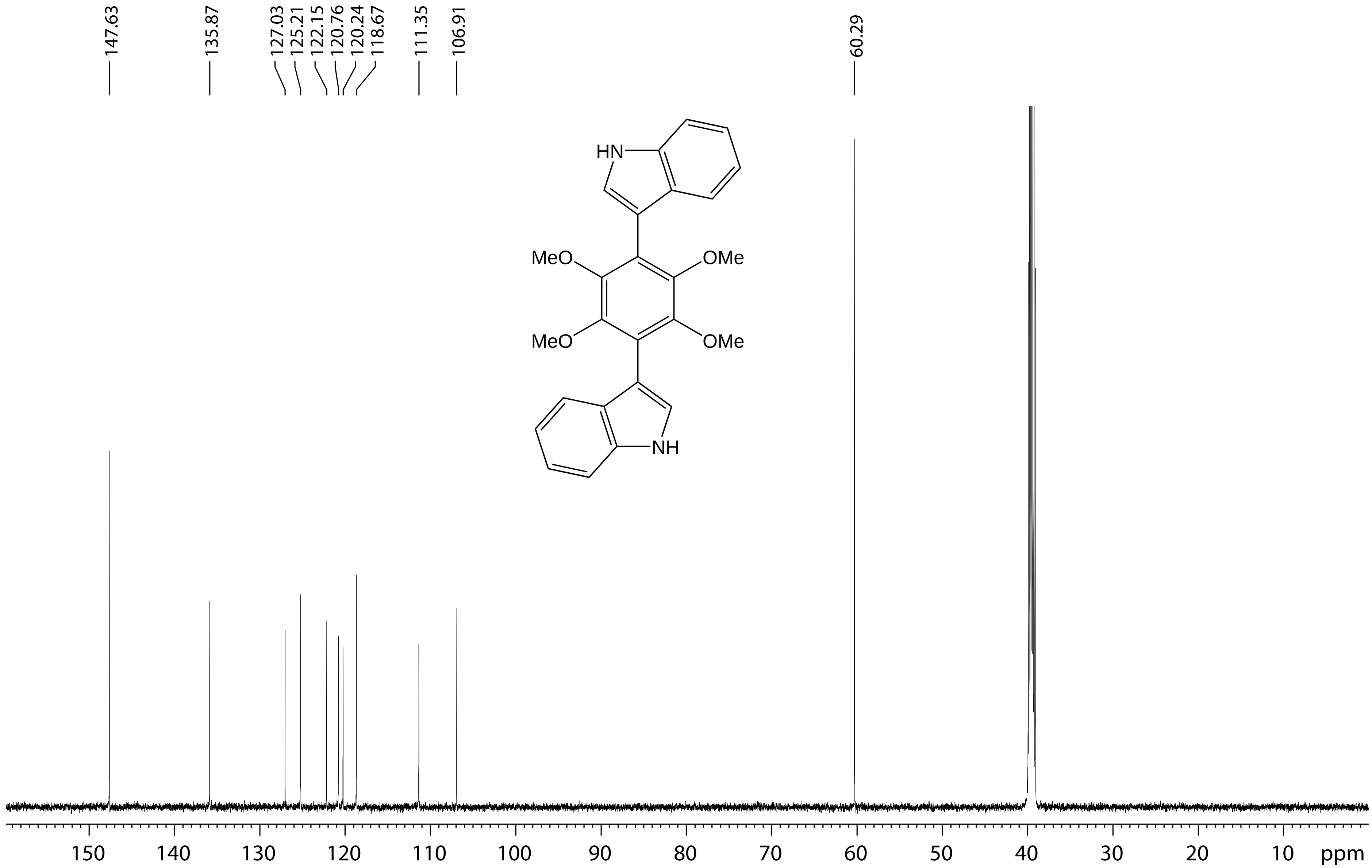


Figure S11. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of asterriquinol D dimethyl ether (**5**)

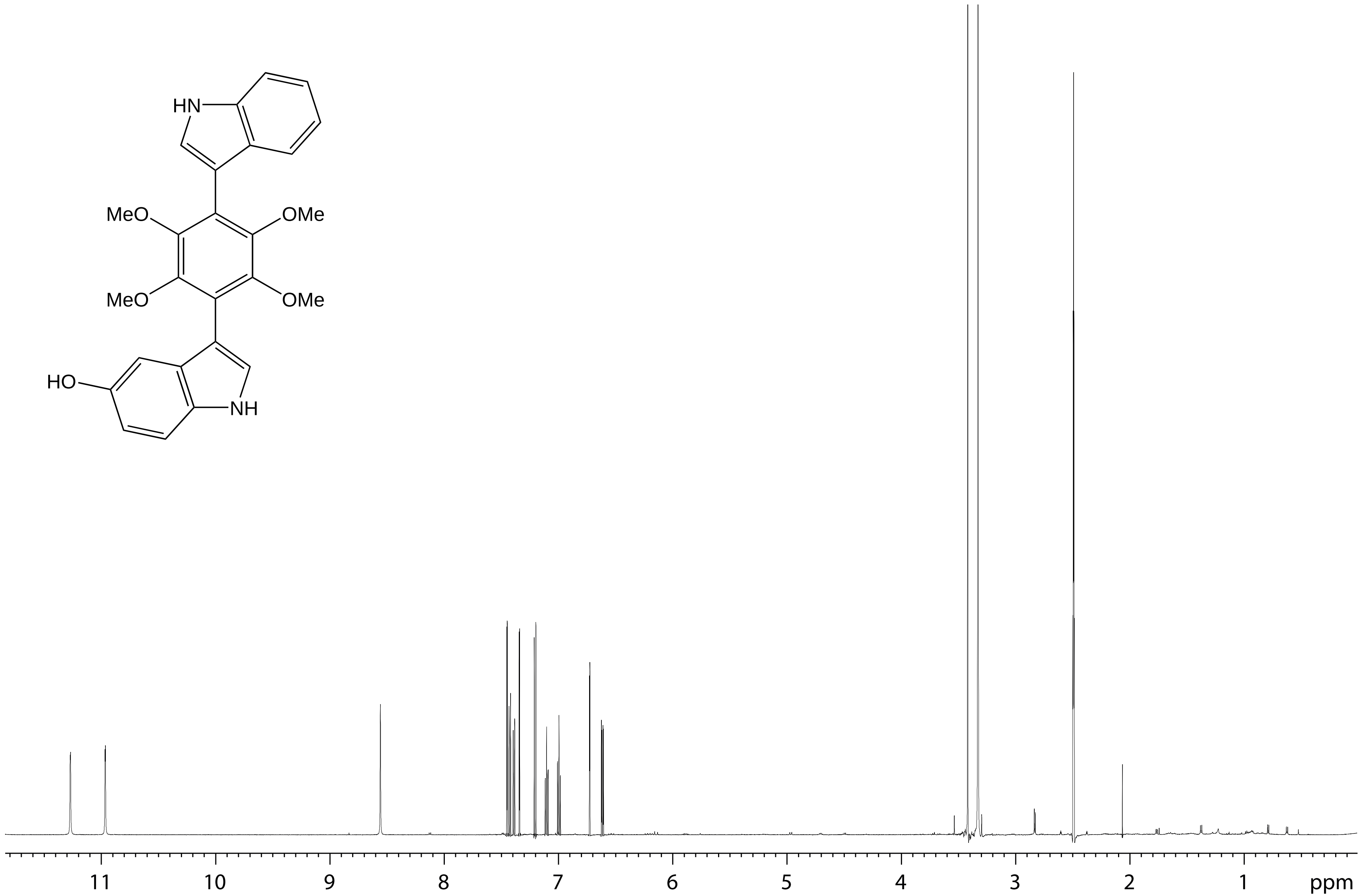


Figure S12. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of petromurin C (**6**)

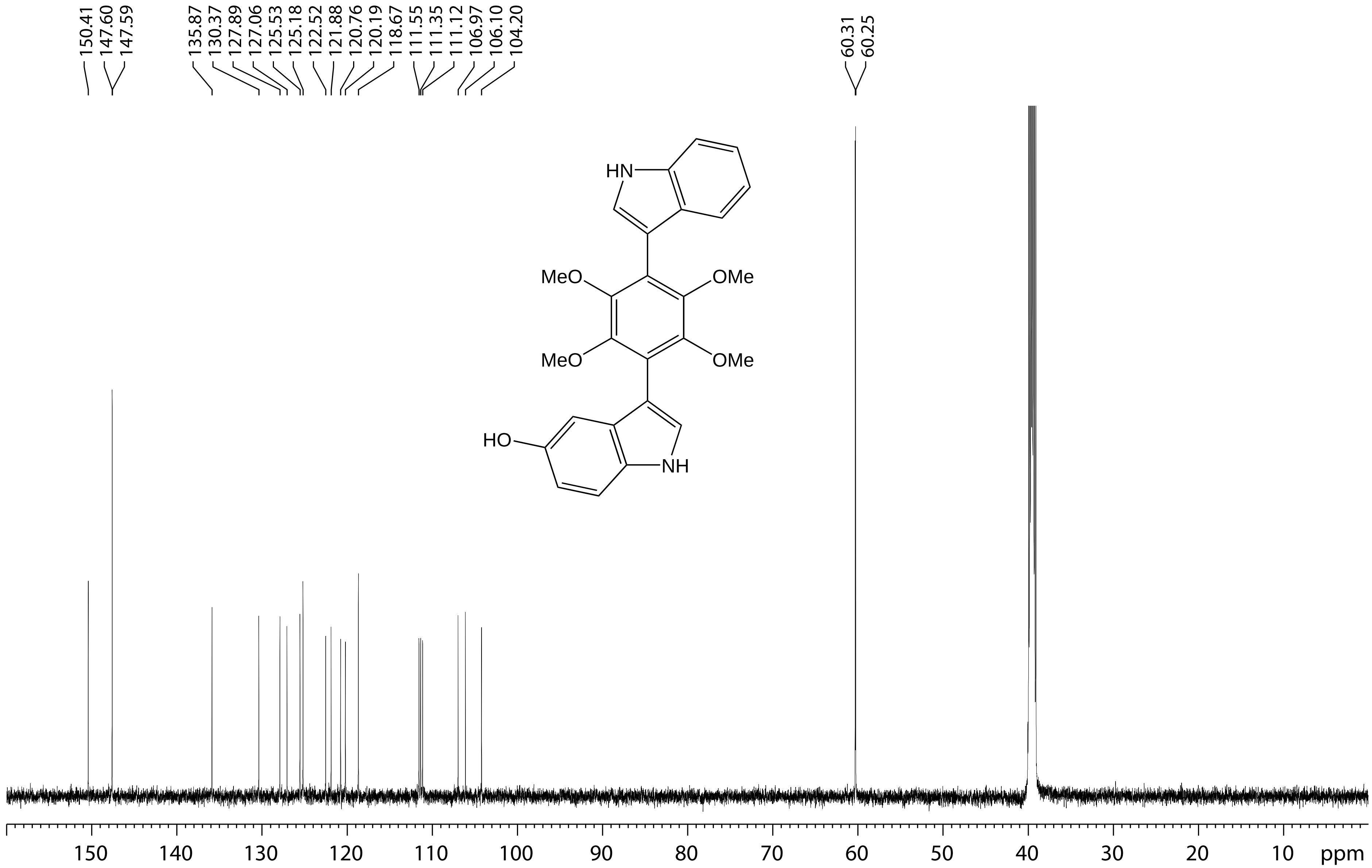


Figure S13. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of petromurin C (**6**)

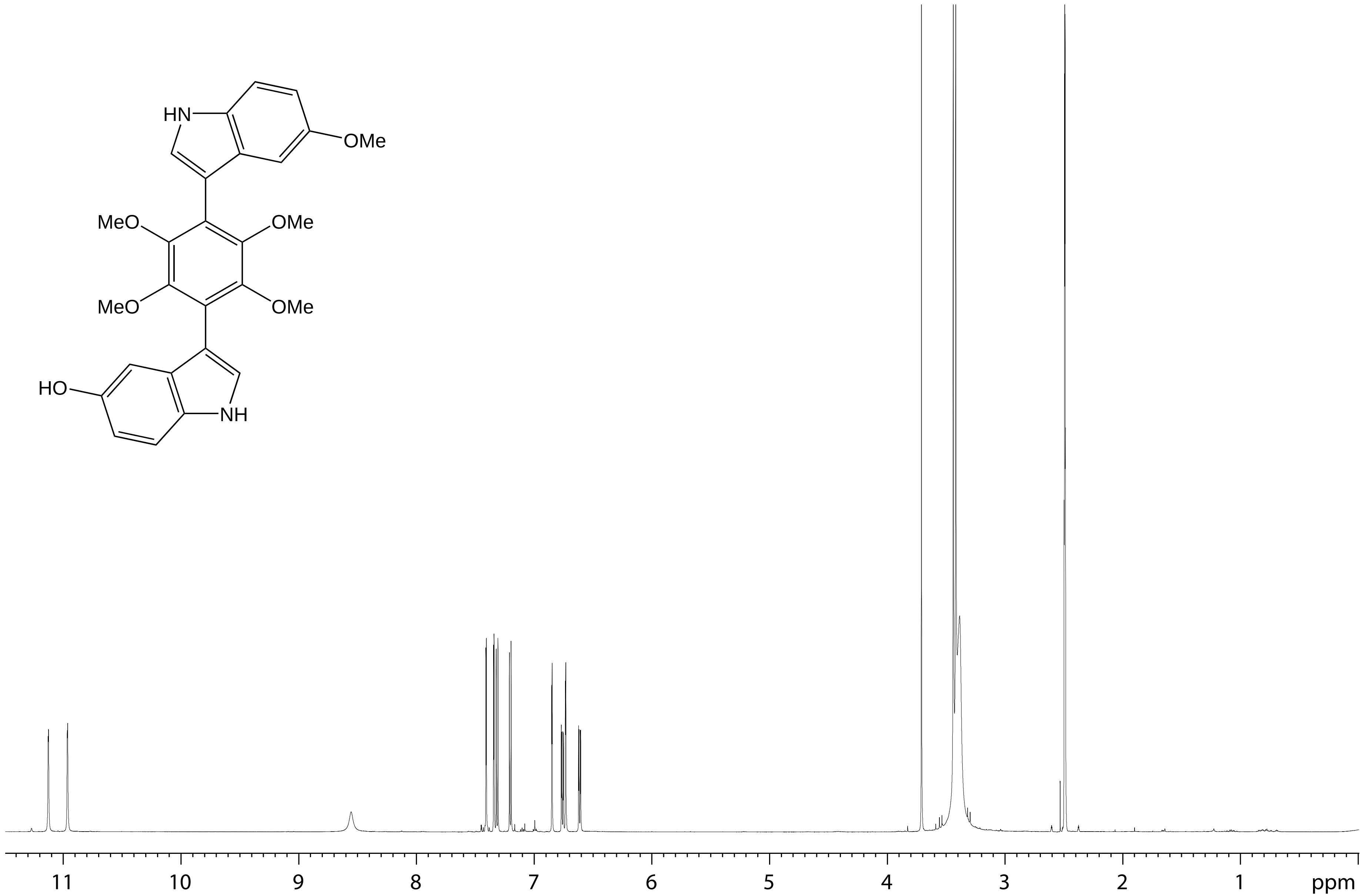


Figure S14. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of petromurin D (**7**)

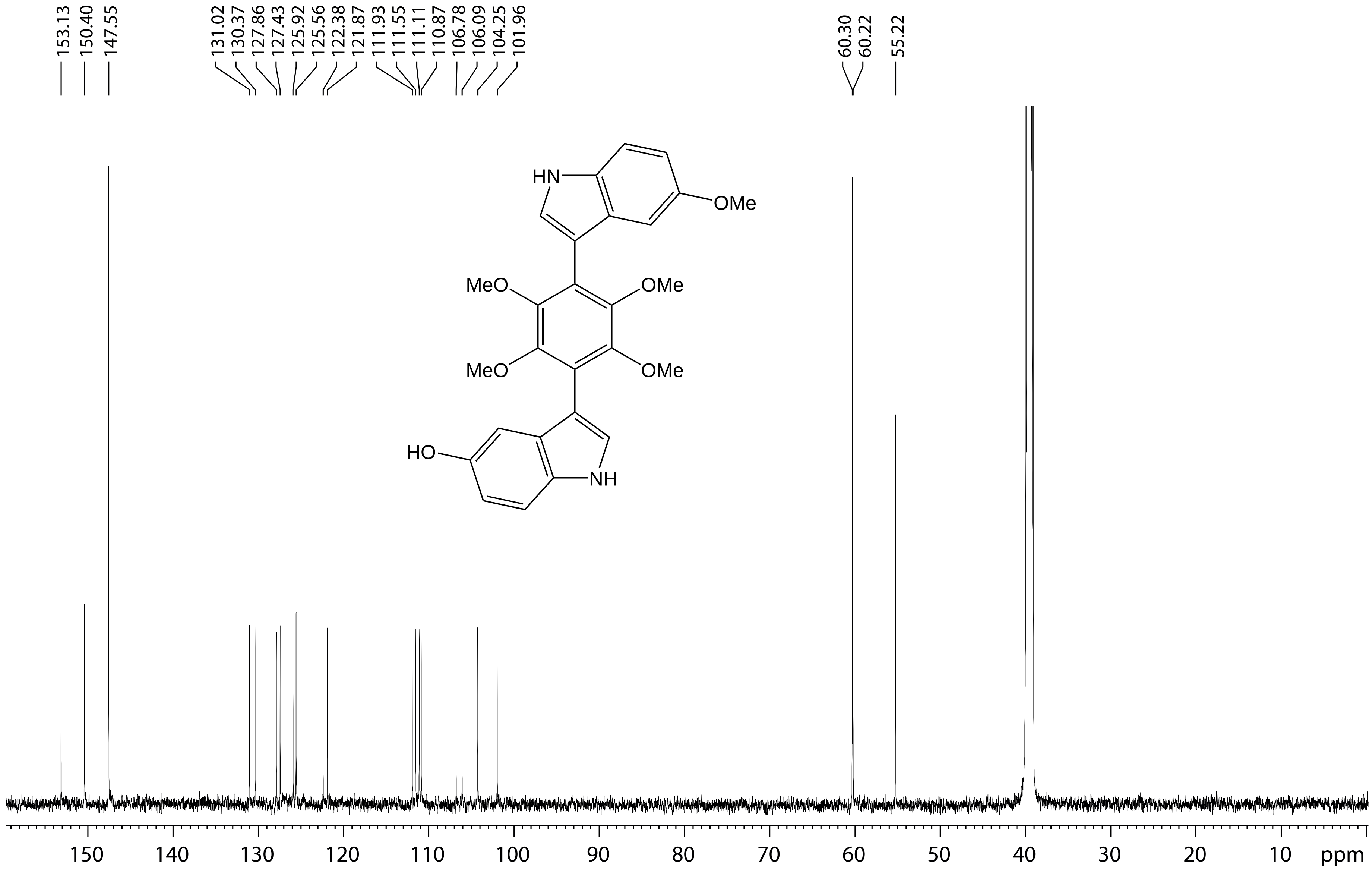


Figure S15. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of petromurin D (**7**)

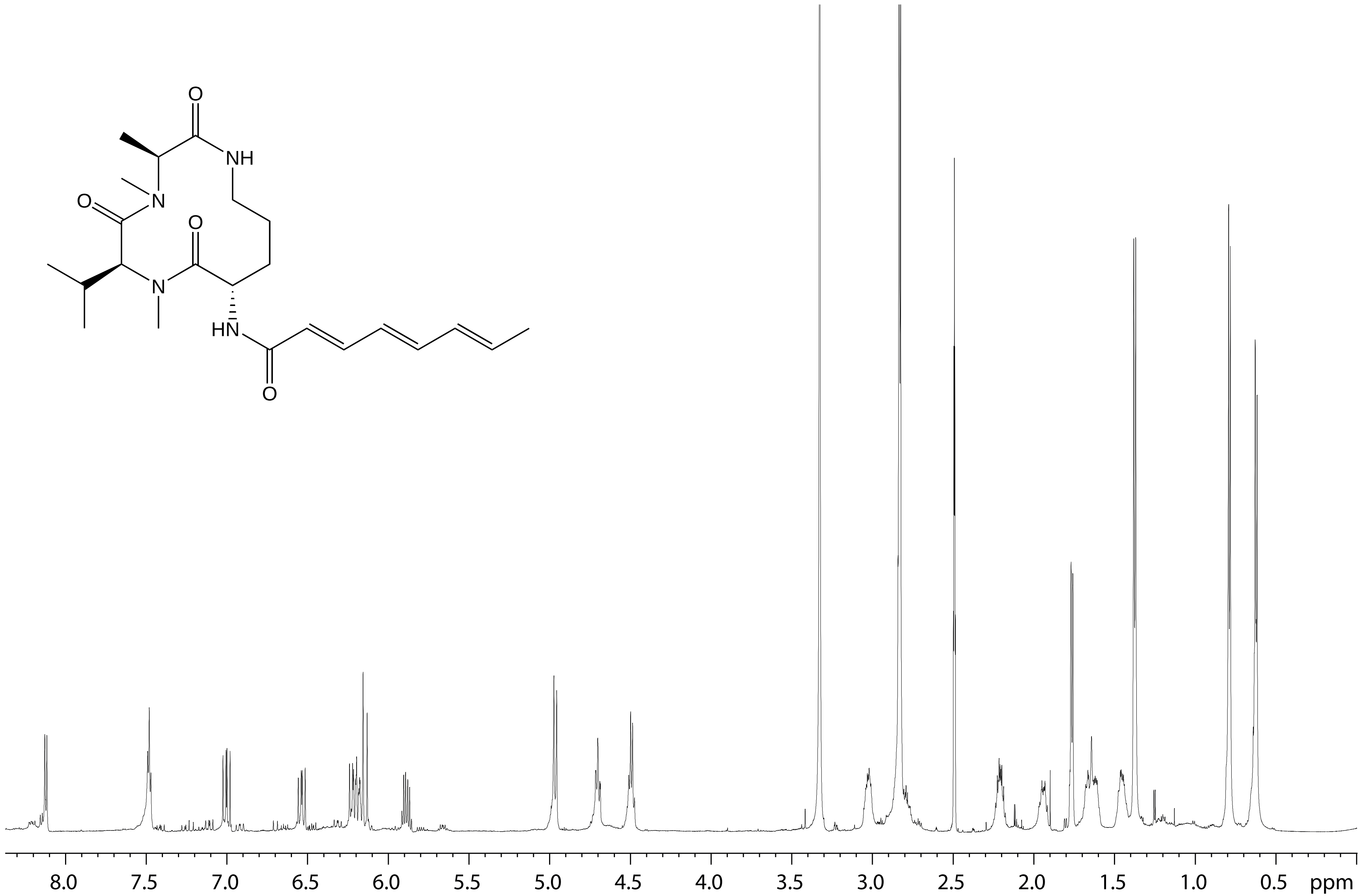


Figure S16. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of aspochracin (**8**)

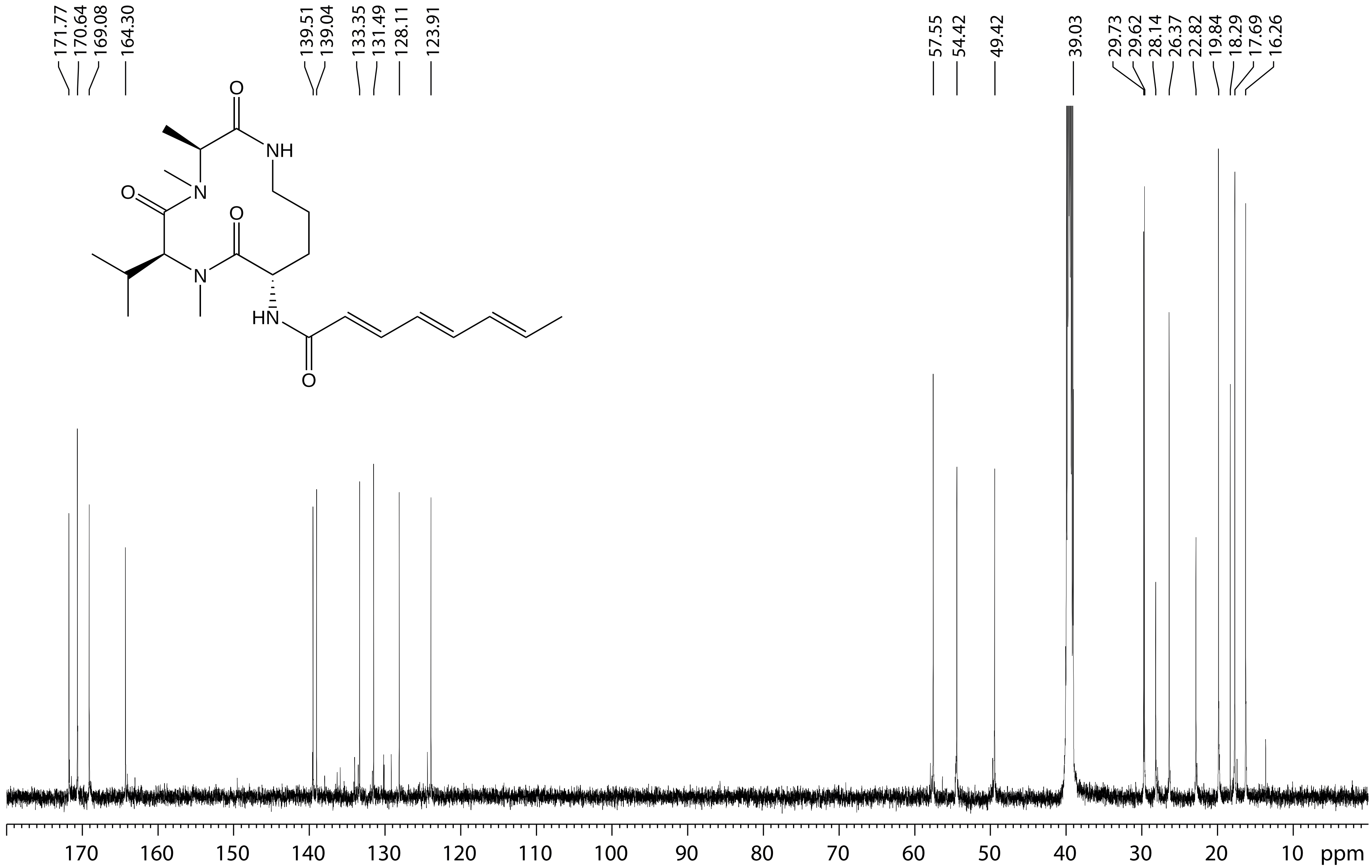


Figure S17. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of aspochracin (**8**)

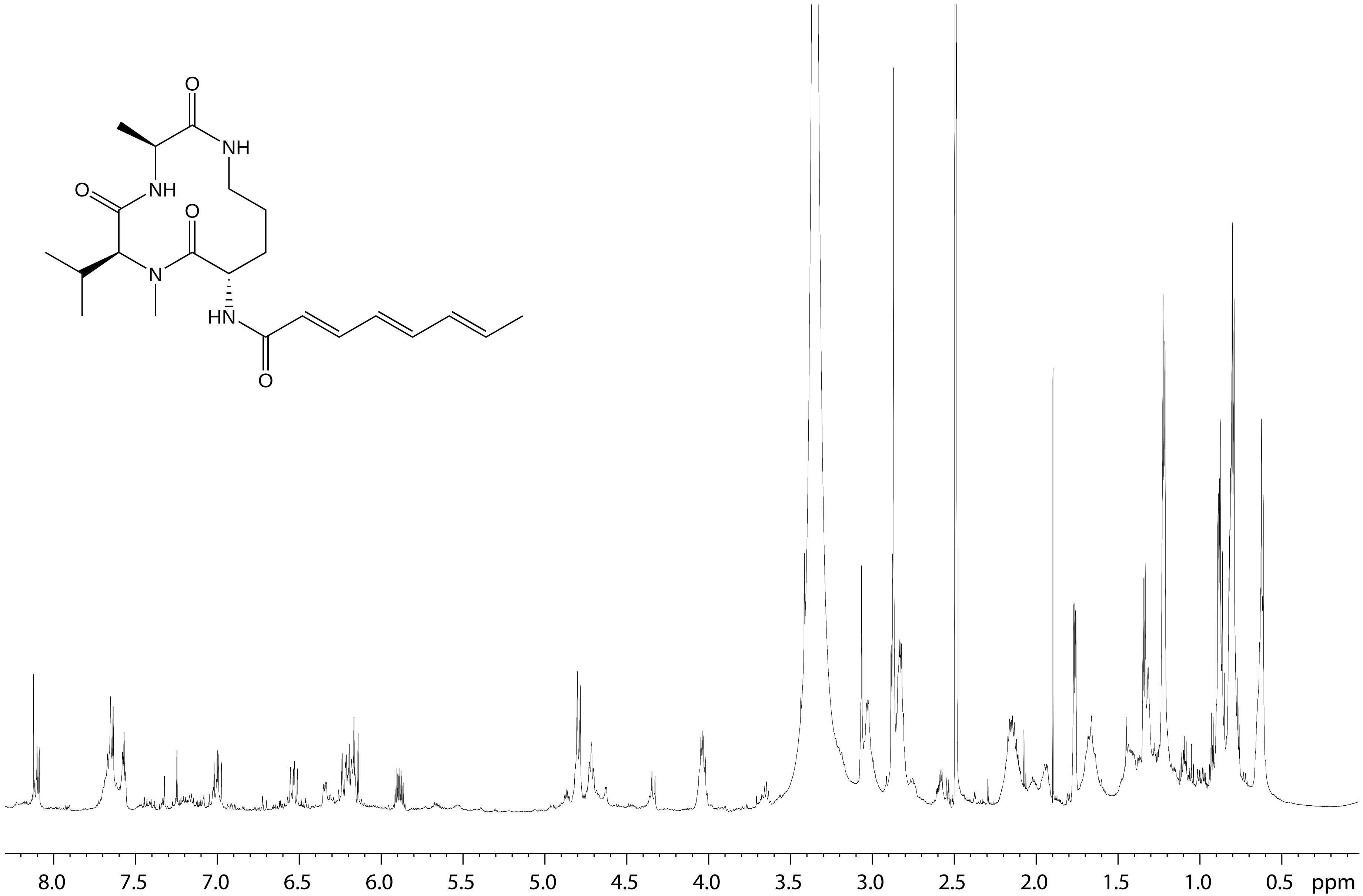


Figure S18. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of JBIR-15 (**9**)

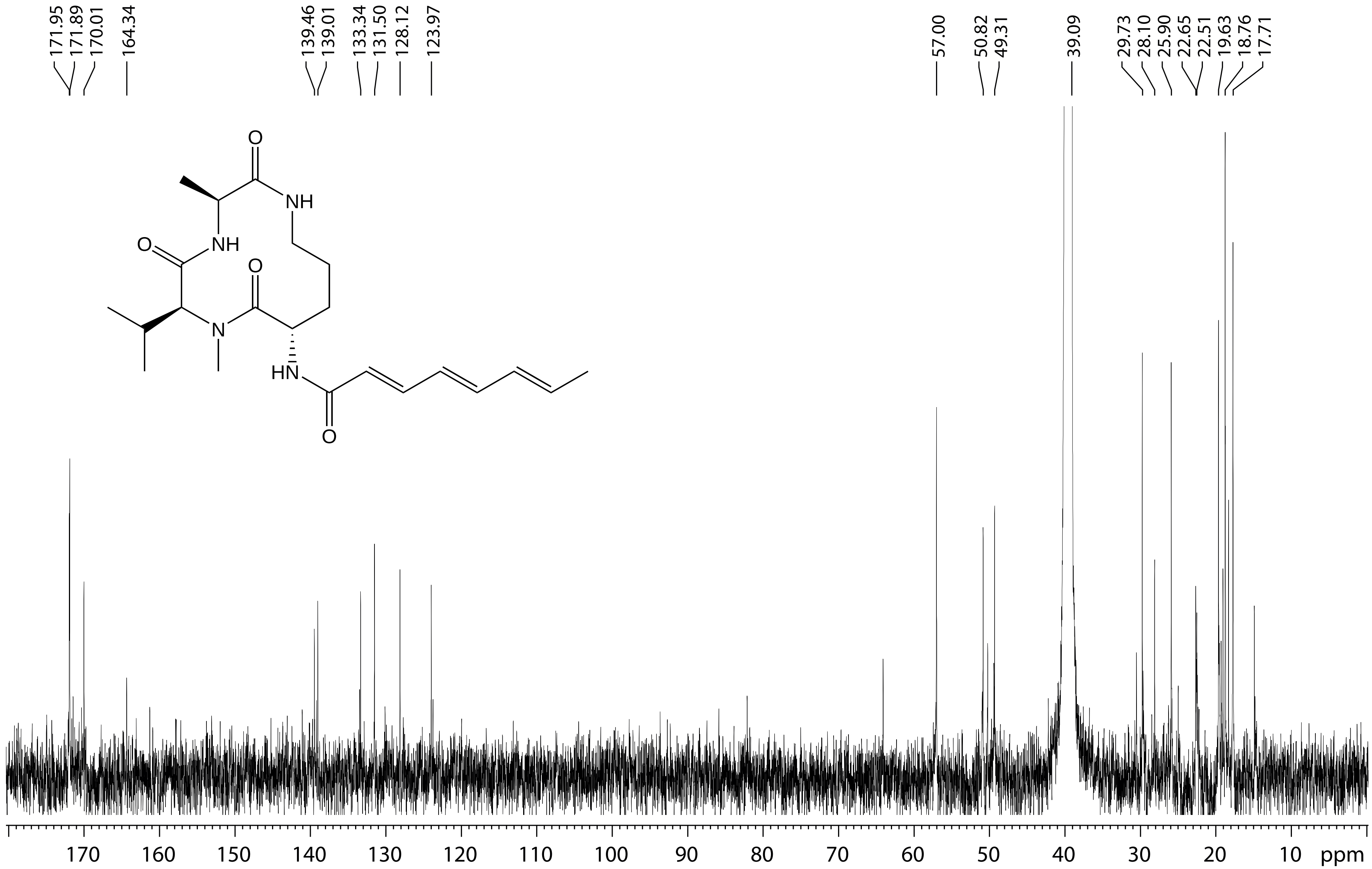


Figure S19. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of JBIR-15 (**9**)

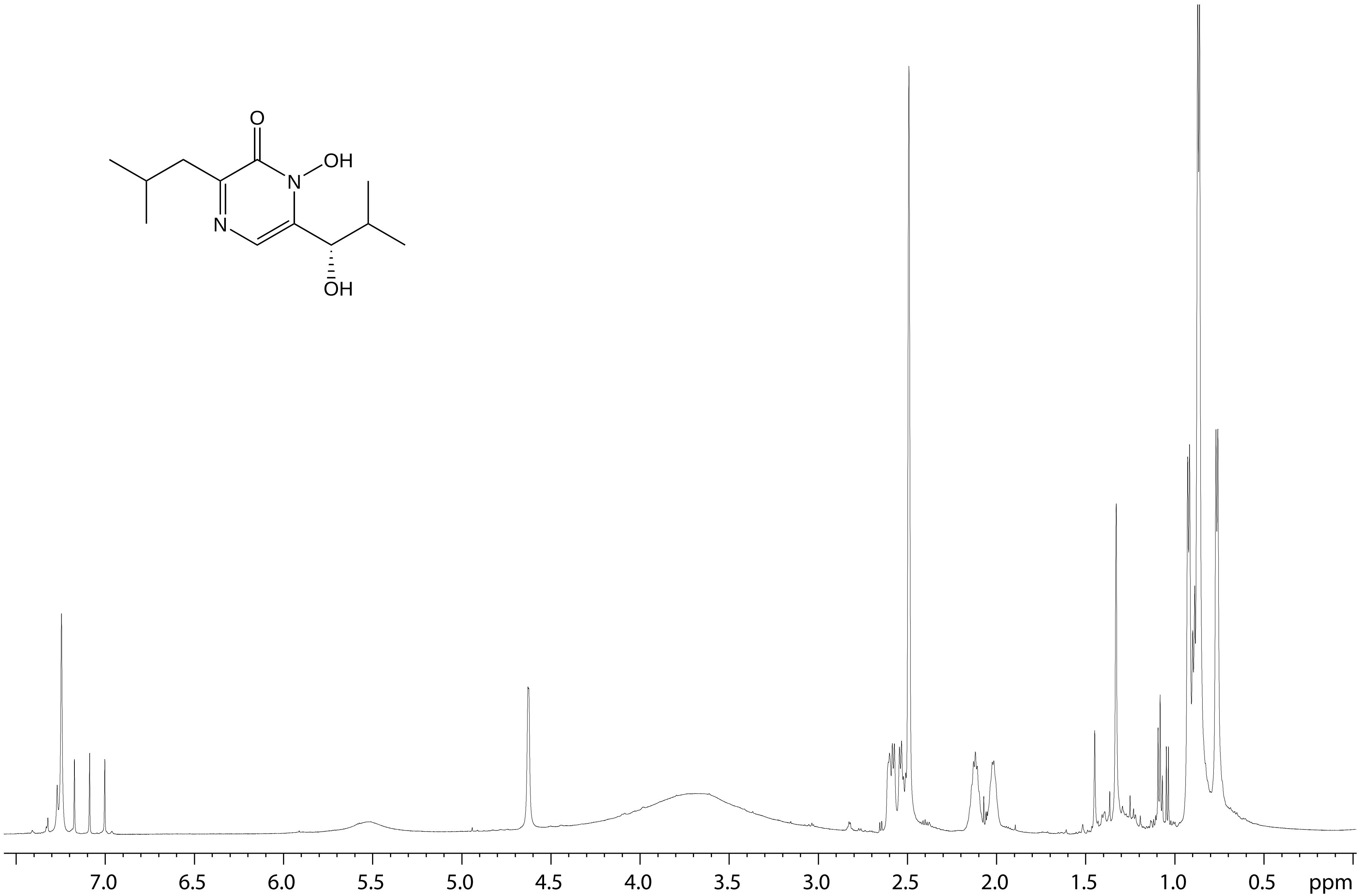


Figure S20. ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of neohydroxyaspergillic acid (**10**)

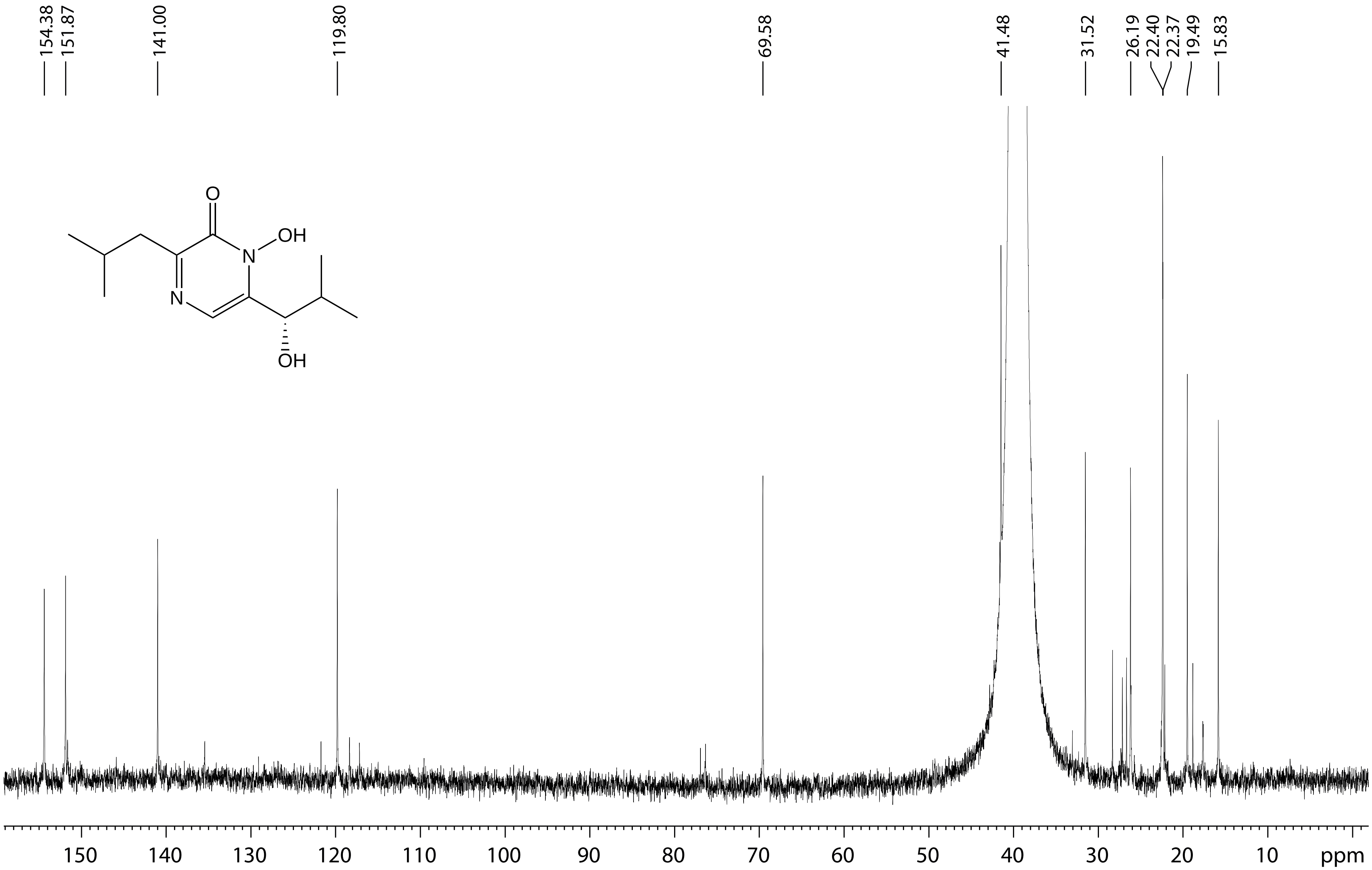


Figure S21. ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of neohydroxyaspergillic acid (**10**)

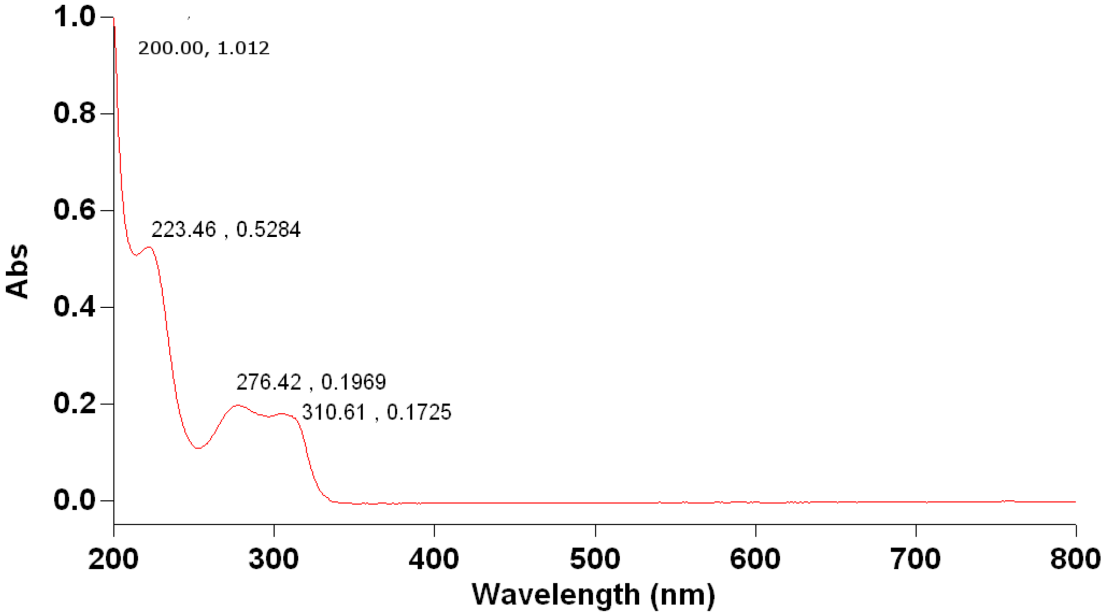


Figure S22. UV-vis spectrum of kumbicin A (**1**) in MeCN

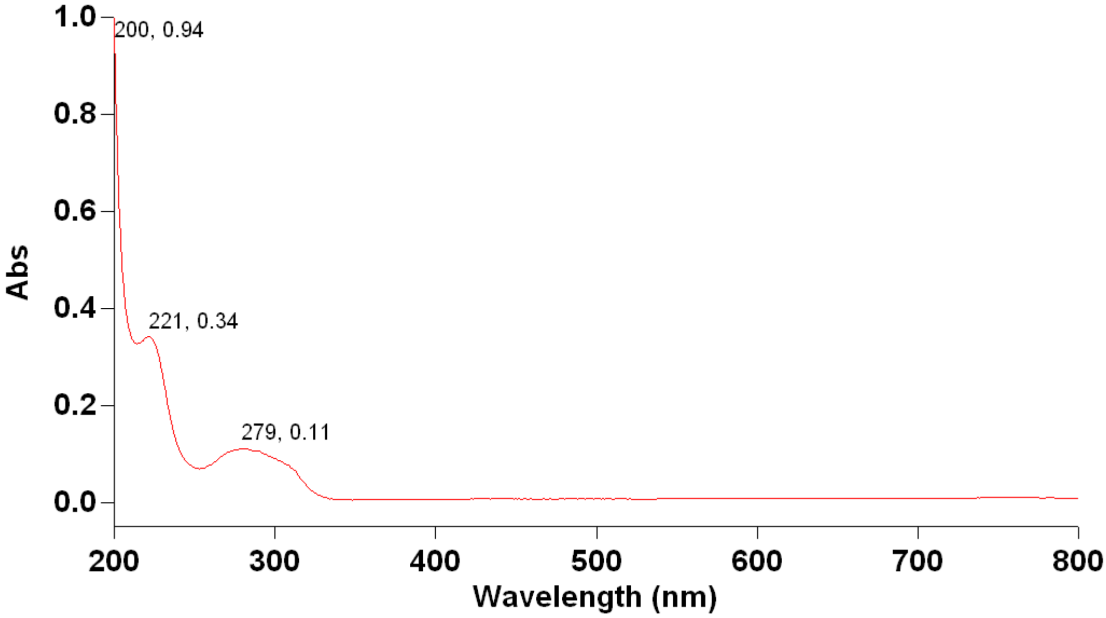


Figure S23. UV-vis spectrum of kumbicin B (**2**) in MeCN

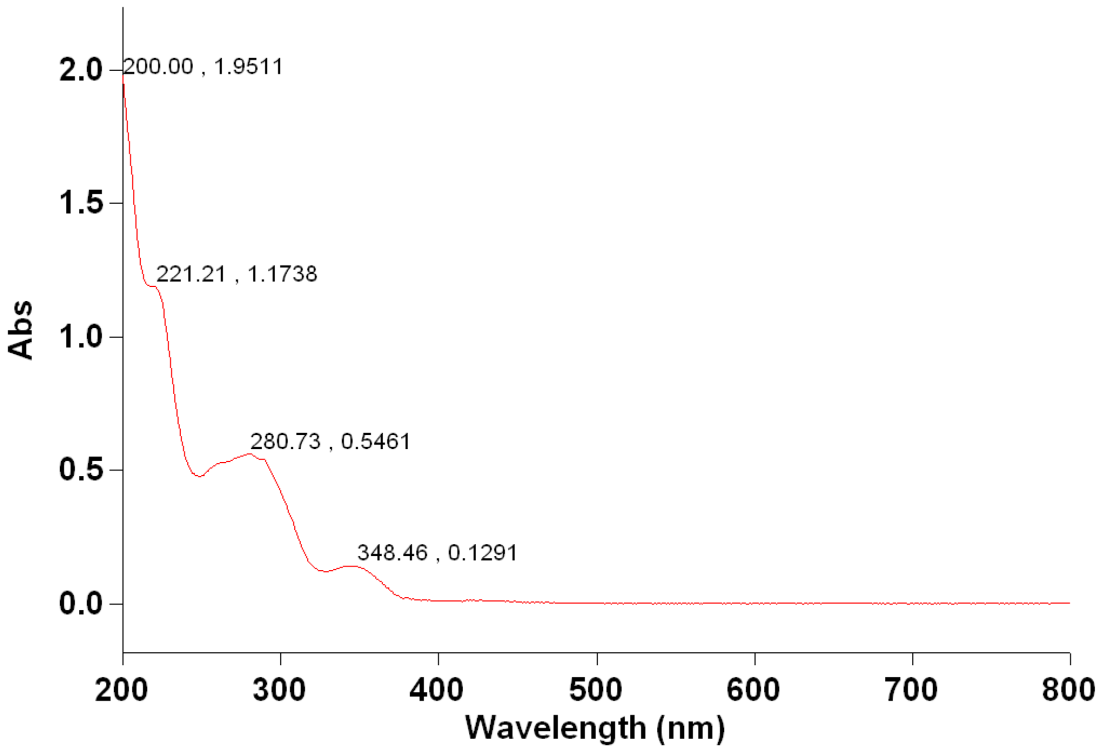


Figure S24. UV-vis spectrum of kumbicin C (**3**) in MeCN

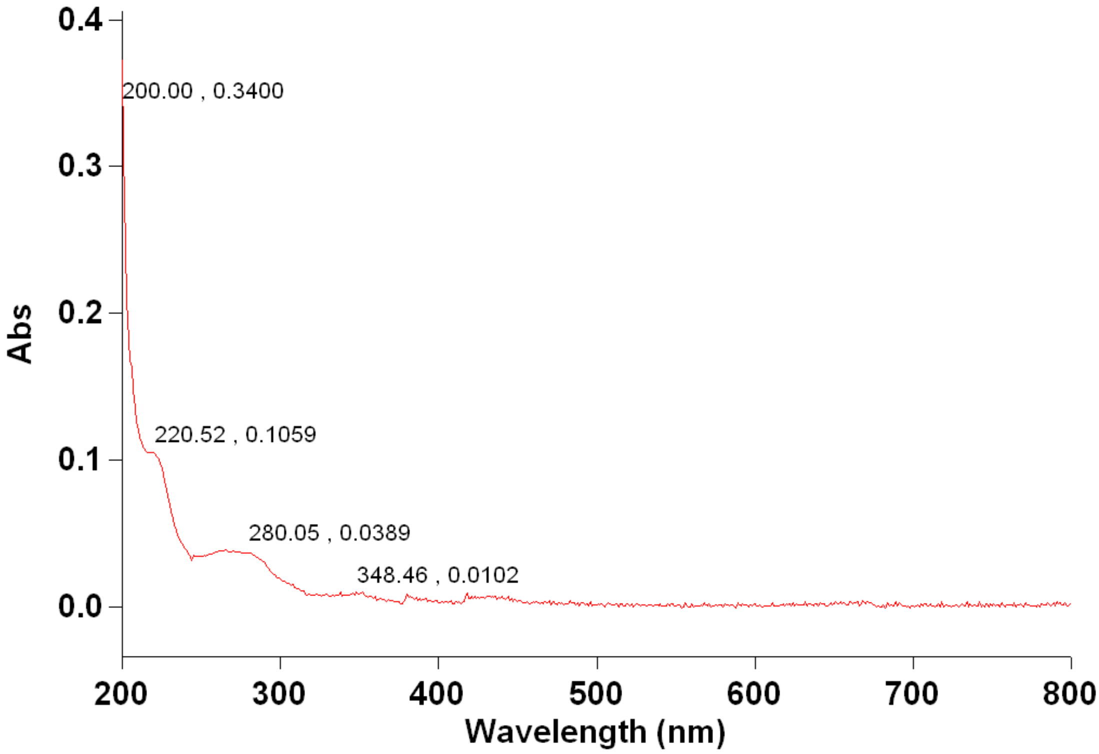


Figure S25. UV-vis spectrum of kumbicin D (**4**) in MeCN

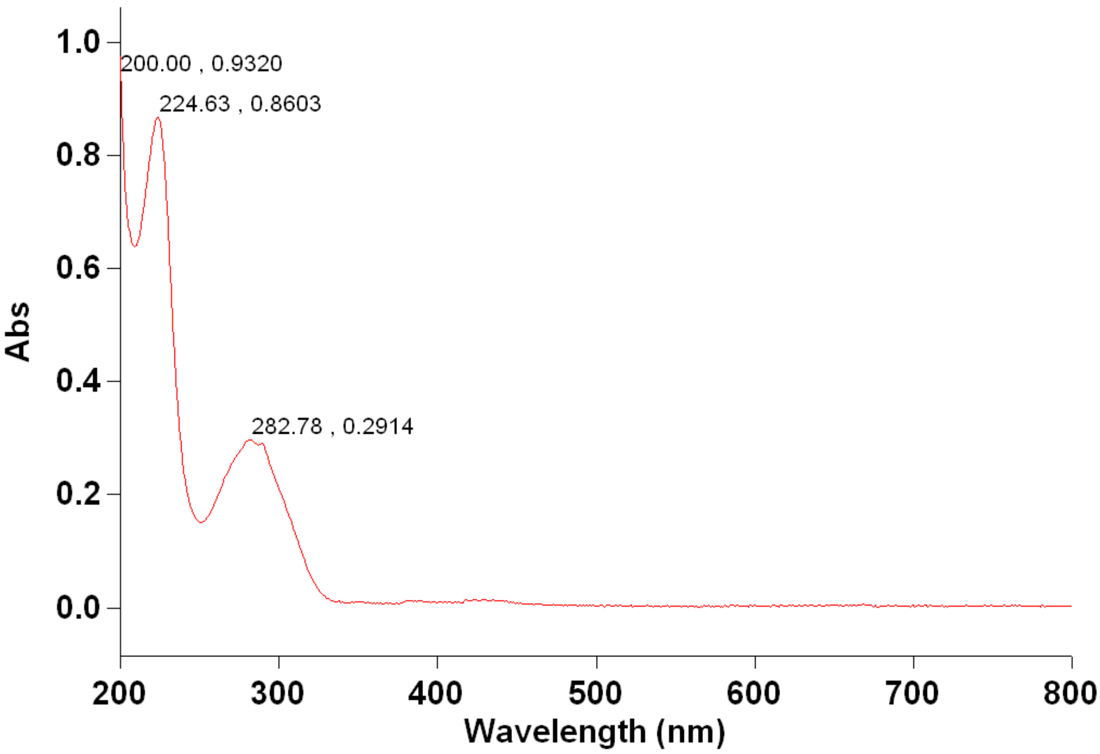


Figure S26. UV-vis spectrum of asterriquinol D dimethyl ether (**5**) in MeCN

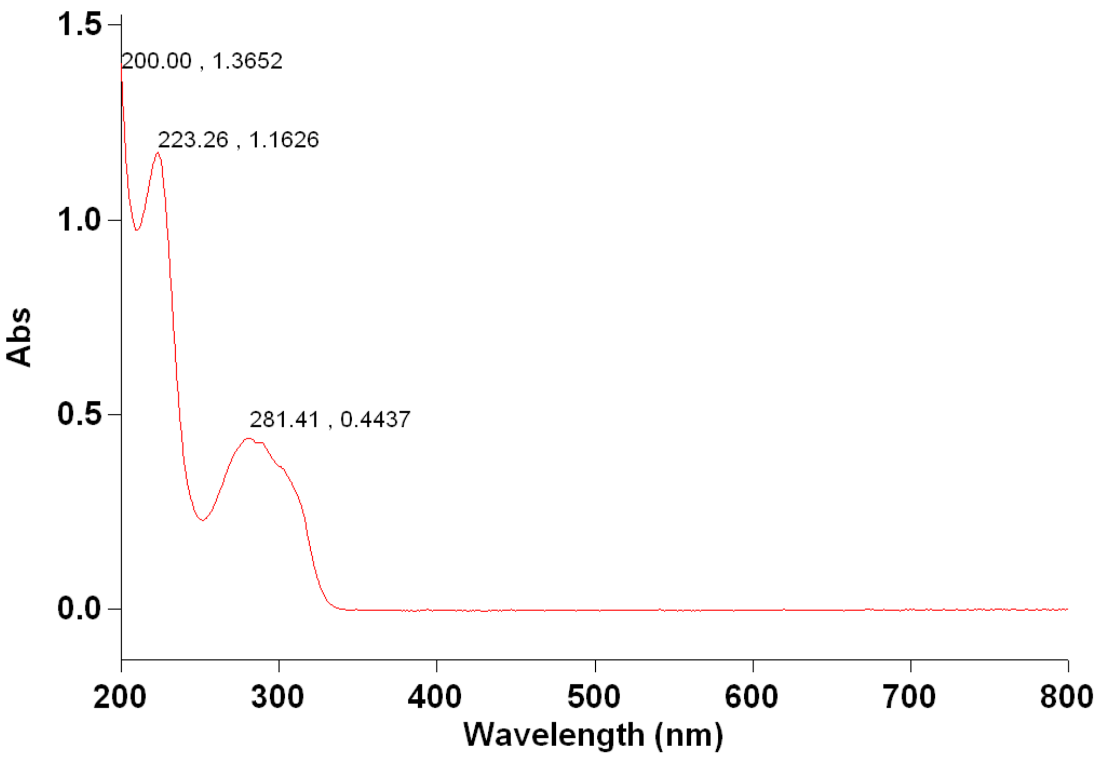


Figure S27. UV-vis spectrum of petromurin C (**6**) in MeCN

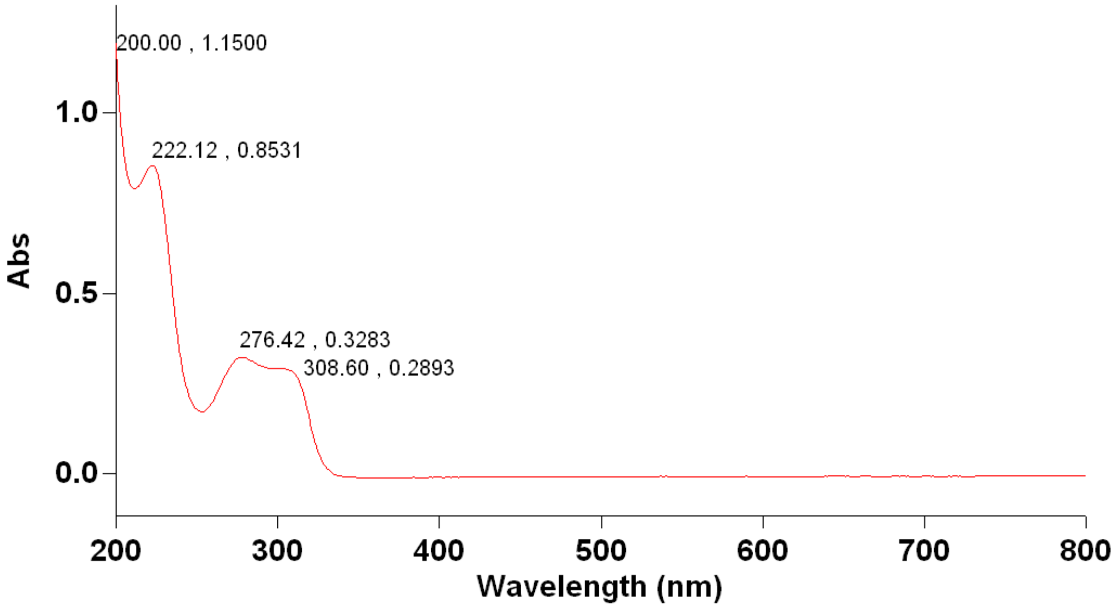


Figure S28. UV-vis spectrum of petromurin D (**7**) in MeCN

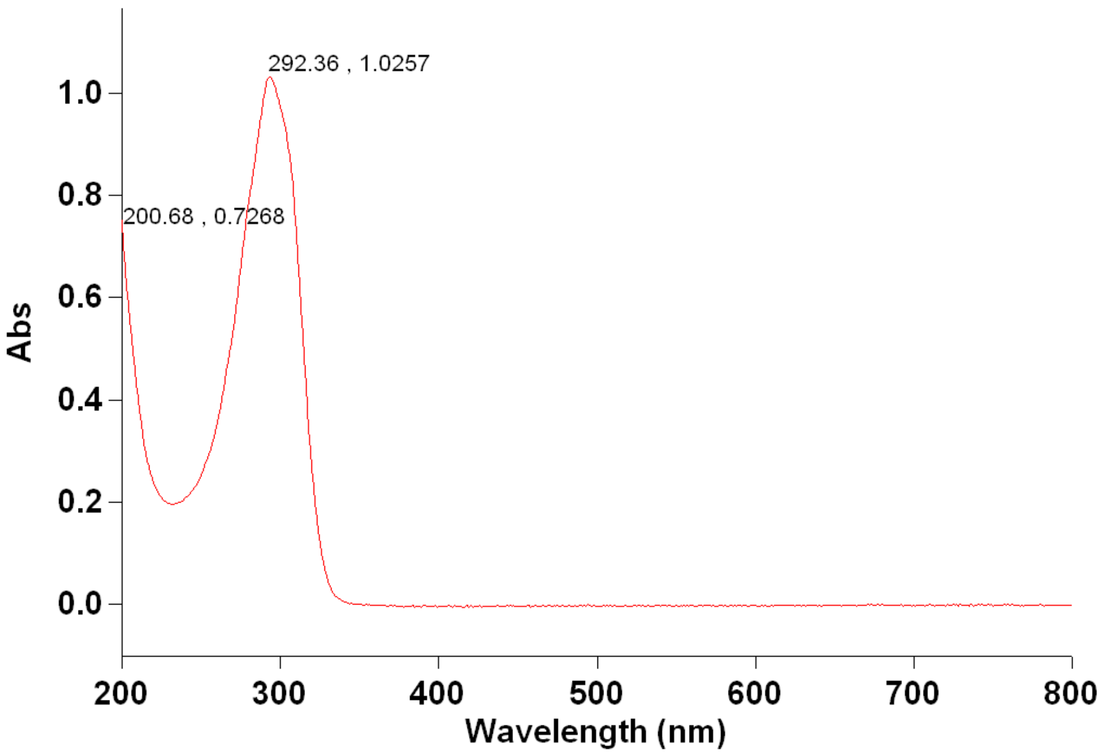


Figure S29. UV-vis spectrum of aspochracin (**8**) in MeCN

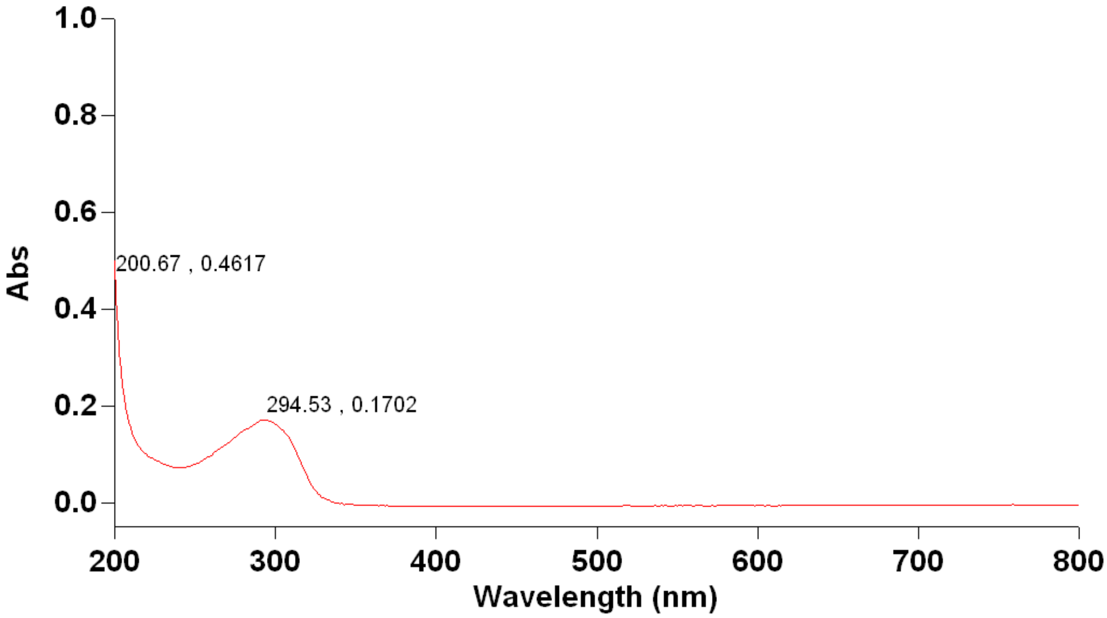


Figure S30. UV-vis spectrum of JBIR-15 (**9**) in MeCN

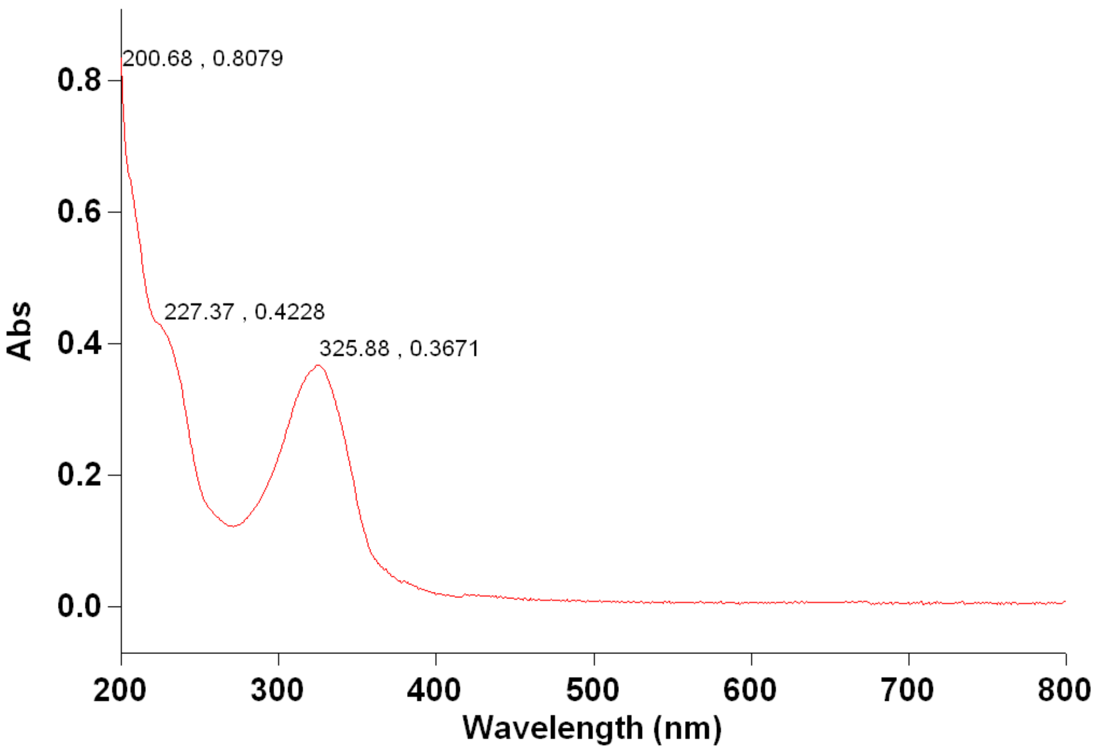


Figure S31. UV-vis spectrum of neohydroxyaspergillic acid (**10**) in MeCN

Table S11. Recipes for microbiological media

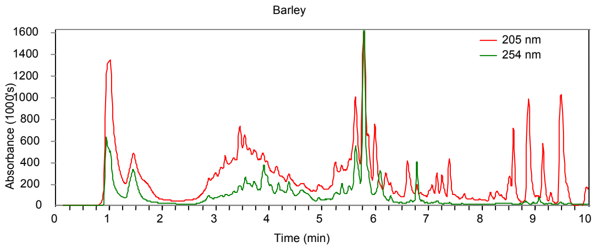
Glycerol Casein Agar (CGA)	
Ingredient	*Quantity*
Glycerol	30 g
Casein peptone (Amyl)	2 g
K_2HPO_4	1 g
NaCl	1 g
$MgSO_4.7H_2O$	0.5 g
Trace element solution*	5 mL
Deionised water	1000 mL
Bacteriological agar (Amyl)	20 g
Autoclave	
***Trace element solution**	
$CaCl_2.2H_2O$	3 g
$FeC_6O_7H_5$	1 g
$MnSO_4$	0.2 g
$ZnCl_2$	0.1 g
$CuSO4.5H_2O$	0.025 g
$Na_2B_4O_7.10H_2O$	0.02 g
$CoCl_2$	0.004 g
$Na_2MoO_4.2H_2O$	0.01 g
Deionised water	1000 mL
Filter sterilize	

Czapeks Agar (CZA)	
Ingredient	*Quantity*
Czapeks Dox Media (Oxoid)	99.88 g
Deionised water	2200 mL

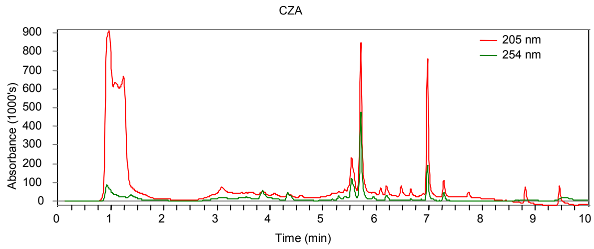
Malt Extract Agar (MEA)	
Ingredient	*Quantity*
Bacteriological peptone (Difco)	3 g
Malt Extract (Amyl)	60 g
Bacteriological glucose (Amyl)	60 g
Distilled water	1000 mL
Adjust pH to 5.5	
Bacteriological agar (Amyl)	20 g
Autoclave	

Yeast Extract Sucrose Agar (YES)	
Ingredient	*Quantity*
Yeast Extract (Difco) (g)	20 g
Sucrose (Amyl) (g)	150 g
Bacteriological Agar (Amyl) (g)	20 g
Deionised water (mL)	1000 mL
Autoclave	

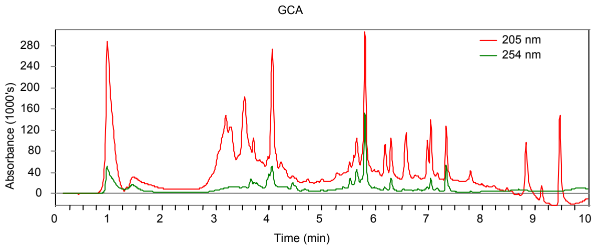
Medium 1: Barley



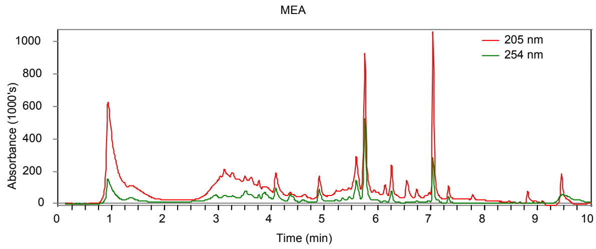
Medium 2: Czapek agar



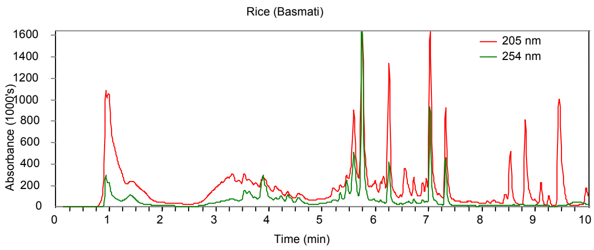
Medium 3: Glycerol Caseine agar



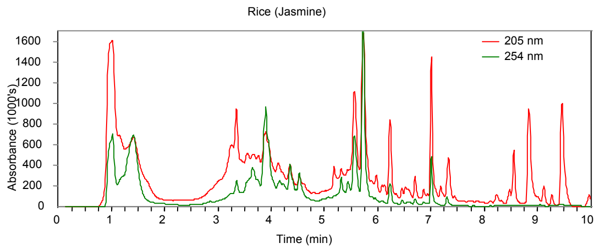
Medium 4: Malt extract agar



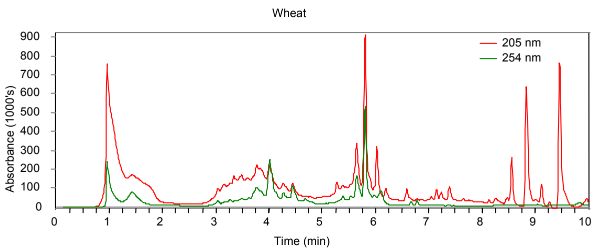
Medium 5: Rice (Basmati)



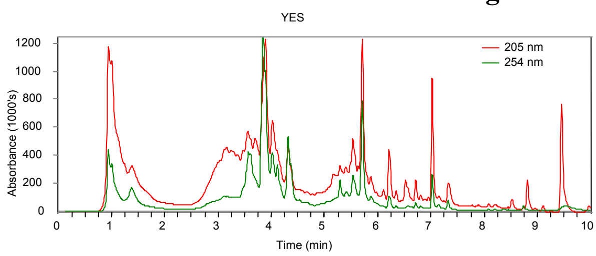
Medium 6: Rice (Jasmine)



Medium 7: Wheat



Medium 8: Yeast extract Sucrose agar



Overlay of the media used for optimisation

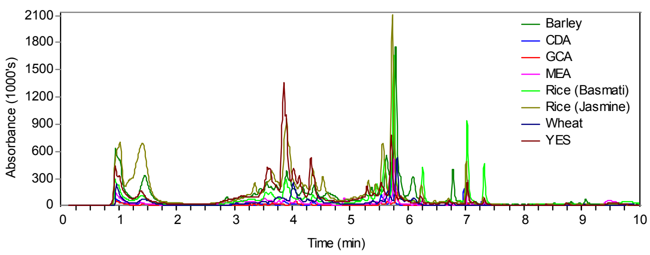


Figure S32. HPLC traces of crude extracts of *Aspergillus kumbius* cultivated on 8 different media

Bioassays

Metabolites were dissolved in DMSO to provide 10,000 μg/mL stock solutions. An aliquot of each stock solution was transferred to the first lane of Rows B to G in a 96-well microtitre plate and two-fold serially diluted across the 12 lanes of the plate to provide a 2,048-fold concentration gradient. Bioassay medium was added to an aliquot of each test solution to provide a 100-fold dilution into the final bioassay, thus yielding a test range of 100 to 0.05 μg/mL. Row A was used as the positive control (no inhibition) and Row H was used as the negative control (complete inhibition).

CyTOX is an indicative bioassay platform for discovery of antitumour actives. NS-1 (ATCC TIB-18) mouse myeloma cells were inoculated in 96-well microtitre plates (190 μL) at 50,000 cells/mL in DMEM (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum (FBS) + 1% penicillin/streptomycin (Life Technologies)) and incubated in 37 °C (5% CO_2) incubator. At 48 h, resazurin (120 μg/mL; 10 μL) was added to each well and the plates were incubated for a further 48 h. Finally, the absorbance of each well at 605 nm was measured using a Spectromax plate reader (Molecular Devices).

NemaTOX is a larval development assay, applicable to all parasitic nematodes with free-living life cycle stages and serves as an *in vitro* bioassay for anthelmintic discovery (Gill & Lacey, 1993; Gill et al.,1995). Nematodes eggs (50 eggs per well), recovered from infected sheep, were applied to the wells of a 96-well microtitre plate, which contained the test compound dispersed in an agar (1.5%, Difco). At 24 h, the hatched larvae were inoculated with *Escherichia coli* and sterile Yeast extract (1%). At 120 h, the numbers of eggs, $L_{1/2}$ larvae and mature L_3 larvae were counted, the % inhibition in larval development calculated, graphed and the LD_{50} determined.

ProTOX is a generic bioassay platform for antibiotic discovery. In the present study *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) were used as indicative species for Gram positive and negative antibacterial activity, respectively. A bacterial suspension (50 mL in 250 mL flask) was prepared in nutrient media by cultivation for 24 h at 250 rpm, 28 °C. The suspension was diluted to an absorbance of 0.01 absorbance units per mL, and 10 μL aliquots were added to the wells of a 96-well microtitre plate, which contained the test compounds dispersed in nutrient agar (Amyl) with resazurin (120 μg/mL). The plates were incubated at 28 °C for 48 h during which time the positive control wells change colour from a blue to light pink colour. MIC end points were determined visually. The absorbance measured using Spectromax plate reader (Molecular Devices) at 605 nm and the IC_{50} values determined graphically.

EuTOX is a generic bioassay platform for antifungal discovery. In the present study, the yeasts *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763) were used as indicative species for antifungal activity. A yeast suspension (50 mL in 250 mL flask) was prepared in 1% malt extract broth by cultivation for 24 h at 250 rpm, 24 °C. The suspension was diluted to an absorbance of 0.005 and 0.03 absorbance units per mL for *C. albicans* and S. *cerevisiae*, respectively. Aliquots (20 μL and 30 μL) *of C. albicans* and S. *cerevisiae,* respectively were applied to the wells of a 96-well microtitre plate, which contained the test compounds dispersed in malt extract agar containing resazurin (120 μg/mL). The plates were incubated at 24 °C for 48 h during which time the positive control wells change colour from a blue to yellow colour. MIC end points were determined visually. The absorbance measured using Spectromax plate reader (Molecular Devices) at 605 nm and the IC_{50} determined graphically.

TriTOX is a bioassay focused on the discovery of inhibitors of the animal protozoan pathogen, *Tritrichomonas foetus*. In the present bioassay *T. foetus* (strain KV-1) were inoculated in 96-well microtitre plates (200 μL) at 4×10^4 cells/mL in *T. foetus* medium (0.2% tryptone, Oxoid; 0.1% yeast extract, Difco; 0.25% glucose; 0.1% L-cysteine; 0.1% K_2HPO_4; 0.1% KH_2PO_4 ; 0.1% ascorbic acid; 0.01% $FeSO_4.7H_2O$; 1% penicillin/streptomycin, 10% new born calf serum (NBCS), Life Technologies). The plates were incubated in anaerobic jars (Oxoid AG25) containing Anaerogen satchel (Oxoid AN25) in 37 °C (5% CO_2) incubator. At 72 h, *T. foetus* proliferation was counted and % Inhibition graphed to determine the IC_{50} values.

References:
J. H. Gill, E. Lacey, *Int. J. Parasitol*. **1993**, *23*, 375.
J. H. Gill, J. M. Redwin, J. A. Van Wyk, E. Lacey, *Int. J. Parasitol*. **1995**, *25*, 463.

Table S12. Bioassay profile of *A.kumbius* crude extracts

Medium	ProTOX	EuTOX	CyTOX	TriTOX	NemaTOX
CGA	0	0	0	0	0
CZA	0	0	0	0	0
YES	0	4	4	0	0
MEA	0	0	0	0	0
Barley	0	8	8	2	0
Wheat	0	8	8	2	0
Rice (Jasmine)	0	0	8	4	0
Rice (Basmati)	0	0	4	0	0

The data are presented as bioassay titres, representing the highest dilution at which biological could be detected in the bioassay

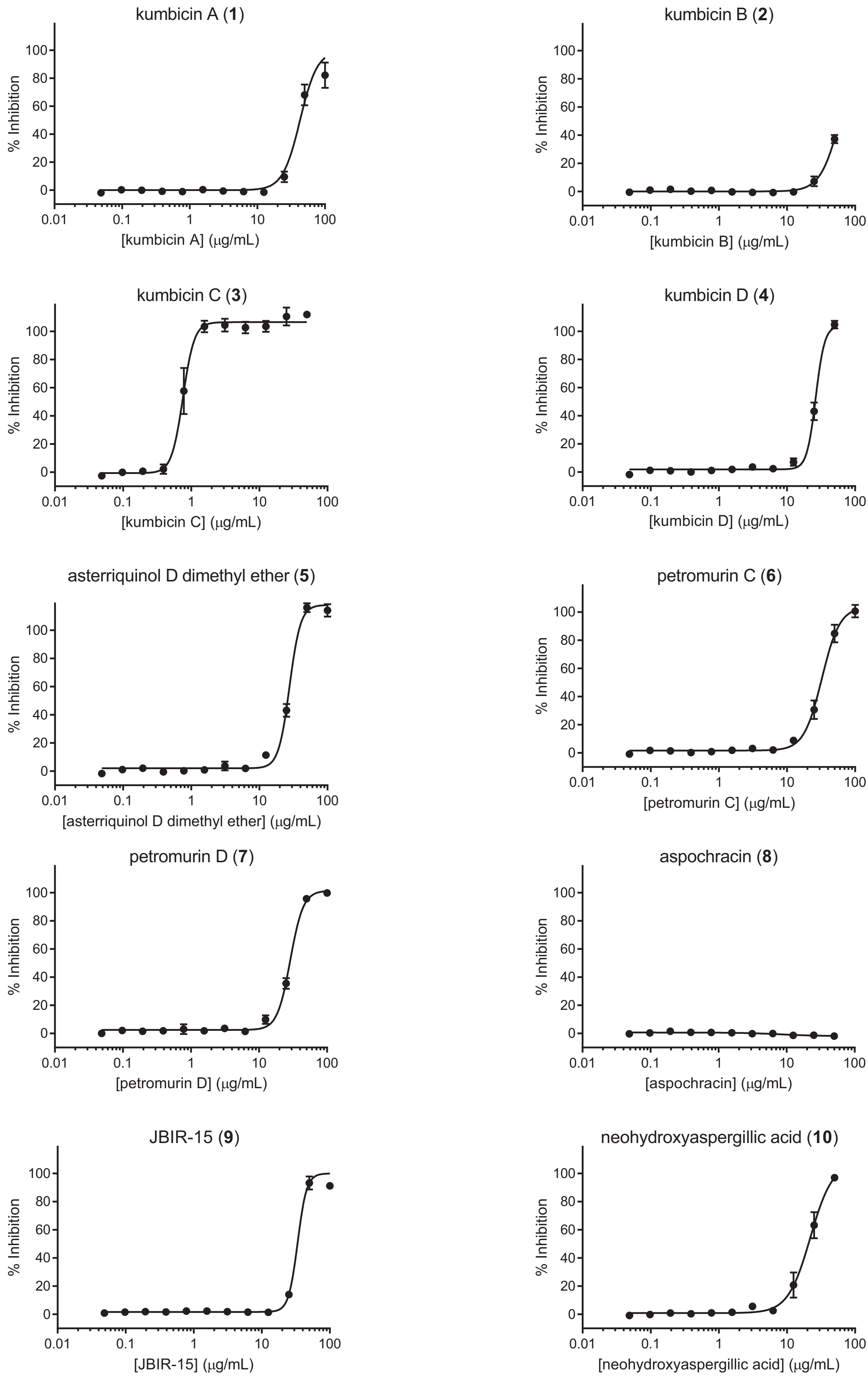
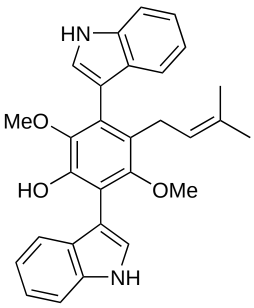
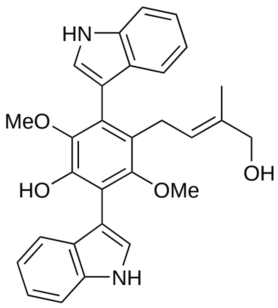


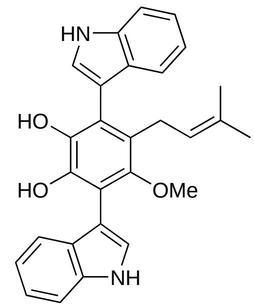
Figure S33. *In vitro* cytotoxicity of **1–10** against the mouse myeloma cell line NS-1 (ATCC TIB-18)



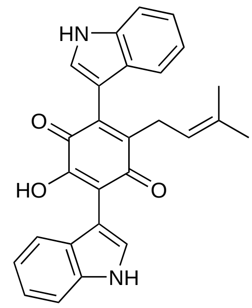
ochrindole A



ochrindole B



ochrindole C



ochrindole D

Figure S34. Structures of the previously reported compounds ochrindoles A–D