สารออกฤทธิ์ทางชีวภาพจากเชื้อราทะเลสายพันธุ์ *Nodulisporium* sp., *Aspergillus unguis* **และ** *Penicillium citrinum*

นายสัญญา สุเรวัมย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

BIOACTIVE COMPOUNDS FROM MARINE FUNGI STRAINS *Nodulisporium* sp., *Aspergillus unguis* AND *Penicillium citrinum*

Mr. Sanya Sureram

A Thesis Submitted in Partial Fulfillment of the Requirements

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Department of Chemistry

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Thesis Title	BIOACTIVE COMPOUNDS FROM MARINE FUNGI STRAINS *Nodulisporium* sp., *Aspergillus unguis* AND *Penicillium citrinum*
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Field of Study	Chemistry
Thesis Advisor	Associate Professor Nattaya Ngamrojnavanich, Ph.D.
Thesis Co-advisor	Prasat Kittakoop, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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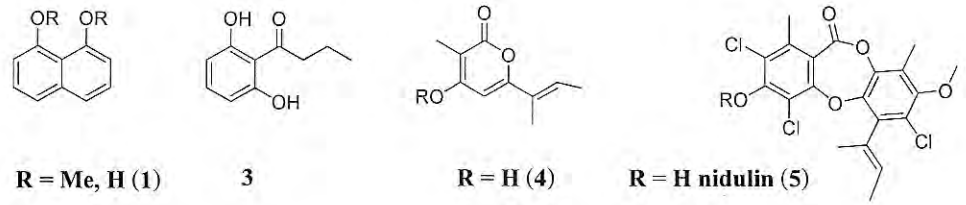
สัญญา สุเรรัมย์ : สารออกฤทธิ์ชีวภาพจากเชื้อราทะเลสายพันธุ์ *Nodulisporium* sp., *Aspergillus unguis* และ *Penicillium citrinum* (BIOACTIVE COMPOUNDS FROM MARINE FUNGI STRAINS *Nodulisporium* sp., *Aspergillus unguis*, AND *Penicillium citrinum*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. นาตยา งามโรจนวณิชย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. ประสาท กิตตะคุปต์, 144 หน้า.

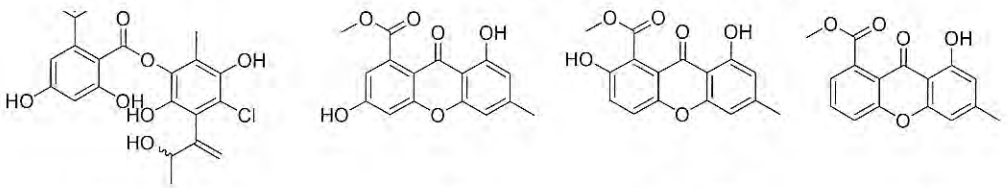
การศึกษาเชื้อราทะเลสายพันธุ์ *Nodulisporium* sp., *Aspergillus unguis*, และ *Penicillium citrinum*, พบว่าเชื้อรา *Nodulisporium* sp. ผลิตสาร **1**, **2** และ **3** ในขณะที่ สาร **4**, niludin (**5**) และ สาร **6** ผลิตโดยเชื้อรา *Aspergillus unguis* นอกจากนี้ได้ทำการสังเคราะห์สารอนุพันธุ์ (**7** และ **8**) และเชื้อรา *Penicillium citrinum* ที่ผลิตสาร **9**, pinselin (**10**) และ **11** โดยโครงสร้างของสารทั้งหมด ทำการพิสูจน์ด้วยเทคนิคทางสเปคโตรโคปี

จากการศึกษาพบว่าบางสาร มีฤทธิ์เป็นพิษต่อเซลล์มะเร็งท่อน้ำดีในตับ, มะเร็งปอด, มะเร็งตับและมะเร็งเม็ดเลือดขาว



R = Me, H (1) **3** **R = H (4)** **R = H nidulin (5)**

R = Me (2) **R = Me (7)** **R = Me (8)**



6 **9** pinselin (**10**) **11**

ภาควิชา เคมี ลายมือชื่อ นิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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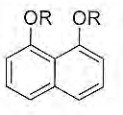
KEY WORDS : M arine fungi / *Nodulisporium* s p./ *Aspergillus u nguis* / *Penicillium citrinum* / Cytotoxicity

SANYA SURERAM : BIOACTIVE COMPOUNDS FROM MARINE FUNGI STRAINS *Nodulisporium* sp., *Aspergillus* sp. AND *Penicillium citrinum* (สารออกฤทธิ์ชีวภาพจากเชื้อราทะเลสายพันธุ์ *Nodulisporium* sp., *Aspergillus unguis* และ *Penicillium citrinum*) THESIS ADVISOR : [ASSOC. PROF. NATTAYA NGAMROJNAVANICH, Ph.D.], THESIS COADVISOR : [PRASAT KITTAKOOP, PH.D.], 144 pp.

Bioactive compounds of the marine-derived fungi *Nodulisporium* sp., *Aspergillus unguis* and *Penicillium citrinum* were explored. The spong-derived fungus *Nodulisporium* sp. produced know natural products **1**, **2** and **3**, while *Aspergillus unguis* produced compound **4**, niludin (**5**) and **6**. Furthermore, *O*-methyl derivatives **7** and **8** were prepared. The marine-derived fungus, *Penicillium citrinum* produced **9**, pinselin (**10**) and compound **11**. All structures were elucidated by extensive spectroscopic analysis.

In this study, some compounds exhibited cytotoxic activity against HuCCA-1, A549, HepG-2 and MOLT-3 cancer cell lines.



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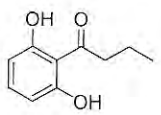

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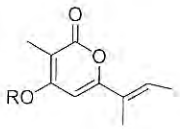
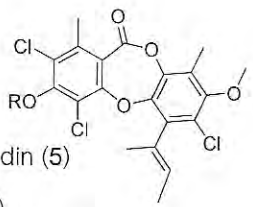


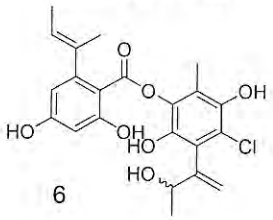
R = H (**4**)

R = Me (**7**)



R = H niludin (**5**)

R = Me (**8**)



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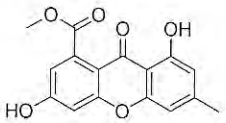

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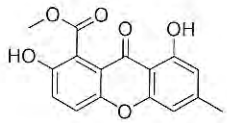
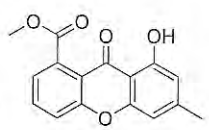


pinselin (**10**)



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Department : Chemistry

Field of Study : Chemistry

Academic Year : 2009

Student's Signature : Sanya Sureram

Advisor's Signature : N. Ngamrojnavanich

Co-advisor's Signature : .....

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Finally, I am deeply grateful to my parents for their, love, care and understanding through out my study.

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LIST OF ABBREVIATIONS

APCI-TOF	atmospheric pressure chemical ionisation-time of flight
actone-d_6	deuterated acetone
br	broad
°C	degree Celsius
$CDCl_3$	deuterated chloroform
CH_2Cl_2	methylene chloride
COSY	correlation spectroscopy
calcd.	calculated
cm^{-1}	wave number unit
DEPT	distortionless enhancement by polarization transfer
DMF	N,N-Dimethylformamide
DMSO-d_6	deuterated dimethylsulphoxide
d	doublet
dq	doublet of quartets
EtOAc	ethyl acetate
g	gram
H_2O	water
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectroscopy
Hz	Hertz
h	hour
IC_{50}	50% inhibitory concentration
IR	infra-red radiation
J	coupling constant
K_2CO_3	Potassium carbonate
L	liter
M	molar (mole/liter)

MHz	megahertz
MS	mass spectroscopy
Me	methyl group
MeI	methyl iodide
MeOH	methanol
m/z	a value of mass divided by charge
mL	milliliter
mg	milligram
m.p.	melting point
mult	multiplicity
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
nm	nanometer
ppm	part per million
ppt	part per thousand
q	quartet
s	singlet
sp.	species
t	triplet
UV	ultraviolet radiation
µL	microliter
µM	micromolar
µg	microgram
ν_{max}	maximum wave number
λ_{max}	maximum absorption wavelength
δ	chemical shift (ppm)
ε	the reciprocating wavelength
$[\alpha]_D$	specific rotation

CHAPTER I

INTRODUCTION

Microorganisms have long been recognized as sources of pharmacologically active metabolites. Fungi have become an important source of natural products for the discovery of new drug candidates. Marine-derived fungi especially are one of most significant resources for structurally diverse natural products. More specifically, fungi isolated from various organisms in the marine environments such sponge, alga, grass plant, tunicate, sediment, coral, wood, fish, mollusc, and other unknown sources[1], have been investigated for their bioactive secondary metabolite contents.

In the present study, marine-derived fungi, *Aspergillus unguis*, (isolated from an unidentified marine spong CRI282), *Nodulisporium* sp., (isolated from an unidentified marine spong CRI352), and *Penicillium citrinum*, (isolated from an unidentified marine spong CRI355) were selected for chemical investigation, because crude extracts of these marine-derived fungi showed interesting chemical profiles (by ^{1}H NMR spectrum) and exhibited antimalarial activity and cytotoxicity against HuCCA-1, A549, HepG-2, and MOLT-3 cell lines as shown **Tables 1.1**.

Figure 1 An unidentified marine spong CRI282

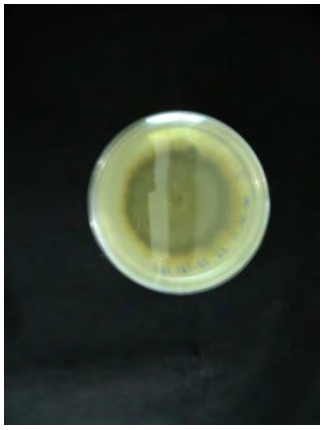


Figure 2 *Aspergillus unguis* CRI282-03

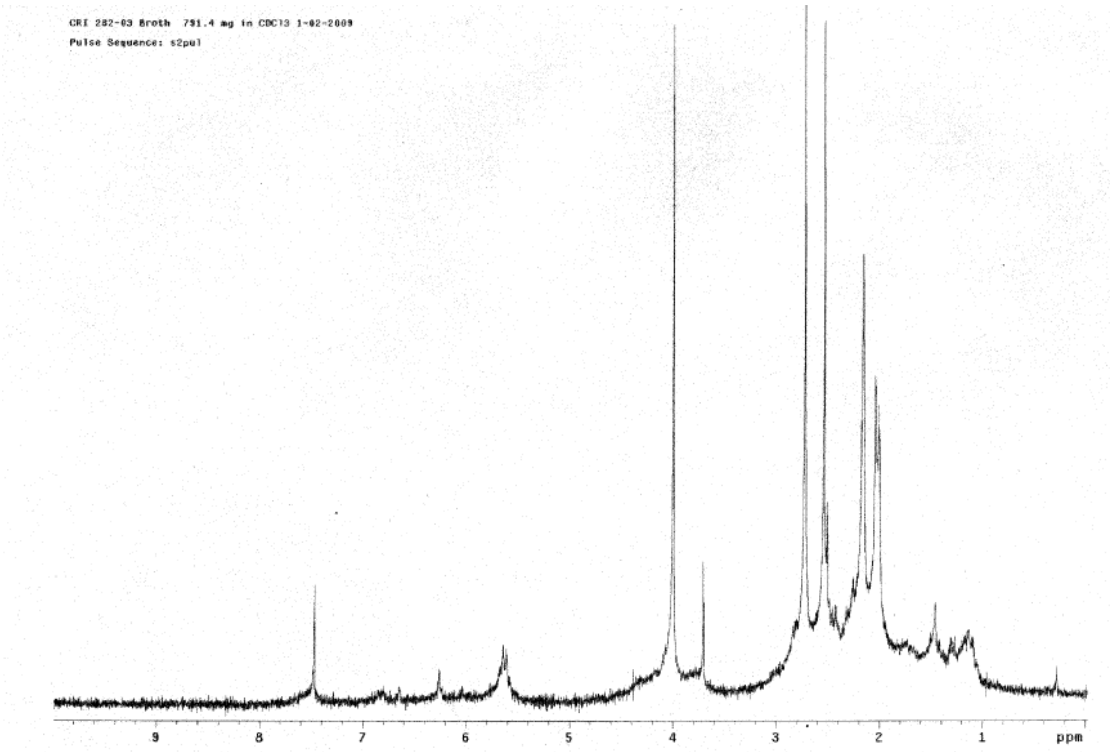


Figure 3 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude broth extract of the fungus *Aspergillus unguis* CRI282-03

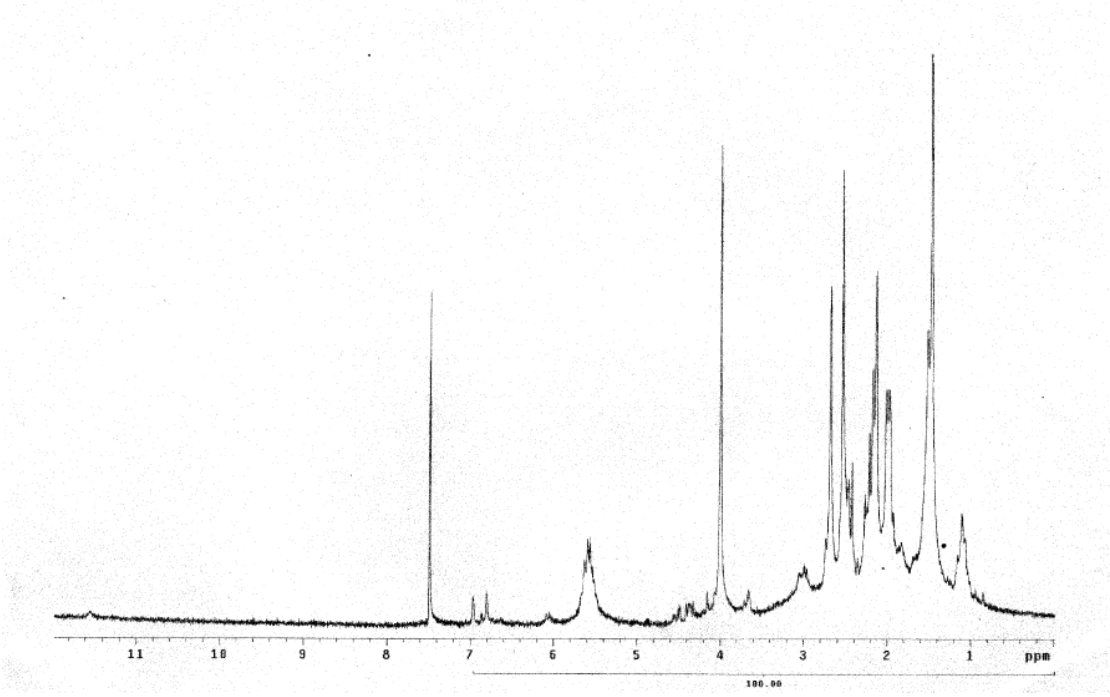


Figure 4 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude cell extract of the fungus *Aspergillus unguis* CRI282-03

Figure 5 An unidentified marine spong CRI352

Figure 6 *Nodulisporium* sp. CRI352-01

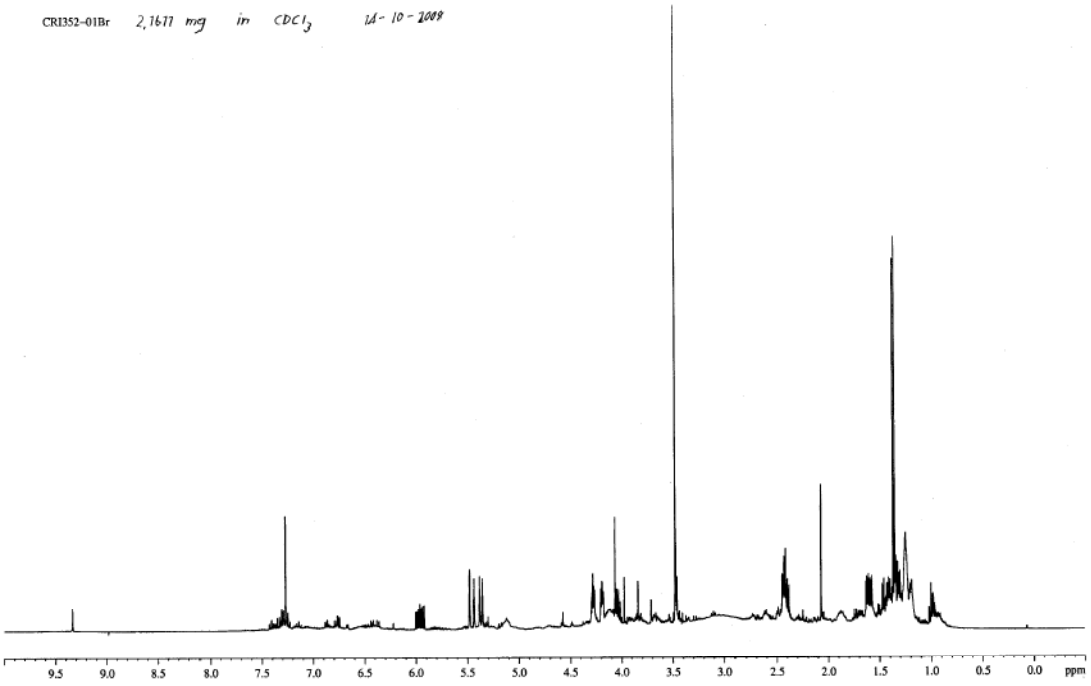


Figure 7 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude broth extract of the fungus *Nodulisporium* sp. CRI352-01

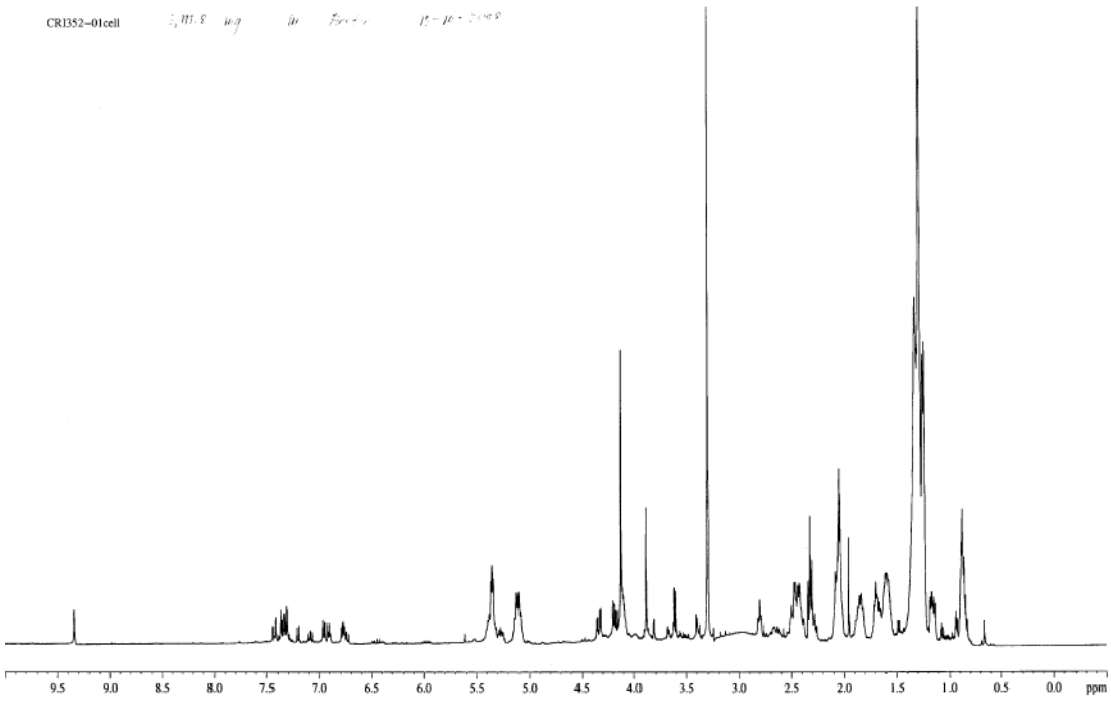


Figure 8 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude cell extract of the fungus *Nodulisporium* sp. CRI352-01

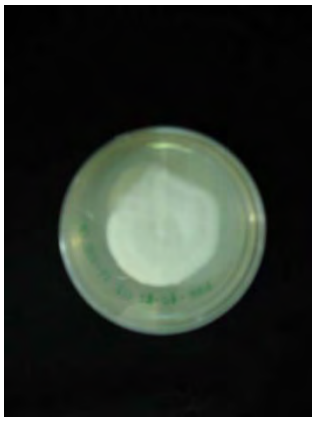


Figure 9 An unidentified marine spong CRI355 **Figure10** *Penicillium citrinum* CRI355-01

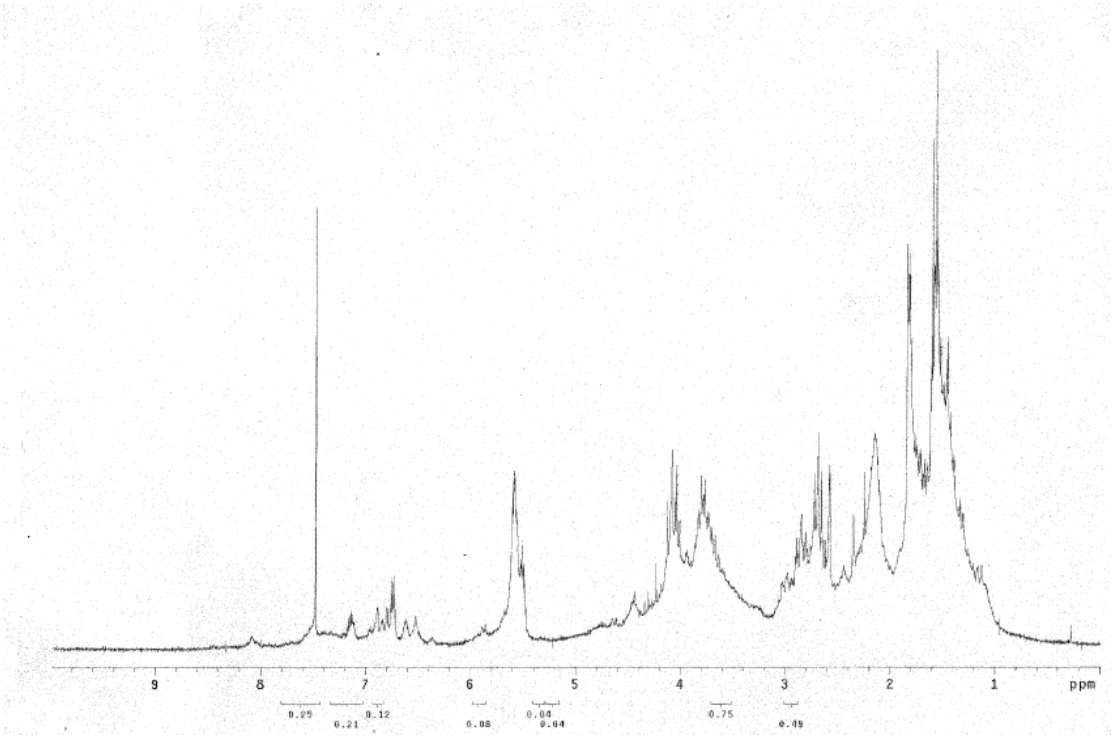


Figure 11 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude broth extract of the fungus *Penicillium citrinum* CRI355-01

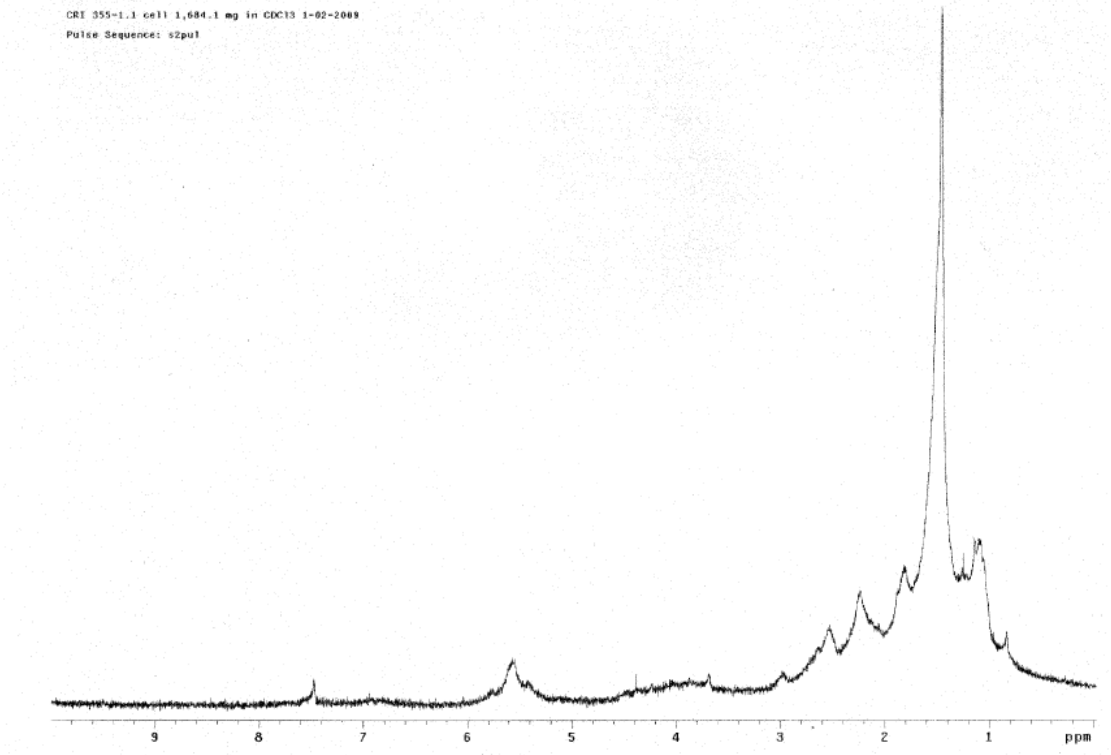


Figure 12 The 200 MHz ^{1}H NMR ($CDCl_3$) spectrum of crude broth extract of the fungus *Penicillium citrinum* CRI355-01

Table 1.1 Antimalarial and cytotoxic activities of crude extracts, *Aspergillus unguis*, *Nodulisporium* sp. and *Penicillium citrinum*.

Fungal strain		Antimalaria activity		Anti-cancer activity				
		Dose µg/mL	% inhibition	Dose µg/mL	Cytotoxicity activity (% inhibition) µg/mL			
					HuCCA-1	A549	HepG-2	MOLT-3
Aspergillus unguis	Broth	1000	Cell lyse	30	18	15	22.8	74.5
		100	69.70	10	15	0	30.06	32.5
	Mycelia	1000	Cell lyse	30	23	28	91.5	30.06
		100	81.81	10	2	0	52	32.61
Nodulisporium sp.	Broth	1000	53.85	30	11	3	0	66
		100	46.15	10	0	0	0	2
	Mycelia	1000	69.23	30	0	0	0	12
		100	30.76	10	0	0	0	2
Penicillium citrinum	Broth	1000	69.70	30	4	20	6.47	45.5
		100	69.70	10	0	5	1.04	9.5
	Mycelia	1000	75.75	30	39	7	54.09	53
		100	45.45	10	33	0	49.57	12.5
Chloroquine hydrochloride (µg/mL)			2.98×10^{-7}		-	-	-	-
Etoposide (µg/mL)			-		0.20	0.30	10	0.022
Dexorubicin (µg/mL)			-		-	-	0.25	-

This thesis research describes the isolation, structure elucidation of the compounds from the selected fungi and cytotoxicities against HuCCA-1 (human lung cholangiocarcinoma cancer cells), A549 (human lung carcinoma cell line), HepG-2 (human hepatocellular liver carcinoma cell line), and MOLT-3 (T-lymphoblast (acute lymphoblastic leukemia) cell line) cell lines.

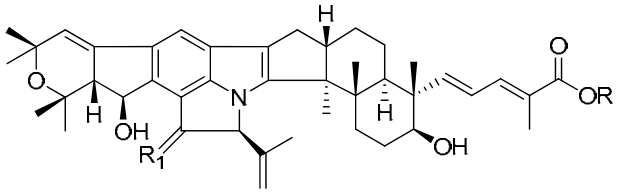
CHAPTER II

LITERATURE REVIEWS

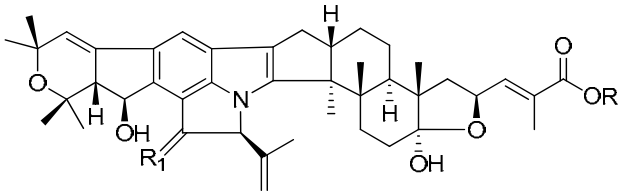
Chemistry and biological activities of secondary metabolites from fungi of the genus *Aspergillus*, *Nodulisporium*, and *Penicillium citrinum* are reviewed below.

2.1 Chemistry and biological activities of secondary metabolites from *Nodulisporium* sp.

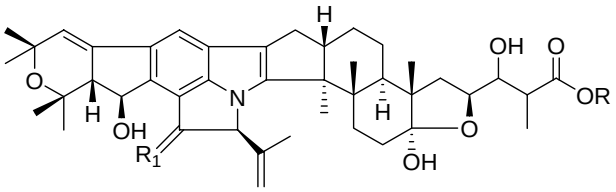
The study on a mutant culture of *Nodulisporium* sp.[2] by J.G. Ondeyka et al. led to the isolation of nodulisporic acids alkaloids which were isolated as sodium salts and methyl esters. It was found that a series of 1'-deoxy-nodulisporic acid derivatives, nodulisporic acid A (**1a**), A_1 (**2a**), A_2 (**3a**), B (**1b**), B_1 (**2b**), B_2 (**3b**) and derivatives exhibited flea killing activities as shown in **Table 2.1**.



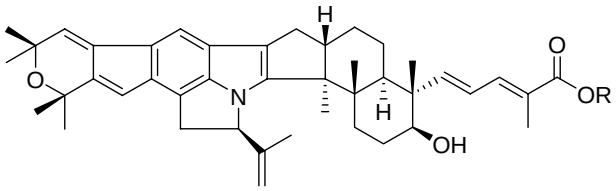
1a : R_1 = O, R = H (Nodulisporic acid A)
1b : R_1 = H, H, R = H (Nodulisporic acid B)
1c : R_1 = H, H, R = Na
1d : R_1 = H, H, R = CH_3
1e : R_1 = O, R = CH_3



2a : R_1 = O, R = H (Nodulisporic acid A_1)
2b : R_1 = H, H, R = H (Nodulisporic acid B_1)
2c : R_1 = H, H, R = Na



3a : R_1 = O, R = H (Nodulisporic acid A_2)
3b : R_1 = H, H, R = H (Nodulisporic acid B_2)
3c : R_1 = H, H, R = Na



4a : R = H
4b : R = CH_3

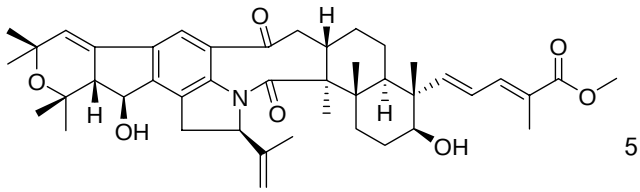


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Table 2.1 Flea killing activities of nodulisporic acids and ivermectin (standard). [2]

compound	Flea assay (LD_{90}, ppm)
Nodulisporic acid A (**1a**)	1
Nodulisporic acid B (**1c**)	100
Nodulisporic acid A_1 (**2a**)	5
Nodulisporic acid B_1 (**2c**)	NA[a]
Nodulisporic acid A_2 (**3a**)	10
Nodulisporic acid B_2(**3c**)	NA
Methyl ester of acid A (**1e**)	10
Methyl ester of acid A (**1d**)	100[b]
Compound **5**	NA
Ivermectin	10

[a] NA (not active at 100 ppm).

[b] Partial activity at 10 ppm.

A series of d-ring-opened nodulisporic acid, nodulisporic acid C (**6a** and **6b**), C_1 (**7a** and **7b**) and C_2 (**8a** and **8b**) were isolated from the fungus *Nodulisporium* sp..[3] These compounds showed insecticidal activity against *Aedes aegypti*, *Lucilia sericata* and flea as shown in **Table 2.2**.

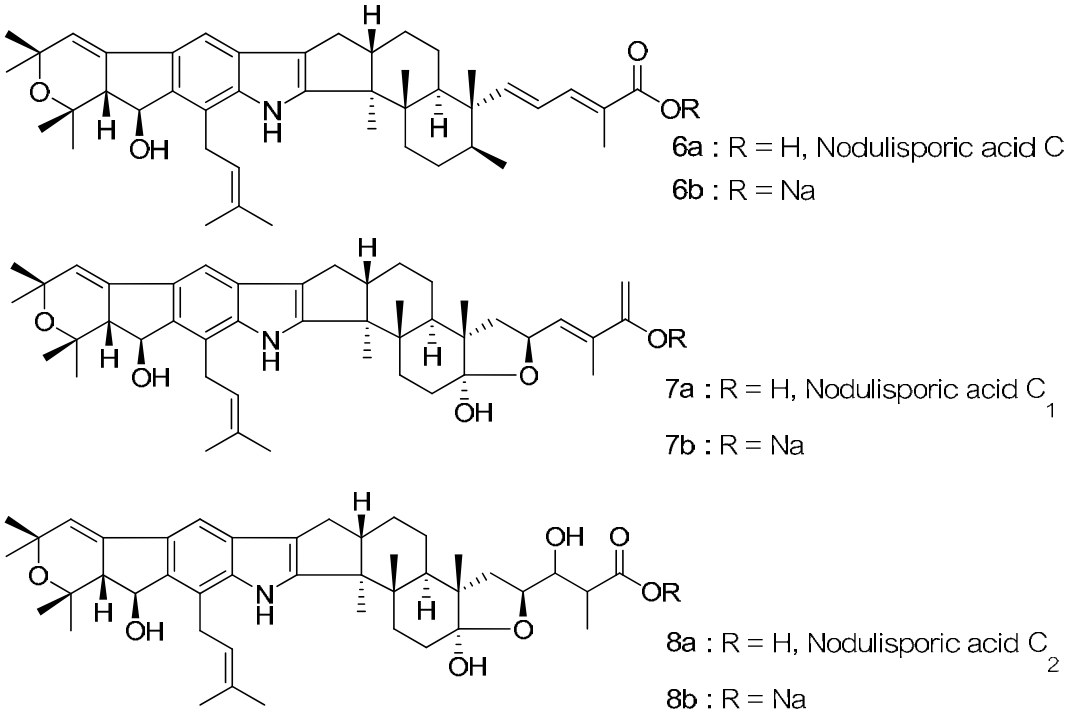
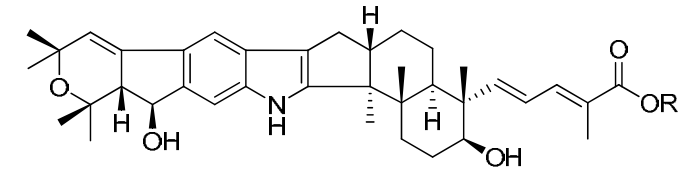


Table 2.2 insecticidal activity of the nodulisporic acids and known insecticides.[3]

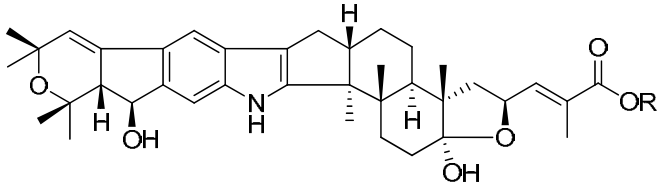
Compound	LD_{90}		
	A. aegypti	*L. sericata*	Flea
	ng/mL	ng/mL	ng/mL
Nodulisporic acid A (**1a**)	500	300	1
Nodulisporic acid A_1 (**2a**)	200	300	5
Nodulisporic acid A_2 (**3a**)	800	600	10
Nodulisporic acid C (**6a**)	10000	10500	10
Nodulisporic acid C_1 (**7a**)	NT[a]	NT	>100
Nodulisporic acid C_2 (**8a**)	NT	NT	>100
paraherquamide	50000	50000	>100
ivermectin	5	40	10

[a] NT (not tested).

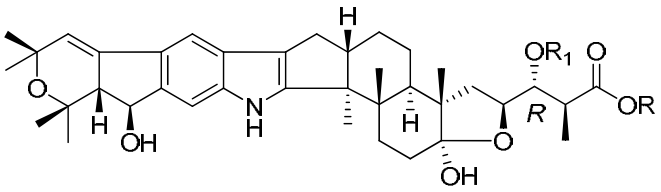
Further study on the mutant *Nodulisporium* [4] strains led to the isolation of nodulisporic acids D-F (**9-14**) and nodulisporic acid A, B and C series (**1a-b**, **1d-e**, **2a-b**, **3a-b**, **6a**, **7a**, **8a**, **15**, **16** and **17**). These fungal metabolites exhibited antiflea activity as shown in **Table 2.3**.



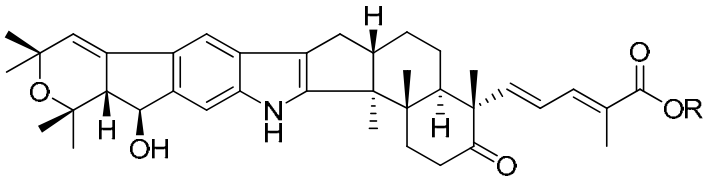
9a : R = H, (Nodulisporic acid D)
9b : R = Me



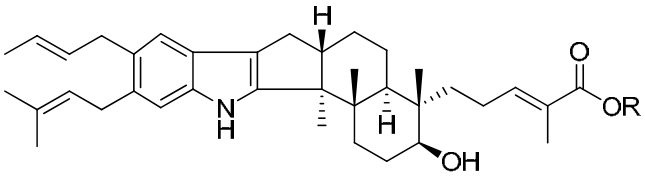
10a : R = H, (Nodulisporic acid D_1)
10b : R = Me



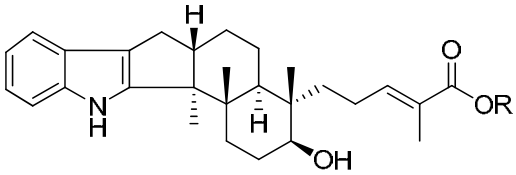
11a : R = R_1 (Nodulisporic acid D_2)
11b : R = Me , R_1 = H
11c : R = Me , R_1 = (*R*)-MTPA
11d : R = Me , R_1 = (*S*)-MTPA



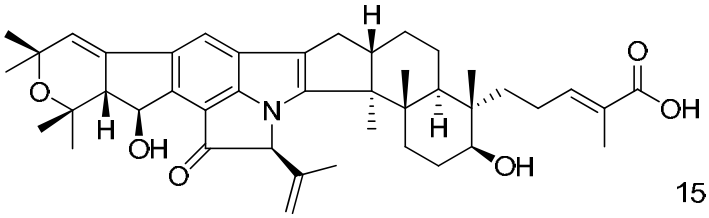
12 : (Nodulisporic acid D_3)



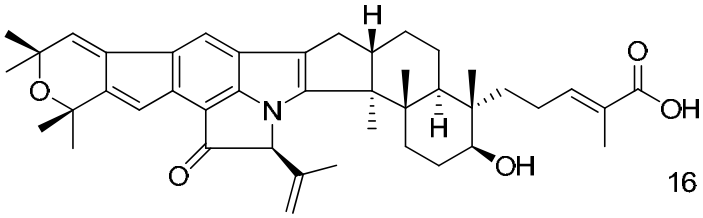
13a : R = H (Nodulisporic acid E)
13b : R = Me



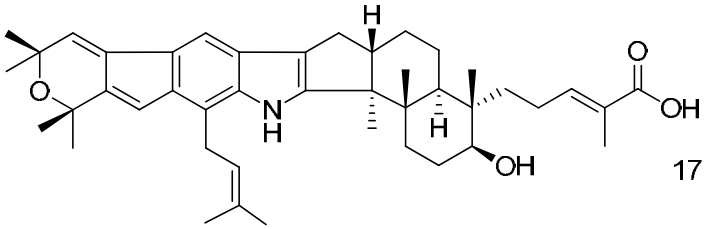
14 : (Nodulisporic acid F)



15 : (Nodulisporic acid A_4)



16 : (Δ^{23}-Nodulisporic acid A_4)



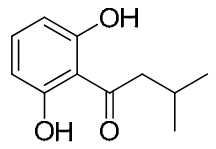
17 : (Δ^{23}-Nodulisporic acid C_4)

Table 2.3 Insecticidal activities of nodulisporic acids. [4]

compound	Flea LD_{90} μM	nodulisporic acid binding IC_{50}, μM
1a	1.5	0.00027
1b	150	0.0174
1e	14.4	0.00043
1d	147.1	0.0026
2a	7.3	0.0105
2b	>147.7	0.121
3a	14.0	0.0700
3b	>142.9	0.101
6a	15.0	0.0052
7a	>146.2	0.0523
8a	>142.5	0.0715
9a	92.6	>1.0
9b	NT	>0.10
10a	NT	>10.0
10b	NT	>0.10
11a	NT	>0.0221
11b	NT	>10.0
12	NT	>1.0
13a	17.5	2.143
13b	NT	>0.10
14	45.9	>10.0
15	14.7	0.0024
16	>15.1	>0.023
17	15.3	>0.10

The endophytic fungus *Nodulisporium* sp.[5] isolated from the twigs of the plant *Juniperus cedre* produced seven new metabolites (**18**, **20**, **23**, **26**, **27**, **29** and **30**) together with ten known compounds (**19**, **21**, **22**, **24**, **25**, **28**, **31**, **32**, **33** and **34**). Some of

these isolated metabolites exhibited antifungal (*Microbotryum violaceum* and *Septoria tritici*), antibacterial (*Bacillus megaterium*) and algicidal (*Chlorella fusca*) activifies in agar diffusion test; the radius of zone of inhibition in mm are shown in Table 2.4.



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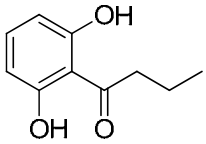

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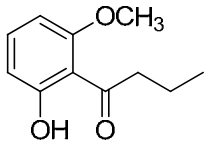

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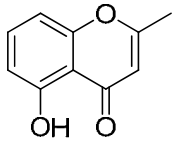

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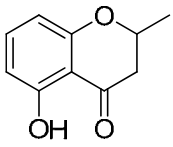

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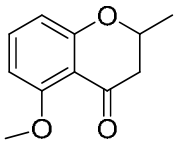

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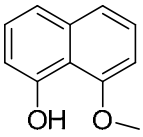

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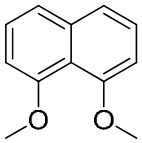

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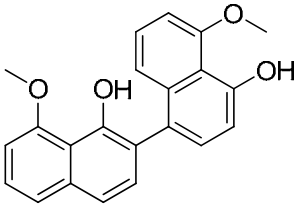
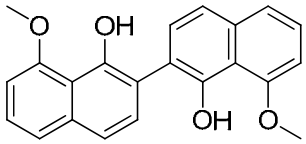
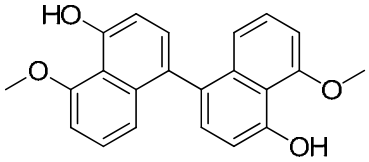


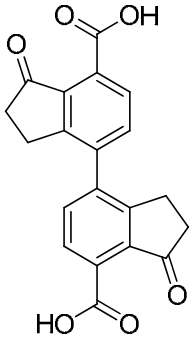
nodulisporin A (26)



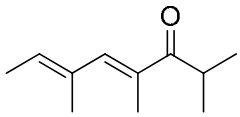
nodulisporin B (27)



daldinol (28)



nodulisporin C (29)



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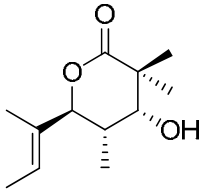
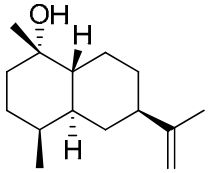
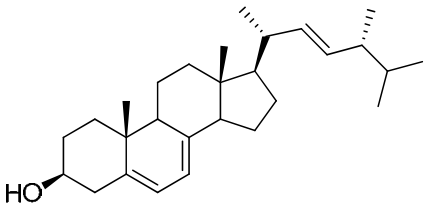


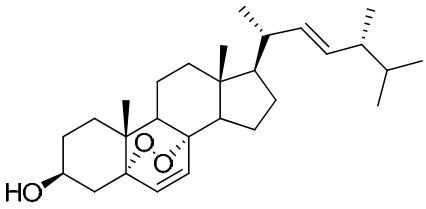
helicascolide A (31)



32



ergosterol (33)



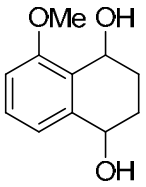
5α,8β-epidioxyergosterol (34)

Table 2.4 Antifungal, antibacterial, and algicidal activities of compounds **18-30**.[5]

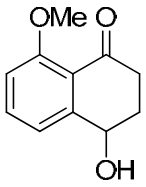
strain	compound												
	18	19	20	21	22	23	24	25	26	27	28	29	30
Bacillus megaterium	0	15	0	0	0	0	0	0	0	0	0	0	0
Microbotryum violaceum	0	3	15	1	12	10PI	6	5	7	6	0	5	8
Septoria tritici	0	17	6	7	0	7PI	0	5	0	0	0	0	7PI
Chlorella fusca	0	20	11	7	1	12	5	6	0	6	5	0	7

PI = partial inhibition within the cleared zone.

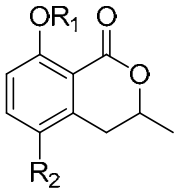
The culture of fungus *Nodulisporium* sp.,[6] which was collected in Chiba prefecture, Japan, was shown to produce two novel tetralols, nodulisporol (**35**) and nodulisporol (**36**) and five know compounds, dihydroisocoumarins (**37-40**) and chromone (**41**). All isolated compounds selectively inhibited the activity of human DNA polymerase λ (pol λ) as shown in **Table 2.5**.



nodulisporol (35)



nodulisporol (36)

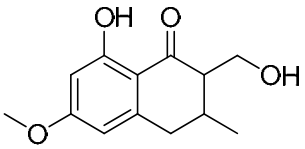


37 : R_1 = Me, R_2 = H

38 : R_1 = H, R_2 = CHO

39 : R_1 = Me, R_2 = Me

40 : R_1 = H, R_2 = H

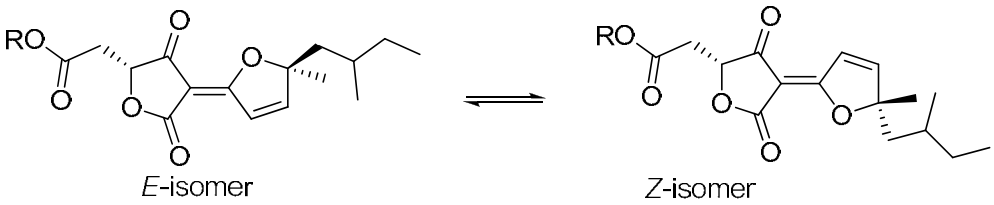


chromone (41)

Table 2.5 IC_{50} values of compounds (**31-37**) for the inhibitory activity of mammalian DNA polymerases.[6]

compound	IC_{50} values of compounds (μM)						
	35	36	37	38	39	40	41
Calf pol α	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Rat pol β	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Human pol γ	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Human pol δ	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Human pol ε	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Human pol λ	168	82	180	275	49	316	454

The marine-derived fugus *Nodulisporium* sp.[7] was isolated from an unidentified of coral CRI258, collected from Surin Island, Phang-nga Province, Thailand, and this fungus produced a new compound, nodulisporacid acid A (**42**). Nodulisporacid A (**42**) and its synthetic derivatives **43** and **44** obtained from respective methylation and benzylation exhibited cytotoxic and antiplasmodial activities as shown in **Table 2.6**.



E-isomer *Z*-isomer

R = H nodulisporacid A (42)

R = Me (43)

R = Bn (44)

Table 2.6 Cytotoxic and antiplasmodial activities of compounds **42-44**. [7]

	Cell line[a]	Cytotoxic activity (IC_{50}, μg/mL)				
		42	**43**	**44**	Etoposide[c]/ Doxorubicin[d]	
	HuCCA-1	>50	2.30±0.10	2.10±0.21	5.30±1.53	
	KB	>50	2.20±0.30	3.20±0.44	0.46±0.15	
	HeLa	>50	2.70±0.17	2.60±0.12	0.40±0.12	
	MDA-B231	>50	2.50±0.46	0.38±0.04	0.40±0.10	
	T47D	>50	1.70±0.25	0.14±0.02	0.04±0.01	
	H69AR	>50	ND[b]	ND[b]	36.0±1.41	
	HepG2	>50	2.30±0.35	2.00±0.42	0.19±0.02	
	A549	>50	7.50±0.71	2.20±0.28	0.48±0.03	
	HCC-S102	>50	6.00±1.41	4.80±0.14	1.20±.14	
	HL-60	>50	1.01±0.20	1.18±0.14	0.77±.35	
	P388	>50	0.77±0.00	0.70±0.06	0.10±0.01	
Antiplasmodial activity (μM)		1-10	1-10	1-10		Chloroquine hydrochloride[e] 0.29

[a] Cancer cell lines are: HuCCA-1 Human cholangiocarcinoma cancer cells; KB Human epidermoid carcinoma of the mouth; HeLa Cervical adenocarcinoma cell line; MDA-MB231 Hormone-independent breast cancer cell line; T47D Hormone-dependent breast cancer cell line; H69AR Multidrug-resistant small cell lung cancer cell line; HepG2 Human hepatocellular liver carcinoma cell line; A549 Human lung carcinoma cell line; HCC-S102 Hepatocellular carcinoma cell line; HL-60 Human promyelocytic leukemia cell line; and P388 Murine leukemia cell line.

[b] ND = Not determined.

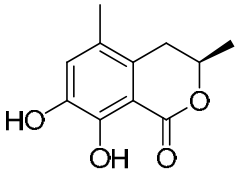
[c] Etoposide was used as the reference drug.

[d] Doxorubicin was used as the reference compound.

[e] Chloroquine hydrochloride was a standard drug.

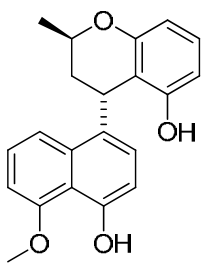
The marine algicolous fungus *Nodulisporium* sp. [8] was isolated from the inner tissue of red alga, *Plocamium* sp., collected from near Helgoland, Germany. Fermentation of this fungus resulted in the production of compound **45**, which showed

antibacterial (*Bacillus megaterium* and *Escherichia coli*), antifungal (*Microbotryum violaceum*, *Eurotium rubrum*, and *Mycotypha microspora*) and antialgal (*chlorella fusca*) activities.

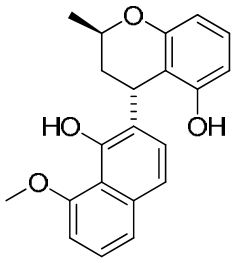


(45)

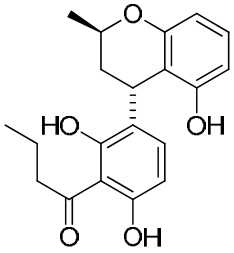
The endophytic fungus *Nodulisporium* sp. [9] was isolated from the plant *Erica arborea*, from Gomera, and this fungus was found to produce six new metabolites (**45-50**) and seven known compounds (**51-52**, **18**, **19**, **21**, **24**, and **25**). Some of these compounds exhibited antibacterial (*Bacillus megaterium*), antialgal (*Chlorella fusca*) and antifungal (*Microbotryum violaceum*) activities as shown in **Table 2.7**.



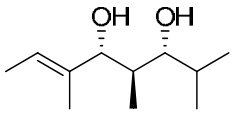
nodulisporin D (**45**)



nodulisporin E (**46**)



nodulisporin F (**47**)



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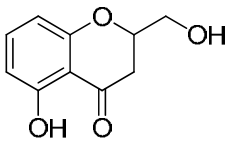
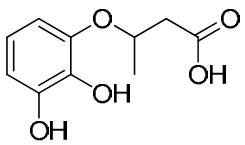


49



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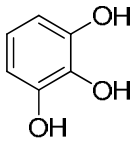

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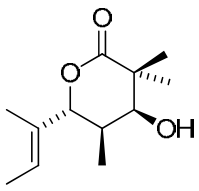


52

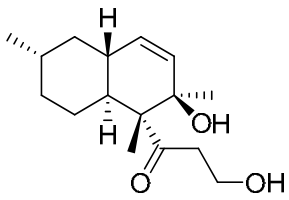
Table 2.7 Biological activity of metabolites **45-51** and reference drugs against microbial test organisms in an agar diffusion assay; results in mm of radius of zone of inhibition.[9]

Compound	*Bacillus megaterium*	*Microbotryum violaceum*	*Chlorella fusca*
45	8	7	8g.i.
46	7	7	5g.i.
47	8	10	8g.i.
48	0	8	6g.i.
49	0	6g.i.	6g.i.
50	0	6g.i.	7g.i.
51	6g.i.	10	7
Penicillin	18	0	0
Tetracycline	18	0	10g.i.
Nystatin	0	20	0
Actidione	0	50	35
Acetone	0	0	0

g.i. = growth inhibition, i.e. there were some growths within the zone of inhibition; concentrations of 0.05 mg/filter disc.

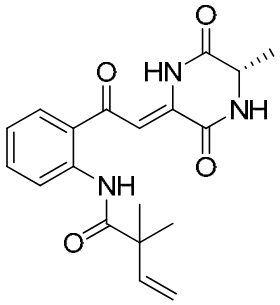
2.2 Chemistry and biological activities of secondary metabolites from *Aspergillus* sp.

A new polyketide, aspermytin A (**53**) was isolated from a marine fungus, *Aspergillus* sp., [10] which was separated from the mussel, *Mytilus edulis*. Aspermytin A induced neuite outgrowth in rat pheochromocytoma (PC-12) cell at concentration of 50 μM.

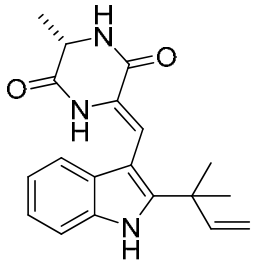


aspermytin A (**53**)

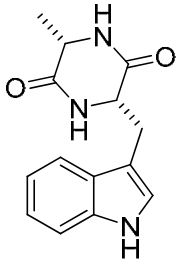
Aspergillus sp. [11] from the marine red alga, *Lomentaria catenata* collected at Golmae Village, Ulsan City, Korea, produced a new diketopiperazine alkaloid, golmaenone (**54**) and related neoechinulin A (**55**) and compound **56**.



golmaenone (**54**)

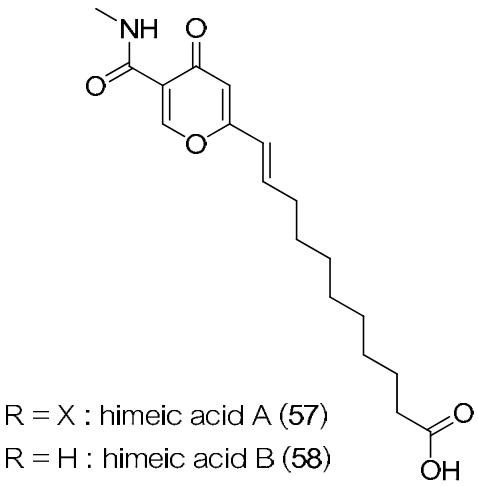


neoechinulin A (**55**)

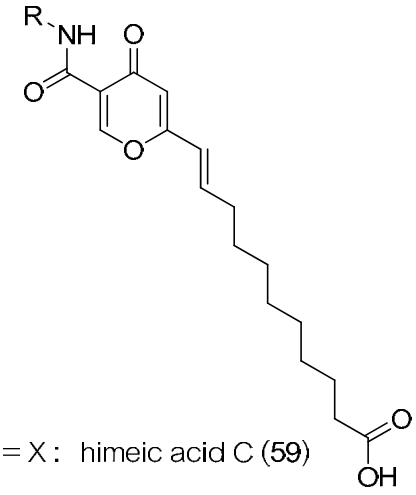


56

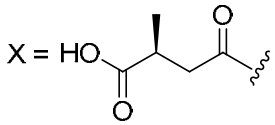
The fungus, *Aspergillus* sp.,[12] from the mussel, *Mytilus edulis*, provided new compounds, himeic acids A-C (**59-61**), which inhibited activities against E1 (the ubiquitin-activating enzyme). The formation of an E1-ubiquitin (Ub) intermediats was 65% inhibited by himeic acid A at concentration of 50 μM, while himeic acids B and C, showed inhibitory activity even at 100 μM.



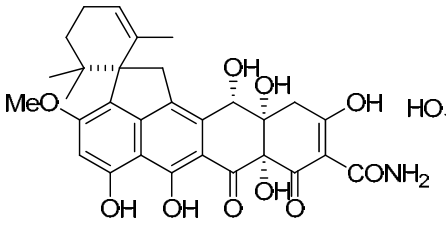
R = X : himeic acid A (**57**)
R = H : himeic acid B (**58**)



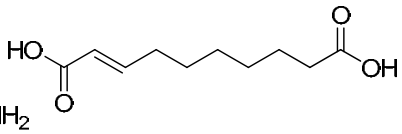
R = X : himeic acid C (**59**)



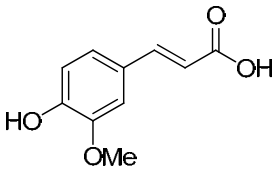
Aspergillus unilateralis, [13] isolated from a soil sample collected near Mount Isa, Queensland, provided viridicatumtoxin (**60**), compound **61**, **64**, ferulis acid (**62**), compound **63**, riboflavin (**65**) and pennicillazine/trichodermamide A (**68**), together with a series of novel dipeptides, aspergillazines A-E (**67-71**). Re-culture of *A. unilateralis* in NaCl (1%) enriched media gave trichodermamide B (**72**). Viridicatumtoxin (**60**) exhibited cytotoxic activity (LD_{99} = 0.78 µg/mL) and antibacterial activity against *B. subtilis* (LD_{99} = 13 µg/mL).



viridicatumtoxin (**60**)



61



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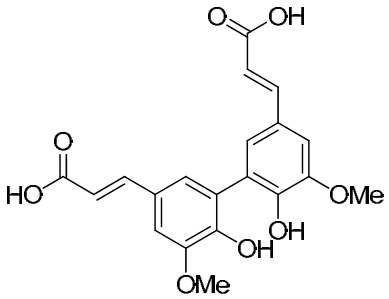

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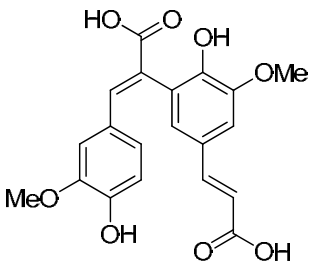

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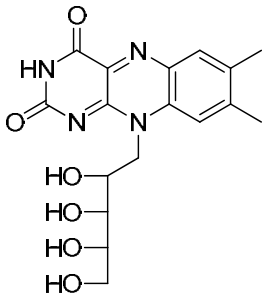
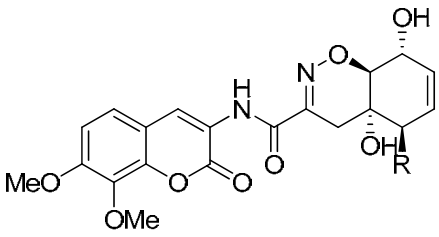
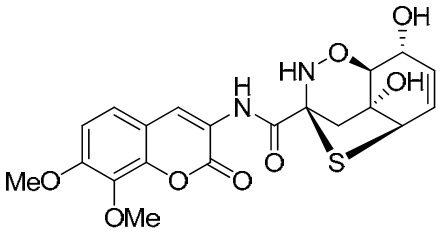


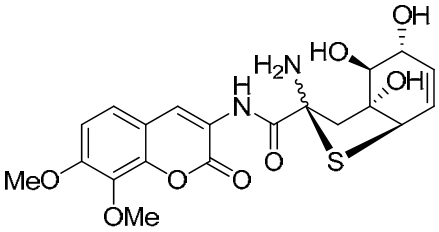
riboflavin (**65**)



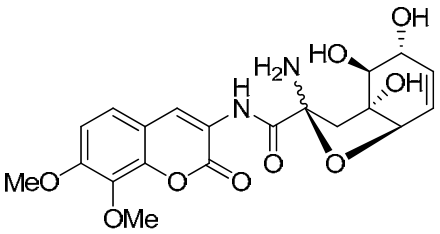
R = OH penicillazine/trichodermamide A (66)
R = Cl trichodermamide B (72)



aspergillazine A (67)

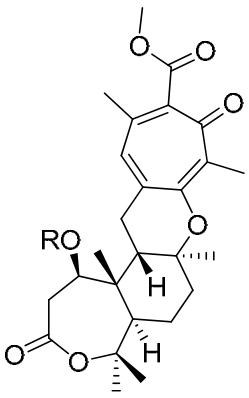


(68) and (69) C-2 epimers
aspergillazine B and C

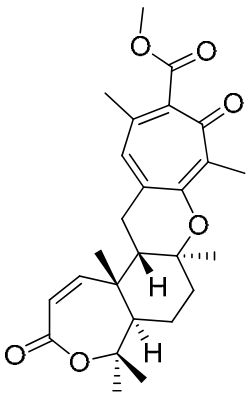


(70) and (71) C-2 epimers
aspergillazine D and E

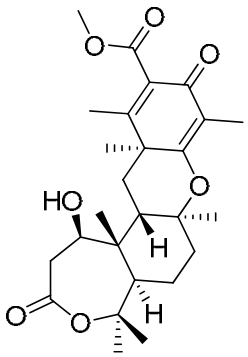
A marine-derved fungus of the genus *Aspergillus*,[14] was isolated from an unidentified sponge collected at Manele Bay, Hawaii, and this fungus produced four new compounds, tropolactones A-C (73-75) and tropolactone D (76). Tropolactones A-C (73-75) showed cytotoxicity against human colon adenocarcinoma cells (HCT-116) with IC_{50} values of 13.2, 10.9 and 13.9 μM, respectively.



R = H tropolactones A (73)
R = $COCH_3$ tropolactones B (74)

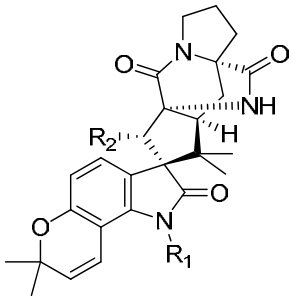


tropolactones C (75)

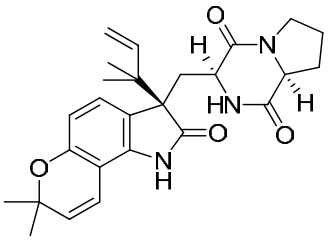


tropolactone D (76)

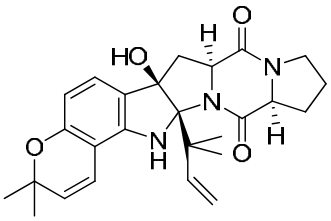
The fungus *Aspergillus* sp. [15] isolated from the common mussel, *Mytilus edulis*, collected off Noto Peninsula in the Sea of Japan, produced four new compounds notoamide A-D (**77-80**), together with stephacidin A (**82**) and dexybrevianamide E (**83**). Notoamides A-D (**77-80**) exhibited cytotoxicity against HeLa and L1210 cells with IC_{50} values in the range of 22-52 μg/mL, but the IC_{50} values in of notoamide D is greater than 100 μg/mL, while notoamide C induced G2/M-cell cycle arrest at a concentration of 6.3 μg/mL.



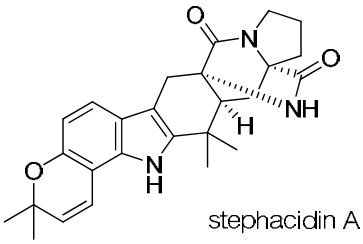
(R_1 = OH, R_2 = H) notoamide A (**77**)
(R_1 = H, R_2 = H) notoamide B (**78**)
(R_1 = H, R_2 = OH) sclerotiamide (**81**)



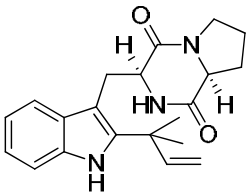
notoamide C (**79**)



notoamide D (**80**)

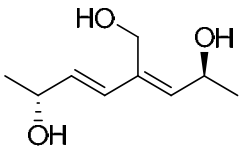


stephacidin A (**82**)

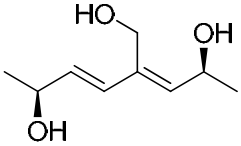


deoxybrevianamide E (**83**)

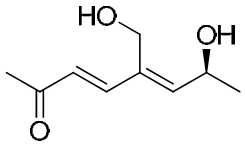
Aspergillus ostianus [16] isolated from an unidentified marine sponge collected in Pohnpei produced three new pentaketides, aspinotriols A (**84**), B (**85**) and aspinonediol (**86**), together with two known compounds, aspinonene (**87**), and compound **88**. New compounds showed no anti-MRSA (methicillin-resistant *Staphylococcus aureus*) at a concentration of 100 μg/dish. But aspinonene (**87**) exhibited toxicity against mouse lymphocytic leukemia cell at 25 ppm (27%), although **84** was inactive.



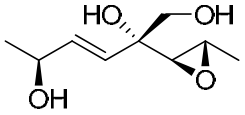
aspinotriols A (84)



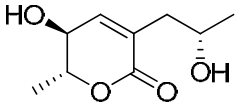
aspinotriols A (85)



aspinonediol (86)

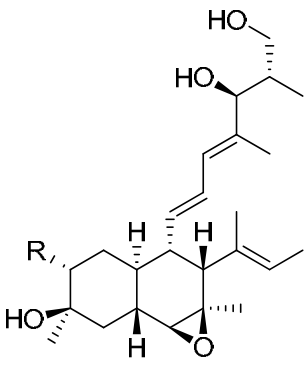


aspinonene (87)

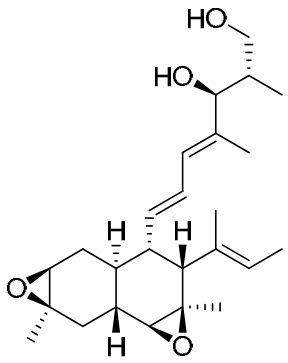


88

A new dehydroxychlorofusarielin B (**89**), and two know compounds fusarielins A (**90**) and B (**91**), have been isolated from the fungus *Aspergillus sp.*,[17] which was separated from surface of the marine brown alga *Sargassum horneri* collected at Gadeok Island, Busan, Korea, in 2001. Compounds **89-91** exhibited an antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC values for each compound towards all strains were as follows: dehydroxychlorofusarielin B (**89**) and fusarielins A (**90**), 62.5 μg/mL for all strains; fusarielins B (**91**), 32.5 μg/mL for *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/mL for multidrug-resistant *S. aureus*.

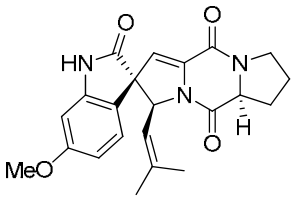


R = Cl dehydroxychlorofusarielin B (**89**)
R = OH fusarielin B (**91**)

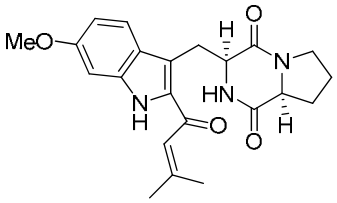


fusarielin A (**90**)

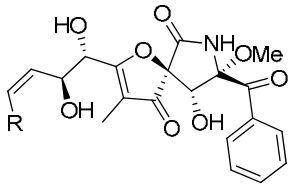
Aspergillus sydowi [18] isolated from a driftwood sample collected from the beach of Baishamen, Hainan, China, provided five new compounds, 6-methoxyspirotryprostatin B (**92**), 18-oxotryprostatin A (**93**), 14-hydroxyterezine D (**94**), 14-norpseurotin A (**95**) and compound **96**, together with twelve known compounds **97-108**. 6-Methoxyspirotryprostatin B (**93**), 18-oxotryprostatin A (**94**), and 14-hydroxyterezine D (**95**) exhibited cytotoxicity against A-549 cell line, with an IC_{50} value of 9.71 μM. Compounds **96** and **97** displayed significant antimicrobial activities against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus*.



6-methoxyspirotryprostatin B (**92**)

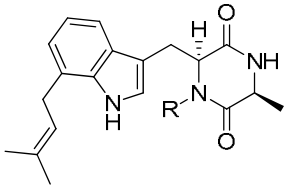


18-oxotryprostatin A (**93**)



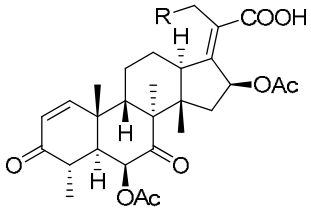
R = Me 14-norpseurotin A (**95**)

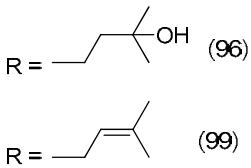
R = CH_2CH_3 (**98**)

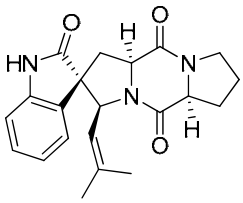


R = OH 14-hydroxyterezine D (**94**)

R = H (**97**)







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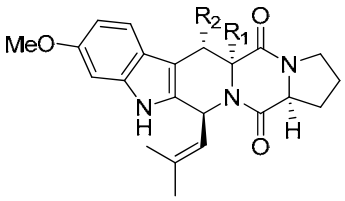
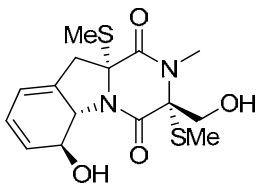


R_1 = OH, R_2 = OH (**101**)

R_1 = R_2 = H (**102**)



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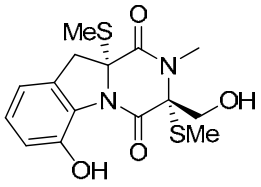
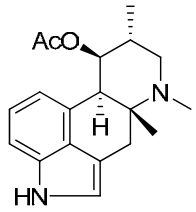


104



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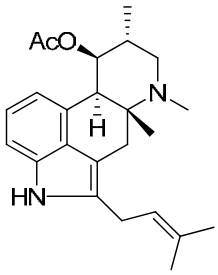

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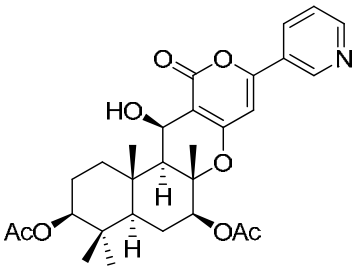

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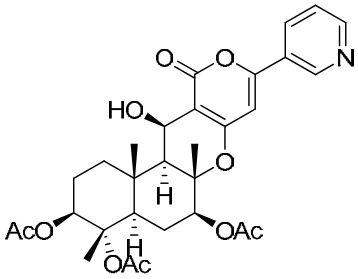
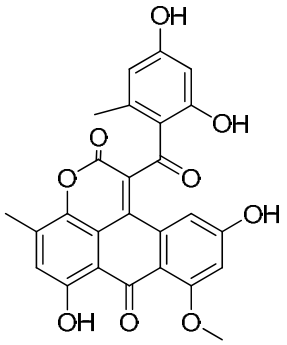
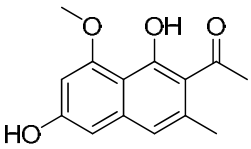


108

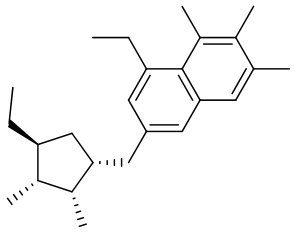
Eight new aromatic polyketides (**109**, **111-113**, **115**, **121**, **123** and **124**) together with eight known compounds (**110**, **114**, **116-120**, and **122**) were isolated from the marine-derived fungus *Aspergillus glaucus*,[19] which was separated from marine sediment surrounding mangrove roots collected the Fujian Province, China. Some of new compounds exhibited cytotoxicity against the HL-60 and A-549 cell lines. Aspergiolide B (**109**) showed cytotoxicity against the HL-60 and A549 cells with IC_{50} values of 0.51 and 0.24 μM, respectively. Conpound **123** exhibited cytotoxicity against the HL-60 and A-549 cell lines with IC_{50} values of 7.8 and 9.2 μM, respectively. Its *cis*-isomer, compound **124**, showed comparable cytotoxicity with the IC_{50} values of 14.2-44.0 μM.



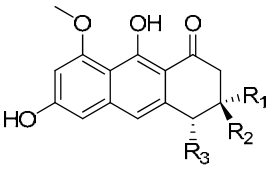
aspergiolide B (**109**)



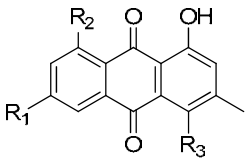
110



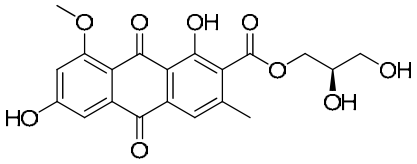
R_1 = acetyl, R_2 = Me (**111**)
R_1 = H, R_2 = Me (**112**)
R_1 = H, R_2 = H (**113**)



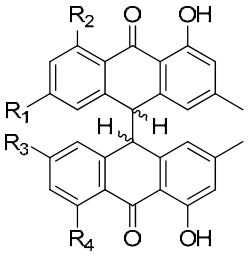
R_1 = Me, R_2 = OH, R_3 = H (**114**)
R_1 = H, R_2 = Me, R_3 = OH (**115**)



R_1 = OH, R_2 = OH, R_3 = H (**116**)
R_1 = OMe, R_2 = OH, R_3 = H (**117**)
R_1 = OH, R_2 = OMe, R_3 = H (**118**)
R_1 = OH, R_2 = OH, R_3 = OH (**119**)
R_1 = OH, R_2 = OMe, R_3 = OH (**120**)

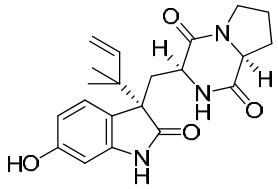


121

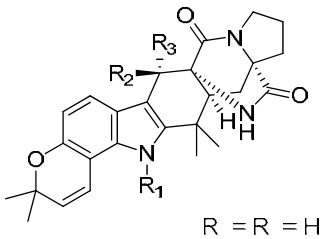


R_1 = R_3 = OMe, R_2 = R_4 = OH, *meso* and (+-)-racemic C-10/C-10' (**123**)
R_1 = R_2 = R_4 = OH, R_3 = OMe, *trans* H-10/H-10' (**124**)
R_1 = R_2 = R_4 = OH, R_3 = OMe, *cis* H-10/H-10' (**125**)

Six new indole alkaloids, notoamides F-K (**125-130**), together with the known alkaloids, sclerotiamide (**81**), deoxybrevianamide E (**83**), and stephacidin A (**82**), were isolated from a marine-derived *Aspergillus* sp.[20] This fungus was separated from the mussel *Mytilus edulis galloprovincialis* collected off Noto Peninsula in the Japan Sea. The cytotoxicity test (Hela cell) of natoamide I (**128**) showed an IC_{50} value of 21 μg/mL, whereas for noloamides F (**81**), J (**129**), and K (**130**) the IC_{50} values were more than 50 μg/mL.



notoamide J (**133**)

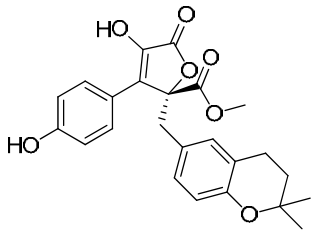


$R_1 = R_2 = H, R_3 = OMe$ notoamide F (**129**)

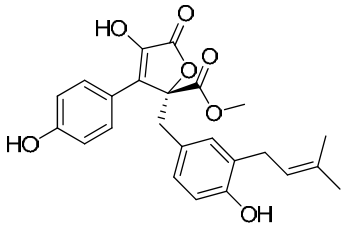
$R_1 = OH, R_2 = H, R_3 = OMe$ notoamide G (**130**)

$R_1 = H, R_2, R_3 = O$ notoamidel (**132**)

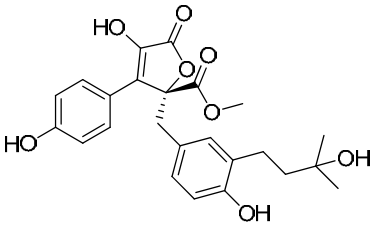
Two new aspernolides A (**131**) and B (**132**), together with butyrolactone I (**133**), were isolated from *Aspergillus terreus*.[21] This fungus was separated as an epiphyte from a soft coral *Sinularia kavarattiensis* collected from the coast of Mandapam, Tamil Nadu, India. Whene tested, aspernolide A (**131**) exhibiled cytotoxicity against H460, ACHN, Calu, Panc1 and HCT116 cell lines with respective IC_{50} values of >88, >103, >147, >130 and 121 μM.



spernolides A (**131**)

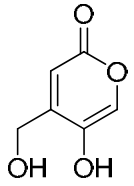


butyrolactone I (**133**)

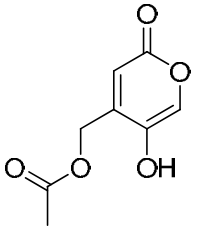


spernolides B (**132**)

A marine-derived fungus *Aspergillus flavus* [22] was separated from a marine algae *Enteromorpha tubulosa*, collected at Putiam Pinghai, China, and this fungus produced two new compounds (**134-135**). Compounds **137** and **138** did not show cytotoxic activity against HL-60 and A-549 cell lines at the concentration of 100 μM.

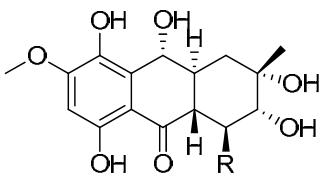


134

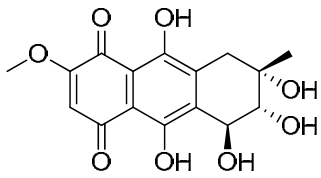


135

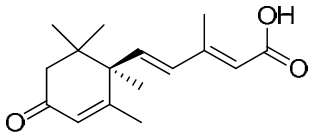
Two new hexahydroanthrones, tetrahydrobostrycin (**136**) and 1-deoxytetrahydrobostrycin (**137**), were isolated from a marine-derived fungus *Aspergillus* sp. [23] collected at the coral reef of Manado, Indonesia, together with bostrycin (**138**) and abscisic acid (**139**). Hexahydroanthrones showed antibacterial activity against *Staphylococus aureus* and *Escherichia coli* (at 100 µg/disc with the inhibition zone of 15 and 9.2 mm in diameter, respectively) and 1-deoxytetrahydrobostrycin aginst *S. aureus* (12 mm at 100 µg/disc).



R = OH tetrahydrobostrycin (**136**)
R = H 1-deoxytetrahydrobostrycin (**137**)

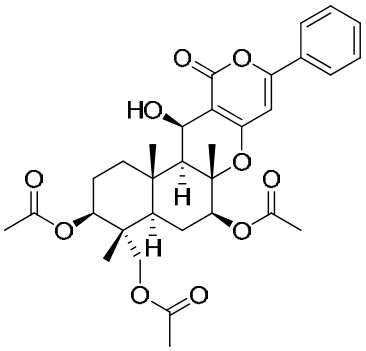


bostrycin (**138**)

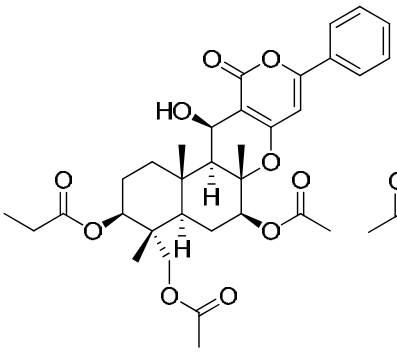


abscisic acid (**139**)

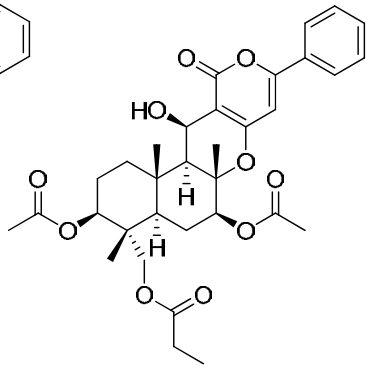
A marine derived fungus of *Aspergillus* sp.[24] was isolated from the marine sediment collected from a depth of 70 m off the Gokasyo Gulf, Mie prefecture, Japan, produced pyripyropenes A (**143**), B (**144**), and D(**145**). The compounds exhibited anti-proliferative agents against HUVECs (human umbilical vein endothelial cells) with IC_{50} values of 1.8 µM.



pyripyropene A (140)

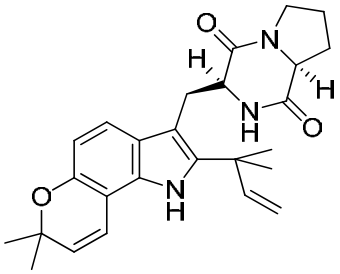


pyripyropene B (141)



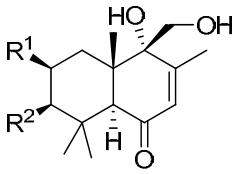
pyripyropene D (142)

A marine-derived fungus *Aspergillus* sp.[25] produced known compounds, notoamide A (**77**), B (**78**), C (**79**), E (**143**), and slephacidin A (**82**).



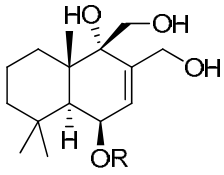
notoamide E (**143**)

Fourteen new compounds, sesquiterpenes (**144-151**), isochromane derivatives (**152-157**), and known compounds, daldinin B (**158**), pergillin (**160**) and compound **159**, were isolated from *Aspergillus ustus*,[26] which was separated from the rhizosphere soil of the mangrove plant *Bruguiera gymnorrhiza* grown in Wenchang, Hainan Province of China. Compounds **147**, **149**, **151**, and **156** showed moderate cytotoxic activity.

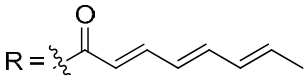


R_1 = H, R_2 = OH (**144**)

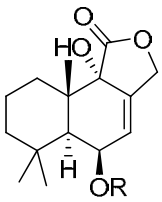
R_1 = OH, R_2 = H (**145**)

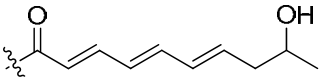


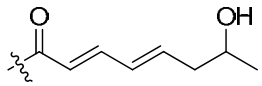
R = Me (**146**)

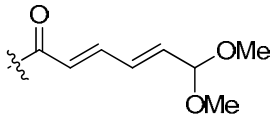


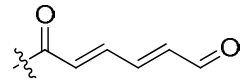
(**147**)

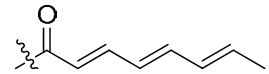


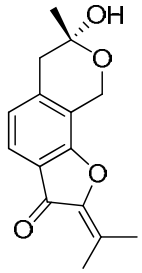
R =  (**148**)

R =  (**149**)

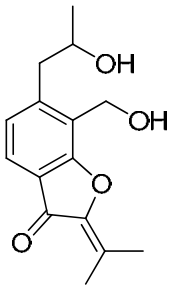
R =  (**150**)

R =  (**151**)

R =  (**159**)



pergillin (**160**)



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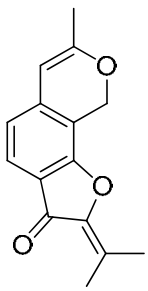

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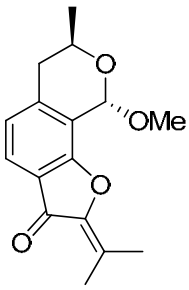

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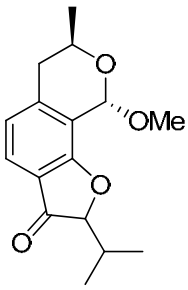

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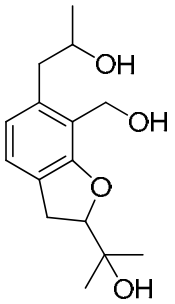

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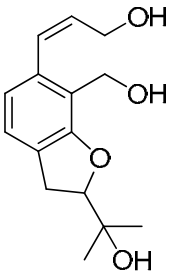

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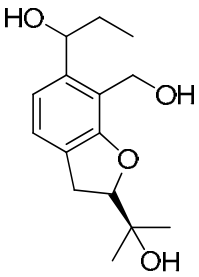
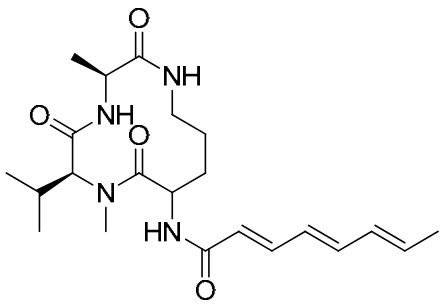


daldinin (158)

Compound **161**, a new aspochracin derivative, together wilh aspochracin (**162**), were isolated from *Aspergillus sclerotiorum*,[27] which was separated from a sponge, *Mycale* sp., collected from Ishigaki Island, Okinawa Prefecture, Japan. Compounds **161** and aspochracin (**162**) did not show any cytotoxic effect even at a concentration of 50 μg/mL.



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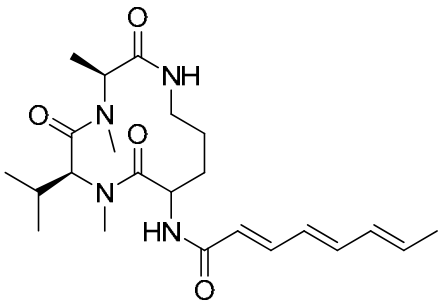
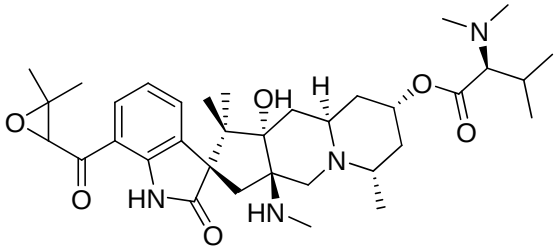


Aspochracin (**162**)

2.3 Chemistry and biological activities of secondary metabolites from *penicilium citrinum*

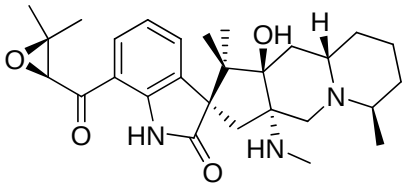
A novel pentacyclic alkaloid, citrinadins A (**163**), was isolated from the fungus *Penicillium citrinum*,[28] which was separated from a marine red alga *Actinotrichia fragilis* collected at Hedo Cape, Okinawa Island. Citrinadin A (**163**) exhibited cytotoxicity

against murine leukemia L1210 and human epidermoid carcinoma KB cells (IC_{50} 6.2 and 10 μg/mL, respectively).

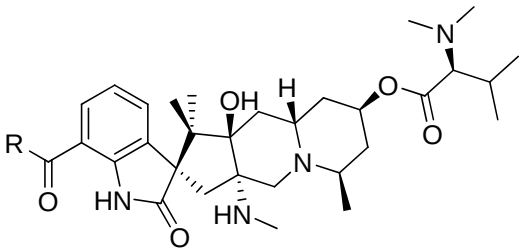


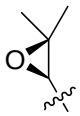
citrinadin A (**163**)

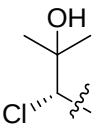
A new pentacyclic indolinone alkaloid, citrinadins B (**164**), known citrinadin A (**165**) and chlorohydrins derivative (**166**), were also isolated from *Penicillium citrinum*.[29] Citrinadins B (**164**) showed cytotoxicity against murine leukemia L1210 cells (IC_{50}, 10 μg/mL).



citrinadin B (**164**)

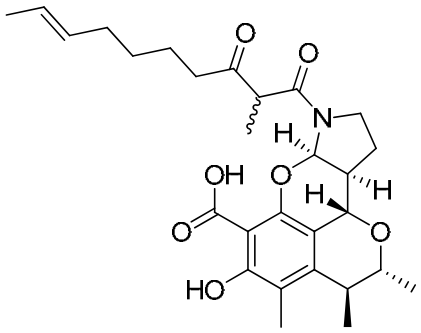


R =  citrinadin A (**165**)

R =  (**166**)

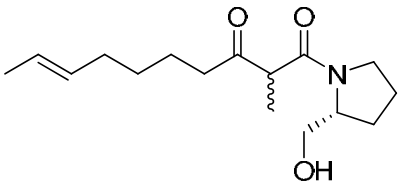
A novel tetracyclic alkaloid, perinadine A (**167**), was isolated from the fungus *Penicillium citrinum*,[30] which was separated from the gastrointestine of parrot fish,

Scalus ovifrons, collected at Hedo Cape, Okinawa Island. Perinadine A (**167**) showed cytotoxicity against murine leukemia L1210 cells (IC_{50}, 20 μg/mL) and antibacterial activity against *Micrococcus luteus* (MIC, 33.3 μg/mL) and *Bacillus subtilis* (MIC, 66.7 μg/mL).

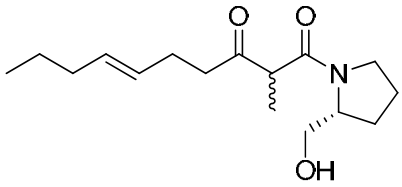


perinadine A (**167**)

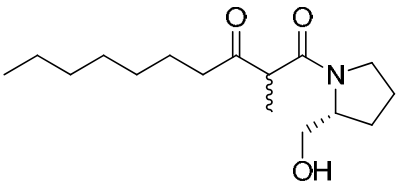
Three new pyrrolidine alkaloids, scalusamides A (**168-170**), together with known pyrrolo [2,1-*b*] oxazines (**171-172**) [31] were alsol isolated from this fungus. Scalusamide A (**168**) exhibited antifungal activity against *Cryptococcus neoformans* (MIC 16.7 μg/mL) and antibacterial activity against *Micrococcus luteus* (MIC 33.3 μg/mL).



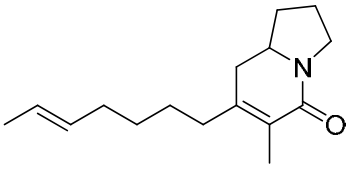
scalusamides A (**168**)



scalusamides B (**169**)



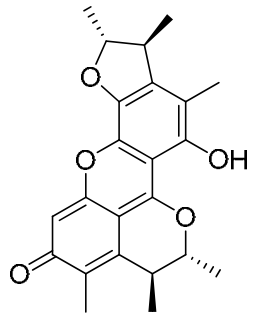
scalusamides A (**170**)



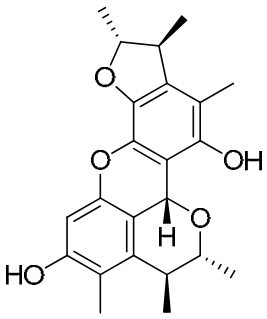
$\Delta^{5'}$ (**171**)

5', 6'-dihydro(**172**)

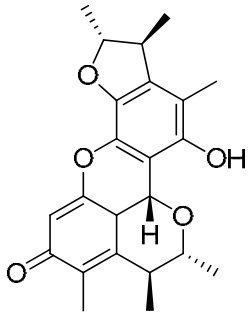
Penicillium citrinum [32] was found to produce three new coupling compounds derived from citrinin (**181**) and 2, 3, 4-trimethyl-5, 7-dihydroxy-2, 3-dihydrobenzofuran (**180**), to generate penicitrinone A (**173**), penicitrinol A (**174**) and penicitrinone B (**175**), and a new citrinin derivative decarboxydihydrocitrinin (**176**), together with quinolactacin A2 (**178**), dihydrocitrinone (**179**), citrinin (**181**), quinolactocin A or B (**182**), decarboxydihydrocitrinone (**183**), compounds **180** and **177**. Quinolactocin A2 (**178**) and citrinin (**181**) showed antifungal activity



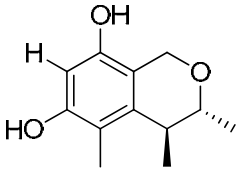
penicitrinone A (**173**)



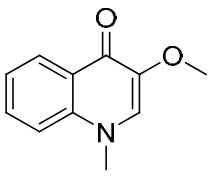
penicitrinol A (**174**)



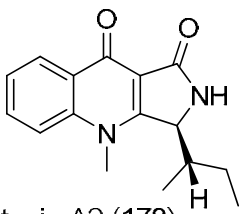
penicitrinone B (**175**)



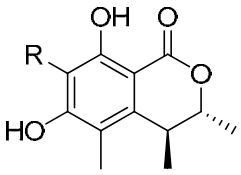
decarboxydihydrocitrinin (**176**)



177

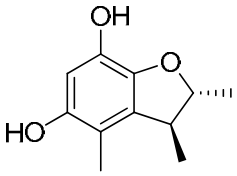


quinolactacin A2 (**178**)

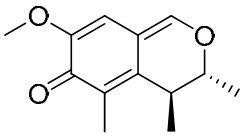


R = H dihydrocitrinone (**179**)

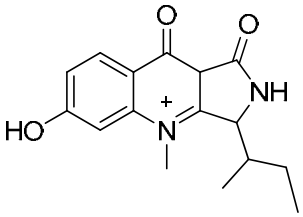
R = CO_2H decarboxydihydrocitrinone (**183**)



2, 3, 4-trimethyl-5, 7-dihydroxy-2, 3-dihydrobenzofuran (**180**)

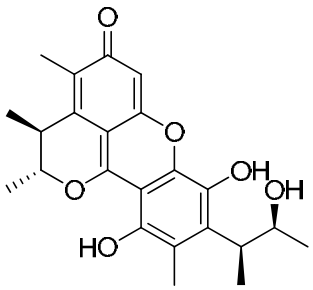


citrinin (**181**)

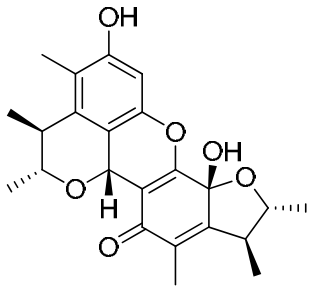


quinolactocin A or B (**182**)

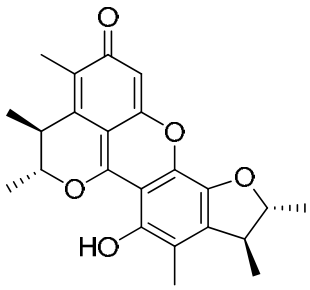
Two new citrinin dimers, pennicitrinone C (**184**) and B (**185**), and eleven known compounds (**186-196**), isolated from the fungus *Penicillium citrinum*,[33] which was separated from sediments. The new compounds did not exhibit cytotoxicity against P388, A-549, BEL-7402, and HL-60 cell lines (IC_{50} > 50 μM). Compounds **184**, **186**, **188**, and **193-195** showed radical-scavenging activities against DPPH with IC_{50} values from 0.8 to 59 μM.



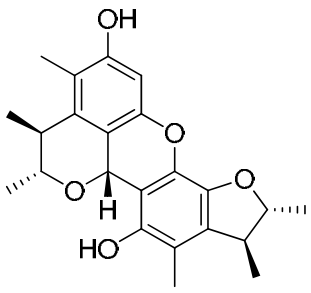
pennicitrinone C (**184**)



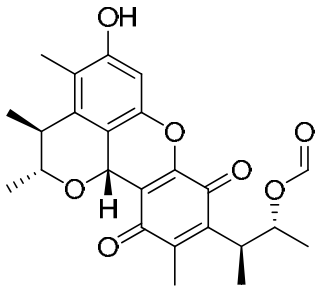
pennicitrinone C (**185**)



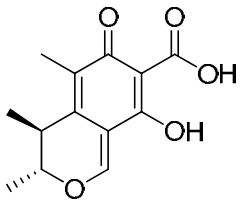
186



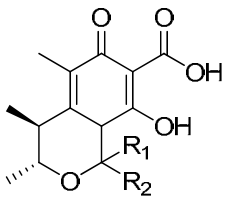
187



188

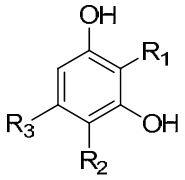


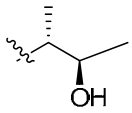
189

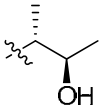


$R_1 = R_2 = O$ (**190**)

$R_1 = R_2 = H_2$ (**191**)

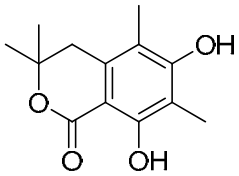


$R_1 = COOH, R_2 = Me, R_3 =$  (**192**)

$R_1 = H, R_2 = Me, R_3 =$  (**193**)

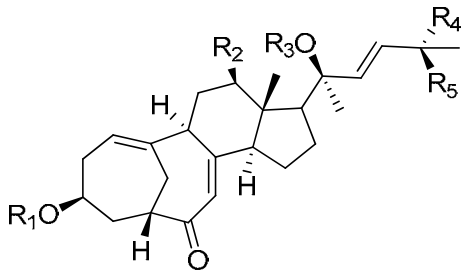
$R_1 = R_2 = H, R_3 = Me$ (**194**)

$R_1 = R_2 = R_3 = Me$ (**195**)



196

Eleven new compounds, 24-*epi*-cyclocitrinols (**197**), 20-*O*-methyl-24-*epi*-cyclocitrinol (**198**), 20-*O*-methylcyclocitrinol (**199**), 24-oxocyclocitrinol (**200**), 12*R*-hydroxycyclocitrinol (**201**), neocyclocitrinols B (**203**) and D (**204**), *erythro*-23-*O*-methylneocyclocitrinol (**205**), *threo*-23-*O*-methylneocyclocitrinol (**206**), isocyclocitrinol B (**209**), and precyclocitrinol B (**212**), together with known cyclocitrinol (**200**), neocyclocitrinols A (**207**) and C (**208**), isocyclocitrinol A (**210**), and 22-*O*-acetylisocyclocitrinol A (**211**), were isolated from fungus *Penicillium citrinum*, [34] which was separated from the crater ash collected from the extinct volcano Huguang in Guangdong, China. 24-*epi*-Cyclocitrinols (**197**), cyclocitrinol (**200**), 20-*O*-methyl-24-*epi*-cyclocitrinol (**198**), neocyclocitrinols C (**208**), *threo*-23-*O*-methylneocyclocitrinol (**206**) could induce the production of cAMP in GPR12+trasfected CHO cells at 10 μM.



$R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$ 24-*epi*-cyclocitrinols (**197**)

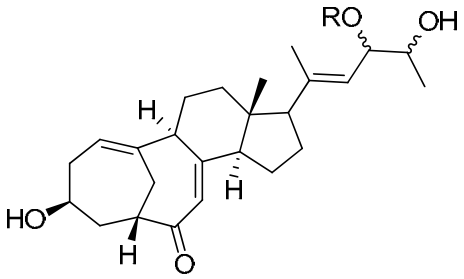
$R_1 = R_2 = R_4 = H$, $R_3 = Me$, $R_5 = OH$ 20-*O*-methyl-24-*epi*-cyclocitrinol (**198**)

$R_1 = R_2 = R_5 = H$, $R_3 = Me$, $R_4 = OH$ 20-*O*-methylcyclocitrinol (**199**)

$R_1 = R_2 = R_3 = H$, $R_4 = R_5 = O$ 24-oxocyclocitrinol (**200**)

$R_1 = R_3 = R_5 = H$, $R_2 = R_4 = OH$ 12*R*-hydroxycyclocitrinol (**201**)

$R_1 = R_2 = R_3 = R_5 = H$, $R_4 = OH$ cyclocitrinol (**202**)



23*S*, 24*S*, R = H neocyclocitrinols B (**203**)

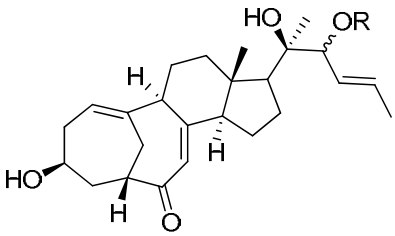
23*S*, 24*R*, R = H neocyclocitrinols D (**204**)

23, 24-*erythro*, R = Me *erythro*-23-*O*-methylneocyclocitrinol (**205**)

23, 24-*threo*, R = Me *threo*-23-*O*-methylneocyclocitrinol (**206**)

23*R*, 24*R*, R = H neocyclocitrinols A (**207**)

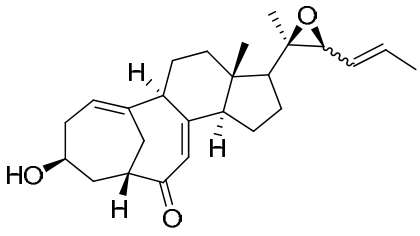
23*R*, 24*S*, R = H neocyclocitrinols C (**208**)



22*R*, R = H isocyclocitrinol B (**209**)

22*S*, R = H isocyclocitrinol A (**210**)

22*S*, R = Ac 22-O-acetylisocyclocitrinol A (**211**)



22*R* or 22*S* precyclocitrinol B (**212**)

CHAPTER III

EXPERIMENTS

3.1 Experimental

^{1}H NMR spectra were recorded on a Varian Gemini 2000 spectrometer (operating at 200 MHz spectrometer). The ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC spectra of pure compounds were recorded on a Bruker AM 400 NMR instrument (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C) and a Bruker AVANCE 600 NMR spectrometer (operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C). APCI-TOF MS were measured on a Bruker MicroTOF$_{LC}$ spectrometer. FTIR specta were recorded on Perkin-Elmer Spectrum One spectrometer. UV spectra were obtained from a Shimadzu UV-vis 2001s spectrophotometer. Melting points were determined by a Büchi 535 and uncorrected.

3.2 Fungal material

The fungus *Nodulisporium* sp. CRI352-01 was isolated from an unidentified marine sponge CRI352 and *Penicilium citrinum* CRI355-01 from an unidentified marine sponge CRI355. These two fungi were collected in November 2006 from Phi Phi Island, Krabi Province, Thailand. The fungus *Aspergillus unguis* CRI282-03 was isolated from an unidentified marine sponge CRI352, collected in December 2005 from the Navi base, Tub-La-Mu Bay, Pang-nga Province, Thailand.

These fungi were identified by Assistant Professor Suthep Wiyakrutta, Department of Microbiology, Faculty of Science, Mahidol University.

3.3 Fermentation, extraction and isolation

3.3.1 *Nodulisporium* sp.

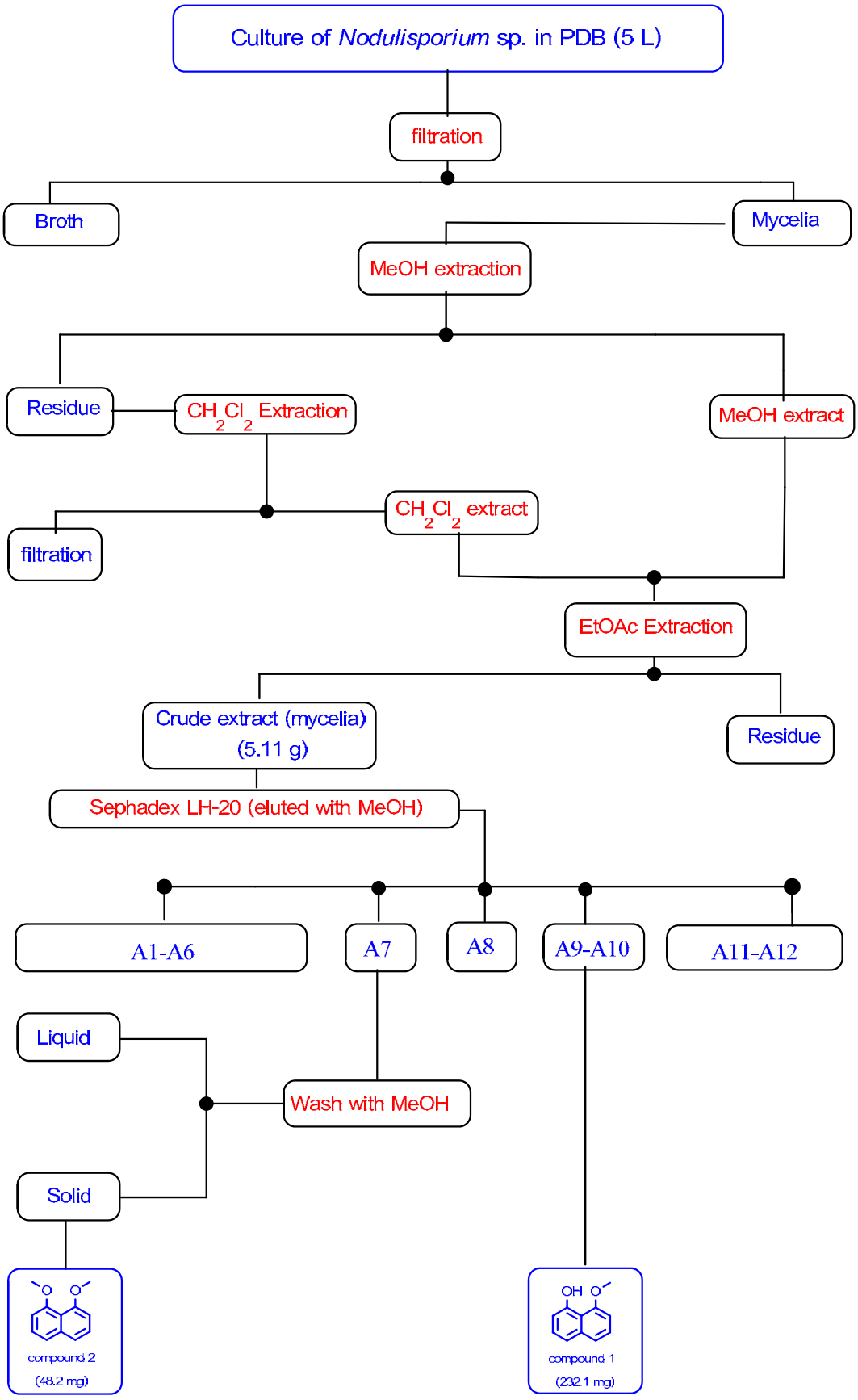
3.3.1.1 Fermentation

The fungus *Nodulisporium* sp. CRI 352-01 was fermented in 20 x 1L Erlenmeryer flask each containing 250 mL of potato dextrose broth (PDB) dissolved in seawater (33 ppt) under static conditions at room temperature of 30 day. The fungal culture was filtered to botain the fermentation broth (5L) and mycelia.

3.3.1.2 Isolation of a mycelial extract of *Nodulisporium* sp. CRI352-01

Mycelia were filtered from the broth and subsequently extracted by soaking in MeOH (500 mL, 2 day) and CH_2Cl_2 (500 mL, 3 day). The MeOH and CH_2Cl_2 extracts were combined and water (100 mL) was added. The mixture was extracted with EtOAc (500 mL x 5) to obtain a black solid (5.11 g). Crude extract from mycelia was passed through a Sephadex LH-20 column (3 x 85 cm) using MeOH as eluent to yield twelve fractions (A1-A12). Fractions A9 and A10 provided pure compound **1** (232.1 mg), while fraction A7 was further purified by washing with MeOH to provide compound **2** (48.2 mg).

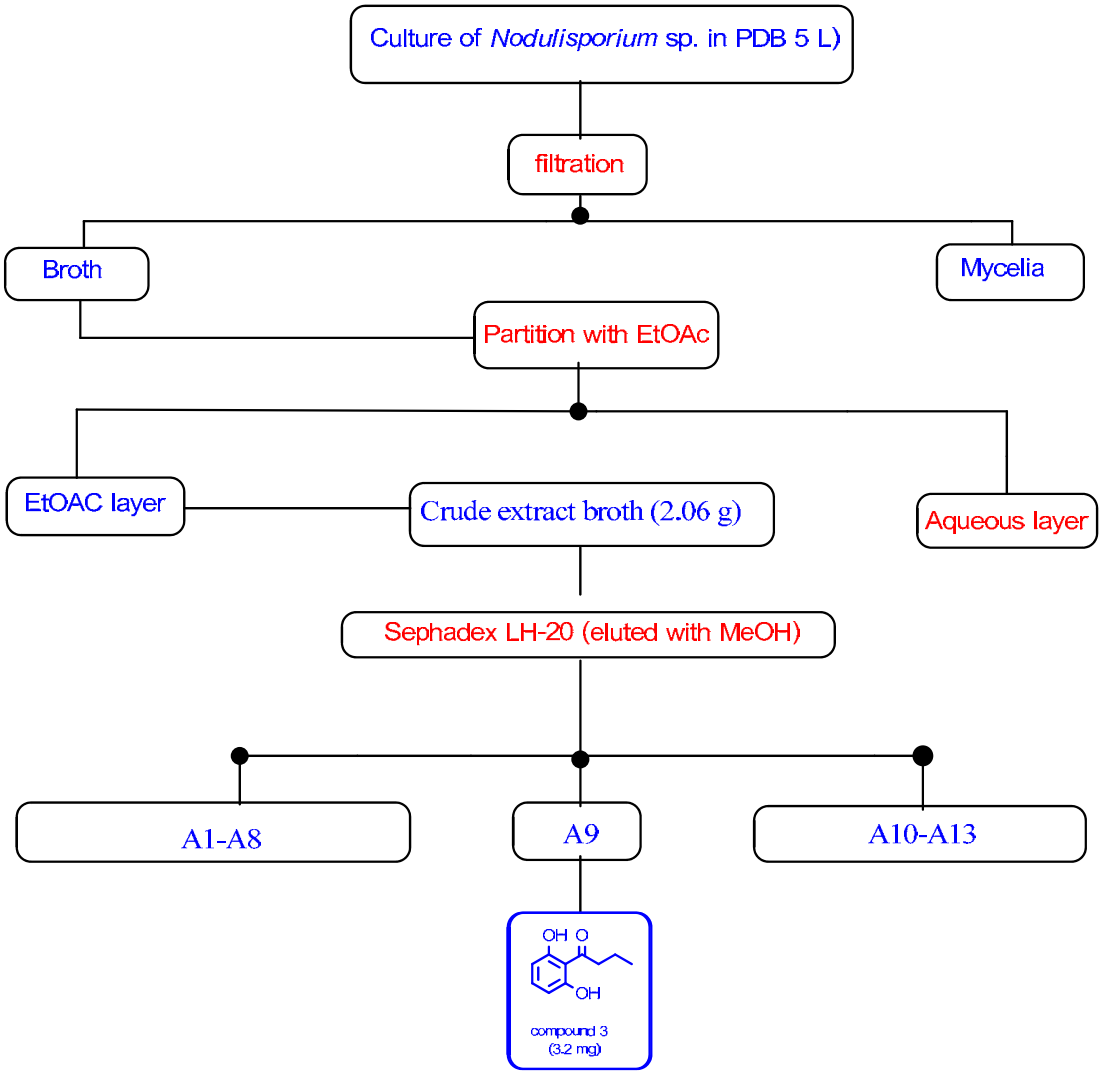
Chart 3.1: The extraction and isolation of compounds **1** and **2** from the fungal extract.



3.3.1.3 Isolation of a broth extract of *Nodulisporium* sp. CRI352-01

A broth extract of the fungus *Nodulisporium* sp. CRI352-01 was partitioned with EtOAc (200 mL x 3) to obtain a black viscous oil (2.06 g). A crude extract was separated by a Sephadex LH-20 column (3x85 cm) using MeOH as eluent to yield thirteen fractions (A1-A13). Fraction A9 was further purified by silica gel column chromatography (EtOAc:hexane, 1:9) to provide compound **3** (3.2 mg) as a white solid.

Chart 3.2: The extraction and isolation of compound **3** from fungal extract.



3.3.2 *Aspergillus unguis*

3.3.2.1 Fermentation

The fungus *Aspergillus unguis* CRI282-03 was fermented in 20 x 1L Erlenmeryer flask each containing 250 mL of potato dextrose broth (PDB) in seawater (33 ppt) under static conditions at room temperature of 30 days. The fungal culture was filtered to botain the fermentation broth (5L) and mycelia.

3.3.2.2 Isolation of a mycelial extract of *Aspergillus unguis* CRI282-03

Mycelia were filtered from broth and subsequently extracted by soaking in MeOH (500 mL, 2 day) and CH_2Cl_2 (500 mL, 3 day). The MeOH and CH_2Cl_2 extracts were combined and water (100 mL) was added. The mixture was extracted with EtOAc (500 mL x 5) to obtain a brown solid (7.19 g). Crude extract from mycelia was passed through a Sephadex LH-20 column (4.0x45 cm) using MeOH as eluent to yield twelve fractions (A1-A12). Fraction A10 provided nidulin (**5**) (159.7 mg). Fraction A6 (1,577.5 mg) was rechromatographed on Sephadex LH-20 (MeOH as eluent) to give eight fractions (B1-B8). Fraction B5 was further purified by washing with CH_2Cl_2 to provide a liquid residue, which was further purified with si gel column chromatography (EtOAc:hexane, gradient elution from 10:90 to 40:60 and MeOH:CH_2Cl_2 (10:90)) to yied fourteen fractions (C1-C4). Fraction C4 was further purified by preparative TLC (EtOAc:hexane (10:90)) to give eight bands (D1-D8); band D6 provided compound **6** (4.6 mg).

Chart 3.3: The extraction and isolation of nidulin (**5**) from the fungal extract.

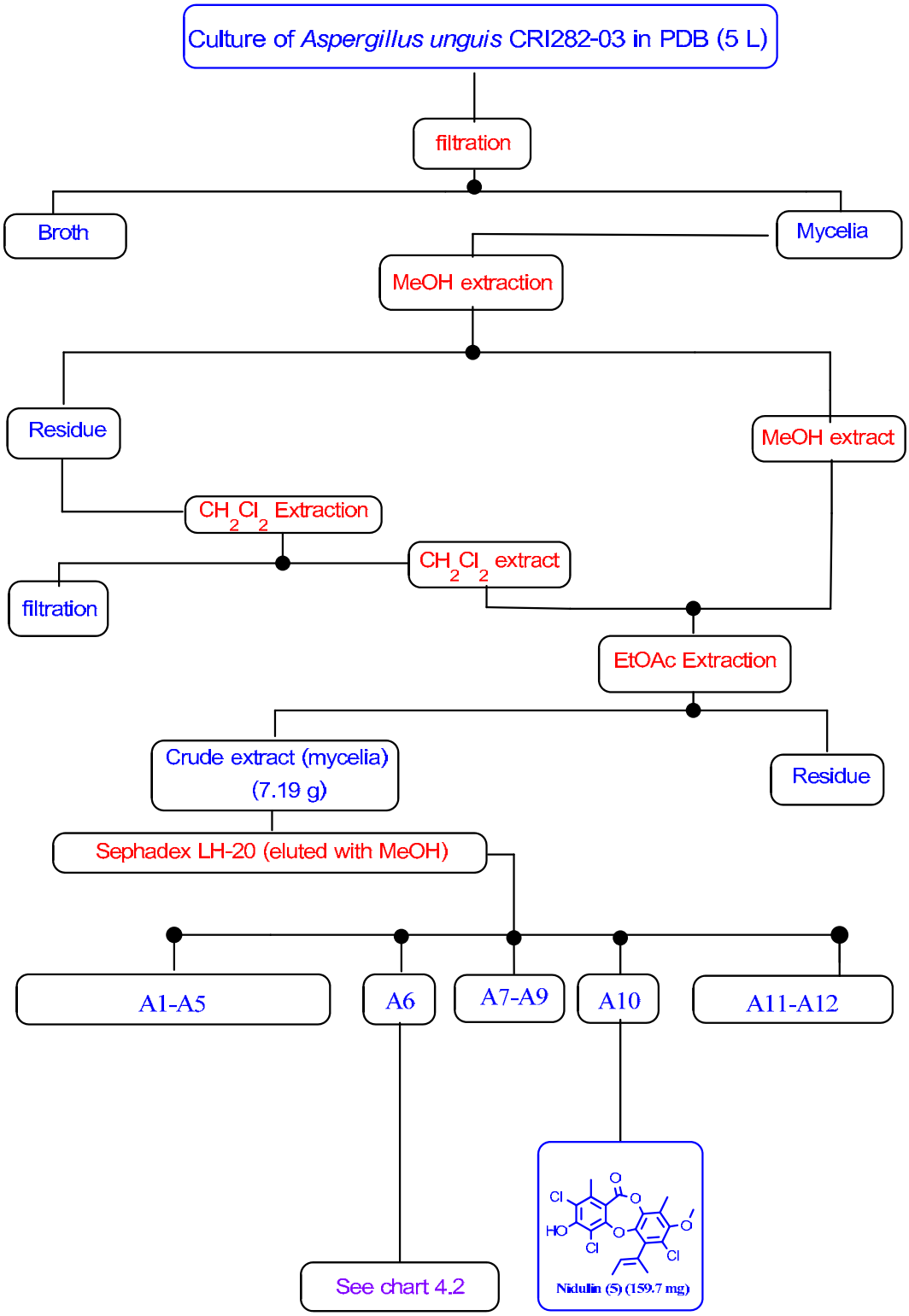
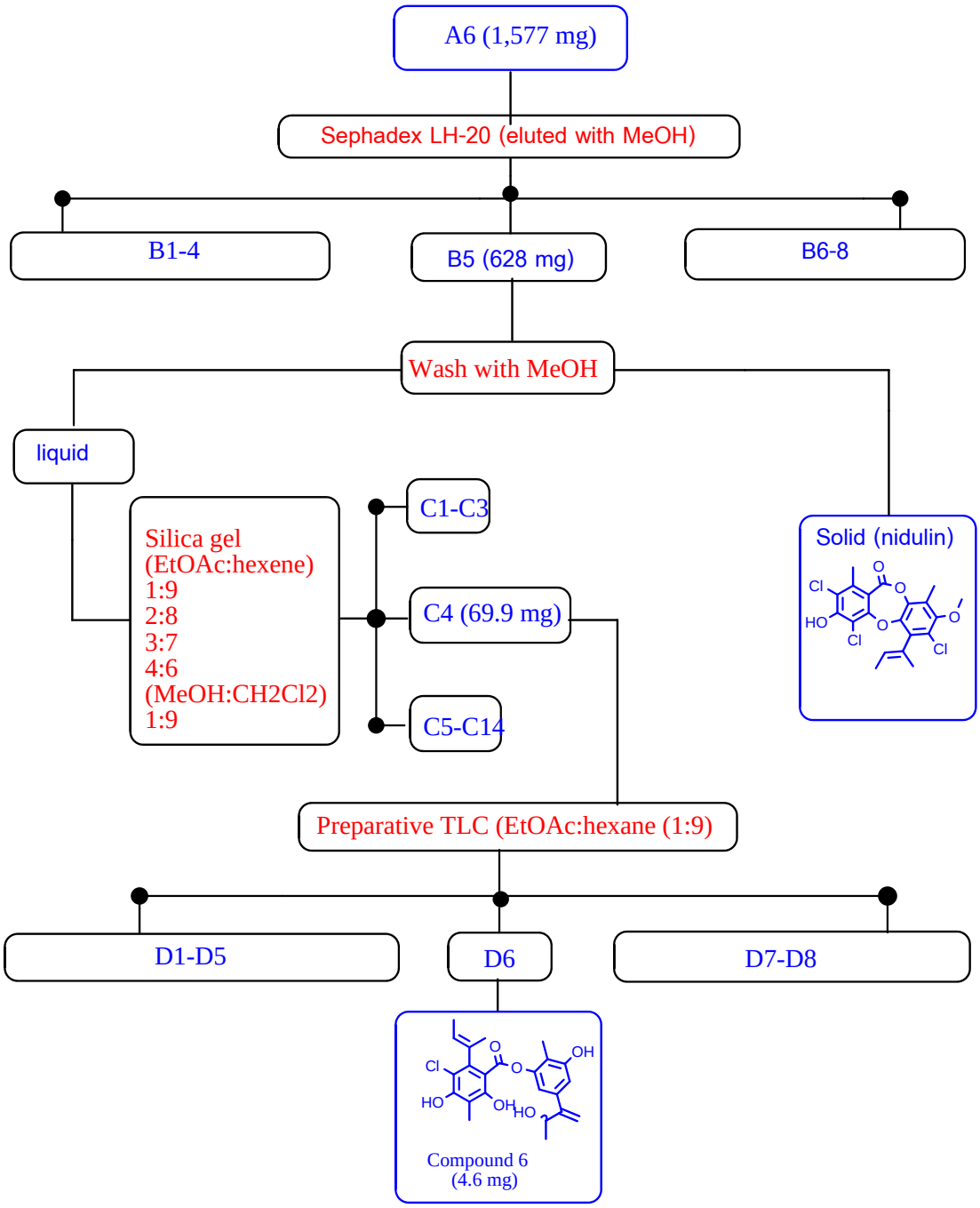


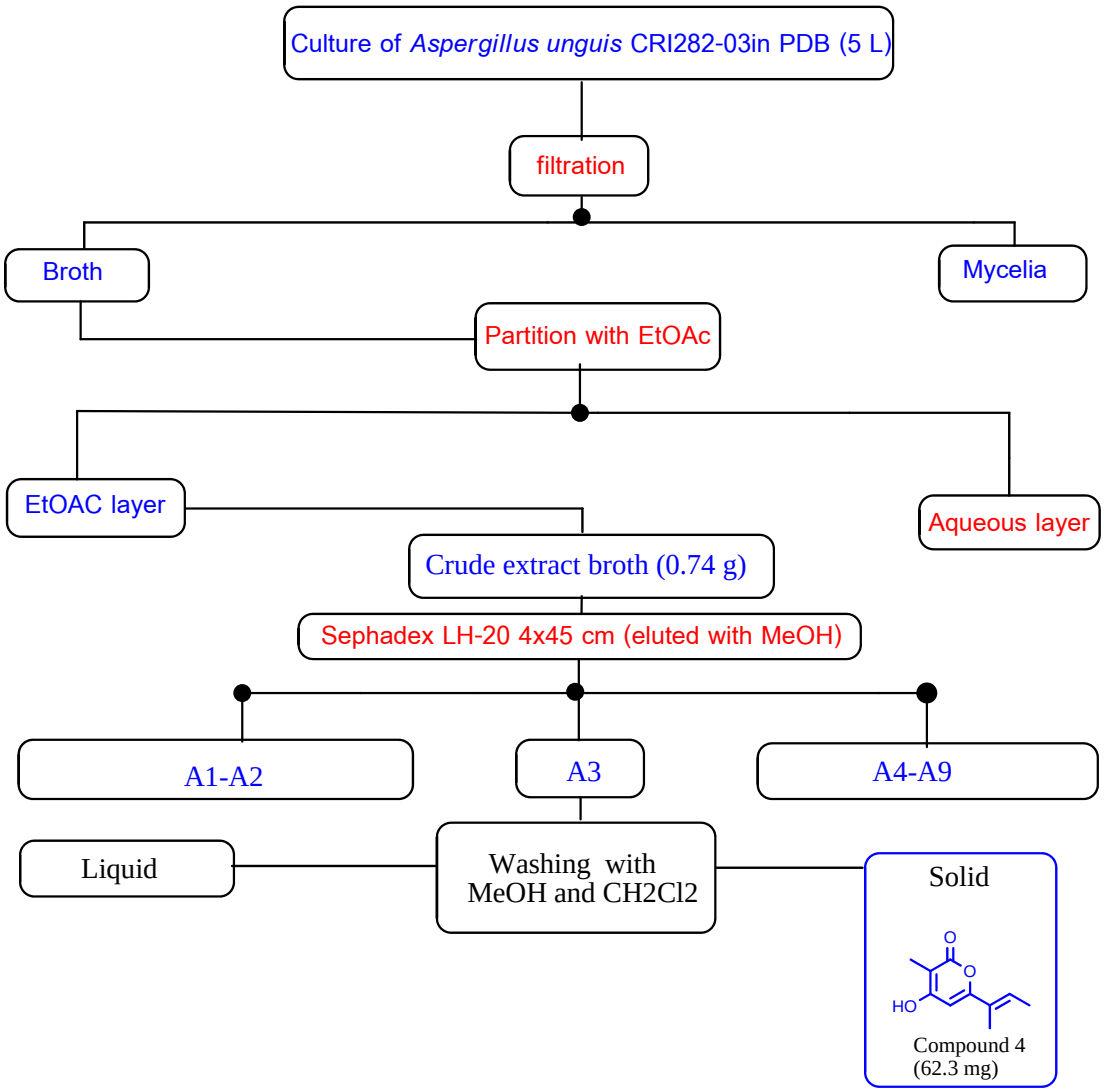
Chart 3.4: The isolation of compound 6.



3.3.2.3 Isolation of a broth extract of *Aspergillus unguis* CRI282-03

A broth extract of the fungus *Aspergillus unguis* CRI282-03 was partitioned with EtOAc (200 mL x 3) to obtain a brown solid (0.74 g). A crude extract was separated by Sephadex LH-20 column (4 x 45 cm) using MeOH as eluent to yield nine fractions (A1-A9). Fraction A3 was further purified by washing with MeOH and CH_2Cl_2 to yield a solid residue identified as compound **4** (62.3 mg).

Chart 3.5: The extraction and isolation of compound **4** from the fungal extract.



3.3.3 Penicilium citrinum

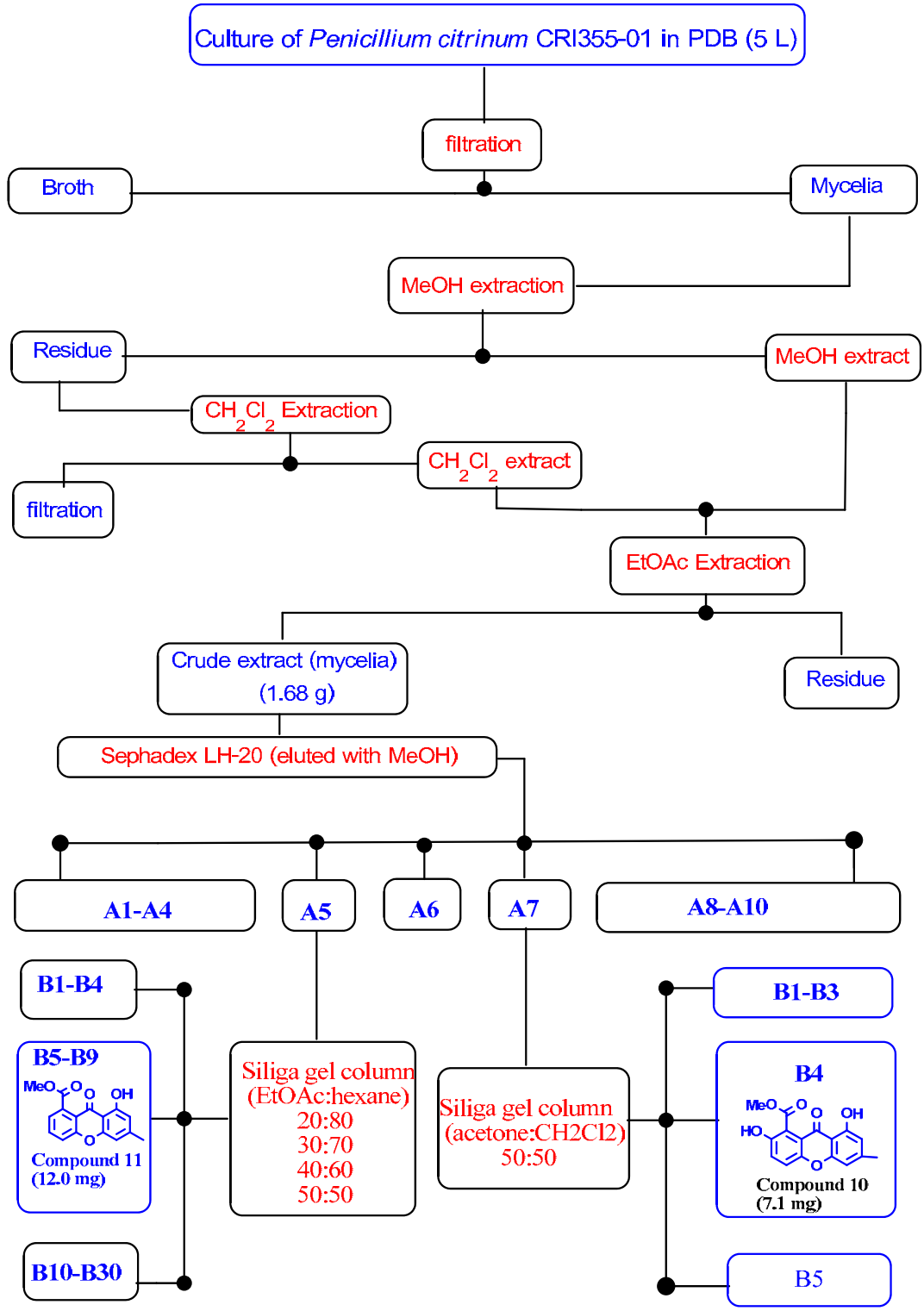
3.3.3.1 Fermentation

The fungus *Penicilium citrinum* CRI355-01 was fermented in 20 x 1L Erlenmeryer flask each containing 250 mL of potato dextrose broth (PDB) dissolved in seawater (33 ppt) under static conditions at room temperature of 30 days. The fungal culture was filtered to obtain the fermentation broth (5L) and mycelia.

3.3.3.2 Isolation of a mycelial extract of *Penicilium citrinum* CRI355-01

Mycelia were filtered from the broth and subsequently extracted by soaking in MeOH (500 mL, 2 day) and CH_2CL_2. The MeOH and CH_2Cl_2 extracts were combined and water (100 mL) was added. The mixture was extracted with EtOAc (500 mL x 5) to obtain a yellow solid (1.68 g). Crude extract from mycelia was passed through a Sephadex LH-20 column (3 x 85 cm) using MeOH as eluent to yield ten fractions (A1-A10). Fraction A5 was further purified by silica column (EtOAc:hexane, gradient elution from 20:80 to 50:50) to yield thirty fractions (B1 to B30). Fractions B5 to B9 provided compound **11** (12.0 mg), while fraction A7 was further purefied by silica column (acetone:CH_2Cl_2, 50:50) to yield five fractions (B1 to B5). Fraction B4 was compound **10** (7.1 mg).

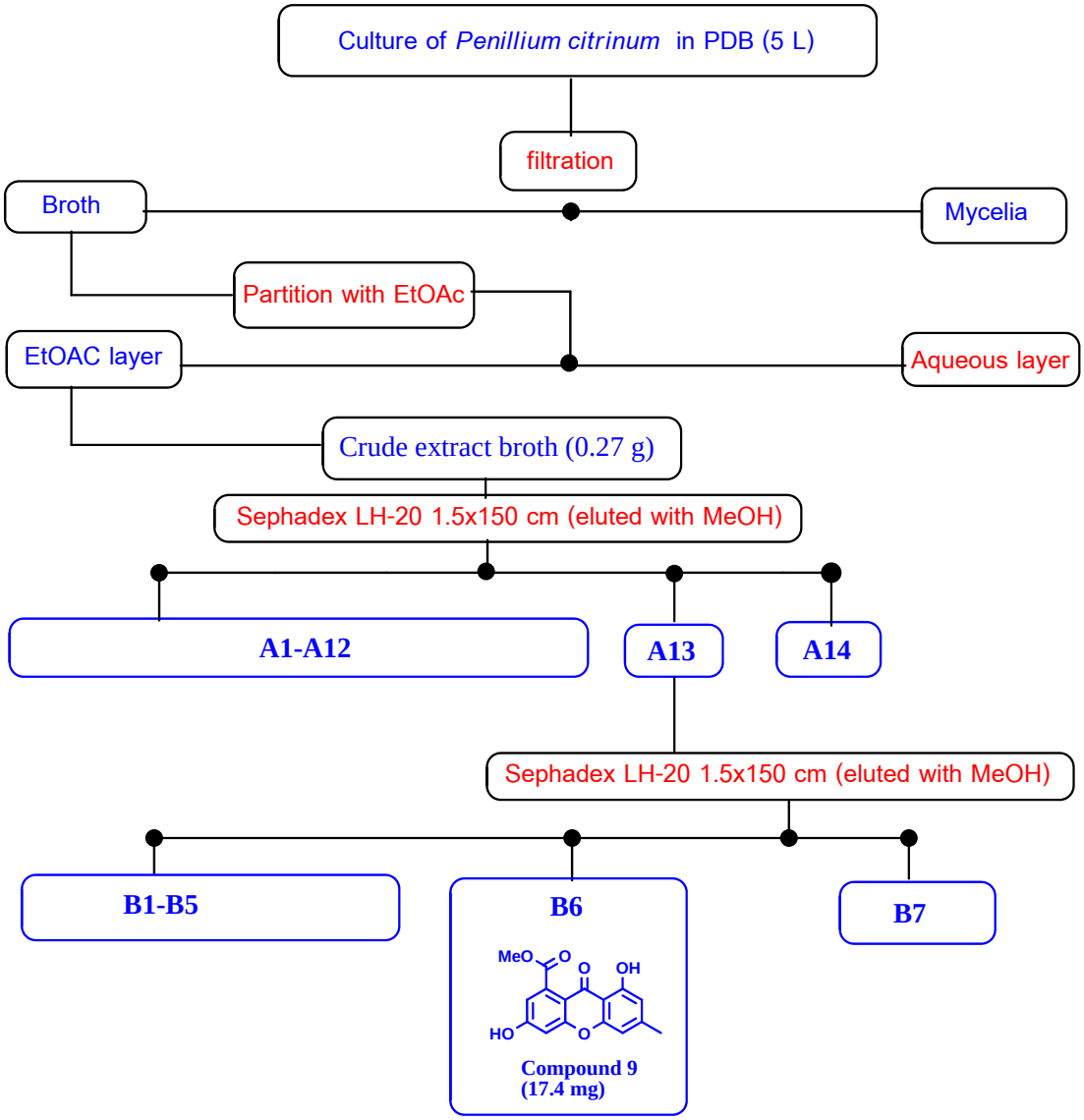
Chart 3.6: The extraction and isolation of pinselin (**10**) and compound **11** from the fungal extract.



3.3.3.3 Isolation of a broth extract of *Penicillium citrinum* CRI355-01

Broth of the fungus *Penicillium citrinum* CRI355-01 was extracted with EtOAc (200 mLx3) to yield fractions (A1-A14). Fraction A13 was rechromatographed on Sephadex LH-20 column (1.5x150 cm) to give seven fractions (B1-B7). Fraction B6 provided compound **9** (17.4 mg). The isolation procedure is shown in **Chart 3.6**.

Chart 3.7: The extraction and isolation of compound **9** from the fungal extract.

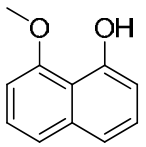


CHAPTER IV

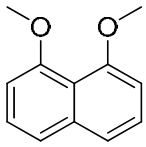
RESULTS AND DISCUSSION

4.1 Bioactive compounds from the fungus *Nodulisporium* sp.

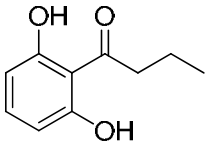
The structures of compounds **1**, **2** and **3** isolated from the fungus *Nodulisporium* sp. CRI352-01 were elucidated by analysis of NMR, IR and MS spectral data.



1



2



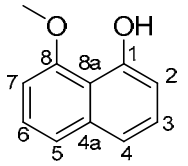
3

Compound **2** showed cytotoxic activity against HepG-2 cell line with IC_{50} value of 20.0 µg/mL while it was inactive (at 50µ g/mL) against HuCCA-1, A549, and MOLT-3 cell lines. Compound **3** showed cytotoxic activity against HepG-2 and MOLT-3 cell line with IC_{50} value of 23.5 and 16.08 µg/mL, respectively. But it was inactive (at 50 µg/mL) against HuCCA-1 and A549 cell lines. Compound **1** was inactive (at 50 µg/mL) towards all cell lines tested (**Table 4.1**).

Table 4.1 Cytotoxicities of compounds **1**, **2** and **3**.

Compound	Cytotoxicity (IC_{50}, µg/mL)			
	HuCCA-1	A549	HepG-2	MOLT-3
1	Inactive	Inactive	Inactive	Inactive
2	Inactive	Inactive	20.0±28.3	Inactive
3	Inactive	Inactive	23.5±2.12	16.0±1.42
Doxorubicin	0.35±0.141	0.225±0.35	0.26±0.08	-
Etoposide	-	-	11.67±2.88	0.021±0.001

4.1.1 Structure elucidation of compound 1



1

Compound **1** was obtained as a black solid. Its molecular $C_{11}H_{10}O_2$ was determined by APCI-TOF-MS (*m/z*: 175.0749 $[M+H]^+$, calcd 175.0759). Its IR spectrum exhibited absorption bands at 3364 and 3059 cm^{-1}, indicative of hydroxyl and arenes groups.The UV spectrum of compound **1** suggested that it contained a typical naphthalene[9] system with maximum absorptions at 227 and 317 nm. The ^1H NMR data (**Table 4.2**) showed characteristic signals of one methoxy group at δ_H 4.02 (3H, s), six aromatic protons at δ_H 6.76 (1H, d, *J*=7.7 Hz), 6.93 (1H, d, *J*=7.6 Hz), 7.31 (1H, t, *J*=8.1 Hz), 7.33 (1H, d, *J*=8.1 Hz), 7.38 (1H, t, *J*=7.8 Hz), and 7.44 (1H, d, *J*=8.3 Hz) and one hydroxyl proton group at δ_H 9.37 (1H, s). The ^1H-^1H COSY spectrum showed a correlation of two pairs of ABC aromatic spin systems. ^{13}C NMR and DEPT data for compound **1** displayed 11 carbon signals comprising 1 methoxy, 6 methines and 4 quaternary carbons.

The ^1H-H COSY spectrum showed the connectivities of H-2/H-3/H-4 and H-5/H-6/H-7. The HMBC spectrum of **1** revealed the correlations from H-2 to C-1, C-3, C-4 and C-8a; H-3 to C-1, C-2, C-4 and C-4a; H-4 to C-4a, C-5 and C-8a; H-5 to C-4, C-4a and C-8a; H-6 to C-4a, C-5, C-7 and C-8; H-7 to C-5, C-6, C-8 and C-8a; 1-OH to C-1, C-2 and C-8a, and 8-OMe to C-8. On the basis of these data, the structure of compound **1**was established as shown. Compound **1** was a known fungal metabolite, known as 8-methoxynaphthalen-1-ol previously from *Nodulisporium* sp.[4],[8]

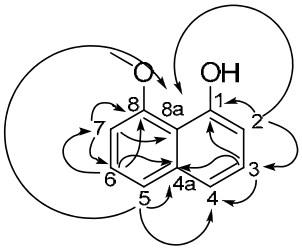
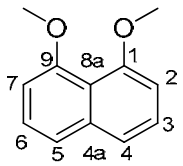


Figure 13 Selected HMBC correlations of compound **1**

Table 4.2 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data ($CDCl_3$) of compound **1**.

position	δ_C	δ_H (mult., *J* in Hz)
1	154.55	-
2	110.44	6.93 (1H, d, 7.6)
3	127.74	7.38 (1H, t, 7.8)
4	118.89	7.33 (1H, d, 8.1)
4a	136.79	-
5	121.86	7.44 (1H, d, 8.3)
6	125.64	7.31 (1H, t, 8.1)
7	103.95	6.76 (1H, d, 7.7)
8	156.18	-
8a	115.11	-
8-OMe	56.08	4.02 (1H, s)

4.1.2 Structure elucidation of compound 2



2

Compound **2** was obtained as a black solid. Its molecular $C_{12}H_{12}O_2$ was determined by APCI-TOF-MS (*m/z*: 189.0908 $[M+H]^+$, calcd 189.0916). Its IR spectrum

exhibited absorption band at 3066 cm^{-1}, indicative C-H stretct of arenes groups. The UV spectrum of compound **2** suggested that it contained a typical naphthalene[9] system with maximum absorptions at 239 and 317 nm. The ^{1}H NMR data of compound **2** (**Table 4.3**) showed characteristic signals of one methoxy group at δ_H 3.98 (3H, s) and three coupled aromatic protons at δ_H 6.86 (1H, d, *J*=7.5 Hz), 7.37 (1H, t, *J*=8.0 Hz) and 7.41 (1H, d, *J*=7.1 Hz), which were mutually coupled on the ^{1}H-^{1}H COSY spectrum. The ^{13}C NMR and DEPT data for compound **2** displayed 7 carbon signals comprising 1 methoxy, 3 methines and 3 quaternary carbons.

The ^{1}H-H COSY spectrum showed a correlation of ABC spin system, H-2/H-3/H-4. The ^{1}H NMR spectrum revealed that signals of H-5, H-6, and H-7 were overlapping. Careful analysis of the ^{13}C NMR spectrum of compound **2** implied that it was a methylated derivative of compound **1**; both compounds **1** and **2** had similar ^{13}C NMR signals except an additional O-Me group in compound **2**. The HMBC spectrum of compound **2** revealed the correlations from H-2 to C-8a, C-1, C-3 and C-4; H-3 to C-1, C-2, C-4 and C-4a; H-4 to C-2, C-3, C-5 and C-8a; and 8-OMe to C-8. Based upon these NMR data, as well as the molecular formular obtained from the mass spectrum, compound **2** was identified as 1,8-dimethoxynaphthalene which was previously isolated from *Nodulisporium* sp. [4],[8]

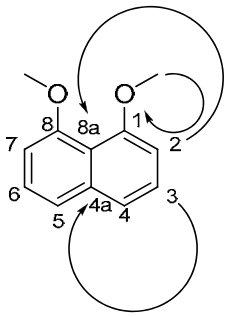


Figure 14 Selected HMBC correlations of compound **2**

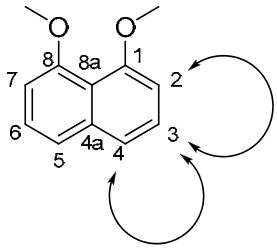
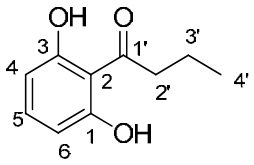


Figure 15 ^{1}H-^{1}H COSY correlations of compound **2**

Table 4.3 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data ($CDCl_3$) of compound **2**.

position	δ_C	δ_H (mult., *J* in Hz)
1	157.12	-
2	106.24	6.86 (1H, d, 7.5)
3	126.35	7.73 (1H, t, 8.0)
4	120.86	7.14 (1H, d, 7.1)
4a	137.41	-
5	120.86	7.14 (1H, d, 7.1)
6	126.35	7.37 (1H, t, 8.0)
7	106.24	6.86 (1H, d, 7.5)
8	157.12	-
8a	117.64	-
1-OMe	56.46	3.98 (3H, s)
8-OMe	56.46	3.98 (3H, s)

4.1.3 Structure elucidation of compound 3



3

Compound **3** was obtained as a white amorphous powder. Its molecular $C_{10}H_{12}O_3$ was determined by APCI-TOF-MS (*m/z*: 181.0858 $[M+H]^+$, calcd 181.0865). Its

IR spectrum exhibited absorption bands at 3278 and 1629 cm^{-1}, indicative of hydroxyl and carbonyl groups. The UV spectrum of compound **1** suggested that it contained a typical dihydroxyphenyl[5] system with the maximum absorption at 269 nm.The ^{1}H NMR data of **3** (**Table 4.4**) showed characteristic signals of one methyl group at δ_H 0.92 (3H, t, *J*=7.4 Hz), two methylene protons at δ_H 1.68 (2H, q, *J*=7.3 Hz) and δ_H 3.05 (2H, t, *J*=7.3 Hz), two aromatic protons at δ_H 6.33 (2H, d, *J*=8.2 Hz) and 7.15 (1H, t, *J*=8.1 Hz), which were mutually coupled on the ^{1}H-^{1}H COSY spectrum. The ^{13}C NMR and DEPT data for compound **3** displayed 10 carbon signals comprising 1 methyl, 2 methylene, 3 methine and 4 quaternary carbons.

The ^{1}H-H COSY spectrum showed a correlation of ABC spin system, H-4/H-5/H-6, and established a partial structure of H-2'/H-3'/H-4' The HMBC spectrum of compound **3** revealed the correlations from H-2' to C-4', C-3', C-1' and C-2'; H-3' to C-4', C-2' and C-1'; H-4' to C-3' and C-2'; H-6 to C-2 and C-4; and H-5 to C-1. On the basis of these data, the structure of compound **3** was established as shown. Compound **3** was a known fungal metabolite, known as 1-(2,6-dihydroxyphenyl) propan-1-one, previously isolated from *Nodulisporium* sp. [4],[8]

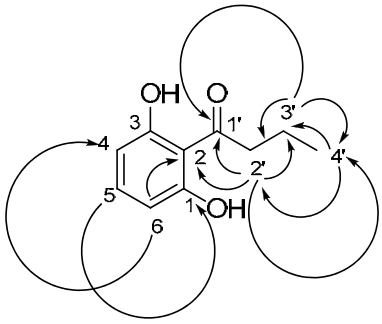


Figure 16 Selected HMBC correlations of compound **3**

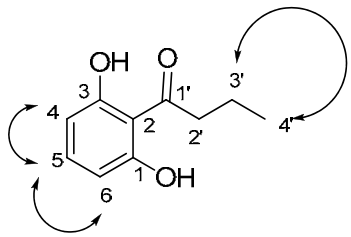
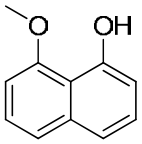


Figure 17 ^{1}H-^{1}H COSY correlations of compound **3**

Table 4.4 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data ($CDCl_3$) of compound **3**.

position	δ_C	δ_H (mult., *J* in Hz)
1	161.17	-
2	110.10	-
3	161.17	-
4	108.43	6.33 (1H, d, 8.2)
5	135.61	7.15 (1H, t, 8.1)
6	108.43	6.33 (1H, d, 8.2)
1'	207.76	-
2'	46.66	3.05 (2H, t, 7.3)
3'	17.79	1.67 (2H, q, 7.3)
4'	13.87	0.92 (3H, t, 7.4)

Compound 1



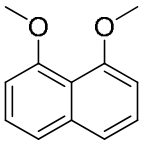
$C_{11}H_{10}O_2$

Black solid

m.p.	53-54 C°
UV (MeOH) λ_{max} (log ε)	332 (3.86), 317 (3.77), 302 (3.77) and 227 (4.50) nm
HRMS (APCI-TOF MS) *m/z*	175.0749 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 175.0759)
IR (UATR-solid) ν_{max}	3386, 3364, 3015, 3059, 2947, 2844, 1920, 1740, 1629, 1583, 1611, 1514, 1452, 1401, 1307, 1258, 1196, 1119, 1074, 1028, 964, 812, 753 and 673 cm^{-1}

^1H NMR and ^{13}C NMR data in $CDCl_3$: see **Table 4.2/ Page 51**

Compound 2



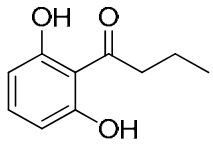
$C_{11}H_{10}O_2$

Black solide

m.p.	161-162 C°
UV (MeOH) λ_{max} (log ε)	331 (3.77), 317 (3.79), 302 (3.80), 239 (3.95) and 214 (3.86) nm
HRMS (APCI-TOF MS) *m/z*	189.0908 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 189.0916)
IR (UATR-solid) ν_{max}	3066, 3003, 2956, 2837, 1581, 1428, 1463, 1387, 1275, 1111, 1055, 1091, 1055, 997, 813, 767, 756 and 685 cm^{-1}

^1H NMR and ^{13}C NMR data in $CDCl_3$: see **Table 4.3/ Page 53**

Compound 3



$C_{10}H_{12}O_3$

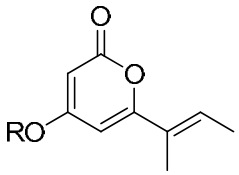
White amorphous powder

UV (MeOH) λ_{max} (log ε)	340 (3.17), 269 (3.74) and 224 (3.83) nm
HRMS (APCI-TOF MS) *m/z*	181.0858 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 181.0865)
IR (UATR-solid) ν_{max}	3278, 2961, 2928, 2873, 1629, 1586, 1448, 1386, 1357, 1339, 1234, 1215, 1166, 1038, 990, 902, 794, 745 and 725 cm^{-1}

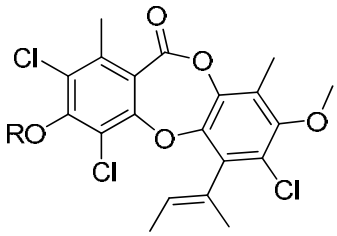
^1H NMR and ^{13}C NMR data in $CDCl_3$: see **Table 4.4/ Page 55**

4.2 *Aspergillus unguis*

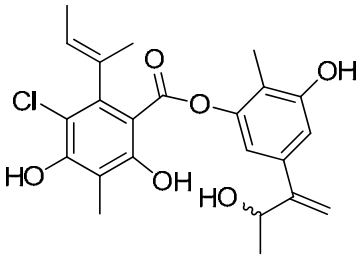
Compounds **4**, nidulin (**5**) and **6** were also isolated from the fungus *Aspergillus unguis* CRI282-03. Its structures were elucidated by analysis of NMR, IR, MS and X-ray spectral data. The functional groups -OH were replaced by –OMe through methylation of compounds **4** and **5**, giving the methylated products **7** and **8**, respectively.



R = H (**4**)
R = Me nectriapyrone (**7**)



R = H nidulin (**5**)
R = Me (**8**)



6

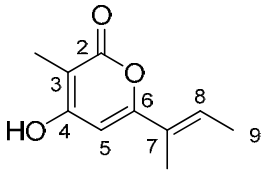
All compounds, except compounds **7** and **6**, were evaluated for their cytotoxicity against HuCCA-1, A549, HepG-2 and MOLT-3 cell lines (IC_{50} values are shown in **Table 4.5**). Nidulin (**5**) showed cytotoxic activity against MOLT-3 cell line (IC_{50} = 9.4 µg/mL), and compound **8** was active against MOLT-3 and HepG-2 cell lines with the IC_{50} values of 23.2 and 23.5 µg/mL, respectively. But compound **4** was inactive towards all lines tested.

Table 4.5 Cytotoxic and antimalarial activities of compounds **4**, nidulin (**5**) and **8**.

Compounds	Antimalarial activity (IC_{50}, μg/mL)	Cytotoxicity activity (IC_{50}, μg/mL)			
		HuCCA-1	A549	HepG-2	MOLT-3
4	-	inactive [a]	inactive	inactive	inactive
Nidulin (5)	Inactive(›10^{-5})	inactive	inactive	9.4±0.2	inactive
6	-	-	-	-	-
7	-	-	-	-	-
8	-	inactive	inactive	23.2±4.4	23.5±6.2
Chloroquine - hydrochloride	$2.98x10^{-7}$	-	-	-	-
Etoposide	-	-	-	11.67±2.88	0.021±0.001
Dexorubicin	-	0.35±0.212	0.38±0.035	0.26±0.08	-

[a] Inactive at 50 μg/mL.

4.2.1 Structure elucidation of compound 4



4

Compound **4** was isolated as a white solid. Its molecular formula was determined as $C_{10}H_{12}O_2$ according to APCI-TOF-MS (found *m/z*: 181.0860 $[M+H]^+$, calcd 181.0865). The IR spectrum of compound **4** showed absorption bands at 1614 cm^{-1}, indicative of a carbonyl group. The UV spectrum of compound **4** suggested that it contained a typical α- pyrone chromphore[37] with the maximum absorption at 280 nm.The ^1H NMR data of compound **4** (**Table 4.6**) showed signals of three methyl groups at δ_H 1.75 (3H, s), 1.77 (3H, s), and 1.78 (3H, d, 7.8 Hz). A set of signals at δ_H 6.09 (1H,

s) and 6.40 (1H, brq, 6.6 Hz) assingned to H-5 and H-8. The ^{1}H-^{1}H COSY spectrum showed a correlation of a methyl group proton at δ_H 1.78 (3H, d, 7.8 Hz, H-9) to a proton at 6.40 (1H, brq, 6.6 Hz, H-8). The ^{13}C NMR (**Table 4.6**) and DEPT data of compound **4** displayed 10 carbon signals, comprising 3 methyls, 2 methines and 5 quaternary carbons

HMBC correlations were observed from H-5 to C-3, C-4 and C-6; H-8 to C-6, C-7 and C-9; H-9 to C-7 and C-8; 3-Me to C-2, C-3 and C-4; and 7-Me to C-6, C-7 and C-8. On the basis of these spectroscopic data, the structure of compound **4** was established. Compound **4** was a derivative of nectriapyrone [38],[39] and its NMR data were similar to that of nectriapyrone.

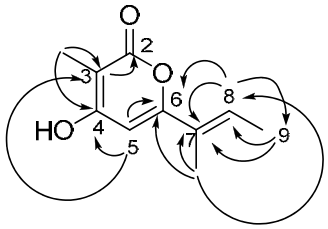
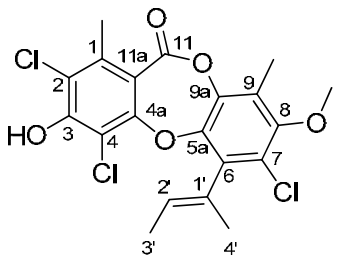


Figure 18 Selected HMBC correlations of compound 4

ble 4.6 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data (DMSO-d_6) of compound **4**.

position	δ_C	δ_H (mult., *J* in Hz)
1	-	-
2	164.16	-
3	97.75	-
4	164.81	-
5	97.06	6.09 (1H, s)
6	157.78	-
7	126.87	-
8	127.64	6.40 (1H, brq, 6.6)
9	11.69	1.78 (3H, d, 7.8)
3-Me	8.48	1.75 (3H, s)
7-Me	13.86	1.77 (3H, s)

4.2.2 Structure elucidation of nidulin (5)



nidulin (**5**)

Nidulin (**5**) was botained a colourless crystal. The molecular ions were observed at *m/z* 443, 445, 447 and 449 with the ratio of 30:29:9:1 suggesting the presence of three chlorine atoms. The molecular formula $C_{20}H_{17}Cl_3O_5$ was deduced from the APCI-TOF-MS spectrum (found *m/z*: 443.0229 $[M+H]^+$, calcd 443.0220). IR spectrum of nidulin (**5**) showed absorptions at 3380 and 1721 cm^{-1} indicative of hydroxyl and carbonyl ester groups. The UV spectrum of nidulin (**5**) suggested that it contained a typical depsidones[40] with maximum absorptions at 320 and 223 nm.The ^1H NMR data of nidulin (**5**) (**Table 4.7**) showed characteristic signals of four methyl groups at δ_H 1.72 (3H, d, 6.7 Hz), 1.87 (3H, s), 2.27 (3H, s) and 2.33 (3H,s), one methoxy proton at δ_H 3.75 (3H, s) and one methine proton at δ_H 5.35 (1H, dq, 1.1, 6.7 Hz), which showed correlation with methyl group proton at δ_H 1.72 in the ^1H-^1H COSY spectrum. The ^{13}C NMR (**Table 4.7**) and DEPT data for nidulin (**5**) displayed 20 carbon signals comprising 5 methyl, 1 methine and 14 quaternary carbons.

HMBC correlations of H-2' to C-1', C-2', C-3', C-4' and C-6; H-3' to C-2' and C-1'; H-4' to C-1', C-2' and C-6; 1-Me to C-1a, C-1 and C-2; 9-Me to C-8, C-9 and C-9a; and 8-OMe to C-8 were observed. On the basis of these data, three substructures were established as shown in **Figure 19**.

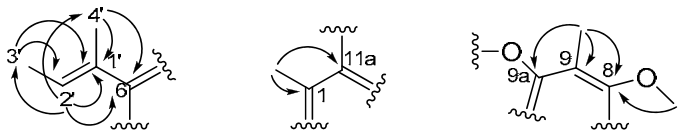


Figure 19 Selected HMBC correlations for substructures of nidulin (**5**)

However, the gross structure of nidulin could not be successfully established based upon available data. Fortunately, nidulin (**5**) could be crystallized to obtain an appropriate crystal for a single crystall X-ray crystallographic analysis; ORTEP plot of nidulin (**5**) is depicted in **Figure 20**. Finally, the structure of nidulin (**5**) was established as shown. Nidulin (**5**) was a known fungal metabolite, previously isolated from *Emericella unguis*. [41]

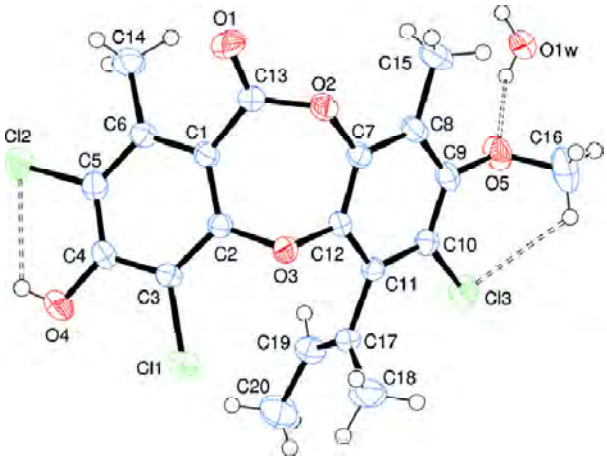
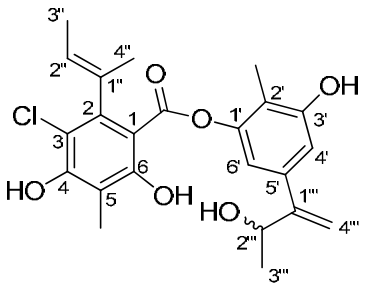


Figure 20 ORTEP plot of nidulin (**5**)

Table 4.7 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data (acetone-d_6) of nidulin (**5**).

position	δ_C	δ_H (mult., *J* in Hz)
1	138.66	-
2	123.16	-
3	162.50	-
4	111.41	-
4a	146.25	-
5a	158.55	-
6	135.99	-
7	123.99	-
8	152.25	-
9	123.36	-
9a	143.48	-
11	164.95	-
11a	103.17	-
1-Me	18.83	2.33 (3H, s)
9-Me	9.76	2.27 (3H, s)
8-OMe	60.08	3.74 (3H, s)
1'	130.04	-
2'	127.31	5.33 (1H, dq, 1.1, 6.7)
3'	13.64	1.71 (3H, d, 6.7)
4'	17.24	1.87 (3H, s)

4.2.3 Structure elucidation of compound 6



6

Compound **6** was botained a yellow solid. The molecular ions were observed at *m/z* 433 and 435 with the ratio of 3:1 implying the presence of one chlorine atom, and the molecular formula $C_{23}H_{25}ClO_6$ was indicated by the APCI-TOF-MS spectrum (found *m/z*: 433.1411 $[M+H]^+$, calcd 433.1412). IR spectrum of compound **6** showed absorptions at 3443 and 1660 cm^{-1} indicative of hydroxyl and carbonyl ester group. The UV spectrum of compound **6** suggested that it contained a typical depsidones[40] with the maximum absorptions at 331, 269 and 219 nm.The ^1H NMR data of compound **6** (**Table 4.8**) showed characteristic signals of five methyl groups at δ_H 1.22 (3H, dd, 6.4, 1.5 Hz), 1.72 (3H, dd,11.8, 5.2 Hz), 1.98 (3H, s), 1.99 (3H, s) and 2.16 (3H, s), two methine protons at δ_H 4.69 (1H, qui, 6.7 Hz) and 5.33 (1H, m), one exomethylene group at δ_H 5.20 (1H, brs) and 5.33 (1H, m), and one pair of meta-coupled aromatic protons at δ_H 6.68 (1H, dd, 8.4,1.2 Hz) and 6.92 (1H, d, 1.1 Hz). The ^1H-^1H COSY of compound **6** showed correlation between the doublet of doublet methyl at δ_H 1.22 (3H, dd, 6.4, 1.5 Hz) and an oxygenated methine at δ_H 4.69 (1H, qui, 6.7 Hz) while doublet of doublet methyl at δ_H 1.72 (3H, dd,11.8, 5.2 Hz) showed the correlation to a methine proton at δ_H 5.33 (1H, m). Two meta compled protons at δ_H 6.68 (1H, dd, 8.4,1.2 Hz) and 6.92 (1H, d, 1.1 Hz) were correlated to each other in the ^1H-^1H COSY spectrum as shown in **Figure 21**.

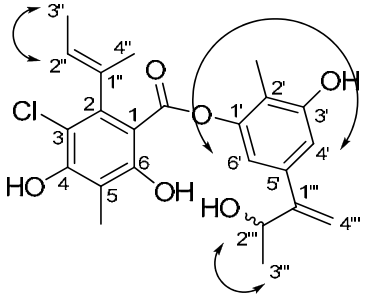


Figure 21 ^1H-^1H COSY correlations of compound **6**

The ^{13}C NMR (**Table 4.8**) and DEPT data for compound **6** displayed 23 carbon signals comprising 5 methyl, 4 methine, 1 methylene and 13 quaternary carbons.

HMBC correlations were observed from H-2" to C-1", C-2, C-3" and C-4'; H-3" to C-1" and C-2"; H-4" to C-1", C-2 and C-2"; H-2"" to C-1"", C-3"", C-4"" and C-5'; H-3"" to C-1"" and C-2""; H-4"" to C-1"", C-2"" and C-5'; H-4' to C-1"", C-2', C-3', C-5 and C-6'; H-6' to C-1', C-1"", C-2', C-4' and C-5'; 5-Me to C-4 and C-6; 2'-Me to C-1' and C-3'. On the basis of these data, four substructures were established as shown in **Figure 22**.

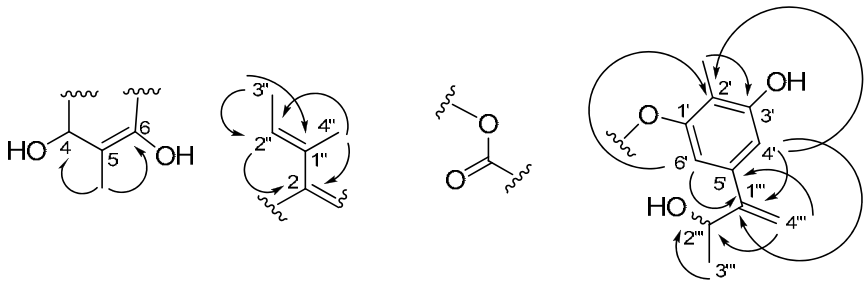


Figure 22 Selected HMBC correlations for substructures of compound **6**

The gross structure of compound **6** was finally established by NMR data correlation of guisinol[42] and compound **6**, assembling the four substructures to form a structure of compound **6** as shown. Compound **6** is new metabolite, whose structure is closely related to guisinol (**Figure 23**) and niludin (**5**).

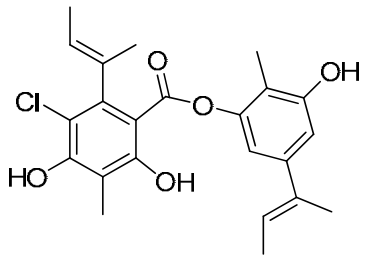


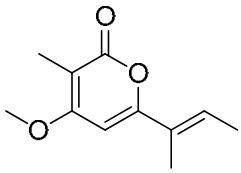
Figure 23 Structure of guisinol

Table 4.8 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data (acetone-d_6) of compound **6**.

position	δ_C	δ_H (mult., *J* in Hz)
1	105.99	-
2	144.96	-
3	112.15	-
4	156.86	-
5	113.20	-
6	161.0[a]	-
1'	156.40	-
2'	117.23	-
3'	150.78	-
4'	111.75	6.68 (1H, dd, 8.4, 1.2)
5'	140.02	-
6'	111.85	6.92 (1H, m)
CO	170.06	-
1''	136.20	-
2''	123.40	5.33 (1H, m)
3''	13.64	1.72 (3H, dd, 11.8, 5.2)
4''	17.21	1.98 (3H, s)
1'''	153.76	-
2'''	69.11	4.69 (1H, dd, 6.4, 1.5)
3'''	23.36	1.22 (3H, dd, 6.4, 1.5)
4'''	111.05	5.20, (1H, brs), 5.33 (1H, m)
5-Me	8.87	2.16 (3H, s)
2'-Me	9.37	1.99, (3H, s)

[a] These signals were assigned from the HMBC correlations, because they did not appear on the ^{13}C NMR spectrum.

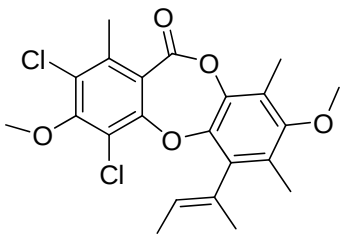
Methylation of compound 4



Methylated product 7

Compound **4** (15.0 mg) was dissolved in a mixture of MeI (1 mL), K_2CO_3 (30.0 mg) and DMF (30 µL). After stirring at room temperature for 18 h, a reaction mixture was evaporated under reduced pressure to obtain a white solid which was subsequently dissolved in EtOAc (20 mL) and washed with H_2O (10 mLX3). The organic layer was evaporated to dryness, yielding compound **7** (11.7 mg).

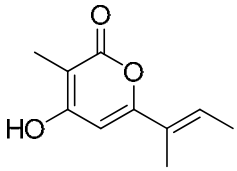
Methylation of nidulin (5)



Methylated product 8

Nidulin (**5**) (101.7mg) was dissolved in a mixture of MeI (1 mL), K_2CO_3 (30 mg) and DMF (30 µL). After stirring at room temperature for 21 h, a reaction mixture was evaporated under reduced pressure to obtain a white solid which was subsequently dissolved in EtOAc (20 mLx3) and washed with H_2O (10 mL). The organic layer was evaporated to dryness, yielding a white solid (66.2 mg). The EtOAc extract was chromatographed on silica gel column eluted with EtOAc:hexane (10:90) to obtain a methylated product **8** (40.8 mg).

Compound 4



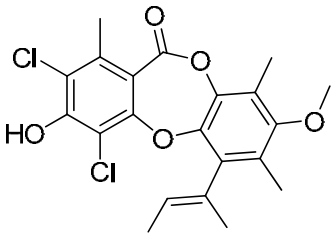
$C_{10}H_{12}O_3$

White solid

m.p	161-162 C° (decomposed)
UV (MeOH) λ_{max} (log ε)	320 (3.98), 280 (3.60), 271 (3.59) and 229 (4.54) nm
HRMS (APCI-TOF MS) *m/z*	181.0860 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 181.0865)
IR (UATR-solid) ν_{max}	2922, 2633, 1614, 1411, 1261, 1227, 1161, 1080, 1020, 866 and 819 cm^{-1}

^1H NMR and ^{13}C NMR data in DMSO-d_6: see **Table 4.6/ Page 60**

Nidulin (5)



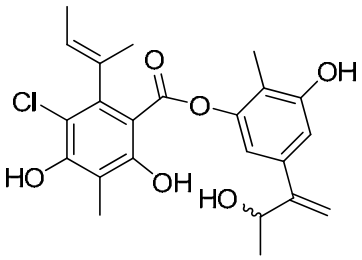
$C_{20}H_{17}Cl_3O_5$

Colourless crystal

m.p.	138-139 C°
UV (MeOH) λ_{max} (log ε)	320 (4.35) and 223 (4.65) nm
HRMS (APCI-TOF MS) *m/z*	443.0229 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 443.0220)
IR (UATR-solid) ν_{max}	3380, 2926, 1721, 1562, 1433, 1403, 1245, 1215, 1117, 1016, 989 and 808 cm^{-1}

^1H NMR and ^{13}C NMR data in acetone-d_6: see **Table 4.7/ Page 63**

Compound 6



6

$C_{23}H_{25}ClO_6$

Yellow solid

UV (MeOH) λ_{max} (log ε) 331 (3.43), 269 (3.91) and 219 (4.38) nm

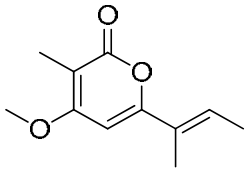
HRMS (APCI-TOF MS) *m/z* 433.1411 $[M + H]^+$

(calcd. for $[C_{11}H_{10}O_2+H]^+$ 433.1412)

IR (UATR-solid) ν_{max} 3443, 2973, 2925, 1660, 1411, 1310, 1266, 1159, 1109, 1068, 914, 799 and 738 cm^{-1}

^1H NMR and ^{13}C NMR data in acetone-d_6: see **Table 4.8/ Page 66**

Compound 7



$C_{11}H_{14}O_3$

Yellow viscous oil

UV (MeOH) λ_{max} (log ε) 326 (3.87) and 280 (4.31) nm

HRMS (APCI-TOF MS) *m/z* 195.1008 $[M + H]^+$

(calcd. for $[C_{11}H_{10}O_2+H]^+$ 195.1021)

IR (UATR-solid) ν_{max} 3400, 3086, 2923, 2853, 1682, 1552, 1380, 1354, 1257, 1234, 1172, 1009, 910, 817 and 748 cm^{-1}

^1H NMR and ^{13}C NMR data in $CDCl_3$

(Assignments are based on data correlation with those of compound **7**)

^{1}H NMR data ($CDCl_3$, 400 MHz)

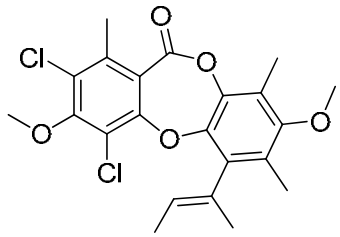
δ 6.69 (1H, dq, *J* = 7.2 Hz, H-8), 6.10 (1H. s, H-5), 3.90 (3H, s, 4-OMe), 1.93 (3H, s, 3-OMe), 1.88 (3H, s), 1.83 (3H, d, 7.1 Hz)

^{13}C NMR data ($CDCl_3$, 100 MHz)

δ 167[a] (C, C-4), 164[a] (C, C-2), 160[a] (C, C-6), 129.68 (CH, C-5), 56.02 (OMe), 1.93 (3H, s, 3-Me), 1.88 (3H, s), 1.83 (3H, d, 7.1 Hz)

[a] These signals were assigned from the HMBC correlations because they did not appear on the ^{13}C NMR spectrum.

Compound 8



$C_{21}H_{118}Cl_3O_5$

White crystal

m.p.	143-144 C°
UV (MeOH) λ_{max} (log ε)	299 (2.85) and 217 (4.31) nm
HRMS (APCI-TOF MS) *m/z*	457.0366 $[M + H]^+$
	(calcd. for $[C_{11}H_{10}O_2+H]^+$ 457.0376)
IR (UATR-solid) ν_{max}	2941, 2853, 1726, 1565, 1407, 1383, 1244, 1215, 1121, 993, 940, 870, 807 and 682 cm^{-1}

^{1}H NMR and ^{13}C NMR data in $CDCl_3$

(Assignments are based on data correlation with those of compound **8**)

^{1}H NMR data ($CDCl_3$, 600 MHz)

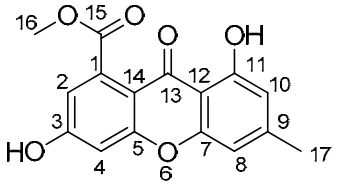
δ 4.42 (1H, dq, *J* = 6.8 Hz), 3.90 (3H, s), 3.79 (3H, s), 2.50 (3H, s), 2.32(3H, s), 1.96 (3H, s), 1.82 (3H, d, *J* = 6.7 Hz)

^{13}C NMR data ($CDCl_3$, 150 MHz)

δ 161.52 (C, C-11), 157.16 (C, C4a), 156.26 (C, C-3), 152.56 (C, C-8), 145.42 (C, C-9a), 141.62 (C, C-5a), 139.92 (C, C-1), 136.11 (C, C-1′), 129.36 (C, C-6), 128.17 (CH,C-2′), 127.94 (C, C-2), 124.39 (C, C-7), 123.35 (C, C-9), 120.01 (C, C-11a), 118.64 (C, C-4), 60.74 (3-OMe), 60.44 (8-OMe), 18.77 (1-Me), 17.43 (4′-Me), 14.08 (3′-Me), 10.35 (9-Me)

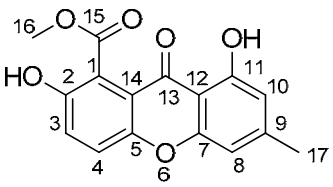
4.3 *Penicillium citrinum*

Chemical studies of the organic extract from the culture broth of *Penicillium citrinum* CRI 355-01 afforded compound **9**.

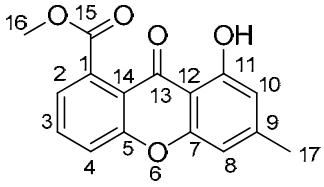


compound **9**

Compounds **10** and **11** were also isolated from the CH_2Cl_2 and MeOH cell extracts of *Penicillium citrinum* CRI355-01, respectively. The isolated compounds were structurally elucidated by analsis of NMR, IR and MS spectral data.



Compound **10**



Compound **11**

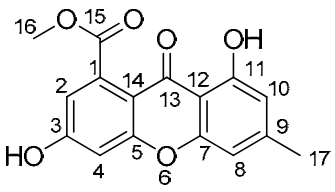
Compounds **9**, **10** and **11** were tested for its cytotoxic activity against HuCCA-1, A549, HepG-2 and MOLT-3 cancer cell lines and antimalarial activity. These bioactivity results are shown in **Table 4.9**

Table 4.9 Cytotoxic and antimalarial activities of compounds **9**, **10** and **11**.

Compounds	Antimalarial activity (IC_{50} µg/mL)	Cytotoxicity activity (IC_{50} µg/mL)			
		HuCCA-1	A549	HepG-2	MOLT-3
9	inactive (›10^{-5})	inactive	inactive	26.6± 3.2	31.5± 0.7
Pinselin (10)	-	inactive	inactive	inactive	inactive
11	Inactive(›10^{-5})	inactive	inactive	inactive	38.0±0.0
Chloroquine hydrochloride	- 2.98x10^{-7}	-	-	-	-
Etoposide	-	0.38±0.035	-	16.5±2.12	0.025±0.001
Dexorubicin	-	0.35±0.212	0.38±0.035	0.24±0.01	-

As shown in **Table 4.9**, compounds **9**, **10** and **11** were mostly inactive toward cancer cell lines tested or showed only marginal activity with IC_{50} ranges of 26.6-38.0 µg/mL. They did not exhibit antimalarial activity.

4.3.1 Structure elucidation of compound 9



Compound 9 was obtained as a pale yellow needle. Its molecular formula $C_{16}H_{12}O_6$ was determined by APCI-TOF-MS (*m/z*: 301.0708 $[M+H]^+$, calcd 301.0712). Its IR spectrum exhibited absorption bands at 3238, 1709 and 1651 cm^{-1}, indicative of hydroxyl and carbonyl groups. The UV spectrum of compound **9** suggested that it contained a typical xanthone[43] with maximum absorptions at 384, 294, 263 and 237 nm.The ^1H NMR data of compound **9** (**Table 4.10**) showed characteristic signals of one

methyl group at δ_H 2.40 (3H, s), one methoxy group at δ_H 3.91 (3H, s), two pairs of meta coupled aromatic protons at δ_H 6.57 (1H, s), 6.78 (1H, s), and 7.52 (1H, s) and a chelated hydroxyl group at δ_H 12.28 (1H, s). The ^{13}C NMR and DEPT data for compound **9** displayed 16 carbon signals comprising 1 methyl, 1 methoxy 4 methine, and 10 quaternary carbons (**Table 4.10**). ^{1}H-^{1}H COSY showed correlation of H-2 and H-4, and that of H-8 and H-10.

There were HMBC correlations from H-4 to C-13; H-8 to C-9, C-10, and C-17; H-10 to C-9, C-11, and C-12; H-17 to C-8, C-9, and C-10; H-16 to C-15; HO-11 to C-10, C-11, and C-12. On the basis of these spectroscopic data, and data comparison of compound **9** with those of 11-hydroxy-1-methoxycarbonyl-9-methyl-xanthone [43] and the structure of compound **9** was established as shown. Compound **9** was a known xanthone, methyl 3,8-dihydroxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylate, previously isolated from *Microspaeropsis* sp.[44]

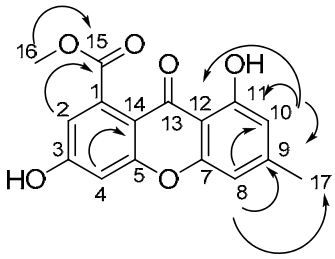


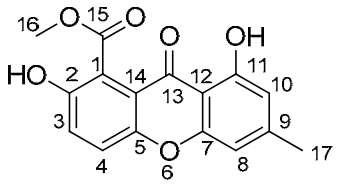
Figure 24 Selected HMBC correlations of compound **9**

Table 4.10 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data (acetone-d_6) of compound **9**.

position	δ_C	δ_H (mult., *J* in Hz)
1	149.58	-
2	124.97	7.52 (1H, s)
3	150.55	-
4	119.08	7.52 (1H, s)
5	150.55	-
6	-	-
7	155.86	-
8	107.09	6.78 (1H, s)
9	149.28	-
10	110.70	6.57 (1H, s)
11	166.56	-
12	107.15	-
13	180.00[a]	-
14	118.05	-
15	166.56	-
16	51.62	3.91 (3H, s)
17	21.49	2.40 (3H, s)
OH-11		12.28 (1H, s)

[a] These signals were assigned from the HMBC correlations, because they did not appear on the ^{13}C NMR spectrum.

4.3.2 Structure elucidation of pinselin (10)



Pinselin (**10**)

Pinselin (**10**) was obtained as a pale yellow needle. Its molecular formula $C_{16}H_{12}O_6$ was determined by APCI-TOF-MS us (*m/z*: 299.0558 $[M-H]^-$, calcd 299.0556). Its IR spectrum exhibited absorption bands at 3507, 1721 and 1650 cm^{-1}, indicative of hydroxyl and carbonyl groups. The UV spectrum of pinselin (**10**) suggested that it contained a typical xanthone[41] with maximum absorptions at 369, 292, 259 and 235 nm.The ^1H NMR data of pinselin (**10**) (**Table 4.11**) showed characteristic signals of an aromatic methyl group at δ_H 2.42 (3H, s), one methoxy group at δ_H 3.92 (3H, s), four coupled aromatic protons at δ_H 6.61 (1H, s), 6.83 (1H, s), 7.59 (1H, d, J = 9.3 Hz) and 8.24 (1H, d, J = 9.4 Hz), which were mutually coupled in the ^1H-^1H COSY spectrum, and a chelated hydroxyl group at δ_H 12.33 (1H, s). The ^{13}C NMR and DEPT data for pinselin (**10**) displayed 16 carbon signals comprising 1 methyl, 1 methoxy, 4 methine, and 10 quaternary carbons (**Table 4.11**). ^1H-^1H COSY showed correlation of H-3 and H-4, and that of H-8 and H-10.

HMBC correlations of H-3 to C-1, C-2 and C-5; H-4 to C-2, C-5 and C-14; H-8 to C-7 and C-10; H-10 to C-8, C-9, C-13 and C-17; HO-11 to C-10, C-11 and C-12; H-16 to C-15 and H-17 to C-8, C-9 and C-10. On the basis of these spectroscopic data, as well as data comparison with those of 11-hydroxy-1-methoxycarbonyl-9-methyl-xanthone,[43] the structure of pinselin (**10**) was established as shown. Pinselin (**10**) was a known ascomycetes metabolite, previously isolated from *Emericella quadrilineata.* [45]

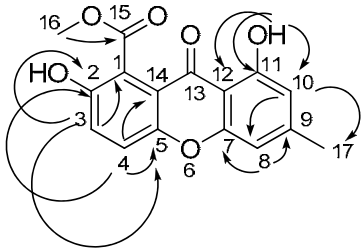


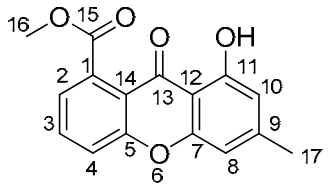
Figure 25 Selected HMBC correlations of pinselin (**10**)

Table 4.11 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data (acetone-d_6) of pinselin (**10**)

position	δ_C	δ_H (mult., *J* in Hz)
1	120.0[a]	-
2	148.0[a]	-
3	129.63	8.24 (1H, d, 9.4)
4	119.24	7.59 (1H, d, 9.3)
5	151.63	-
6	-	-
7	156.0[a]	-
8	107.24	6.83 (1H, s)
9	149.54	-
10	110.97	6.61 (1H, s)
11	161.28	-
12	106.0[a]	-
13	180.0[a]	-
14	116.0[a]	-
15	168.0[a]	-
16	52.07	3.92 (3H, s)
17	21.51	2.42 (3H, s)
OH-11	-	12.23 (1H, s)

[a] These signals were assigned from the HMBC correlations, because they did not appear on the ^{13}C NMR spectrum.

4.3.3 Structure elucidation of compound 11



11

Compound **11** was obtained as a white solid. Its molecular formula $C_{16}H_{12}O_5$ was determined by APCI-TOF-MS (*m/z*: 285.0762 $[M+H]^+$, calcad 285.0763). Its IR spectrum exhibited absorption bands at 1739 and 1651 cm^{-1}, indicative of carbonyl groups. The UV spectrum of compound **11** suggested that it contained a typical xanthone[41] with maximum absorptions at 365, 303, 259 and 239 nm.The ^{1}H NMR data of compound **11** (**Table 4.12**) showed characteristic signals of an aromatic methyl group at δ_H 2.42 (3H, s), one methoxy group at δ_H 4.03 (3H, s), aromatic protons at δ_H 6.62 (1H, d, *J* = 0.6 Hz), 6.74 (1H, d, *J* = 0.6 Hz), 7.30 (1H, dd, *J* = 1, *J* = 7.3 Hz), 7.52 (1H, t, *J* = 8.5 Hz) and 7.75 (1H, t, *J* = 7.3 Hz), which were mutually coupled in the ^{1}H-^{1}H COSY spectrum, and a chelated hydroxyl group at δ_H 12.14 (1H, s). The ^{13}C NMR and DEPT data for compound **11** displayed 16 carbon signals comprising 1 methyl, 1 methoxy, 5 methine, and 9 quaternary carbons (**Table 4.12**).^{1}H-^{1}H COSY showed correlation of H-8 and H-10.

HMBC correlation of H-2 to C-14 and C-15; H-3 to C-1 and C-5; H-4 to C-2, C-5 and C-14; H-8 to C-7, C-9 and C-17; H-10 to C-8, C-9, C-13 and C-17; HO-11 to C-10, C-11 and C-12; H-16 to C-15 and H-17 to C-8, C-9 and C-10. On the basis of these data, the structure of compound **10** was established as shown. Compound **11** was a known fungal metabolite, known as 11-hydroxy-1-methoxycarbonyl-9-methylxanthone, previously isolated from *Chalara* sp..[43]

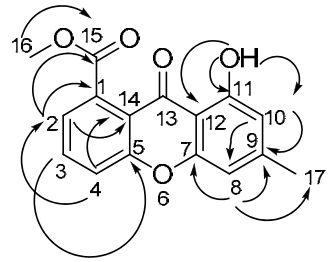
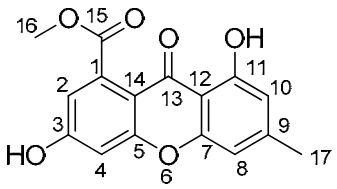


Figure 26 Selected HMBC correlation of compound **11**

Table 5.4 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$) of **11**).

position	δ_C	δ_H (mult., *J* in Hz)
1	133.05	-
2	122.44	7.30 (1H, dd, 1, 7.3)
3	134.73	7.75 (1H, t, 7.3)
4	119.36	7.52 (1H, dd, 1, 8.5)
5	155.61	-
6	-	-
7	155.91	-
8	107.35	6.74 (1H, d, 0.6)
9	149.36	-
10	11.67	6.62 (1H , d, 0.6)
11	161.38	-
12	106.90	-
13	180.38	-
14	117.49	-
15	169.65	-
16	53.08	4.03 (3H, s)
17	22.59	2.42 (3H, s)
OH-11		12.14 (1H, s)

Compound 9



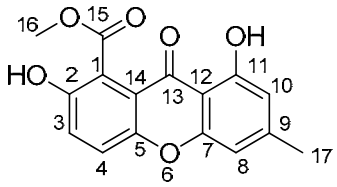
$C_{16}H_{12}O_6$

A pale yellow needle

m.p.	194-195 C°
UV (MeOH) λ_{max} (log ε)	384 (4.68), 294 (4.80), 263 (4.22), 237 and (4.06) nm
HRMS (APCI-TOF MS) *m/z*	301.0708 $[M+H]^+$ (calcd. for $[C_{16}H_{12}O_5+H]^+$ 301.0712)
IR (UATR-solid) ν_{max}	3238, 2953, 1709, 1651, 1608, 1502, 1435, 1366, 1319, 1290, 1231, 1208, 1084, 1017, 825, 720, and 737 cm^{-1}

^1H NMR and ^{13}C NMR data in acetone-d_6: see **Table 4.10/ Page 75**

Pinselin (10)



$C_{16}H_{12}O_6$

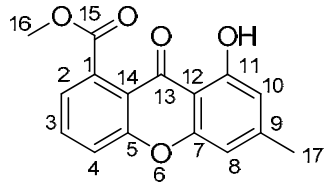
A pale yellow needle

m.p.	170-171 C°
UV (MeOH) λ_{max} (log ε)	369 (3.53), 292 (4.49), 259 (4.28), 235 (4.34) and 206 (4.49) nm
HRMS (APCI-TOF MS) *m/z*	299.0558 $[M-H]^-$ (calcd. for $[C_{16}H_{12}O_6-H]^-$ 299.0556)

IR (UATR-solid) ν_{max} 3507, 3093, 2925, 2854, 1721, 1650, 1619, 1602, 1483, 1440, 1368, 1248, 1206, 1172, 1137, 1060, 1018, 929, 851, 831, 754, 722, and 688 cm^{-1}

^{1}H NMR and ^{13}C NMR data in acetone-d_6: see **Table 4.11/ Page 77**

Compound 223



$C_{16}H_{12}O_5$

White solid

m.p. 189-190 C°

UV (MeOH) λ_{max} (log ε) 365 (3.84), 303 (4.24), 259 (4.61) and 239 (4.50) nm

HRMS (APCI-TOF MS) *m/z* 285.0762 $[M+H]^+$

(calcd. for $[C_{16}H_{12}O_5+H]^+$ 285.0763)

IR (UATR-solid) ν_{max} 2953, 2920, 1739, 1651, 1621, 1600, 1569, 1489, 1435, 1371, 1283, 1207, 1135, 1087, 1020, 912, 823, and 760 cm^{-1}

^{1}H NMR and ^{13}C NMR data in $CDCl_3$: see **Table 4.12/ Page 79**

CHAPTER V
CONCLUSION

In this thesis, the isolated bioactive compounds from marine-derived fungi (*Nodulisporium* sp., *Aspergillus unguis* and *Penicillium citrinum*) were evaluated for their antimalarial and cytotoxic activities.

Compounds **2** and **3** were produced by the fungus *Nodulisporium* sp.. Compound **3** showed cytotoxic activity against HepG-2 and MOLT-3 cell lines with IC_{50} values of 23.5 and 16.0 μg/mL, respectively. Compound **2** showed cytotoxic activity against HepG-2 cell line (IC_{50} 20.0 μg/mL).

Nidulin (**5**) and its *O*-methyl derivative **8** were produced by the fungus *Aspergillus unguis*. Anti-cancer activity of nidulin (**5**) against HepG-2 cell line was at IC_{50} 9.4 μg/mL. While compound **8** showed cytotoxic activity against HepG-2 and MOLT-3 cell lines with IC_{50} values of 23.2 and 23.5 μg/mL, respectively.

Compounds **9** and **11** were isolated from *Penicillium citrinum*. Compuond **9** exhibited cytotoxic activity against HepG-2 and MOLT-3 cell lines with IC_{50} values of 26.6 and 31.5 μg/mL, respectively. Compound **11** was active against MOLT-3 cell line (IC_{50} 38.0 μg/mL).

The present study proves that marine-derive fungi are rich sources of pharmacologically active compounds and structurally diverse metabolites. The marine-derived fungi are therefore an important source of natural products for the discovery of new drug candidates.

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APPENDIX

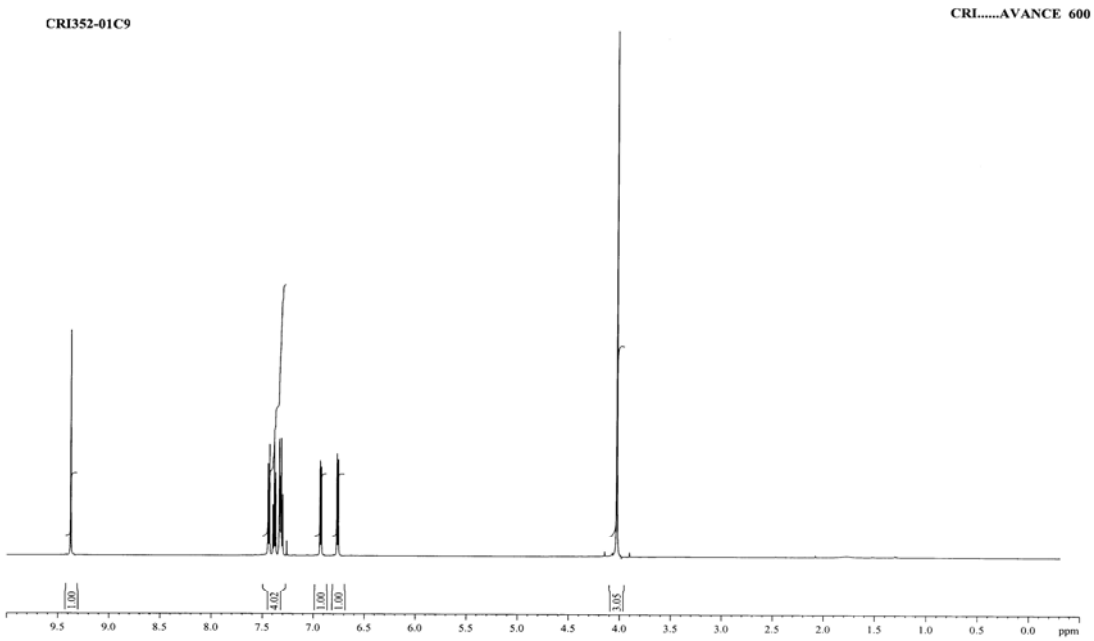

Figure 27 600 MHz ^{1}H-NMR ($CDCl_3$) spectrum of compound **1**

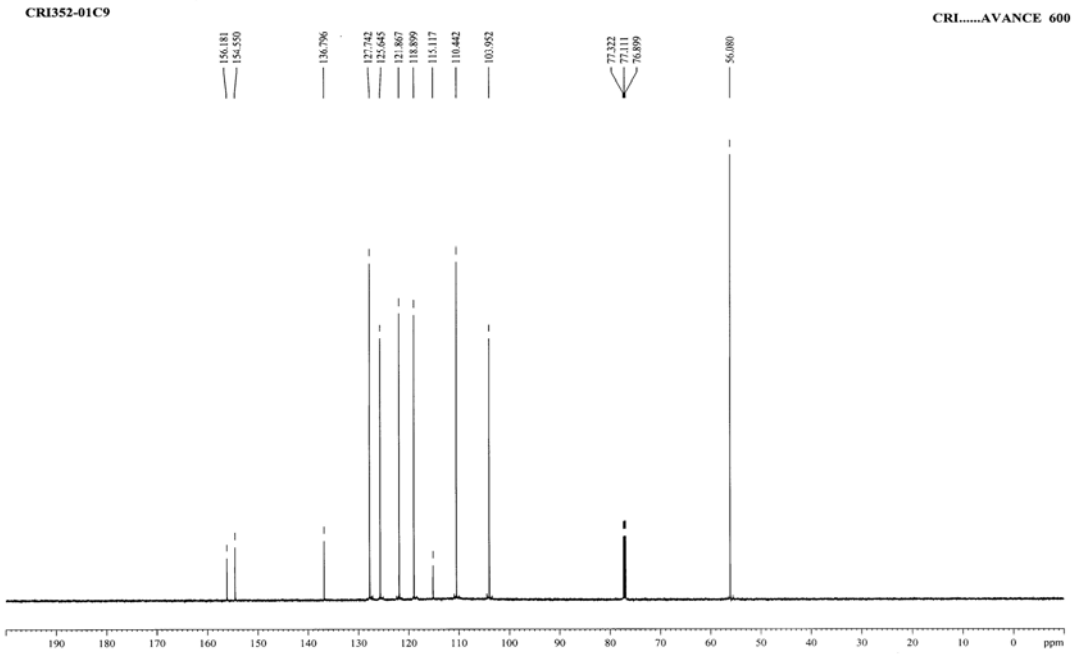

Figure 28 150 MHz ^{13}C-NMR ($CDCl_3$) spectrum of compound **1**

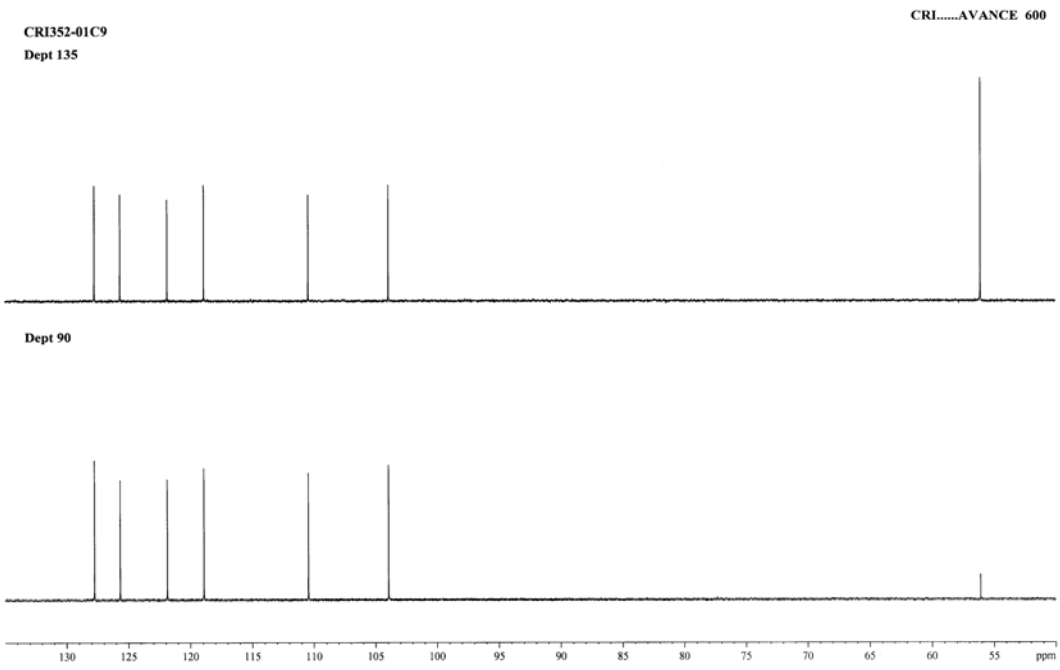

Figure 29 DEPT 90 and 135 spectra of compound **1**

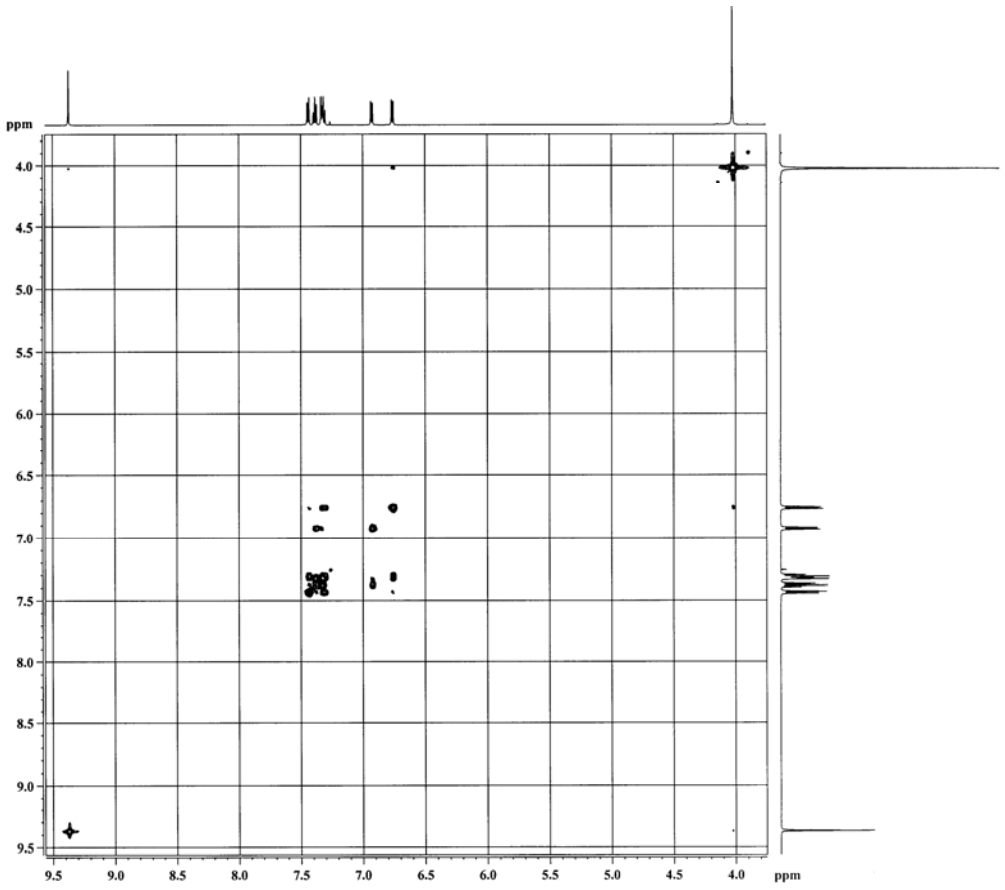

Figure 30 ^{1}H-^{1}H COSY spectrum of compound **1**

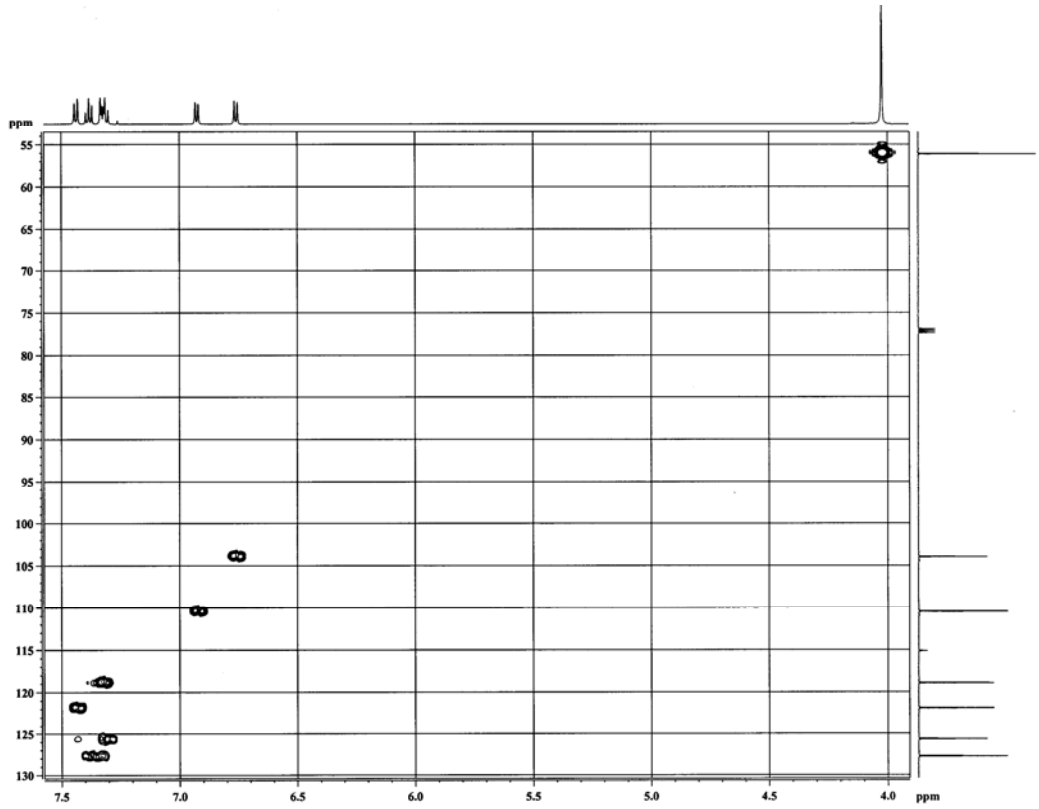

Figure 31 HMQC spectrum of compound 1

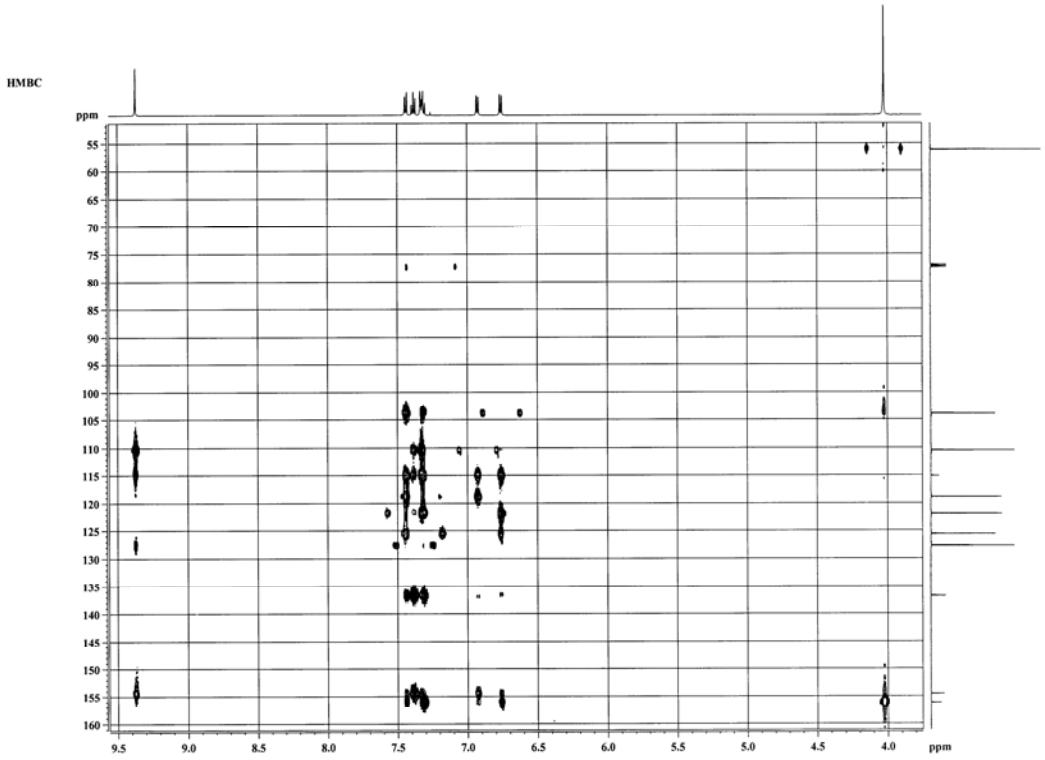

Figure 32 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound 1

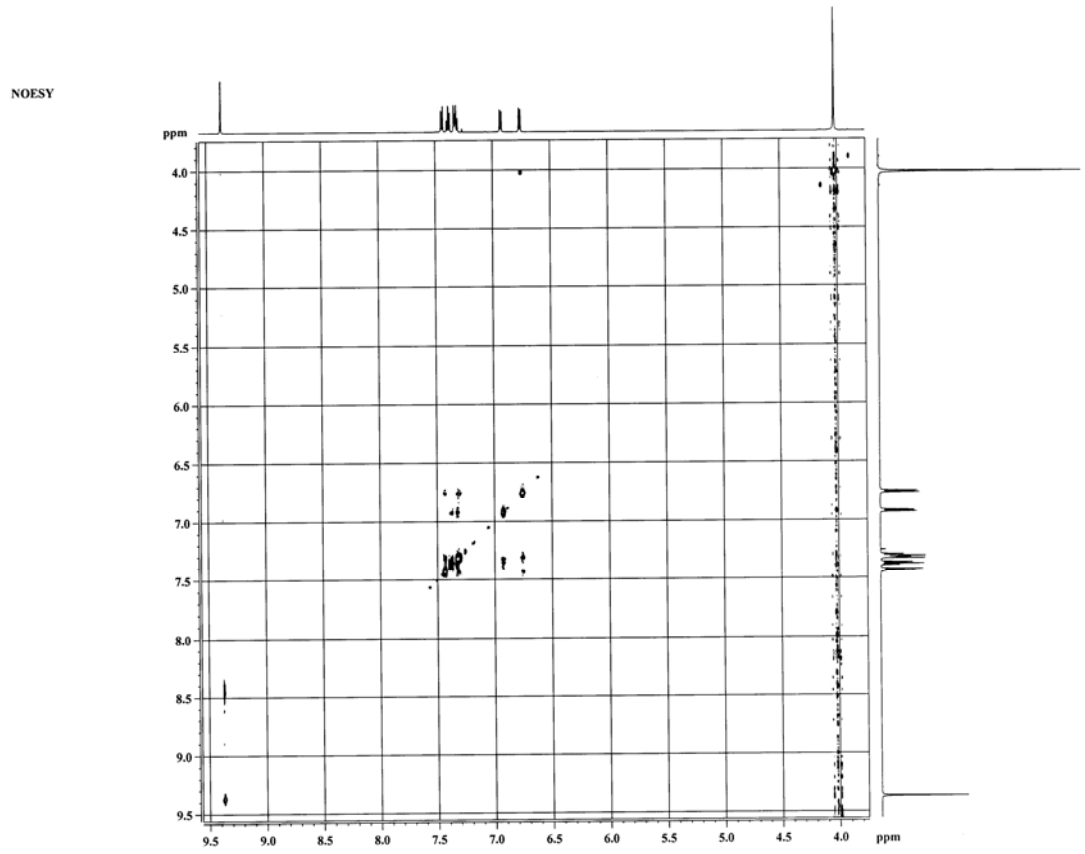

Figure 33 NOESY spectrum of compound **1**

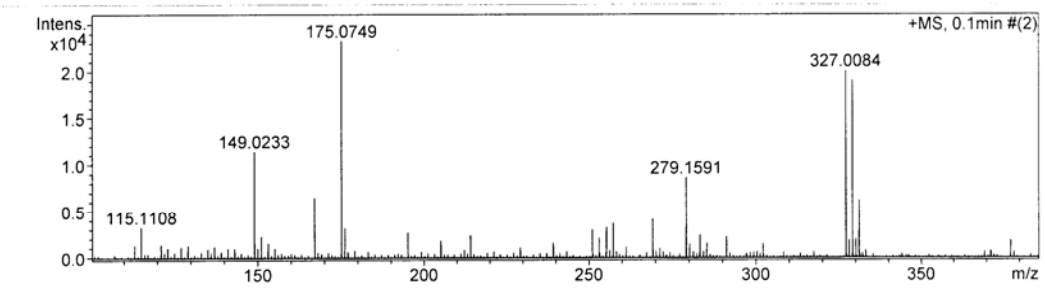

Figure 34 Mass spectrum of compound **1**

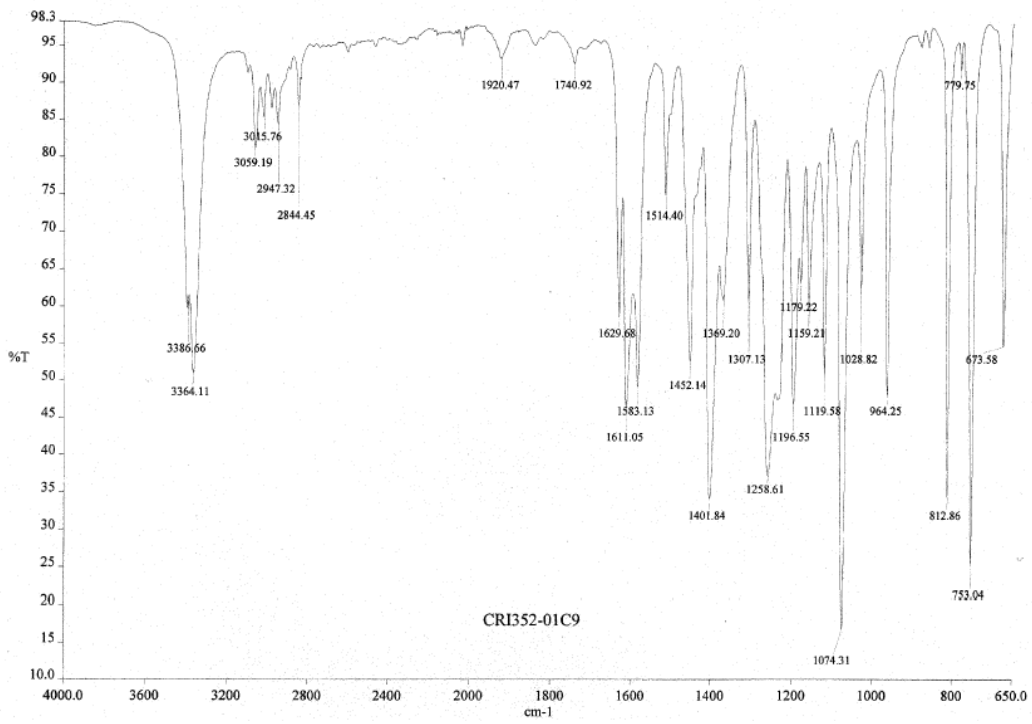

Figure 35 IR spectrum of compound 1

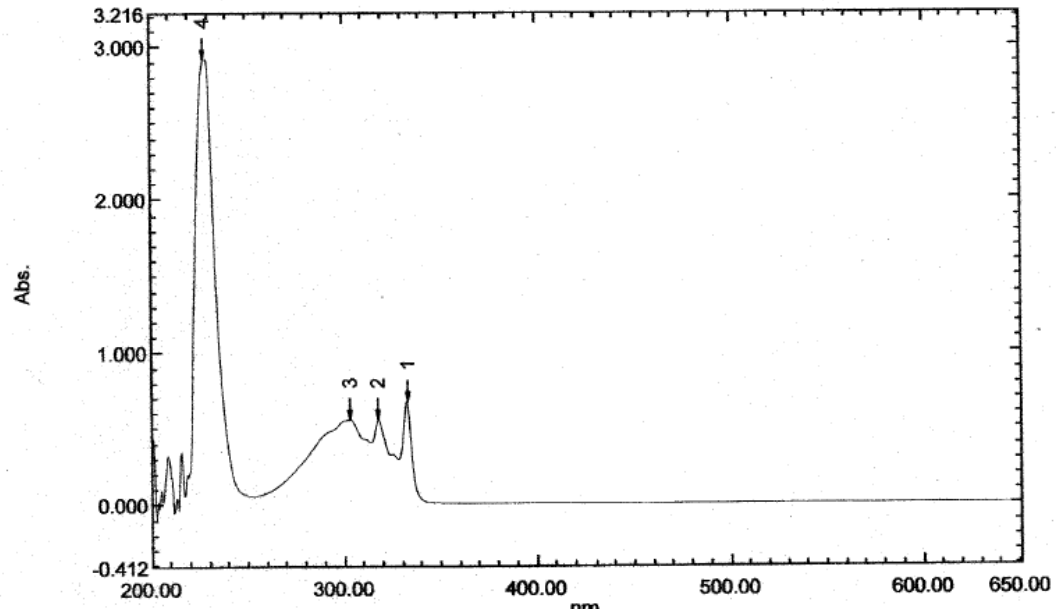

Figure 36 UV spectrum of compound 1

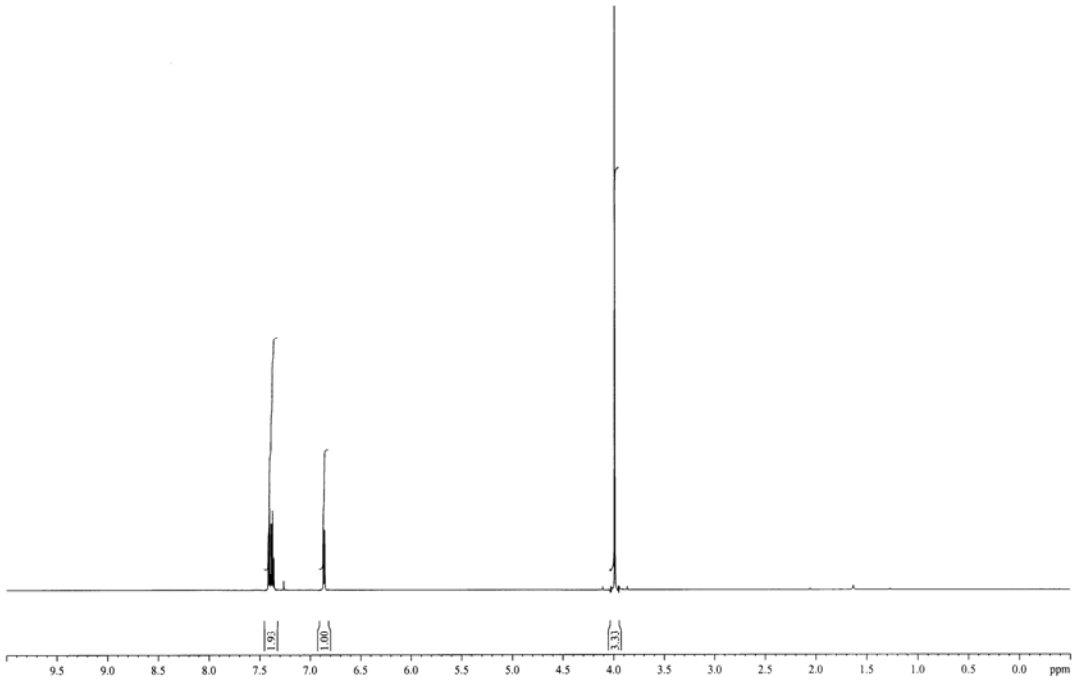

Figure 37 600 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound 2

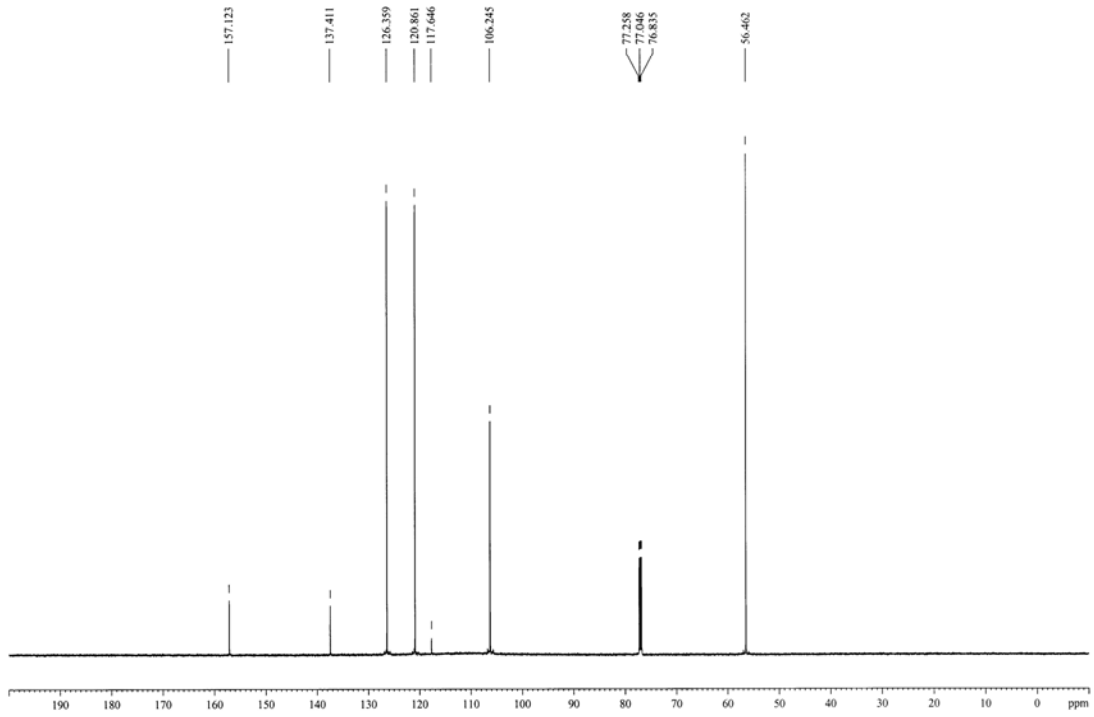

Figure 38 150 MHz ^{13}C NMR ($CDCl_3$) spectrum of compound 2

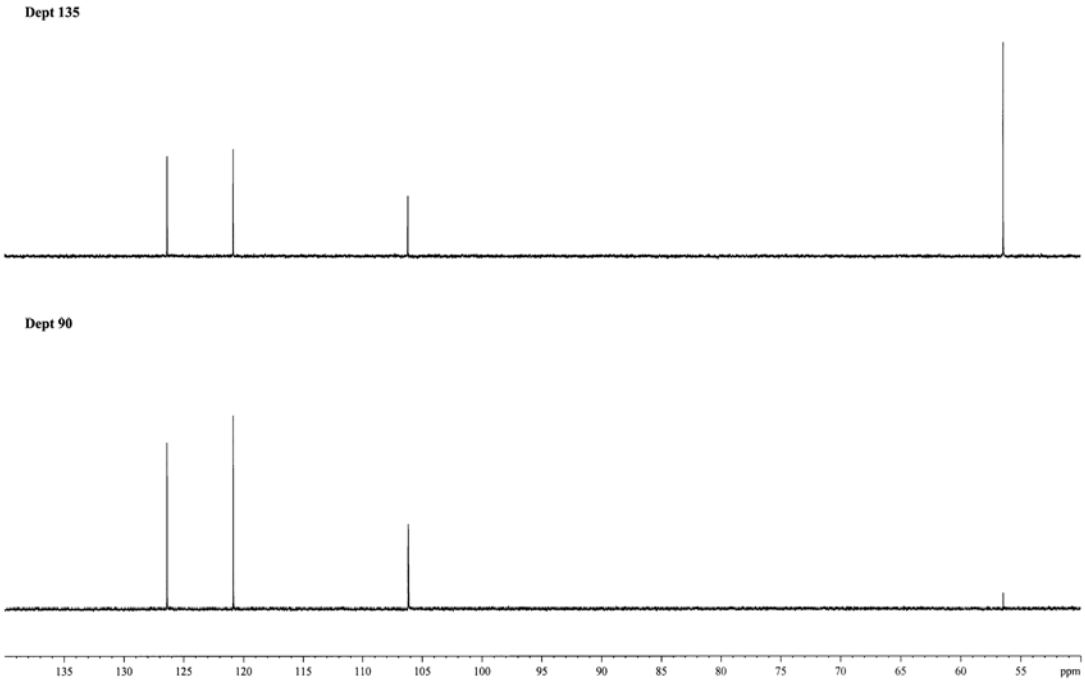

Figure 39 DEPT 90 and 135 spectra of compound 2

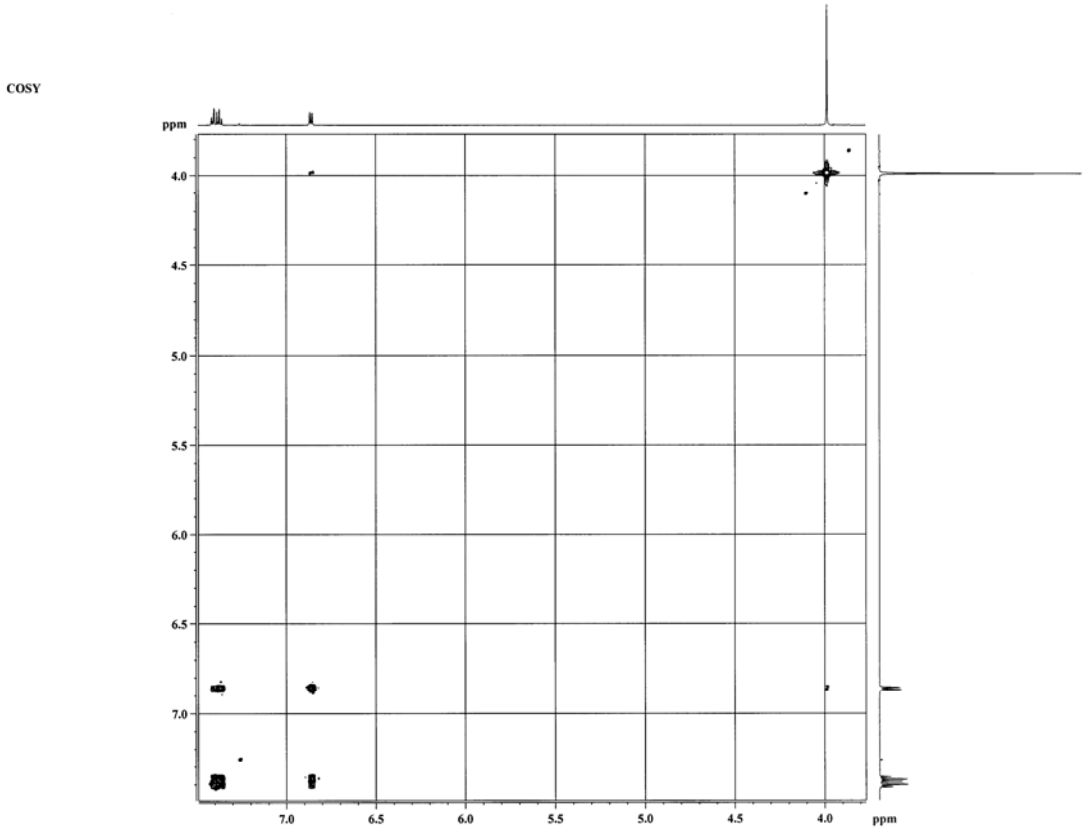

Figure 40 ^{1}H-^{1}H COSY spectrum of compound 2

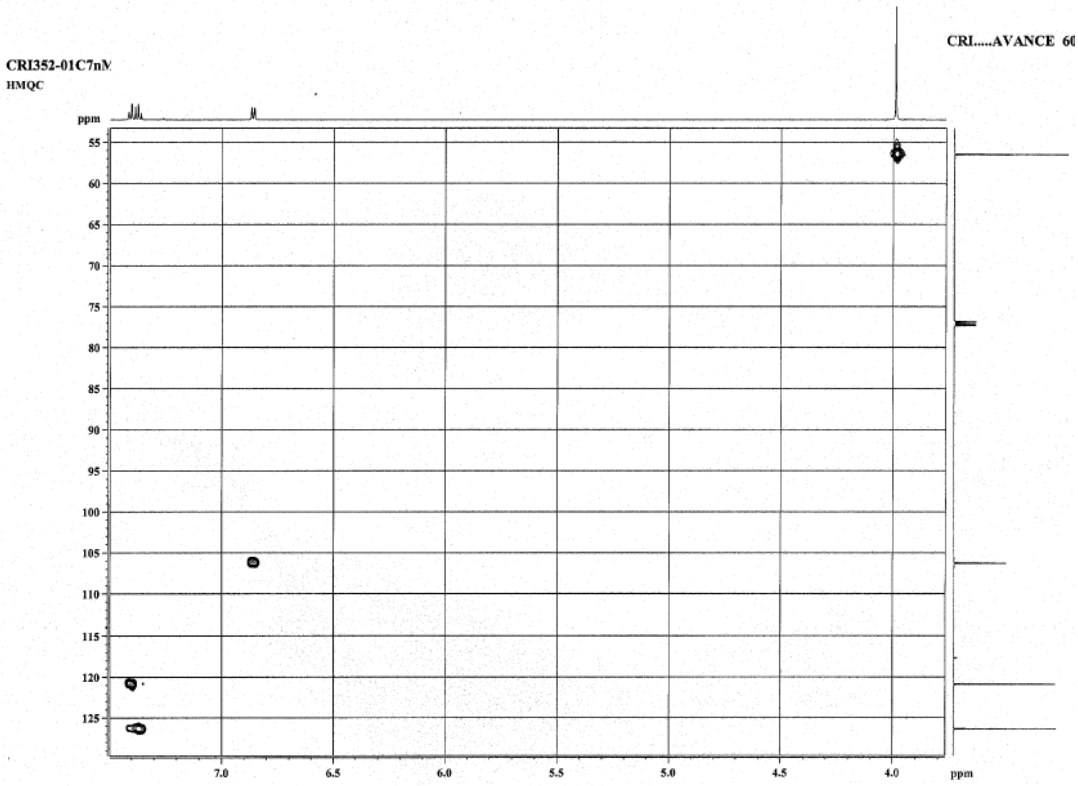

Figure 41 HMQC spectrum of compound 2

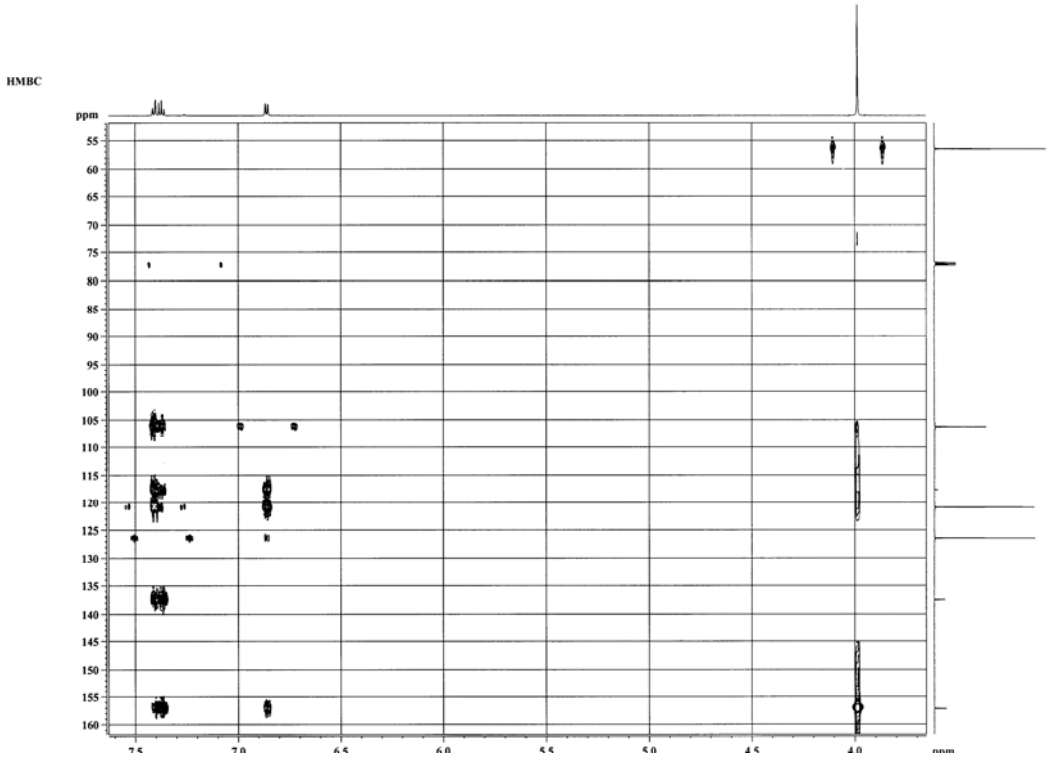

Figure 42 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound 2

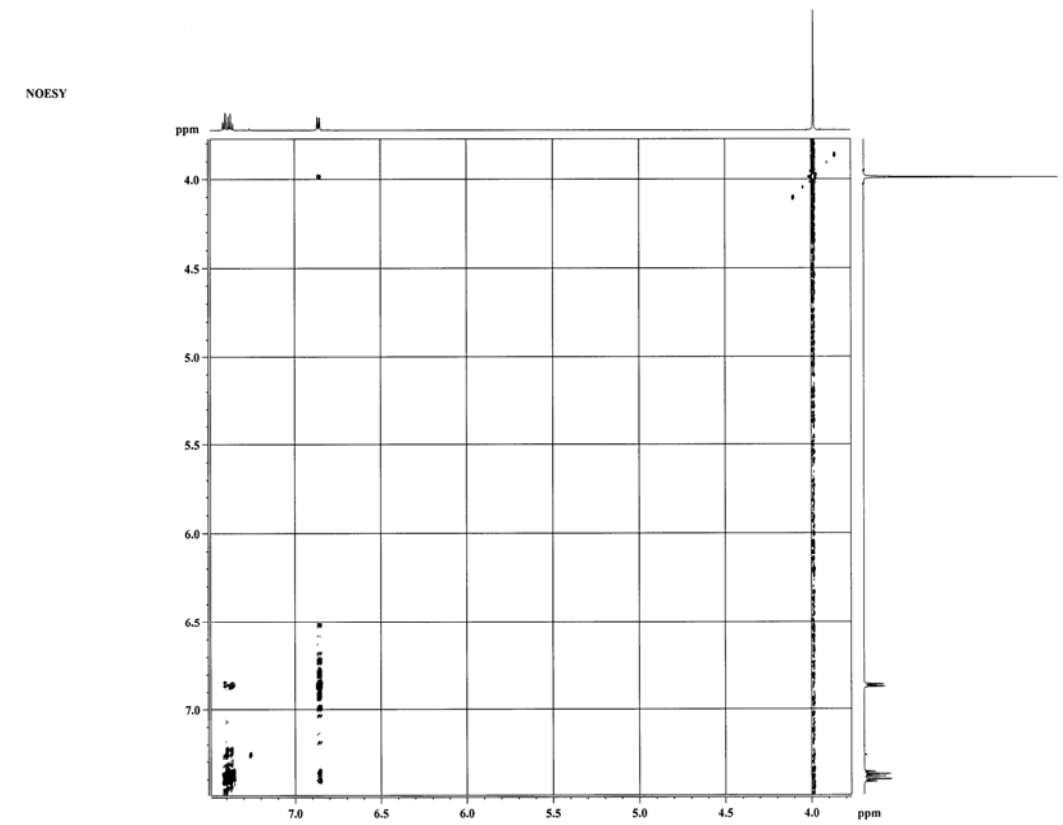

Figure 43 NOESY spectrum of compound 2

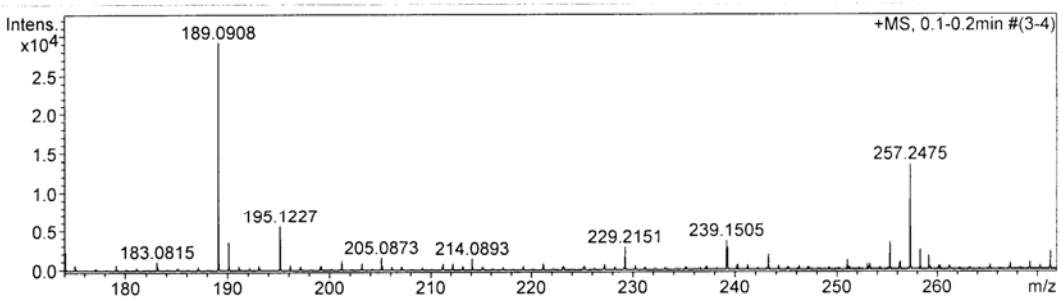

Figure 44 Mass spectrum of compound 2

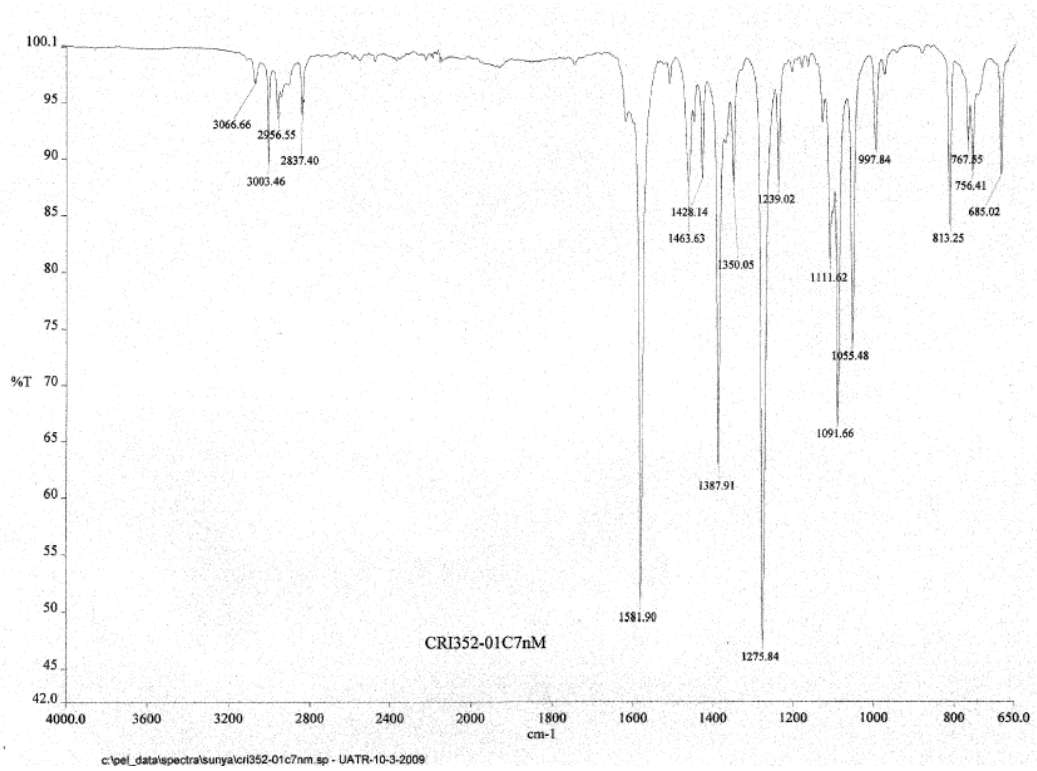

Figure 45 IR spectrum of compound 2

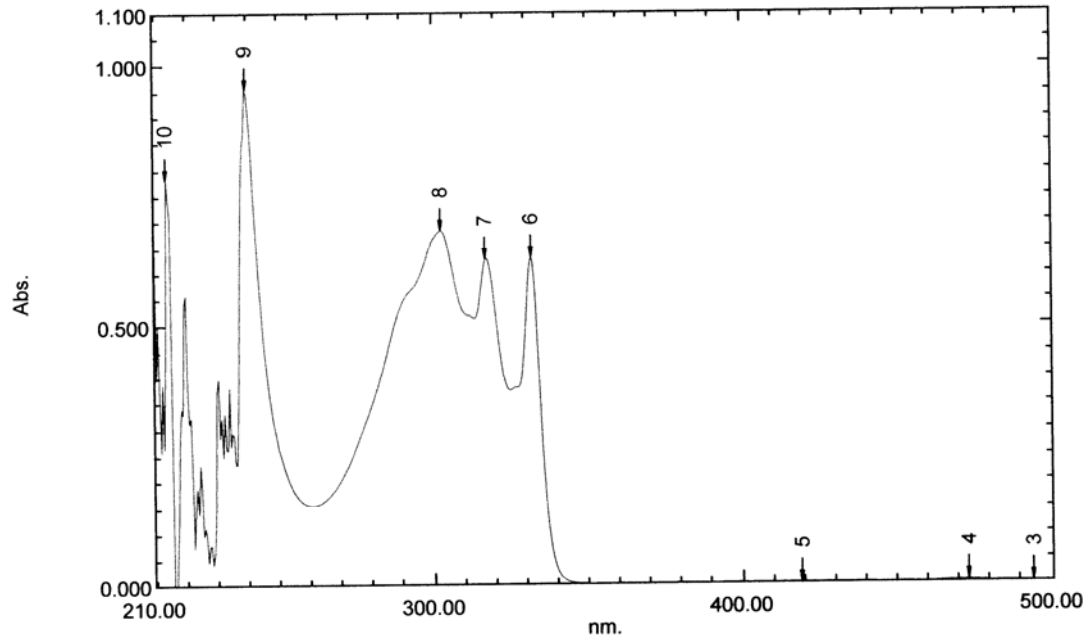

Figure 46 UV spectrum of compound 2

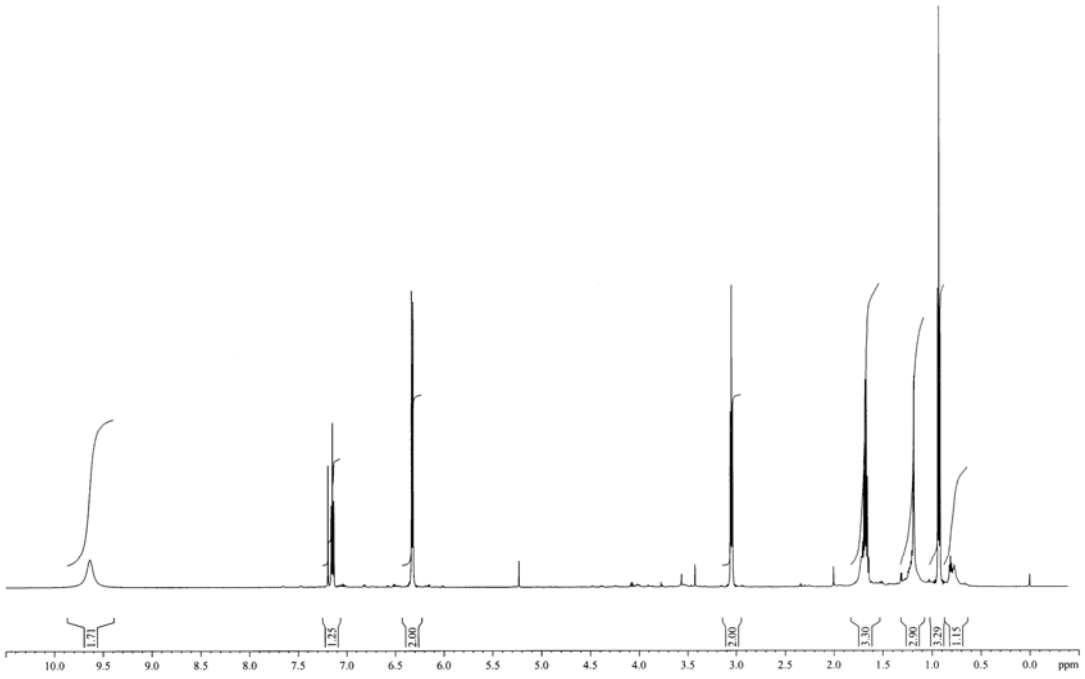

Figure 47 600 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **3**

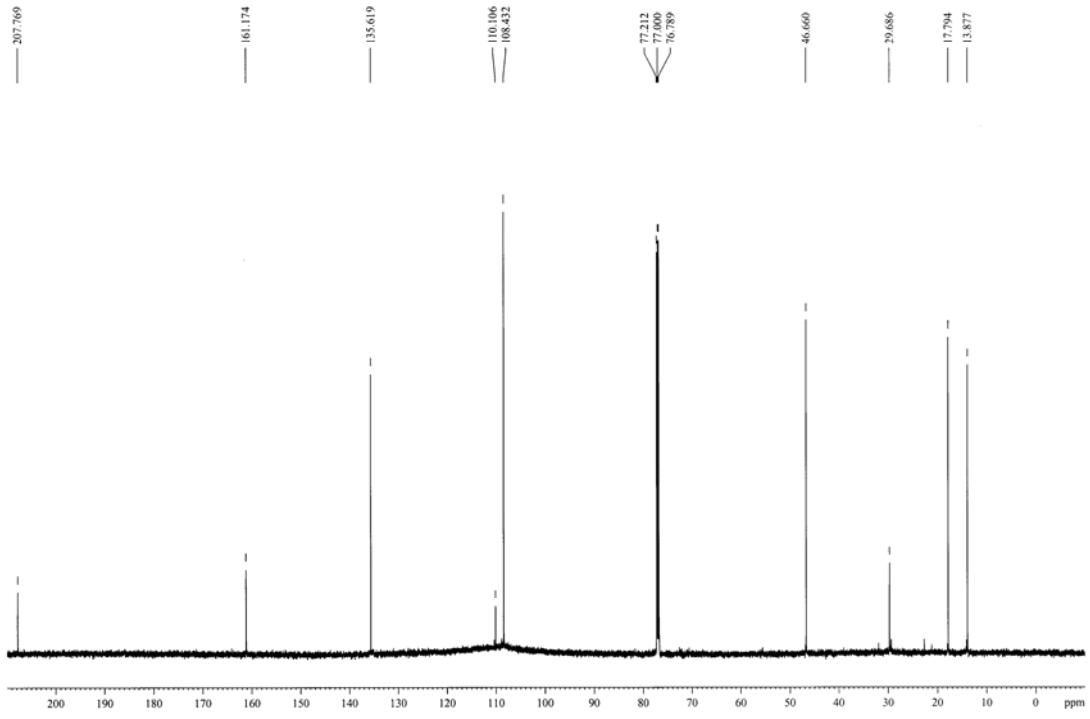

Figure 48 150 MHz ^{13}C NMR ($CDCl_3$) spectrum of compound **3**

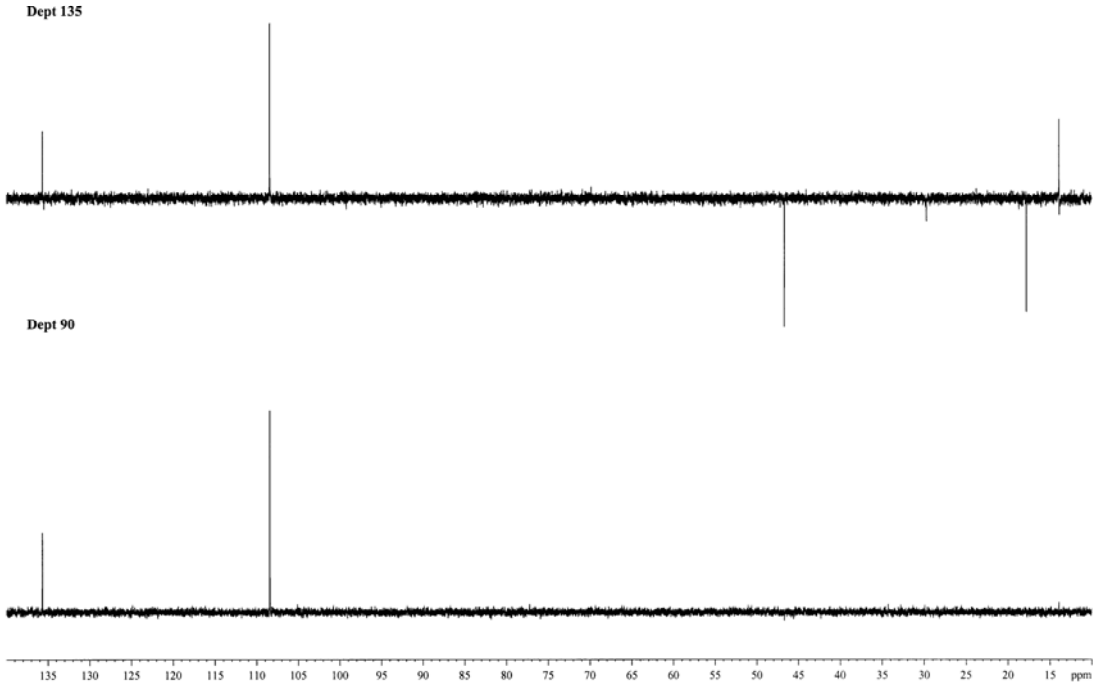

Figure 49 DEPT 90 and135 spectra of compound 3

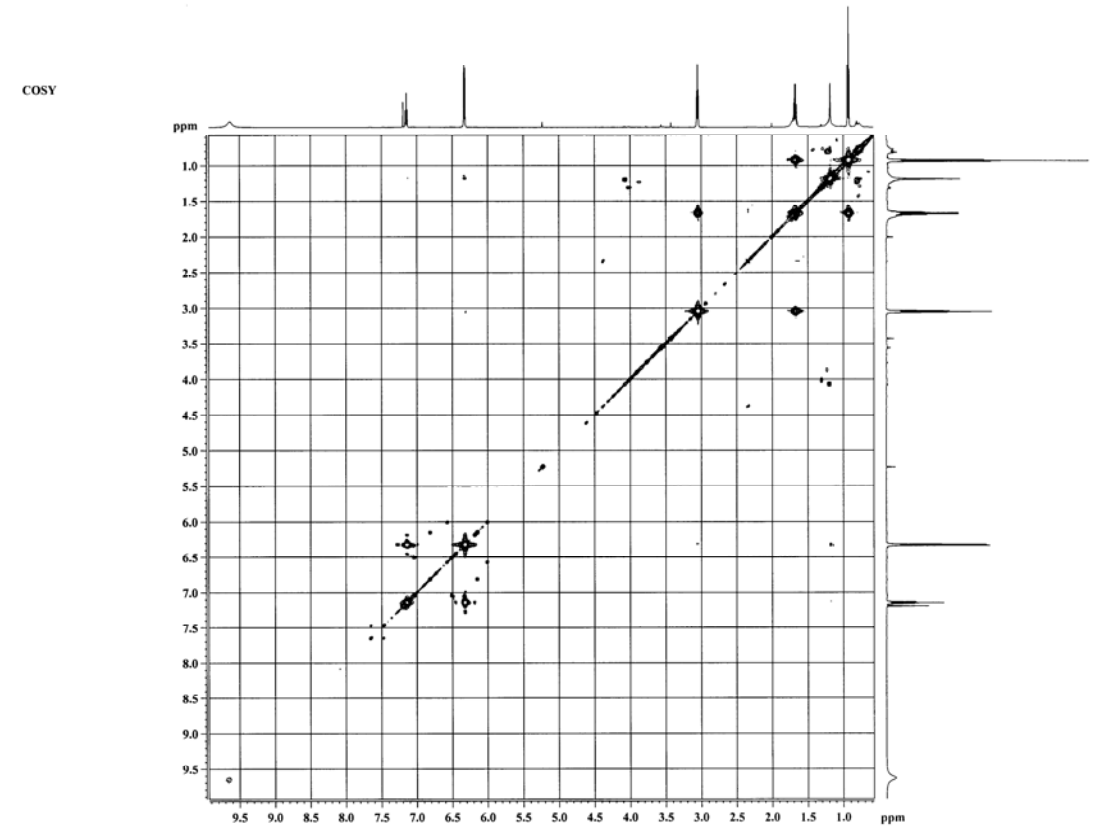

Figure 50 ^{1}H-^{1}H COSY spectrum of compound 3

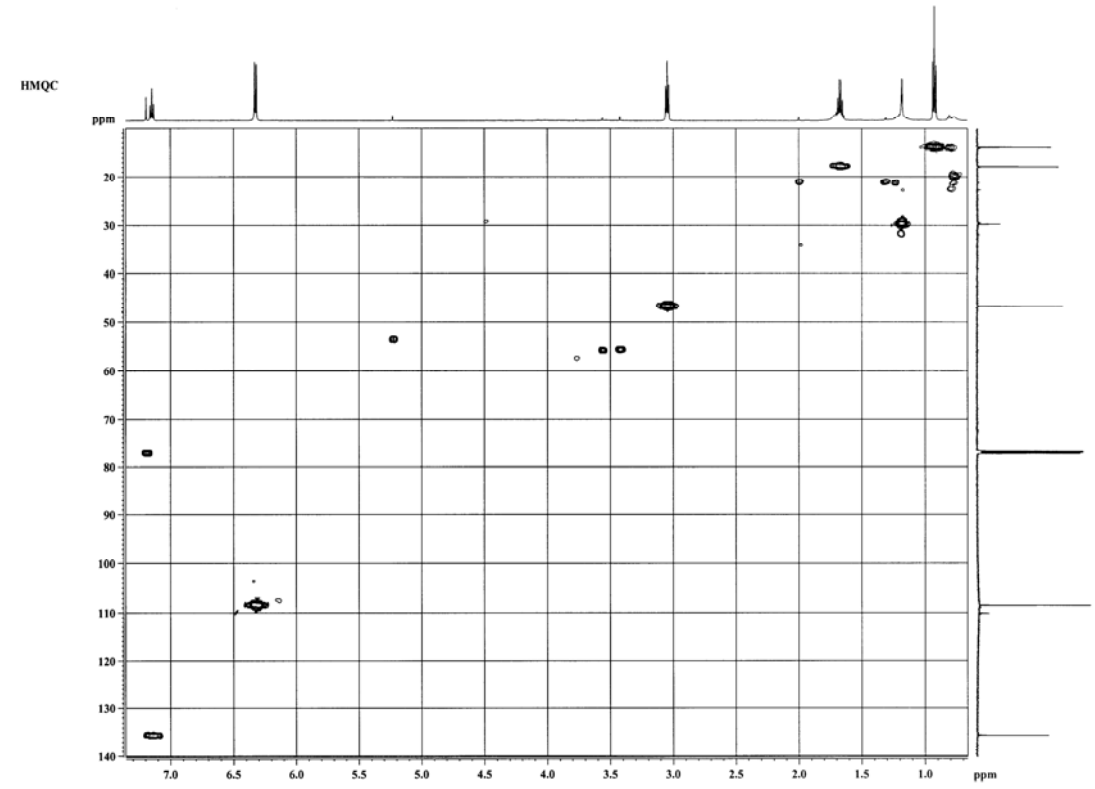

Figure 51 HMQC spectrum of compound **3**

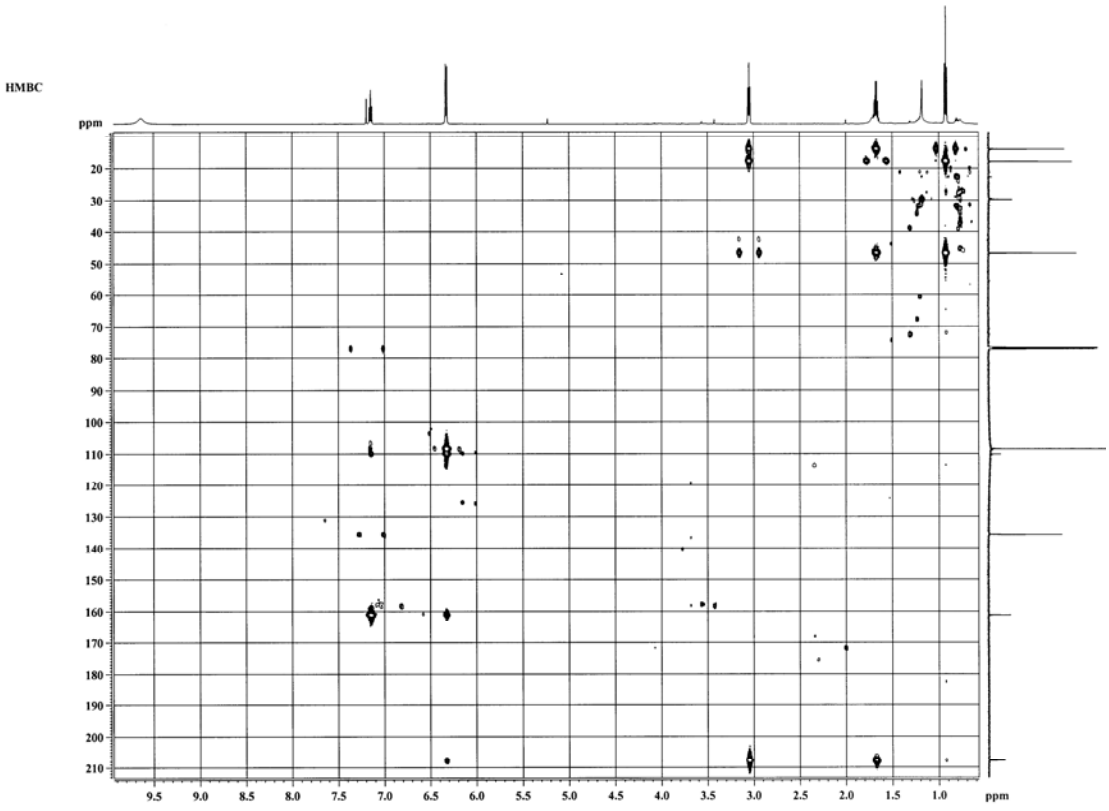

Figure 52 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound **3**

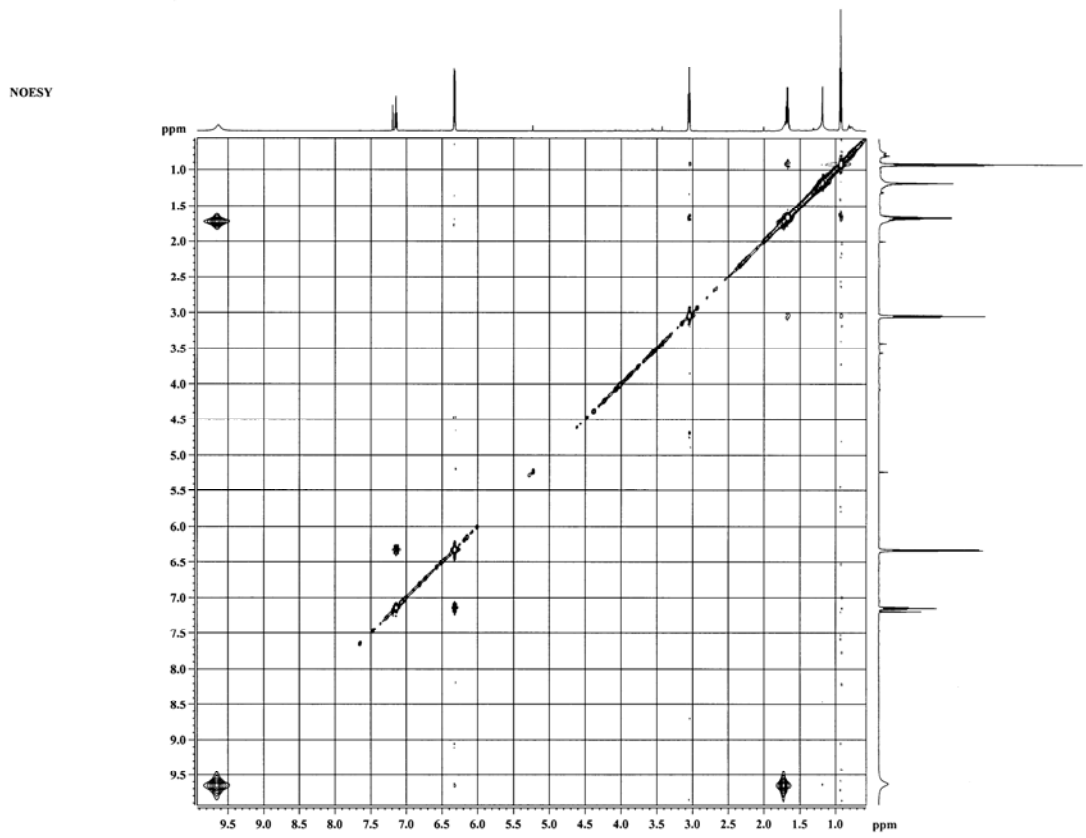

Figure 53 NOESY spectrum of compound **3**

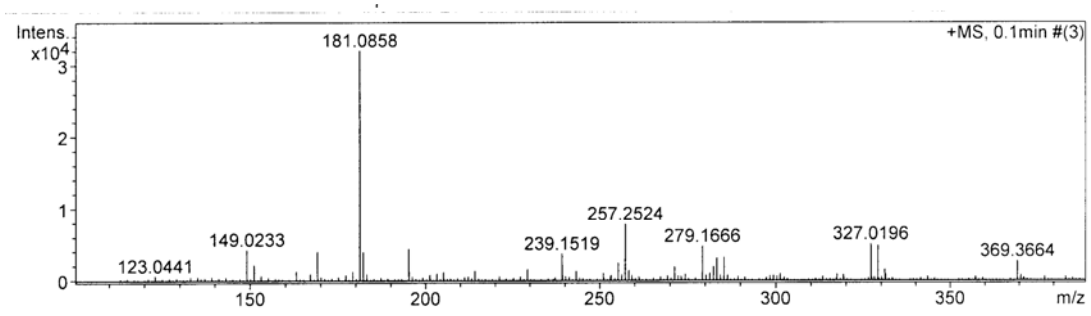

Figure 54 Mass of compound **3**

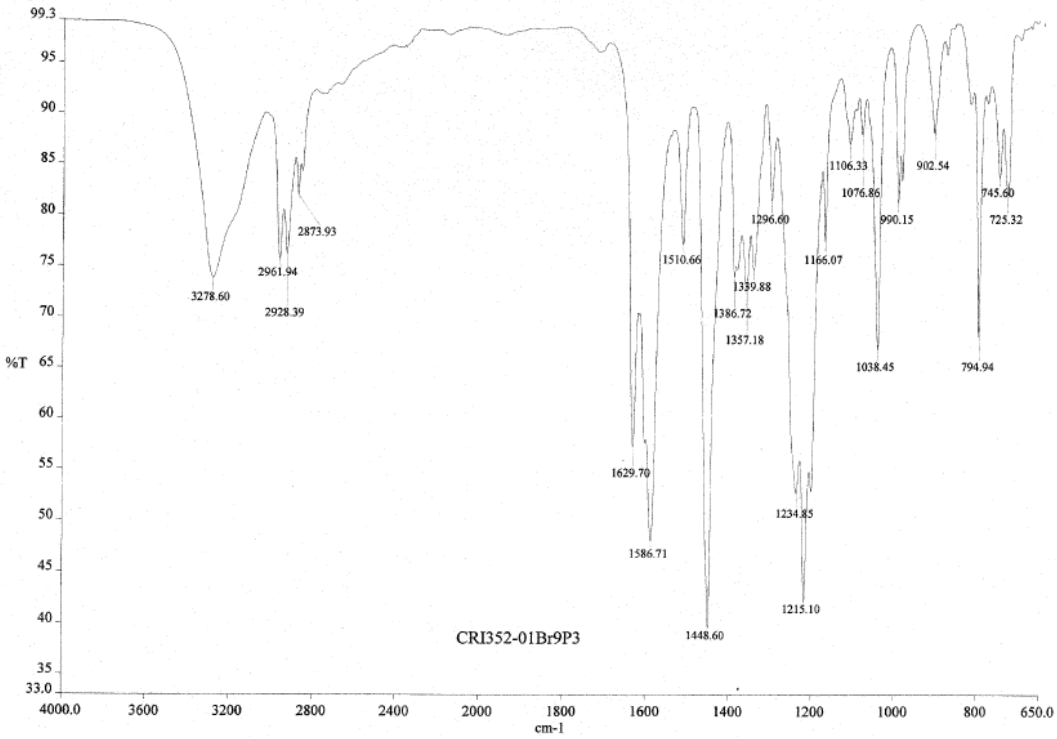

Figure 55 IR spectrum of compound 3

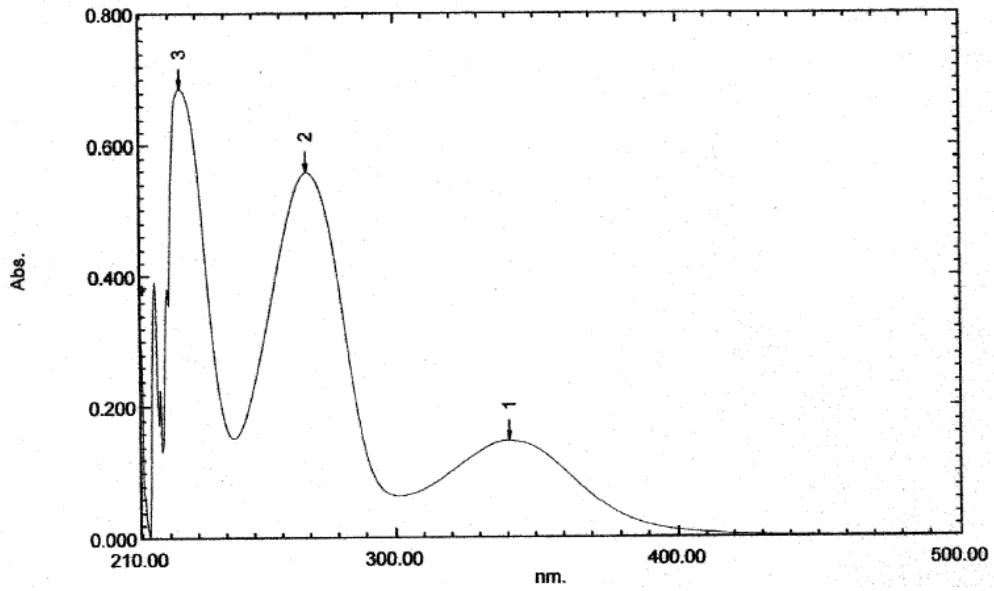

Figure 56 UV spectrum of compound 3

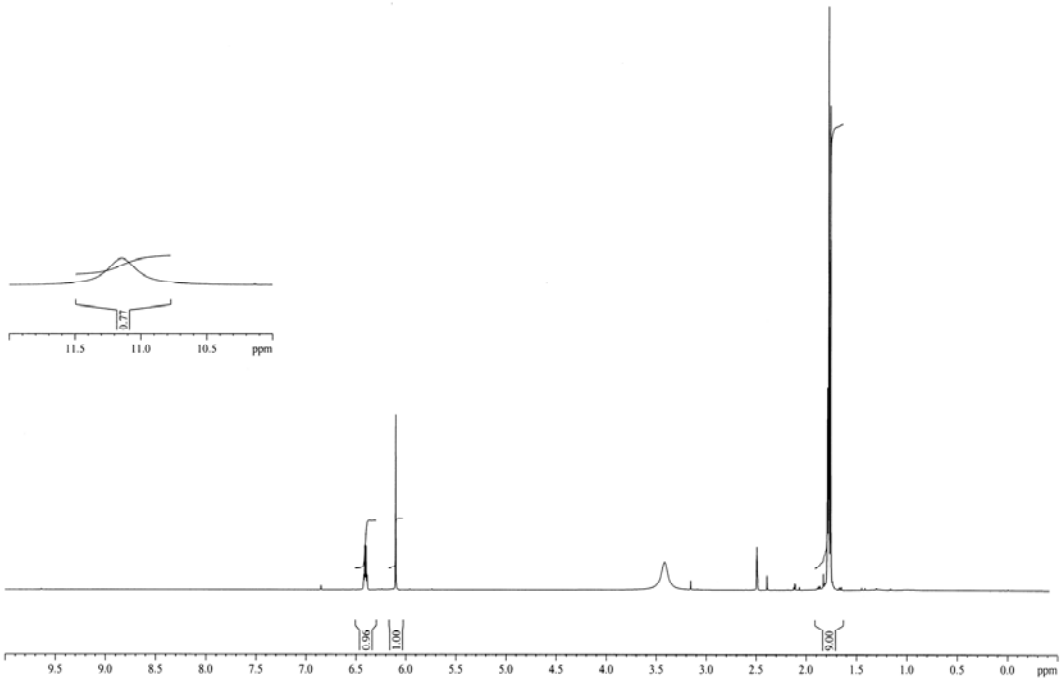

Figure 57 600 MHz ^{1}H NMR (DMSO-d_6) spectrum of compound **4**

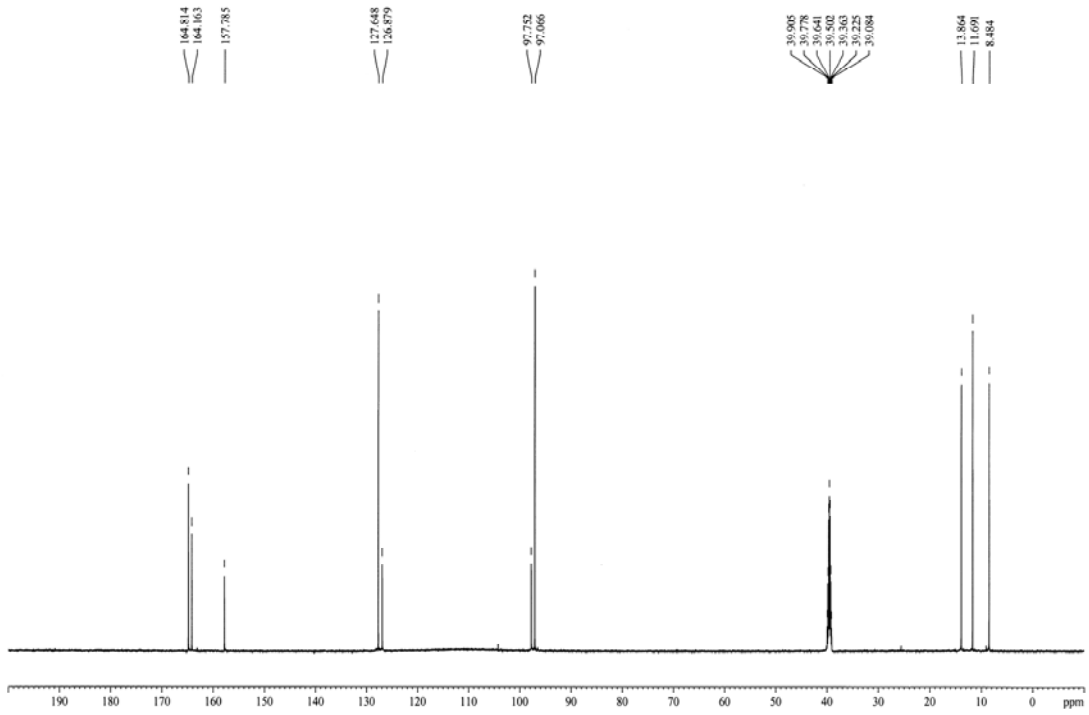

Figure 58 150 MHz ^{13}C NMR (DMSO-d_6) spectrum of compound **4**

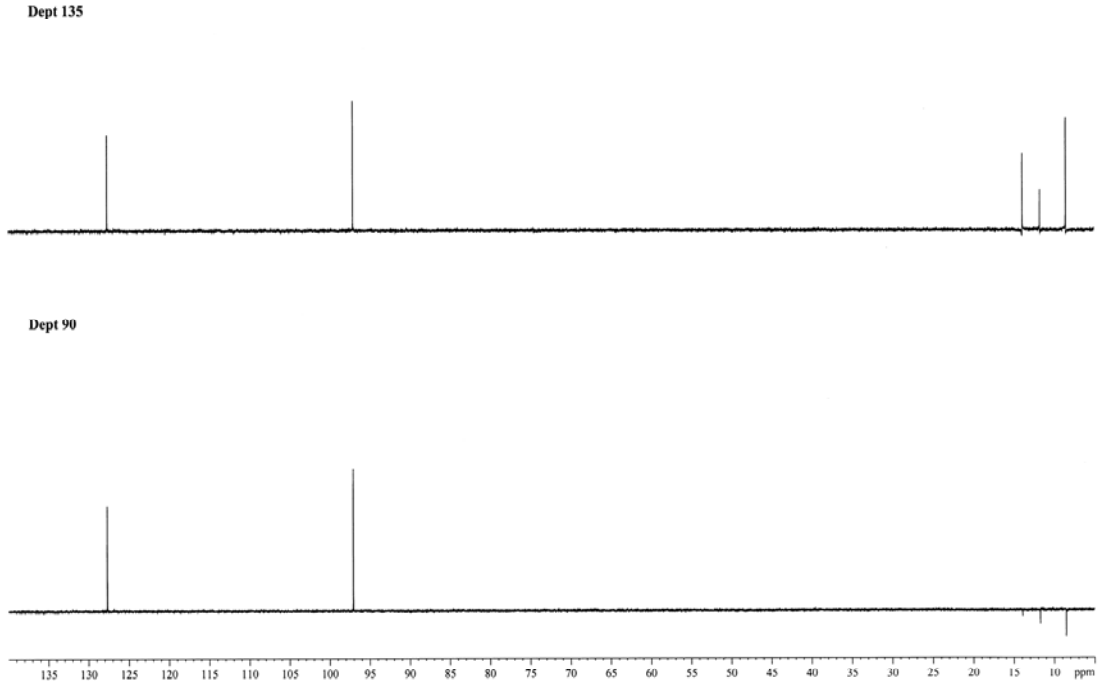

Figure 59 DEPT 90 and 135 of spectra of compound **4**

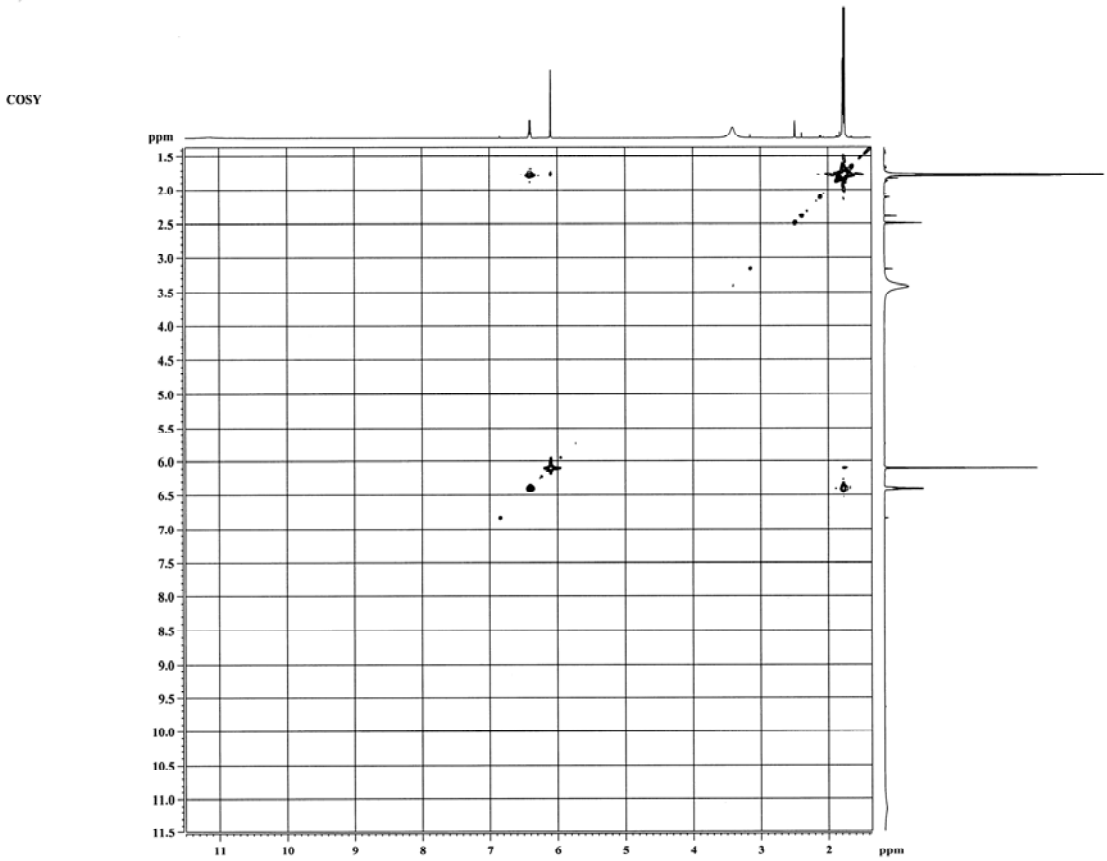

Figure 60 ^{1}H-^{1}H COSY spectrum of compound **4**

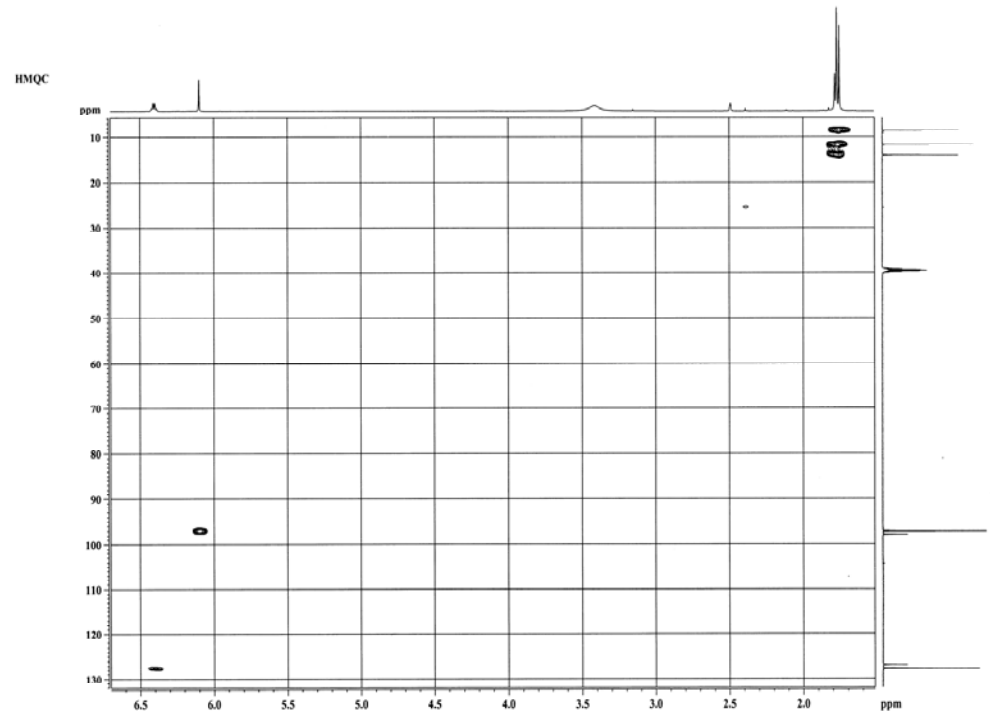

Figure 61 HMQC spectrum of compound **4**

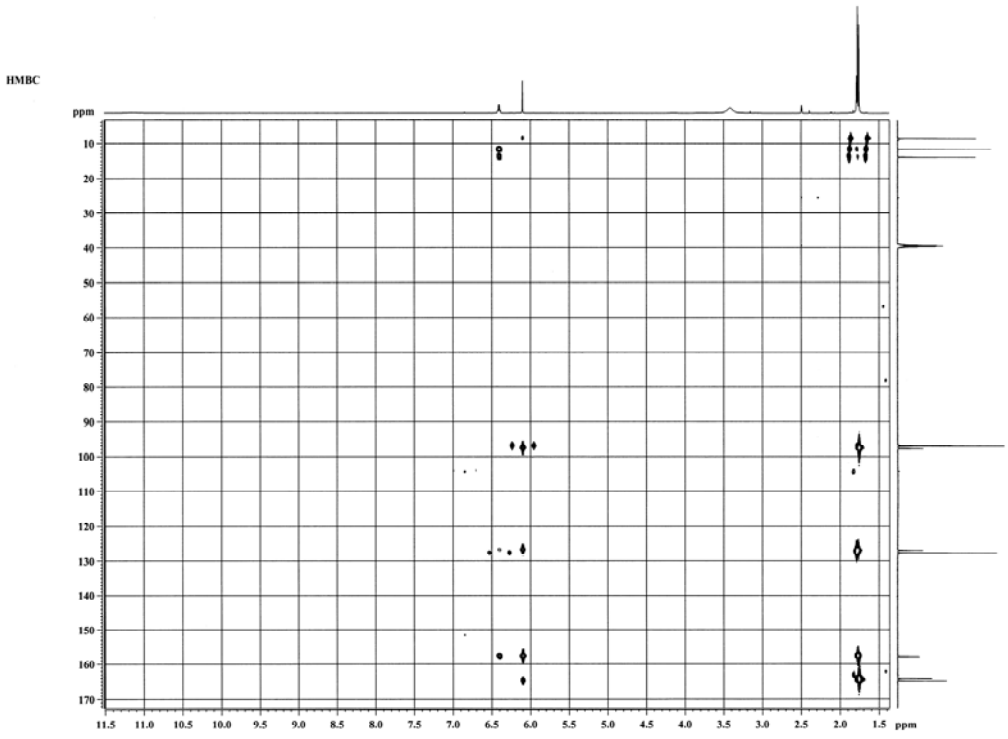

Figure 62 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound **4**

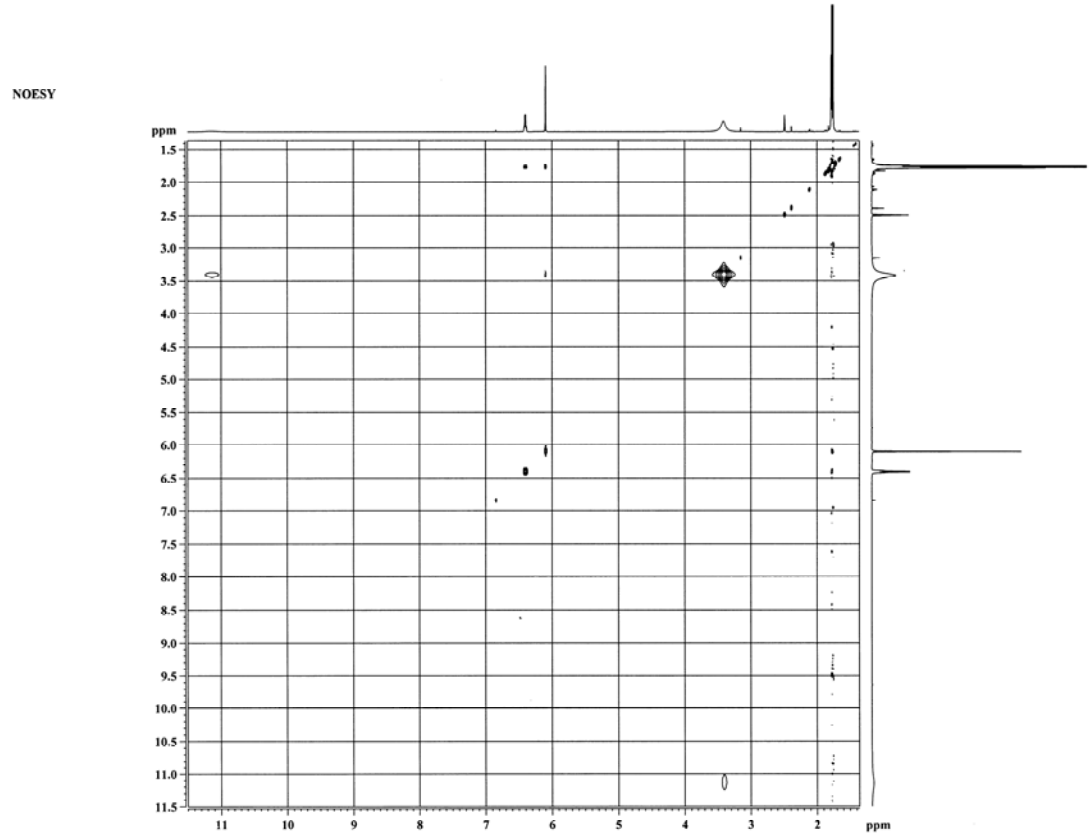

Figure 63 NOSEY spectrum of compound **4**

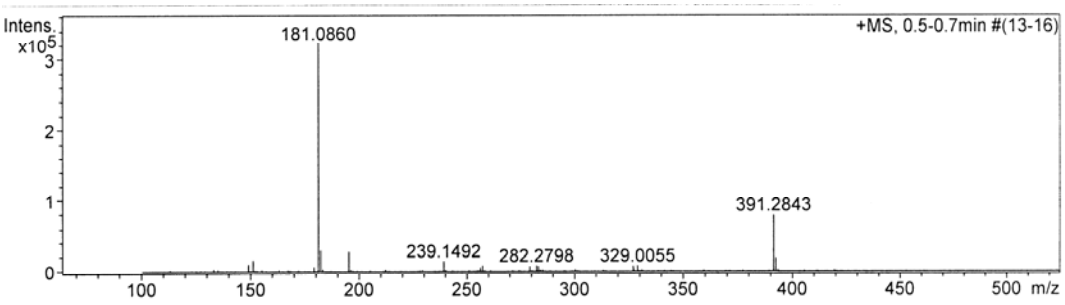

Figure 64 Mass spectrum of compound **4**

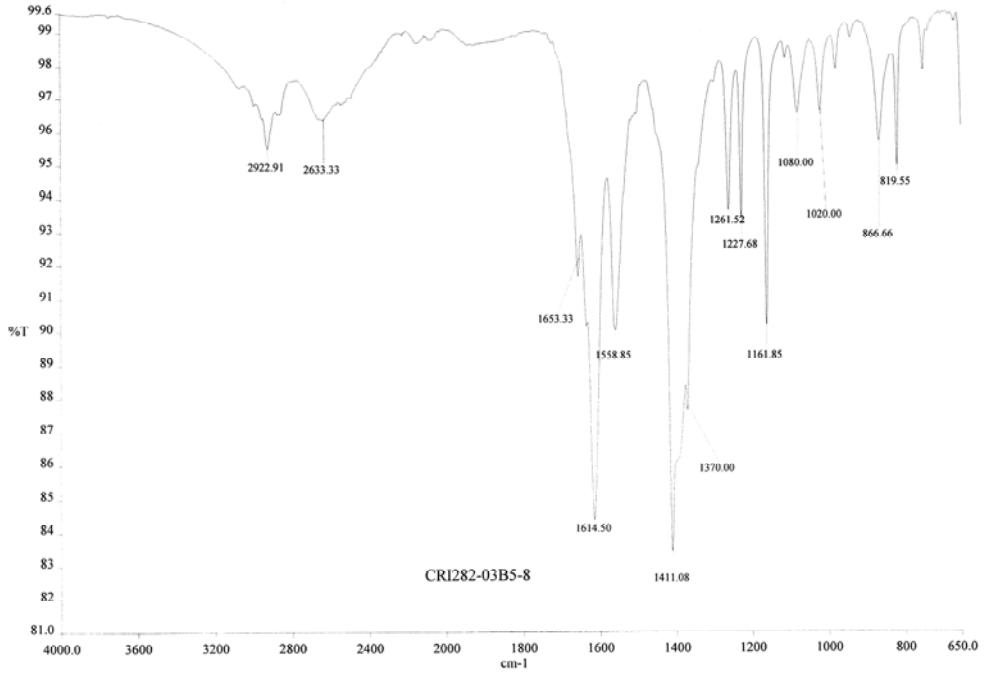

Figure 65 IR spectrum of compound **4**

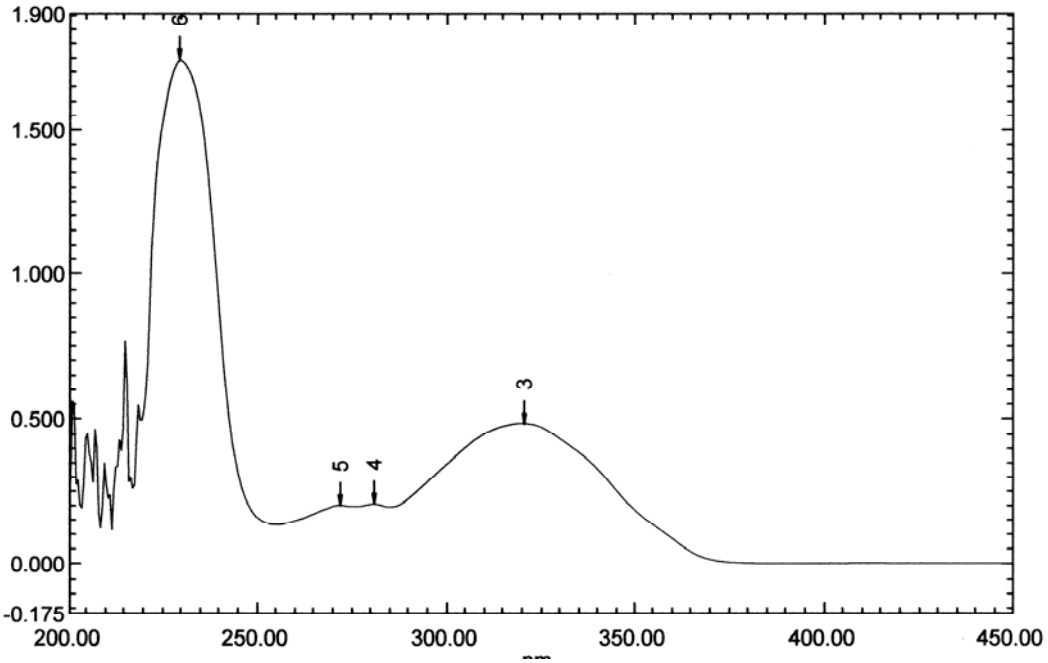

Figure 66 UV spectrum of compound **4**

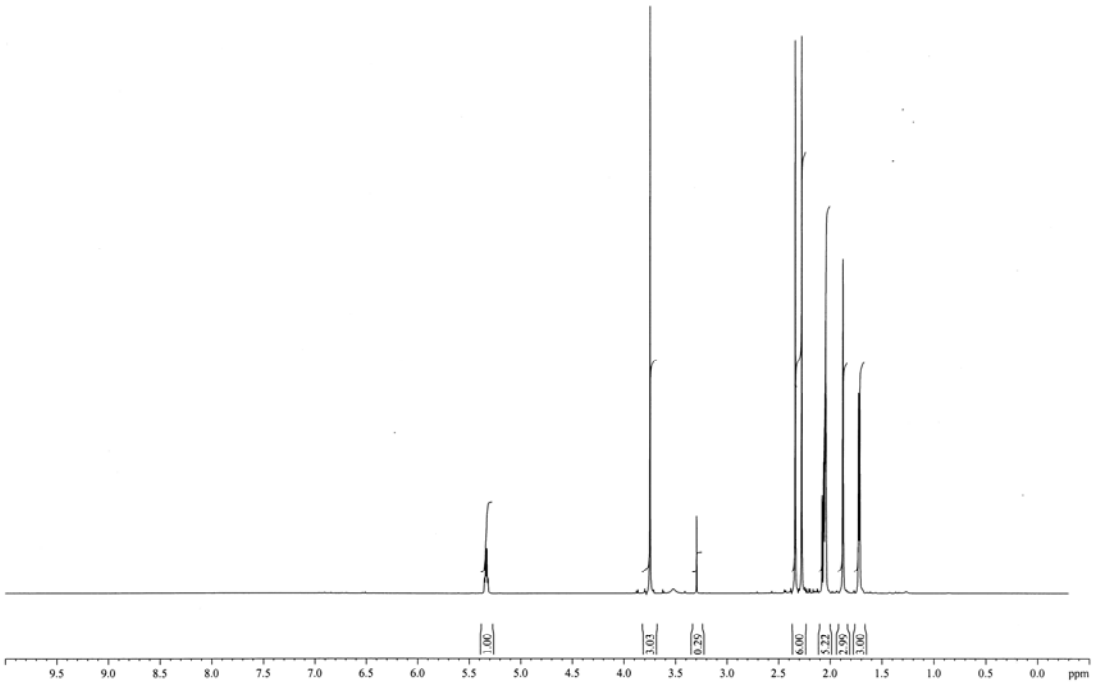

Figure 67 600 MHz ^{1}H NMR (acetone-d_6) spectrum of nidulin (**5**)

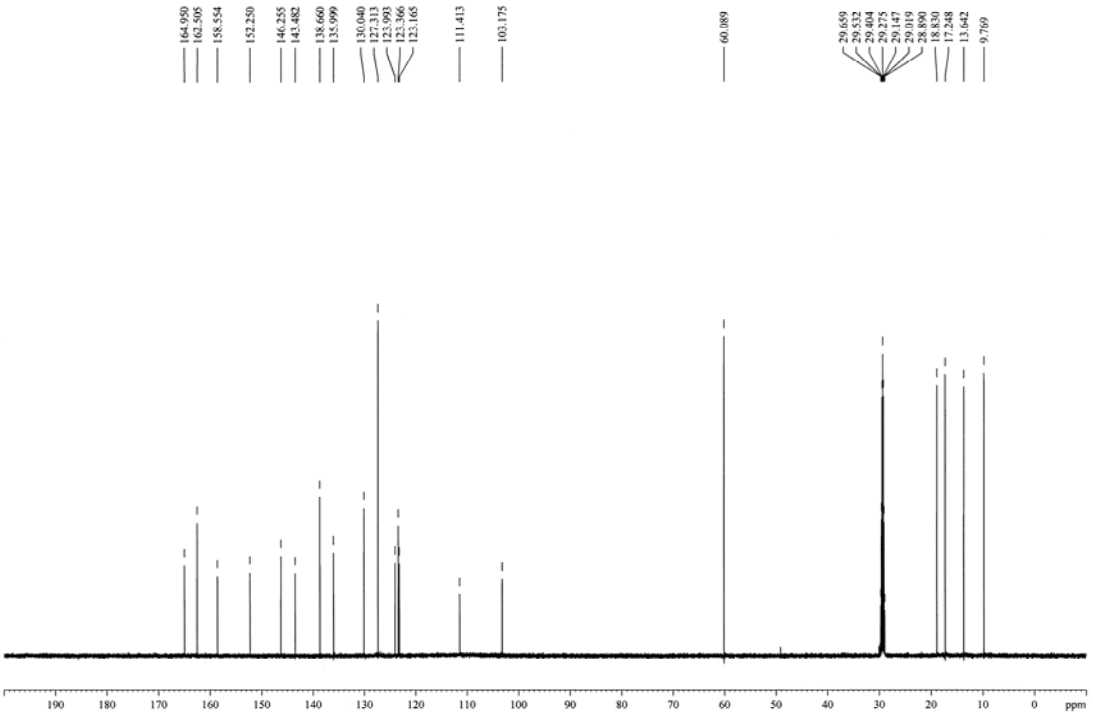

Figure 68 150 MHz ^{13}C NMR (acetone-d_6) spectrum of nidulin (**5**).

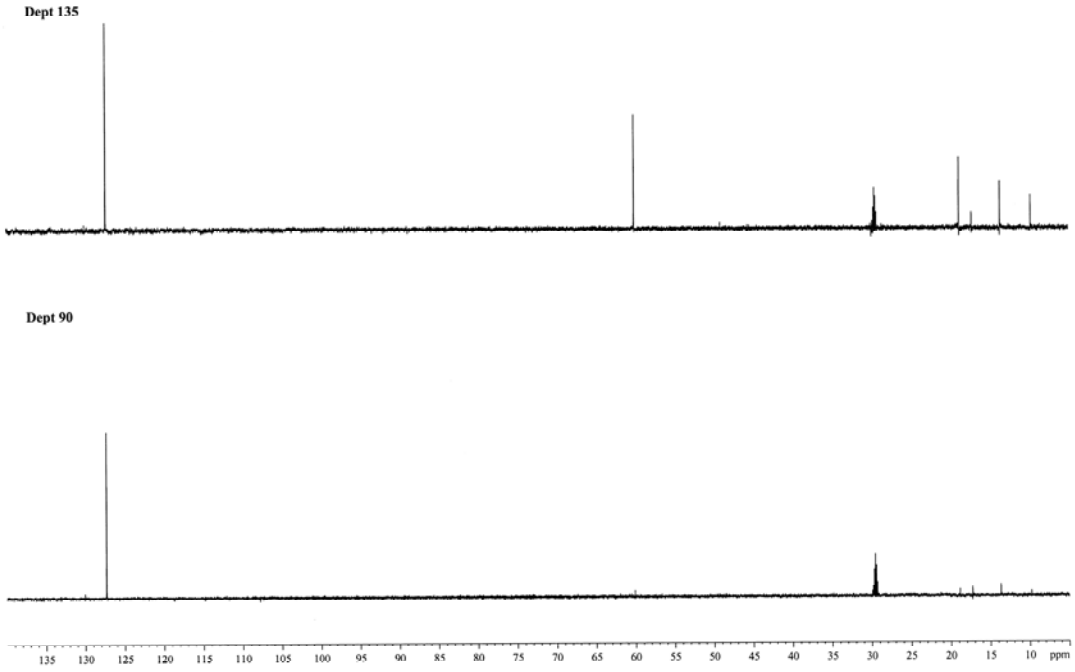

Figure 69 DEPT 90 and 135 spectra of nidulin (**5**)

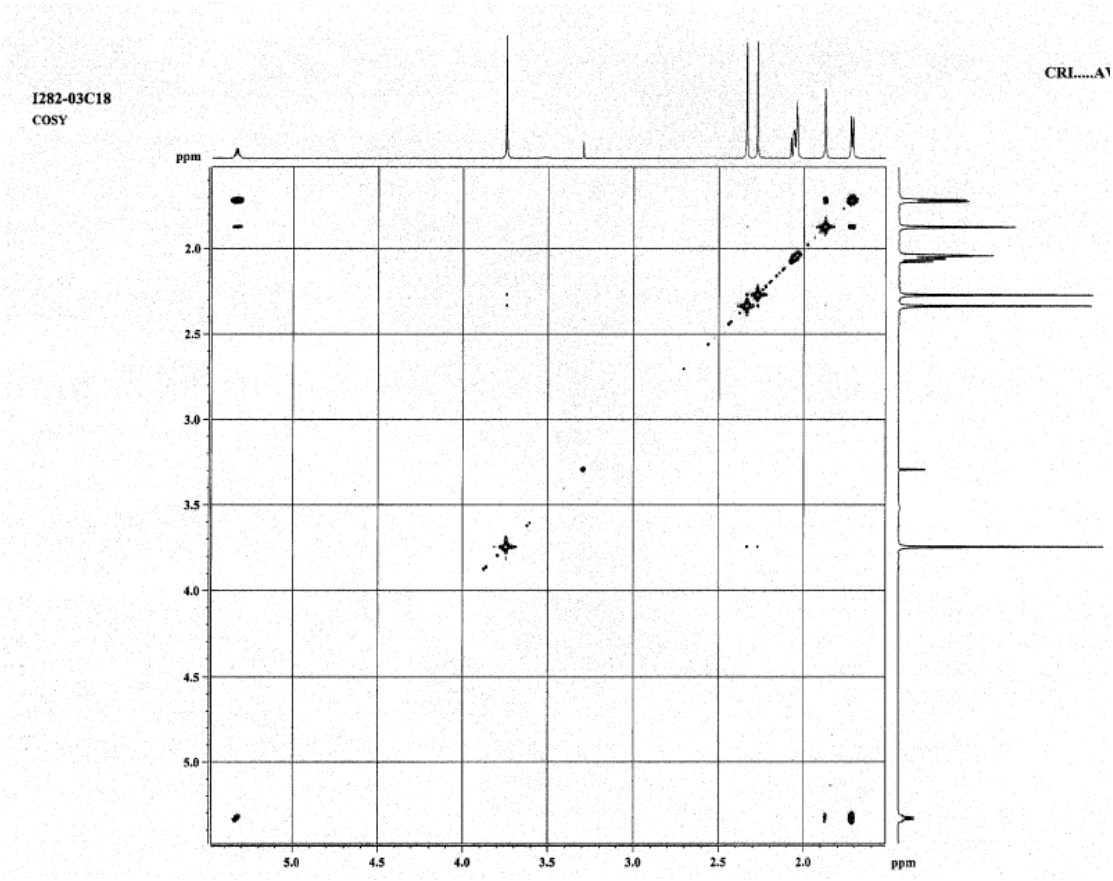

Figure 70 ^{1}H-^{1}H COSY spectrum of nidulin (**5**)

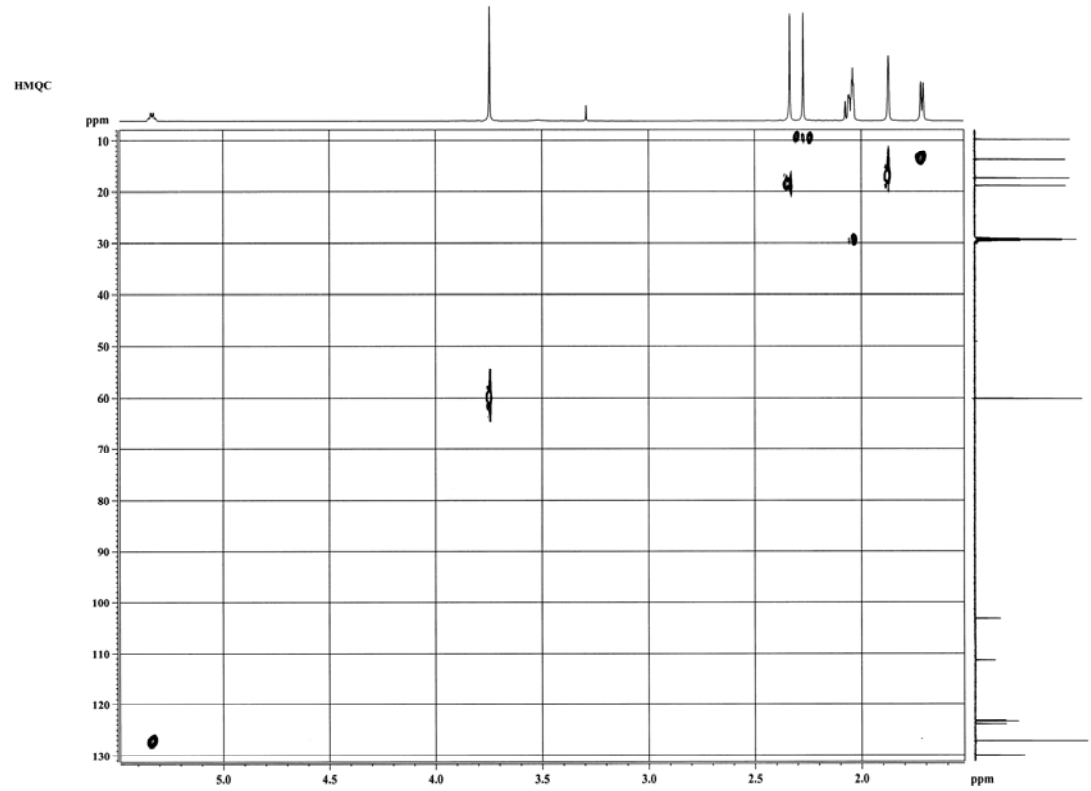

Figure 71 HMQC spectrum of nidulin (**5**)

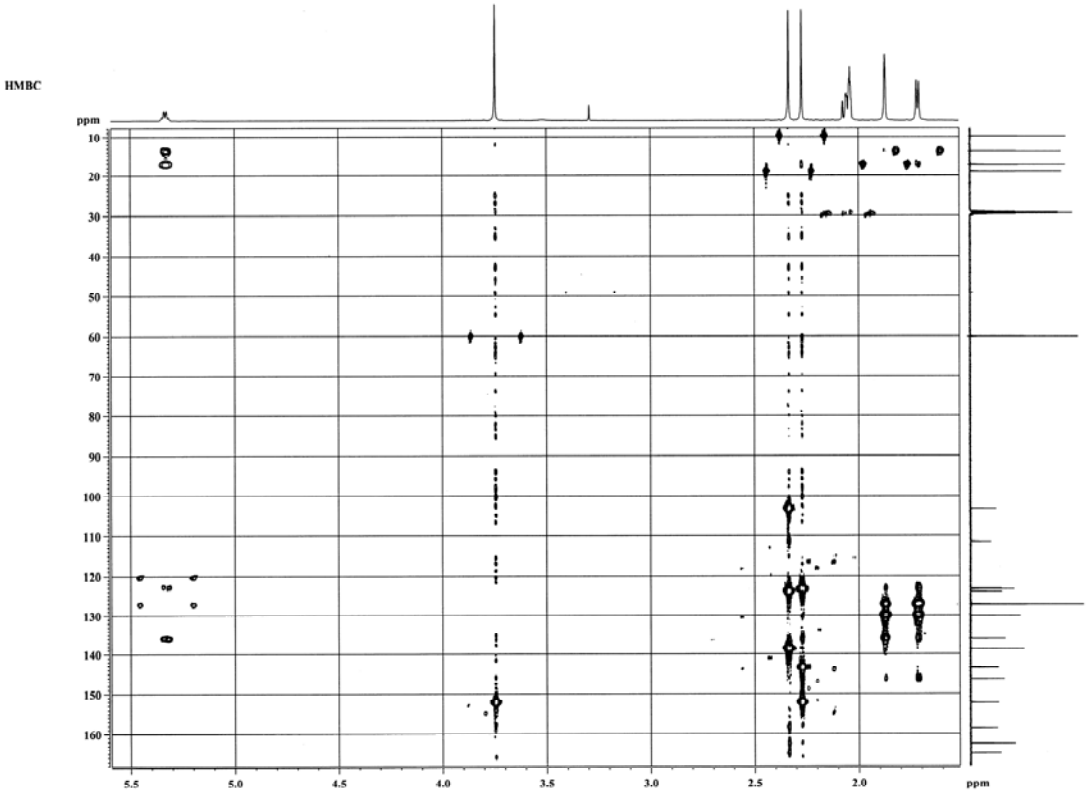

Figure 72 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of nidulin (**5**)

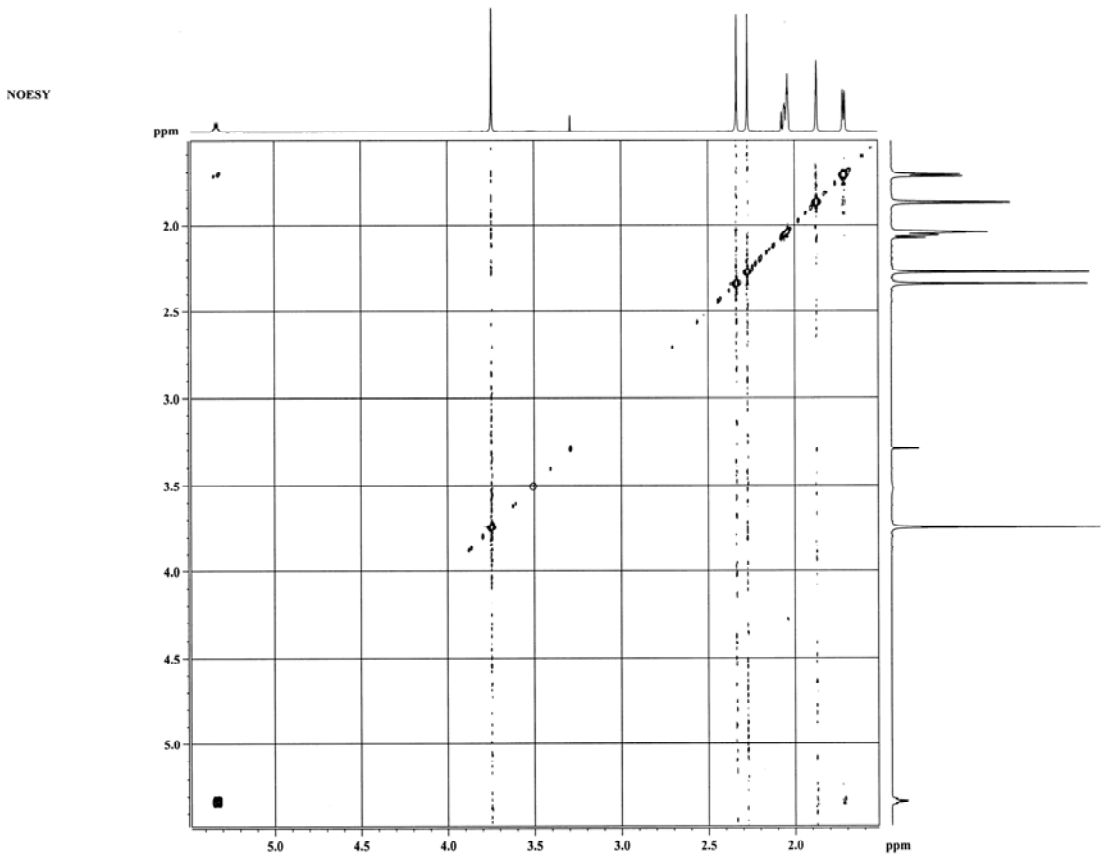

Figure 73 NOESY spectrum of nidulin (**5**)

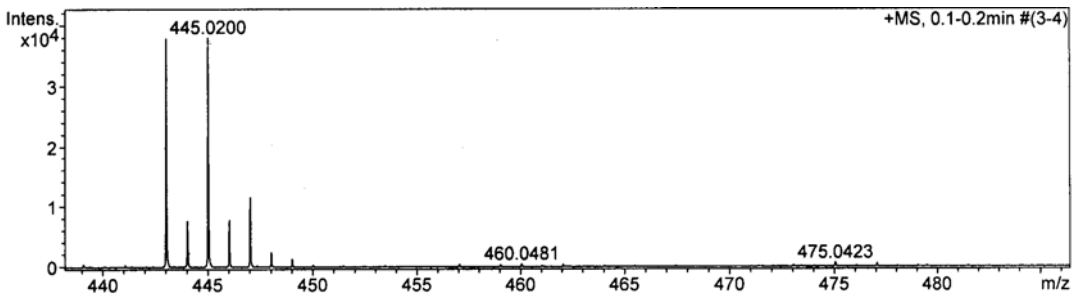

Figure 74 Mass spectrum of nidulin (**5**)

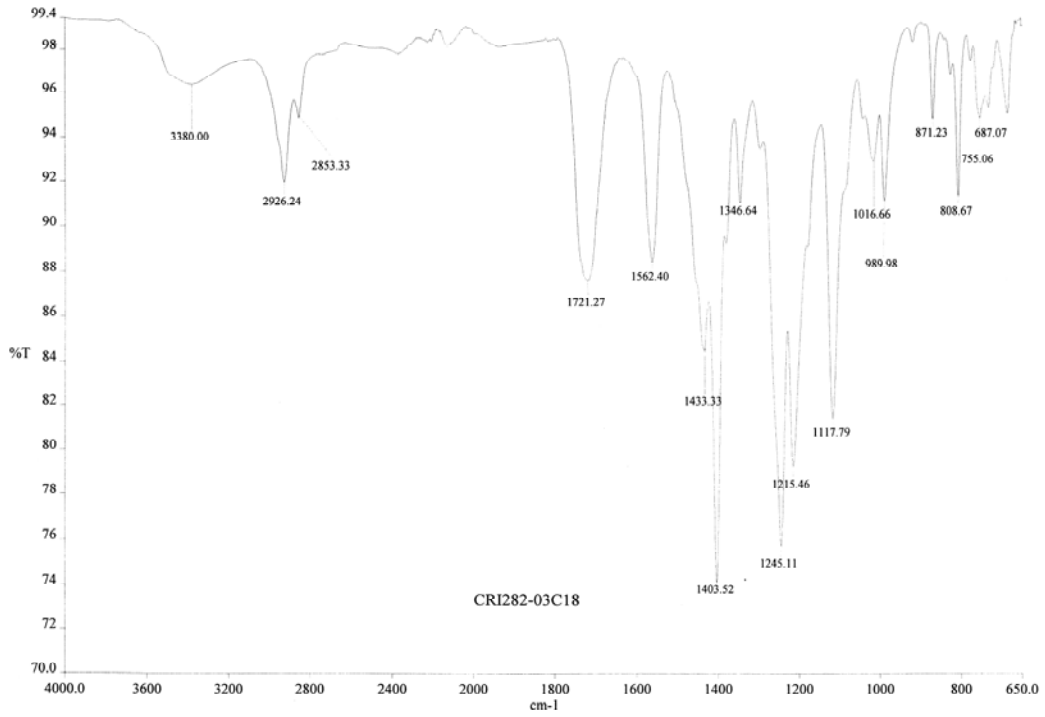

Figure 75 IR spectrum of nidulin (5).

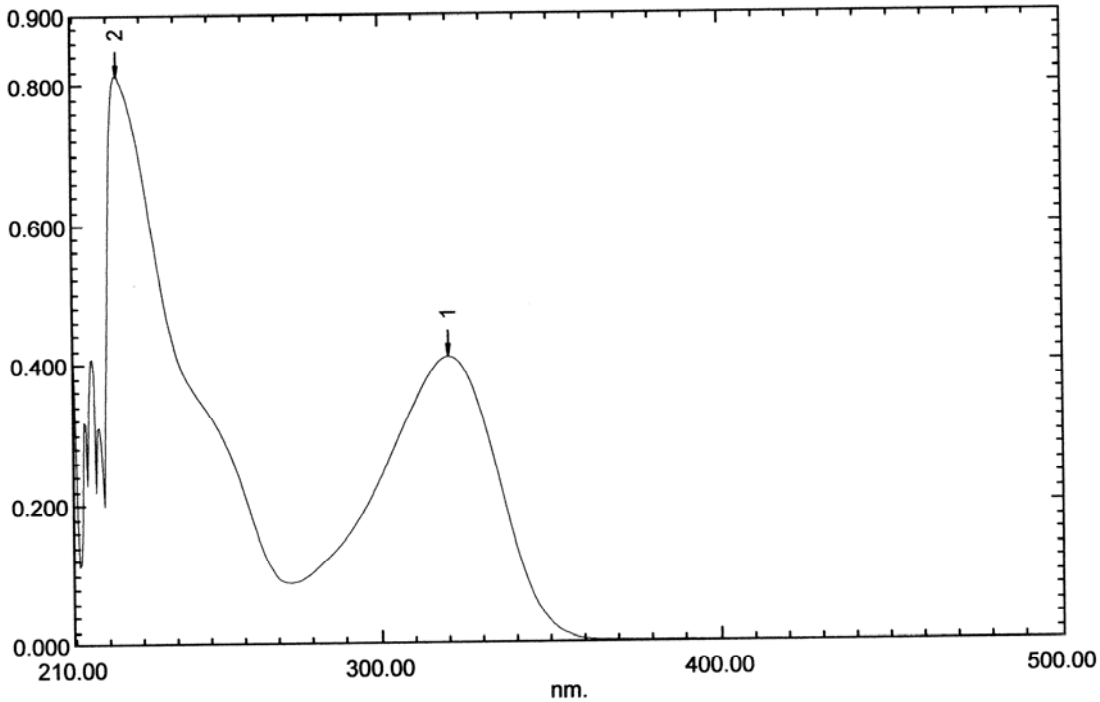

Figure 76 UV spectrum of nidulin (5)

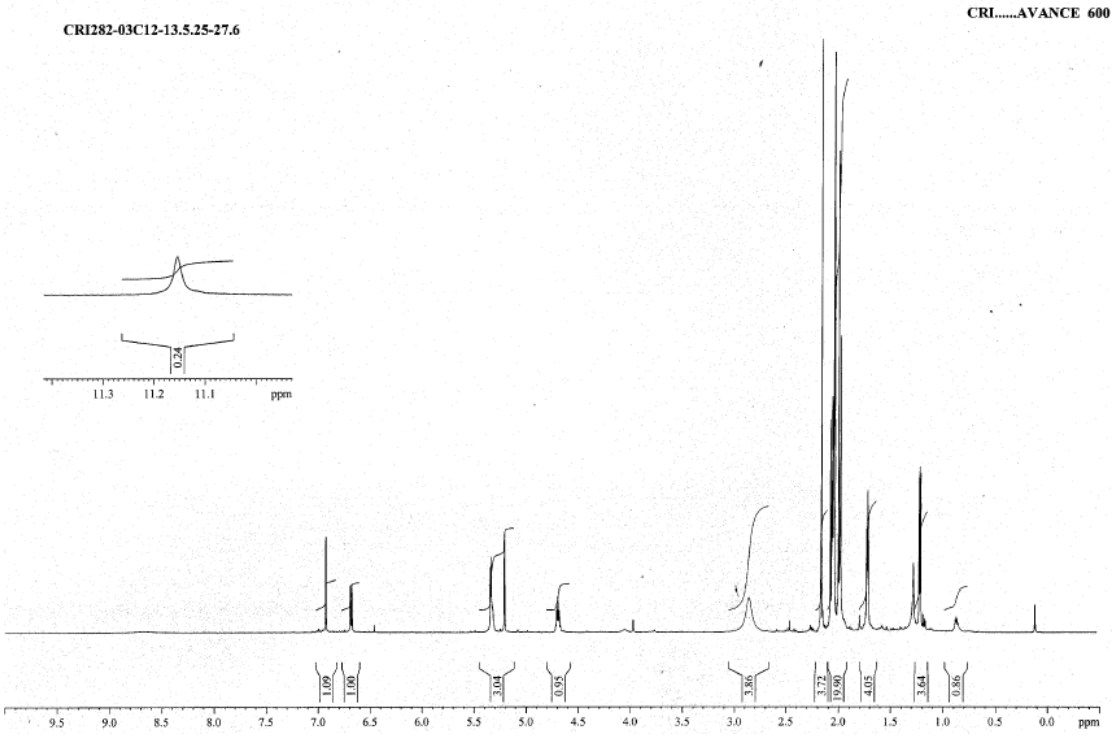

Figure 77 600 MHz ^{1}H NMR (acetone-d_6) spectrum of compound **6**

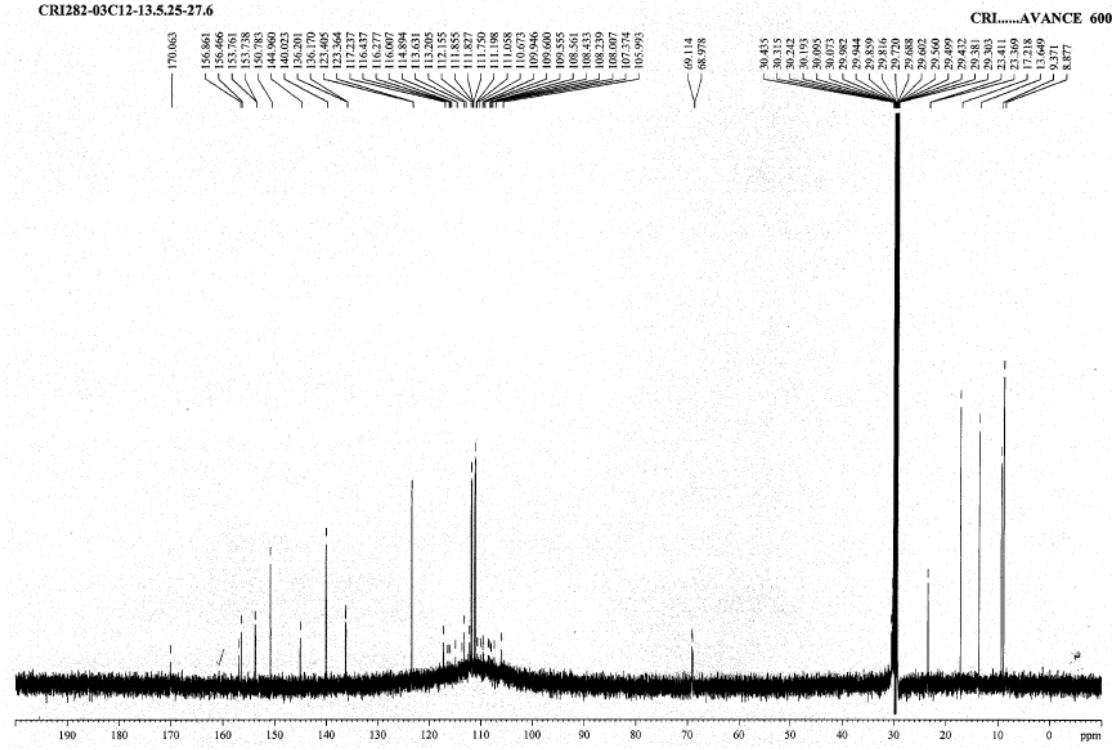

Figure 78 150 MHz ^{13}C NMR (acetone-d_6) spectrum of compound **6**

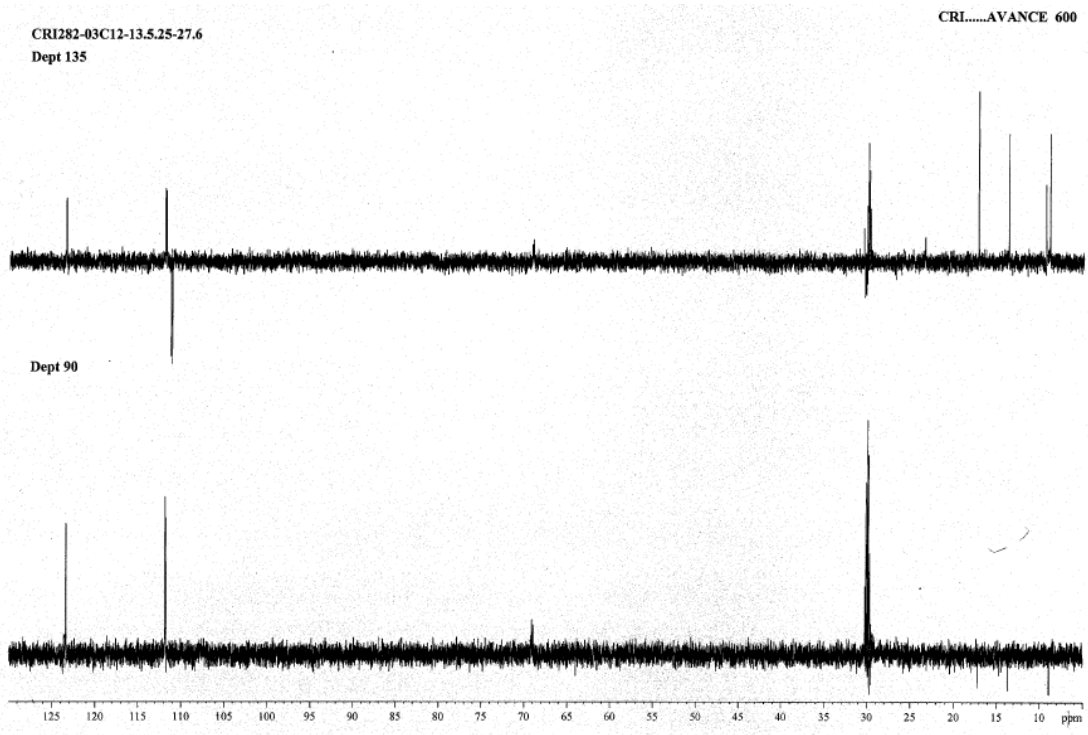

Figure 79 DEPT 90 and 135 spectra of compound 6

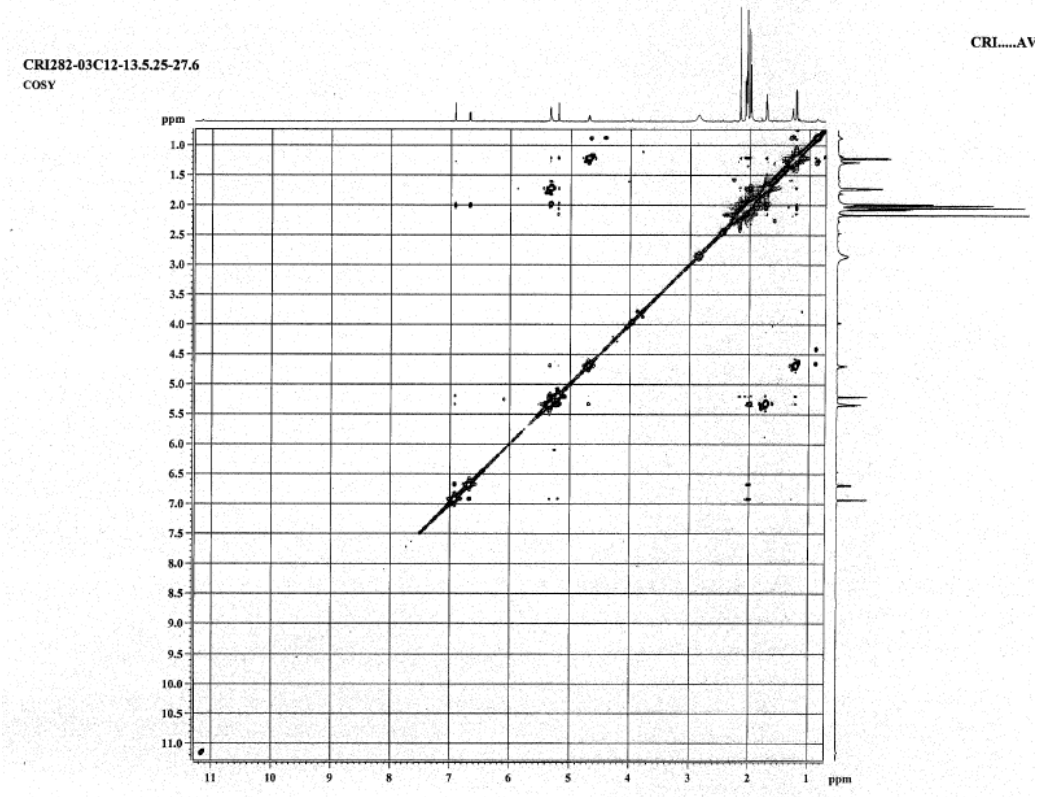

Figure 80 ^{1}H-^{1}H COSY spectrum of compound 6

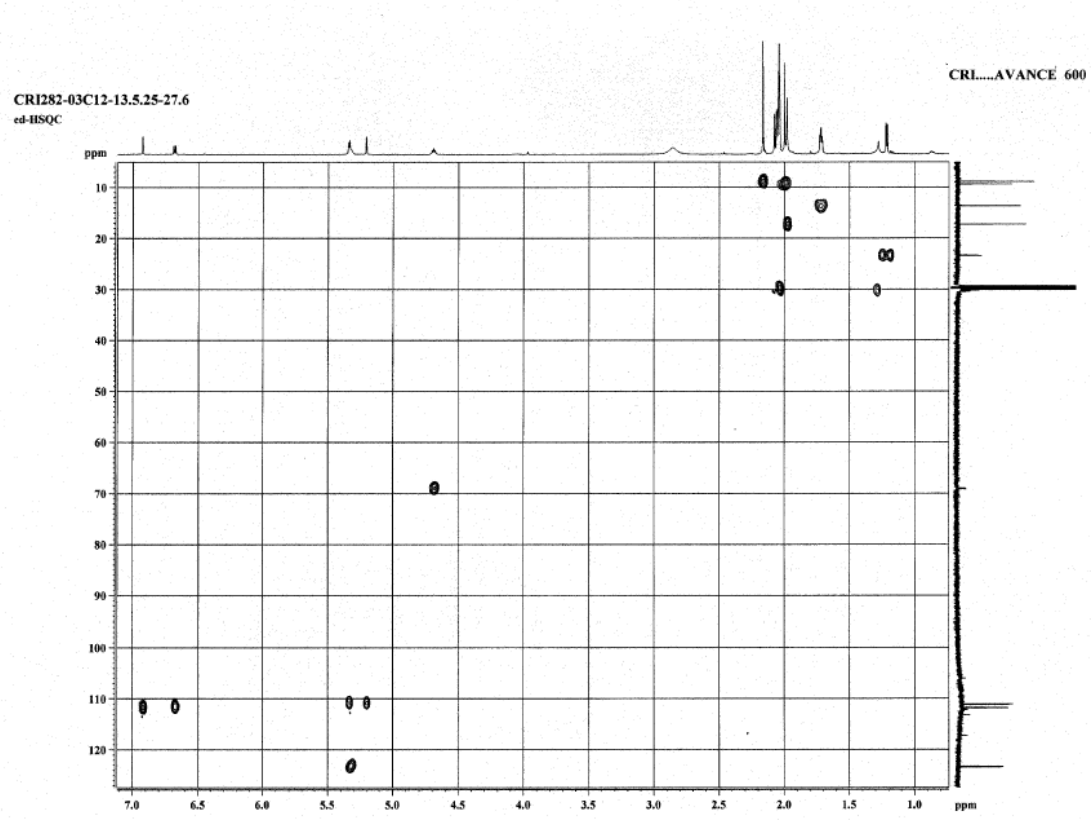

Figure 81 HMQC spectrum of compound 6

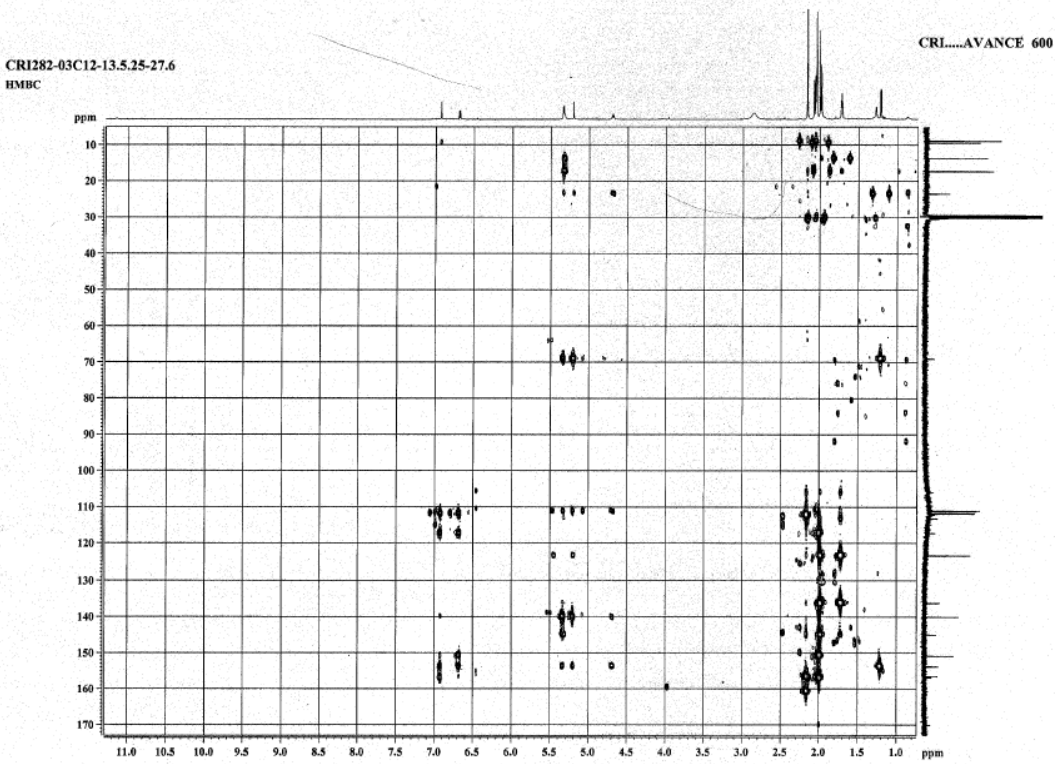

Figure 82 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound 6

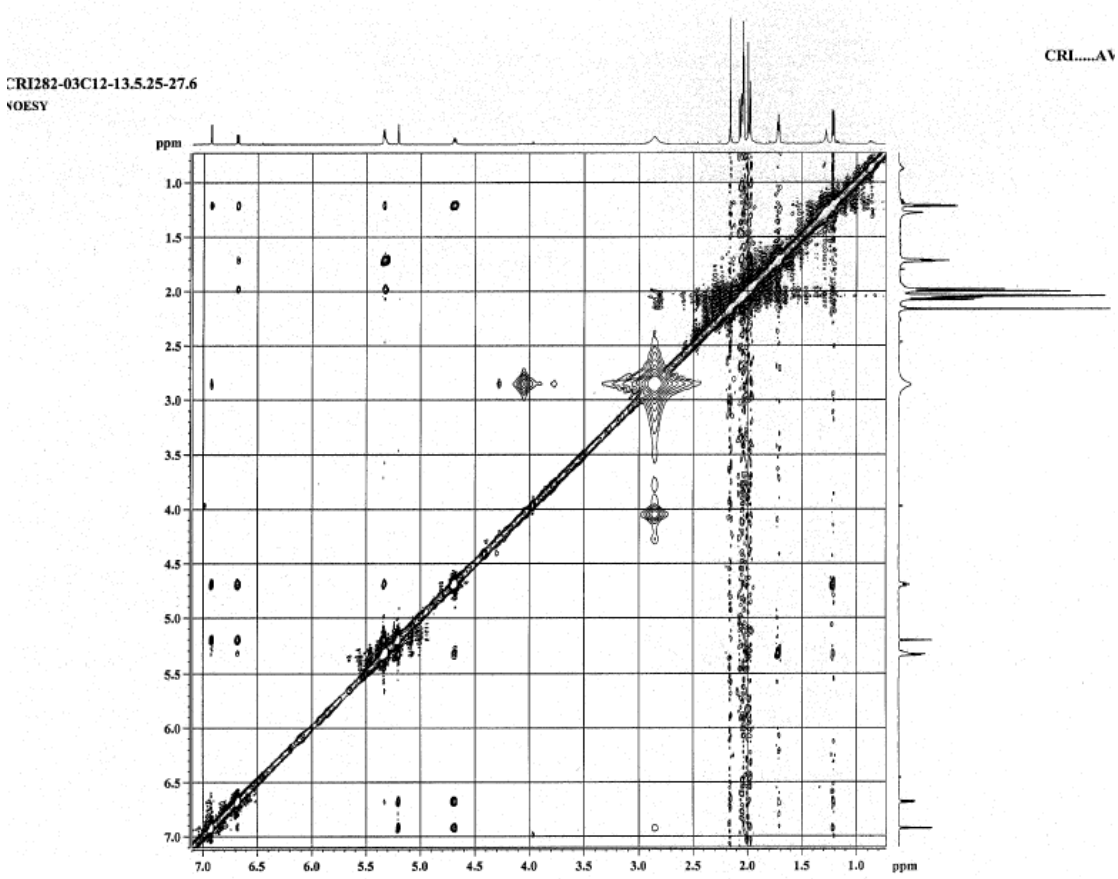

Figure 83 NOESY spectrum of compound **6**

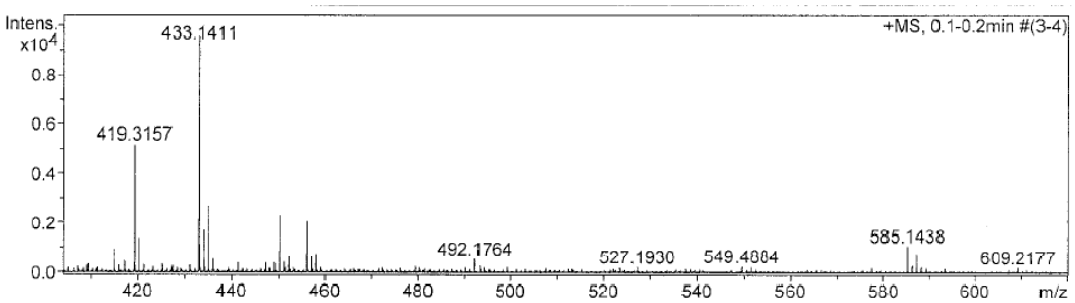

Figure 84 Mass spectrum of compound **6**

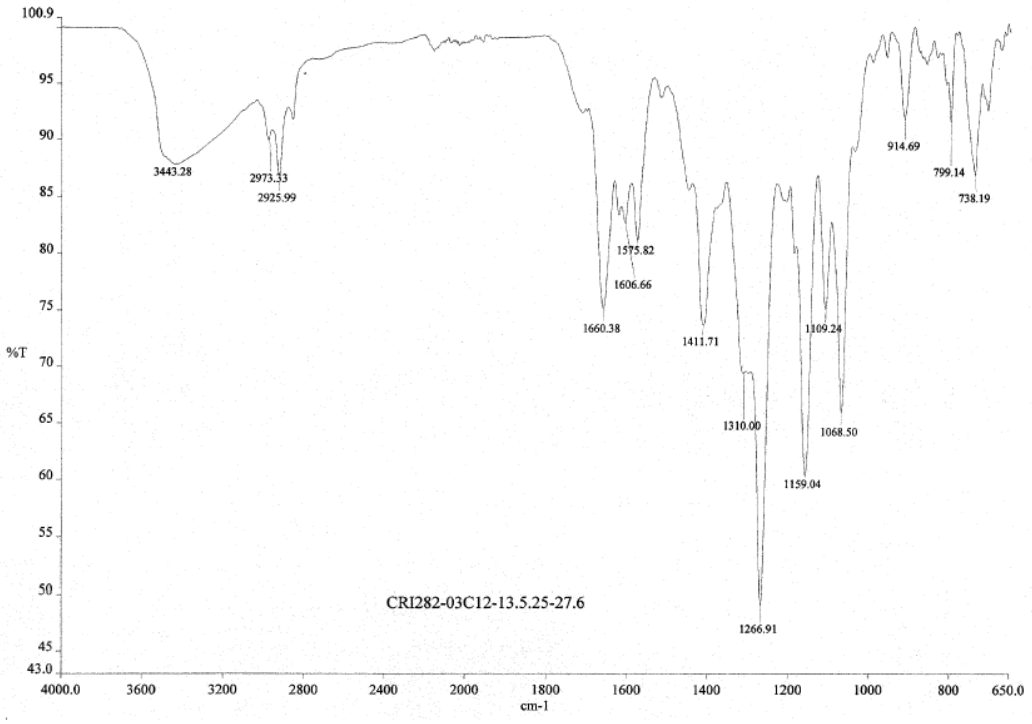

Figure 85 IR spectrum of compound 6

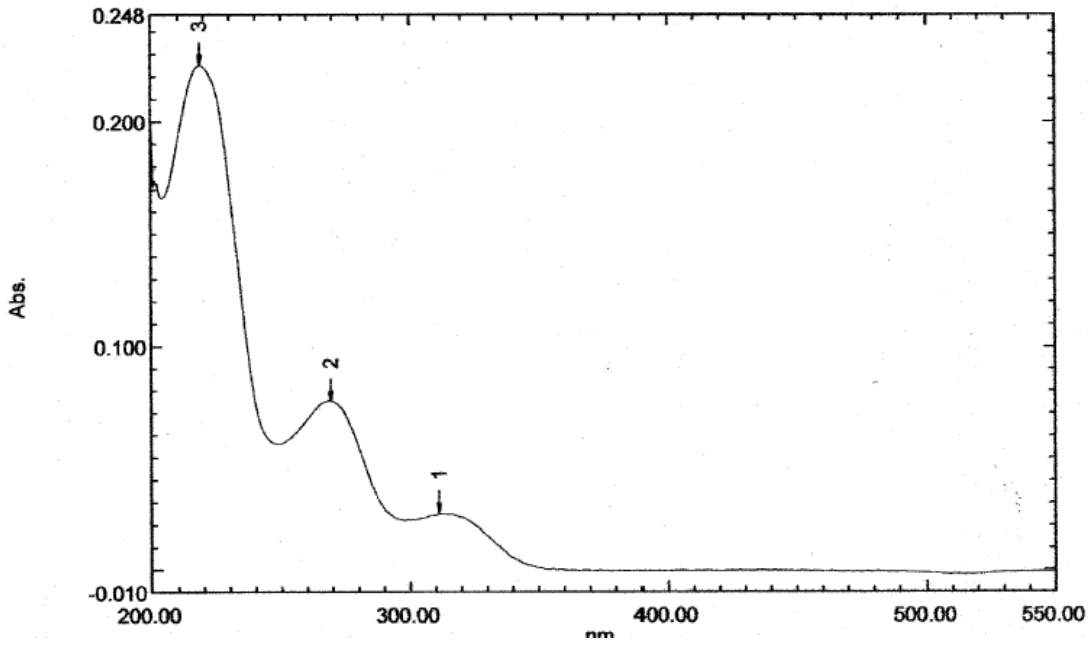

Figure 86 UV spectrum of compound 6

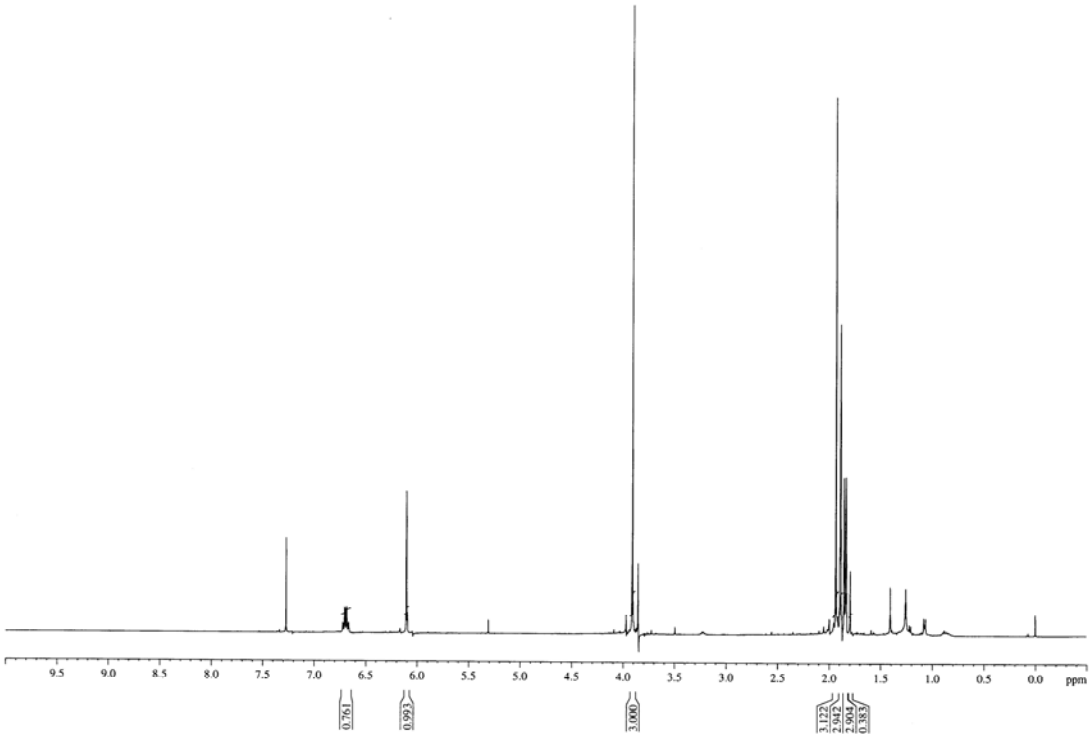

Figure 87 400 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **7**

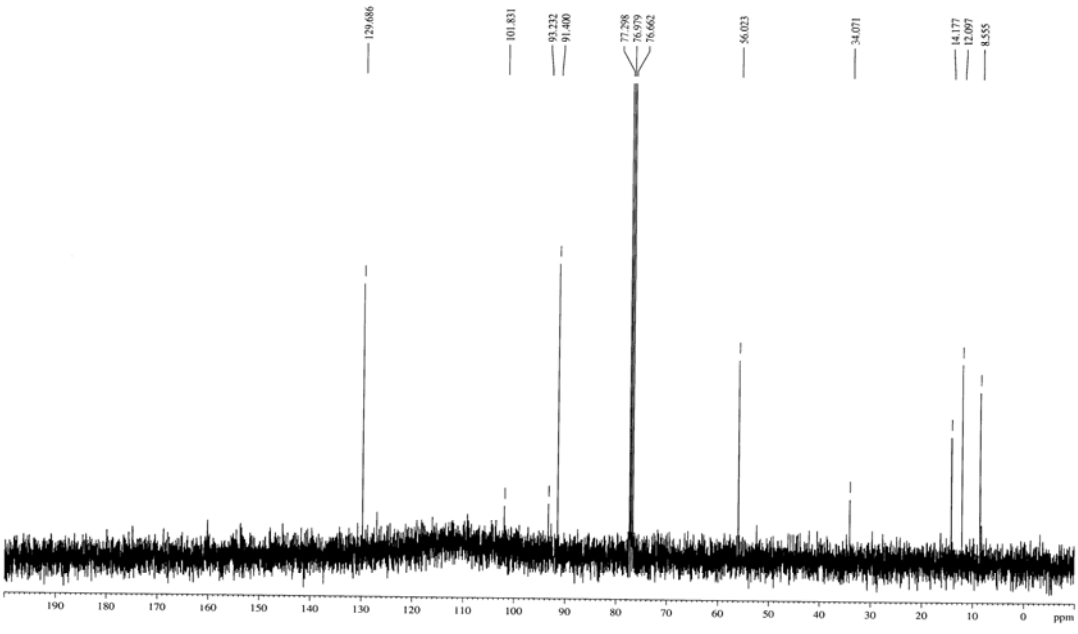

Figure 88 100 MHz ^{13}C NMR ($CDCl_3$) spectrum of compound **7**

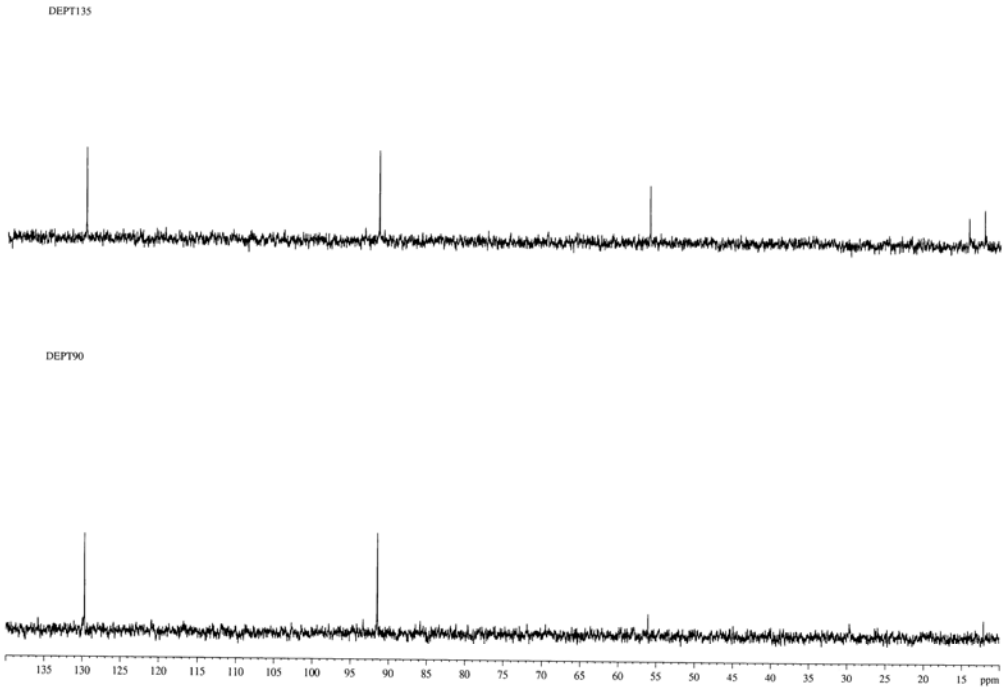

Figure 89 DEPT 90 and 135 spectra of compound **7**

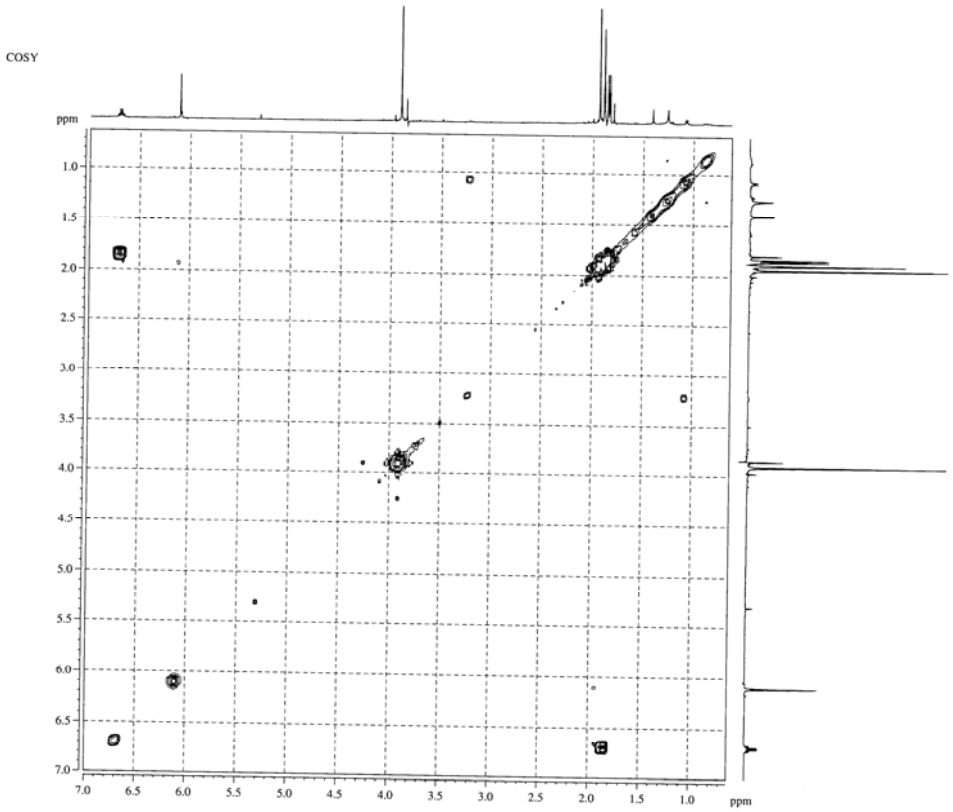

Figure 90 ^{1}H-^{1}H COSY spectrum of compound **7**

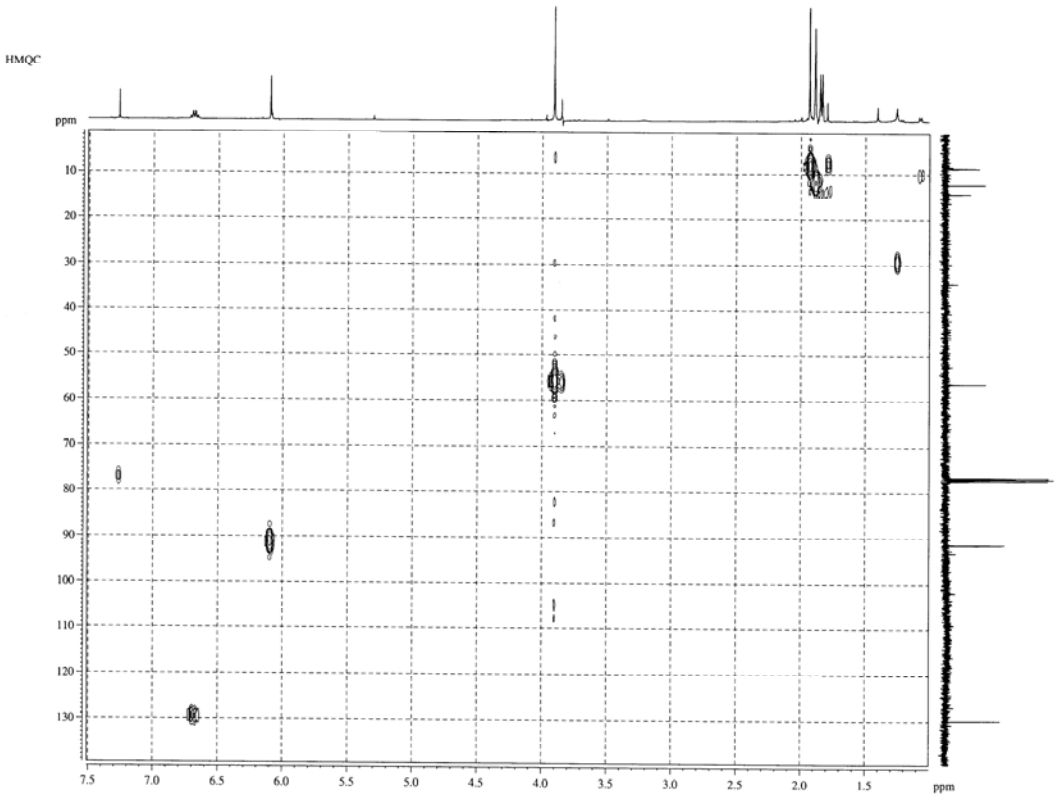

Figure 91 HMQC spectrum of compound 7

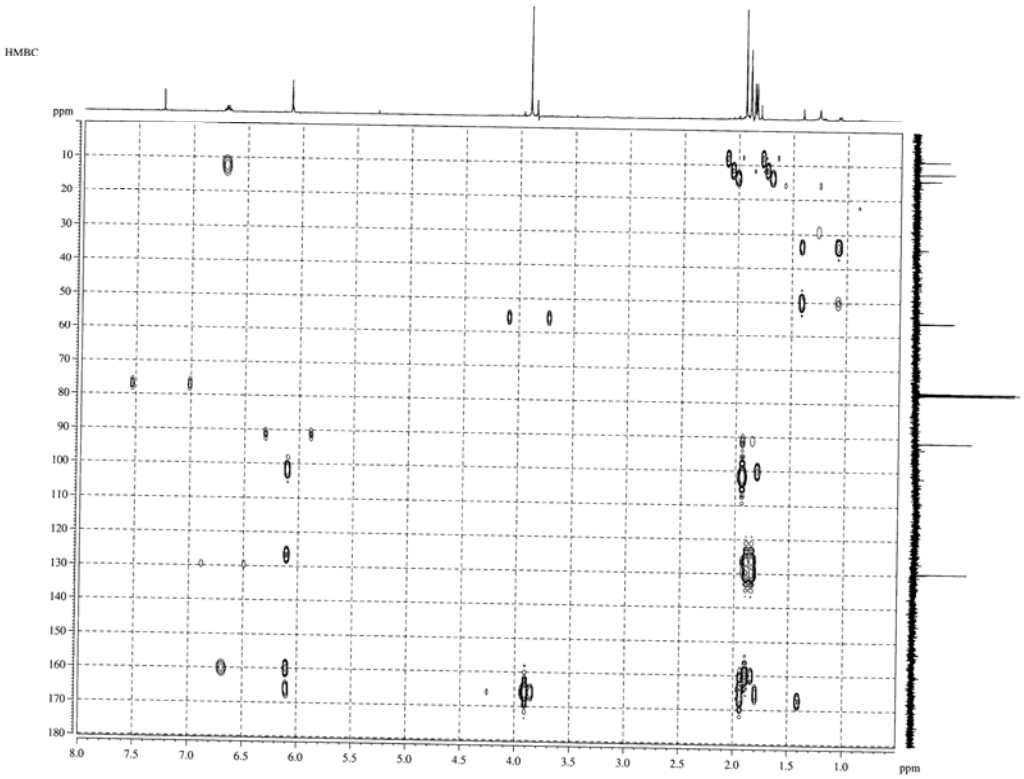

Figure 92 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound 7

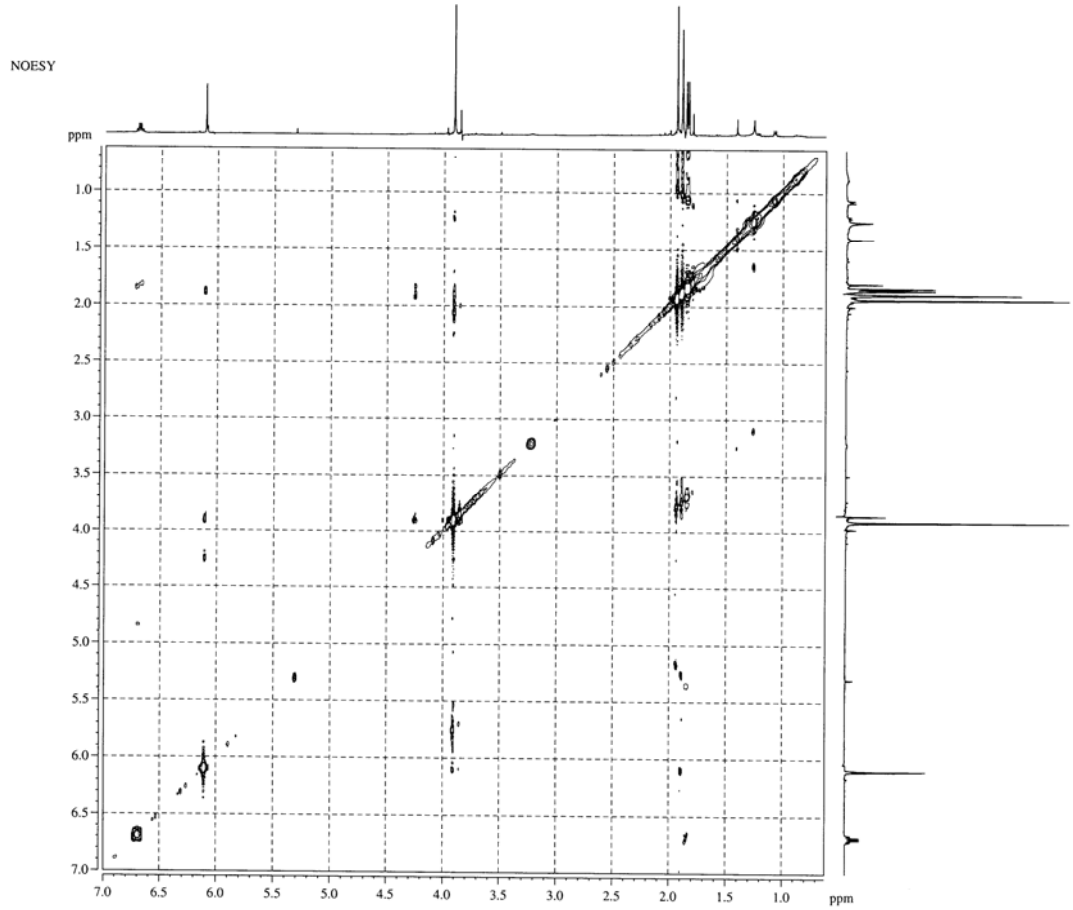

Figure 93 NOESY spectrum of compound **7**

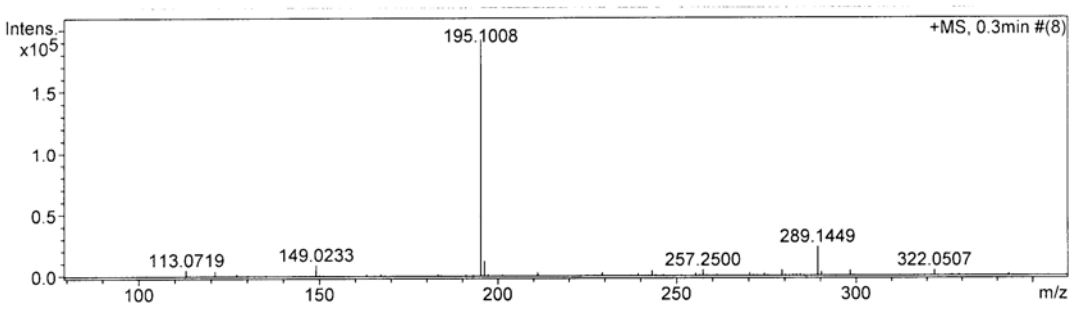

Figure 94 Mass spectrum of compound **7**

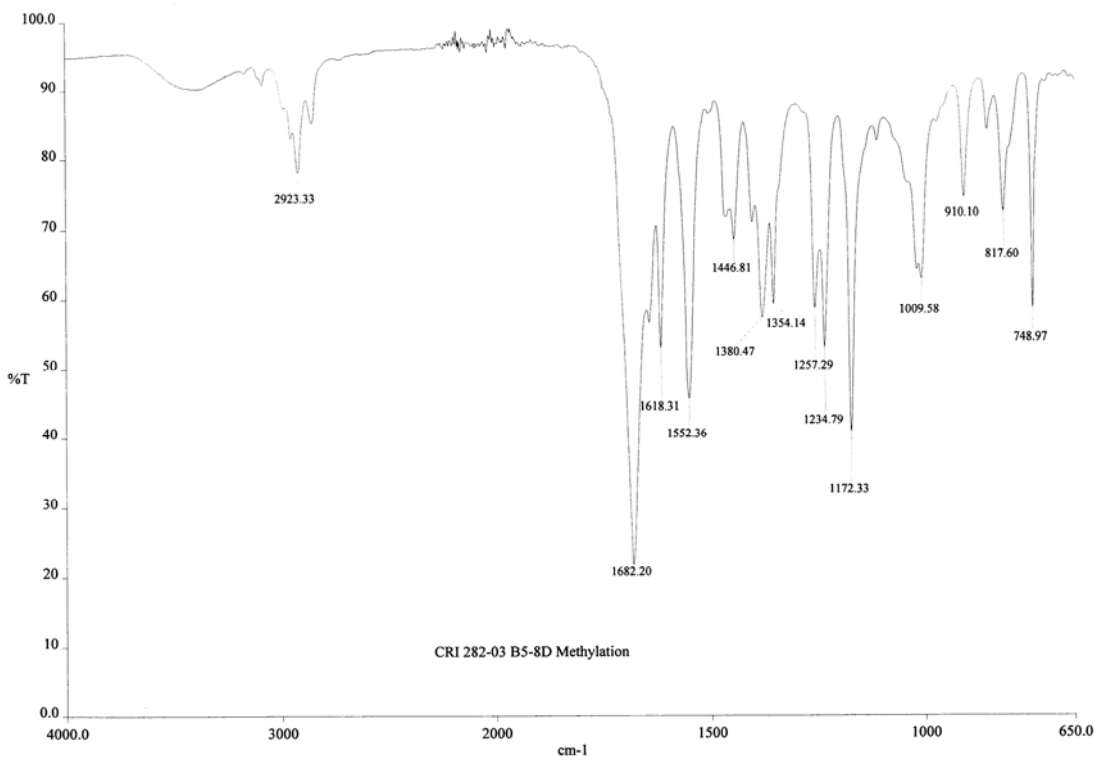

Figure 95 IR spectrum of compound **7**

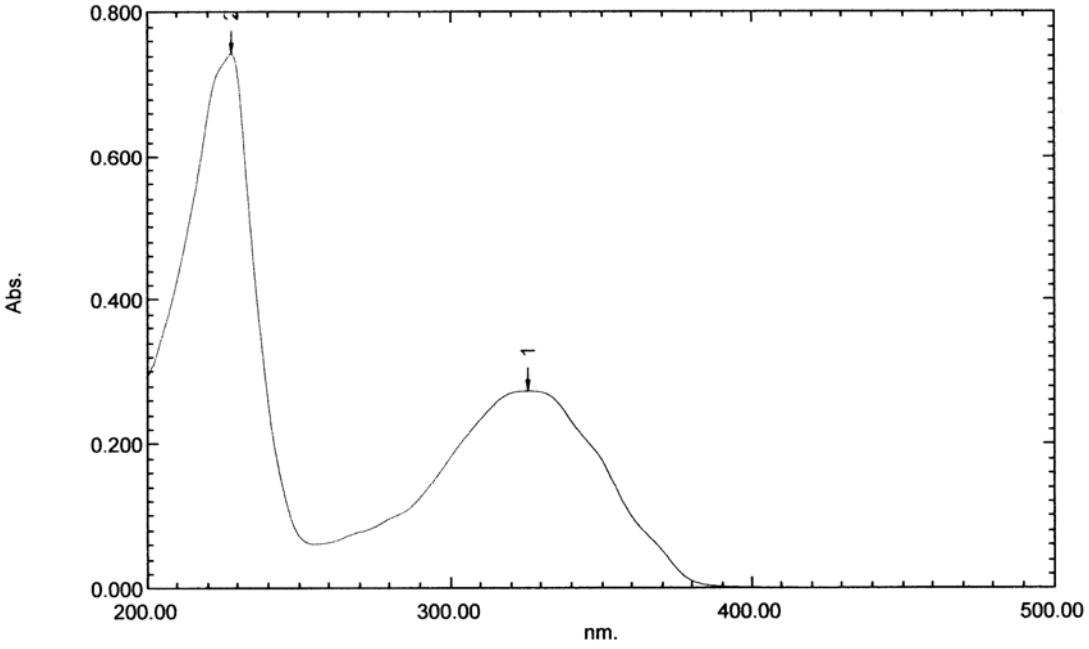

Figure 96 UV spectrum of compound **7**

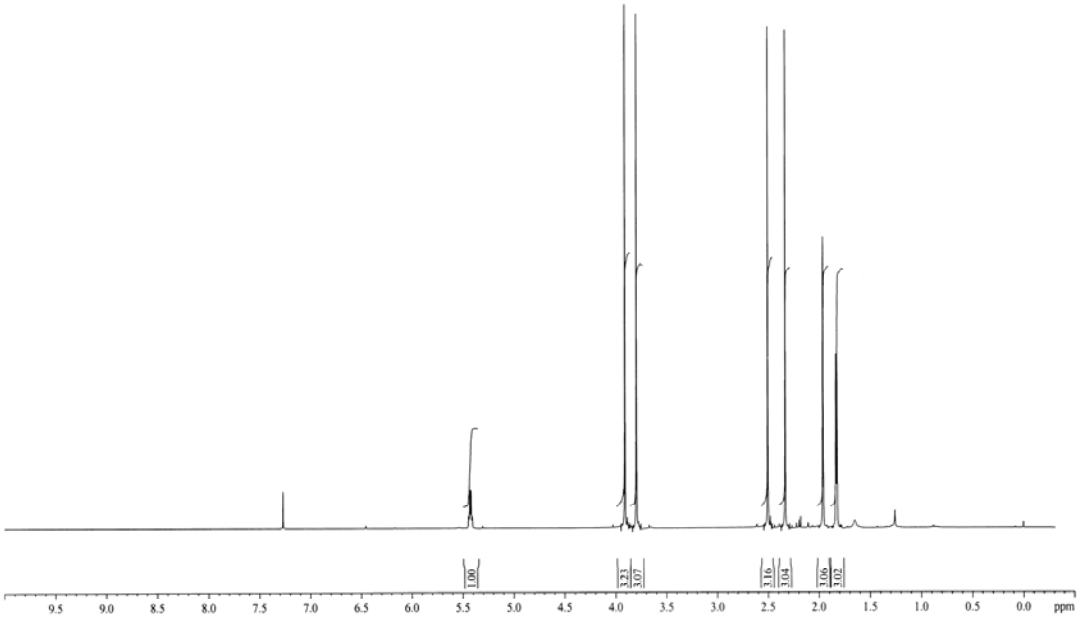

Figure 97 600 MHz ^{1}H NMR ($CDCl_3$) spectrum of compound **8**

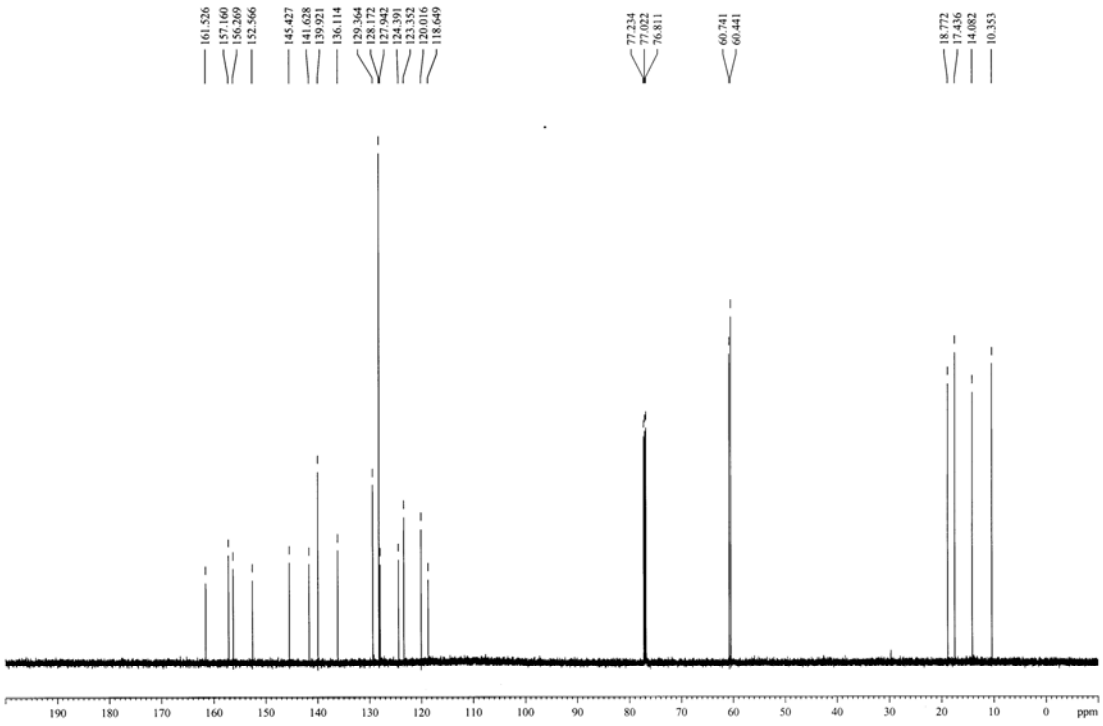

Figure 98 150 MHz ^{13}C NMR ($CDCl_3$) spectrum of compound **8**

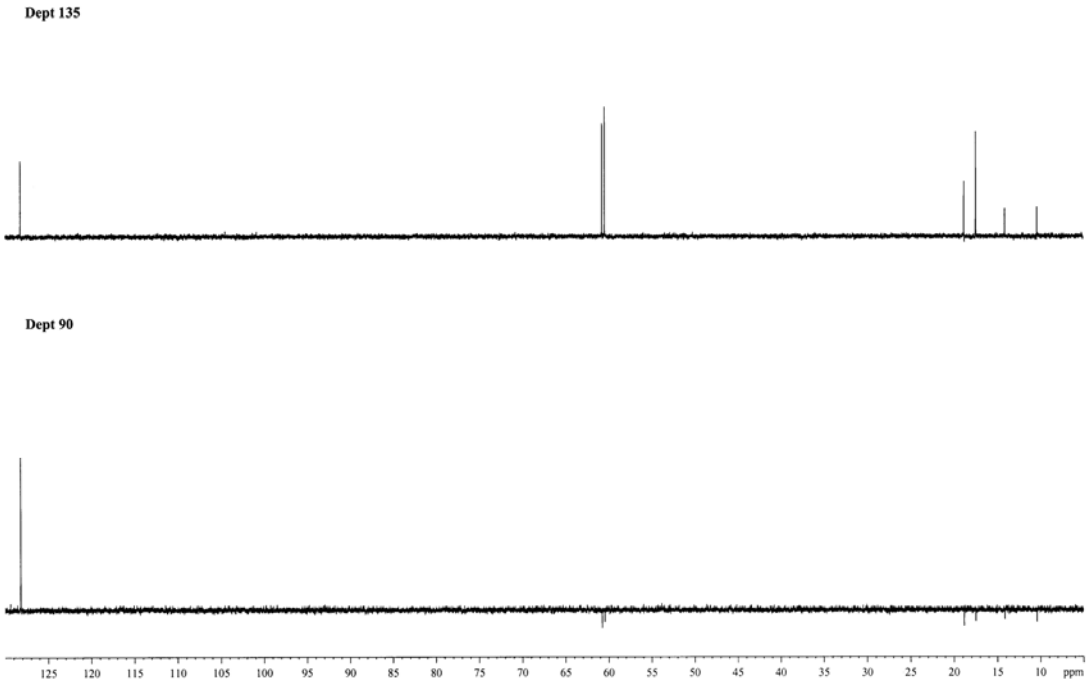

Figure 99 DEPT 90 and 135 spectra of compound 8

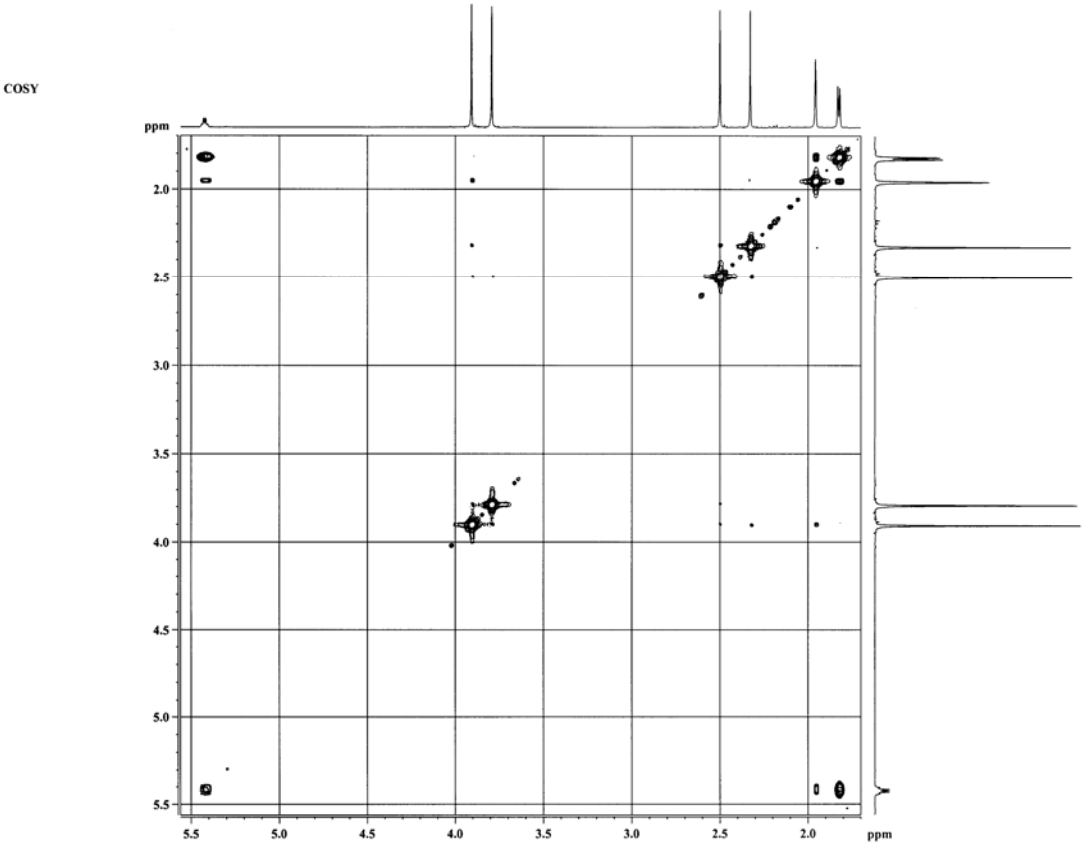

Figure 100 ^{1}H-^{1}H COSY spectrum of compound 8

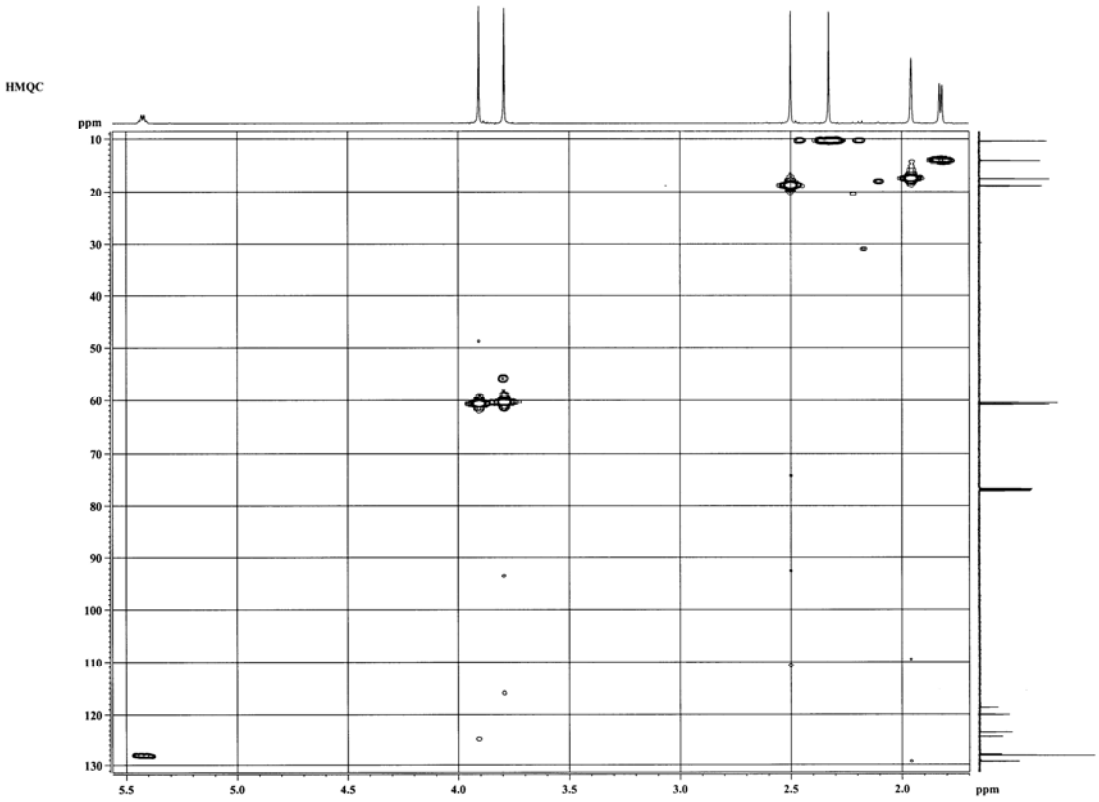

Figure 101 HMQC spectrum of compound **8**

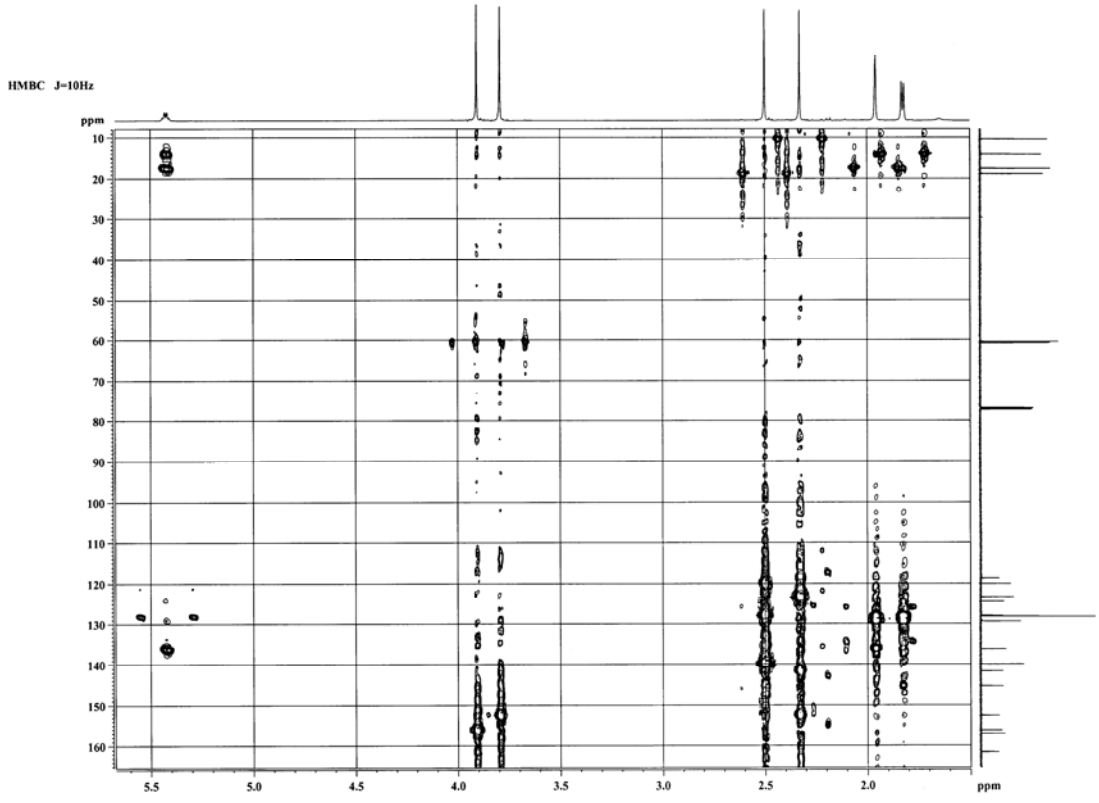

Figure 102 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound **8**

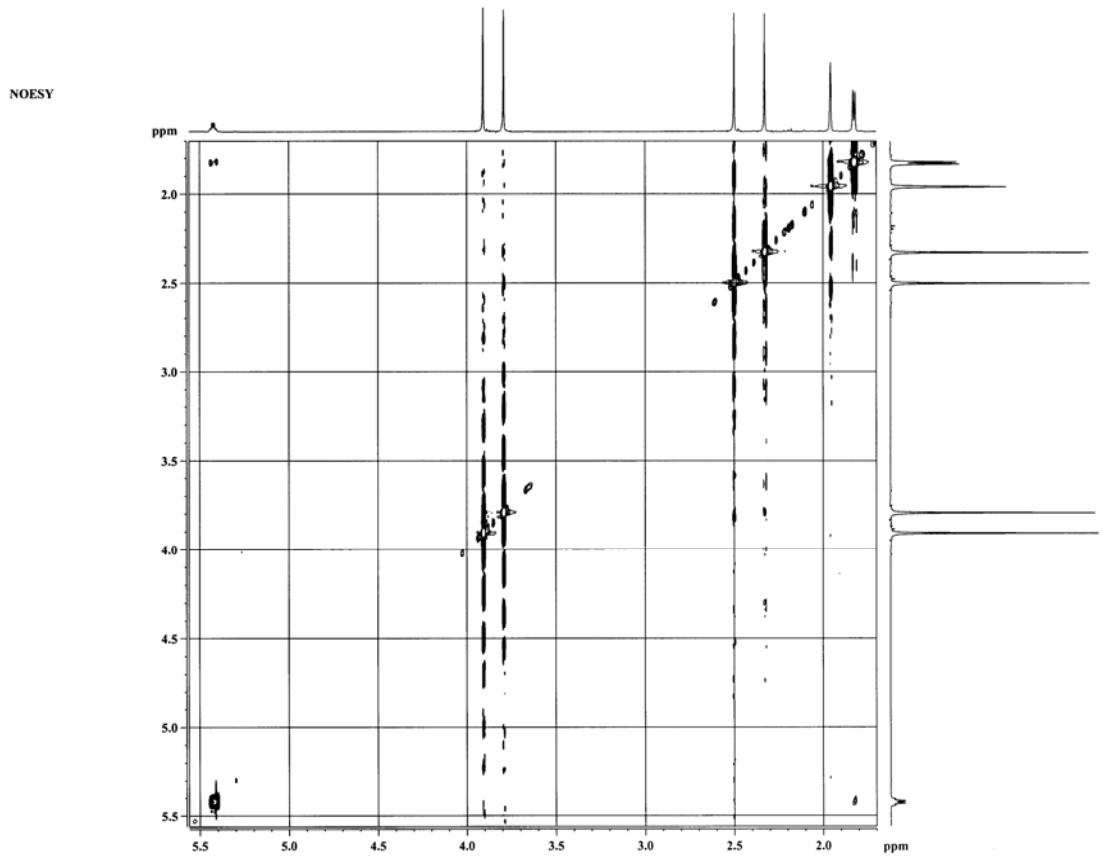

Figure 103 NOESY spectrum of compound **8**

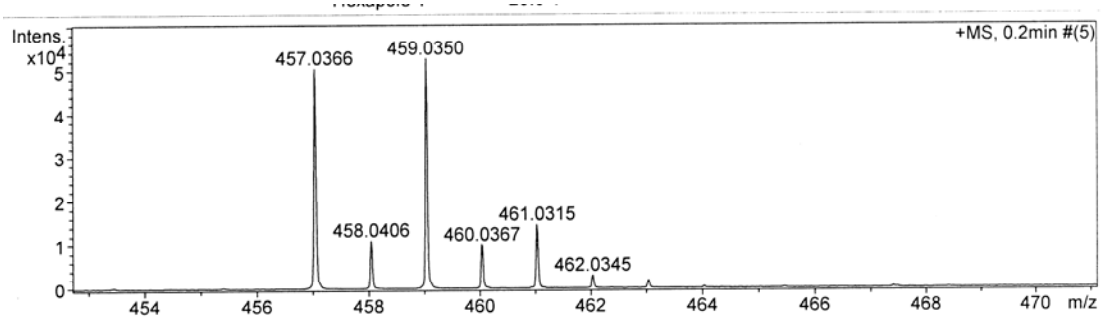

Figure 104 Mass spectrum of compound **8**

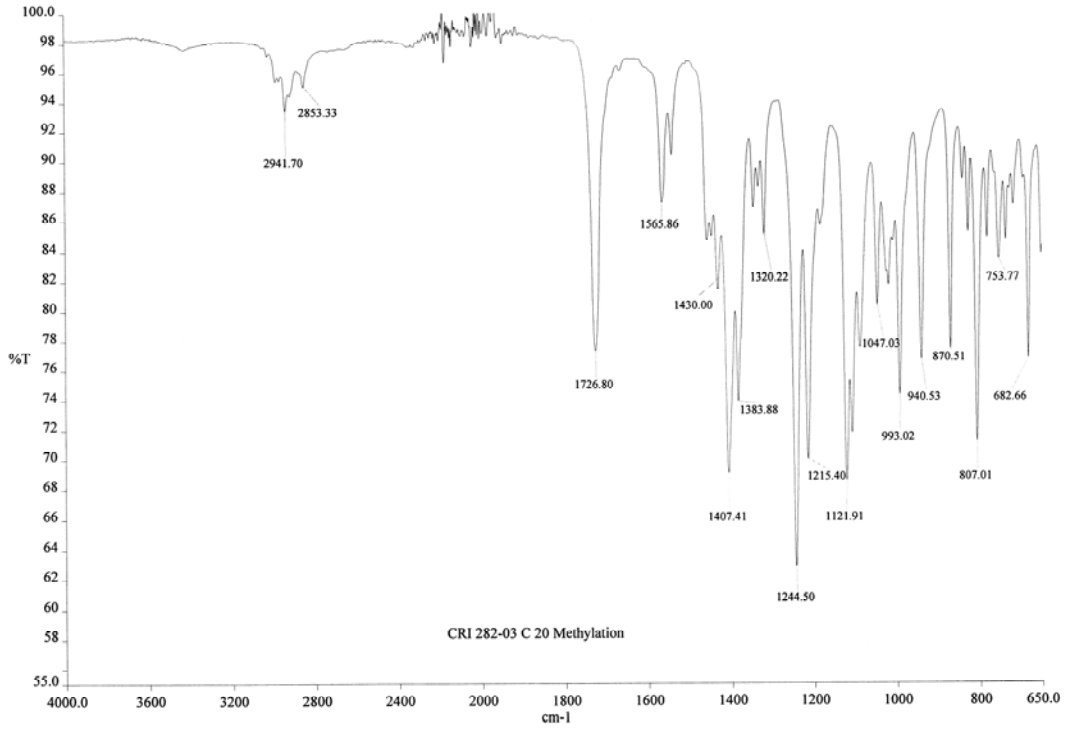

Figure 105 IR spectrum of compound 8

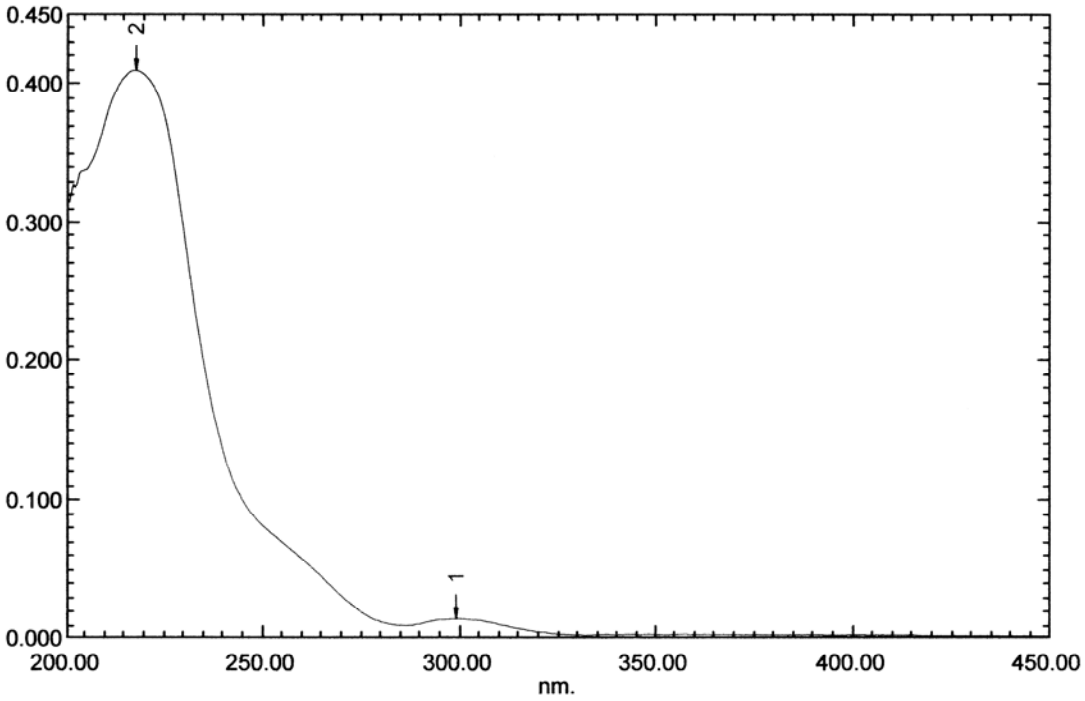

Figure 106 UV spectrum of compound 8

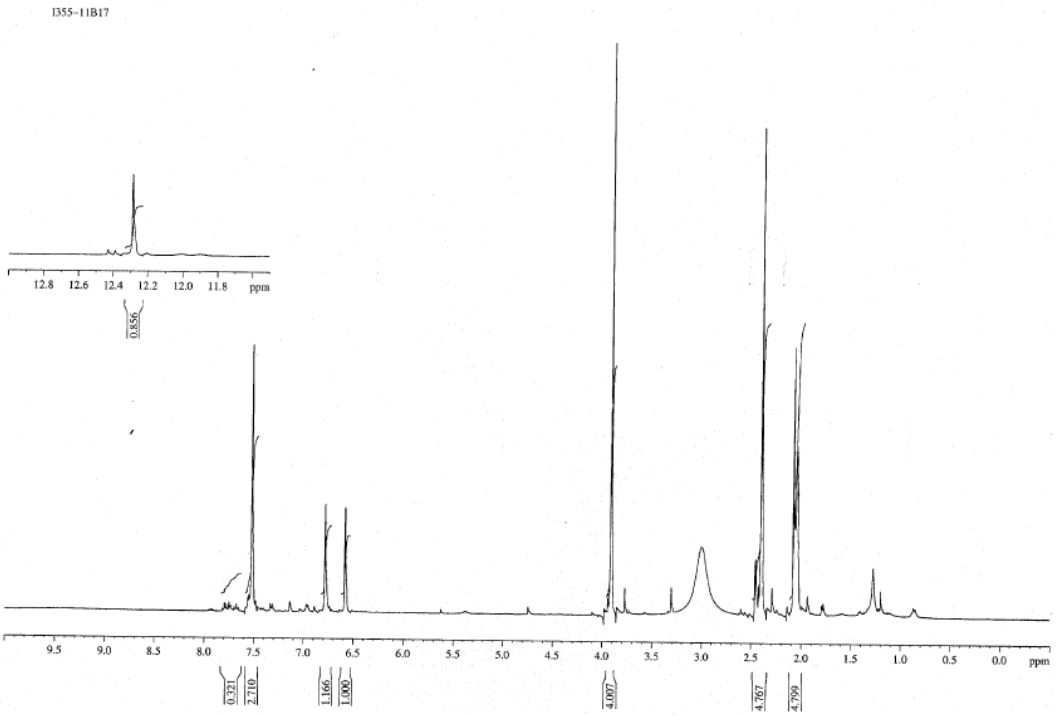

Figure 107 400 MHz ^{1}H NMR (acetone-d_6) spectrum of compound **9**

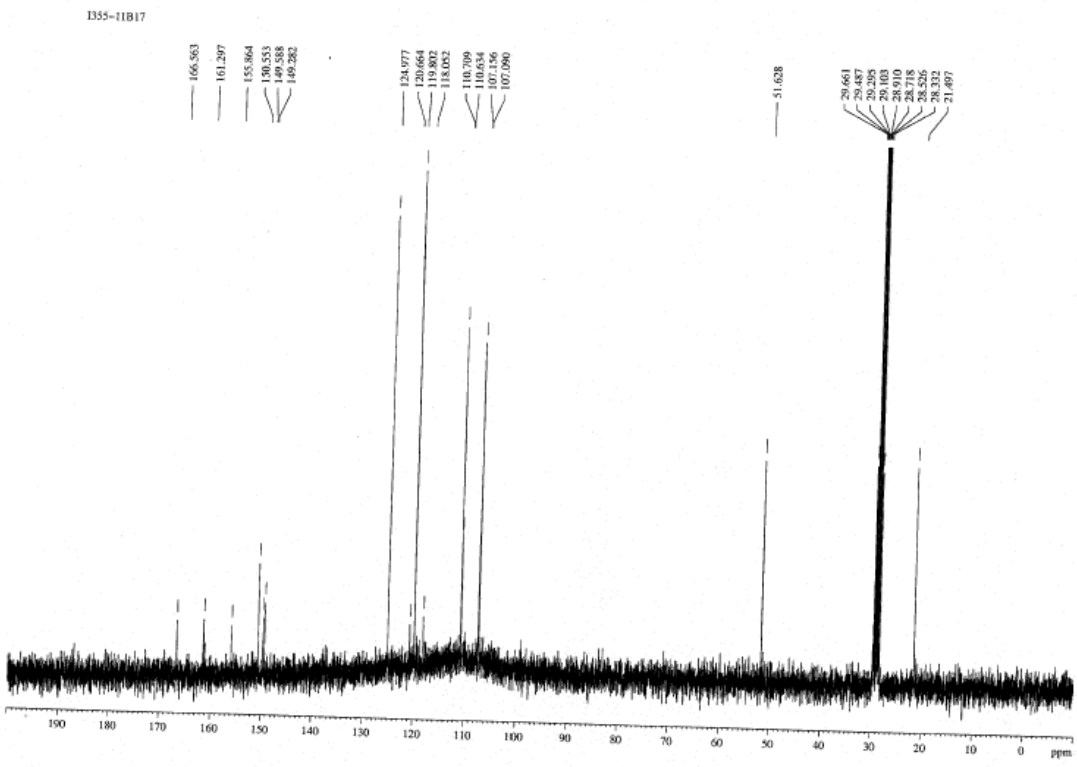

Figure 108 ^{13}C NMR (acetone-d_6) spectrum of compound **9**

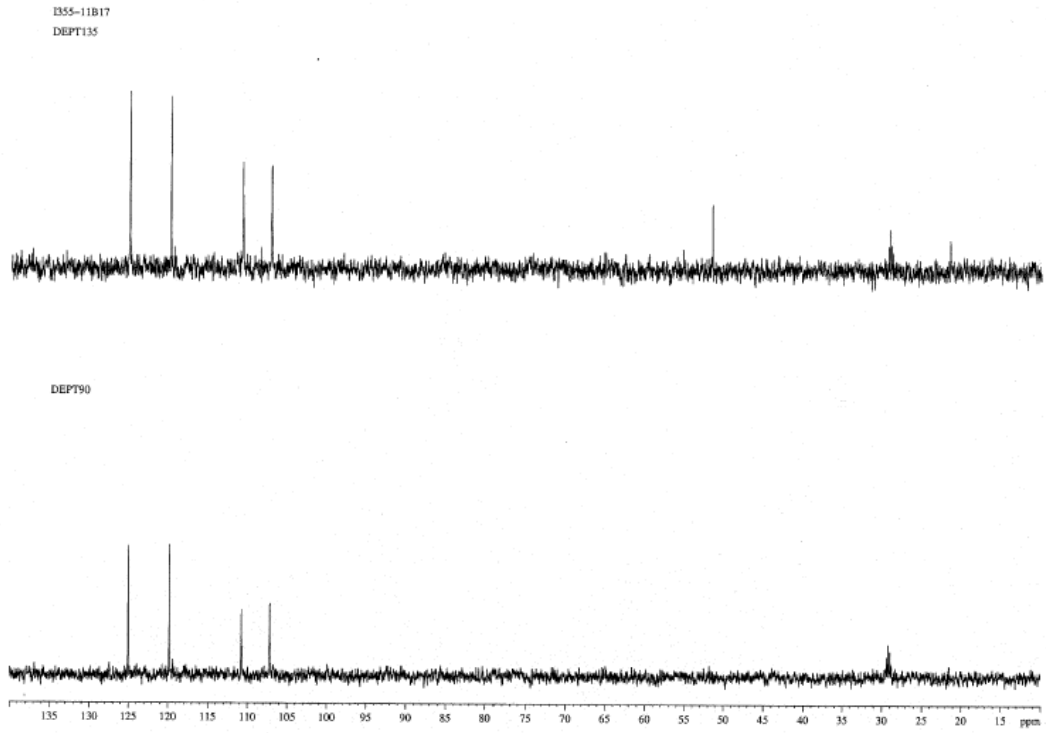

Figure 109 DEPT 90 and 135 spectra of compound **9**

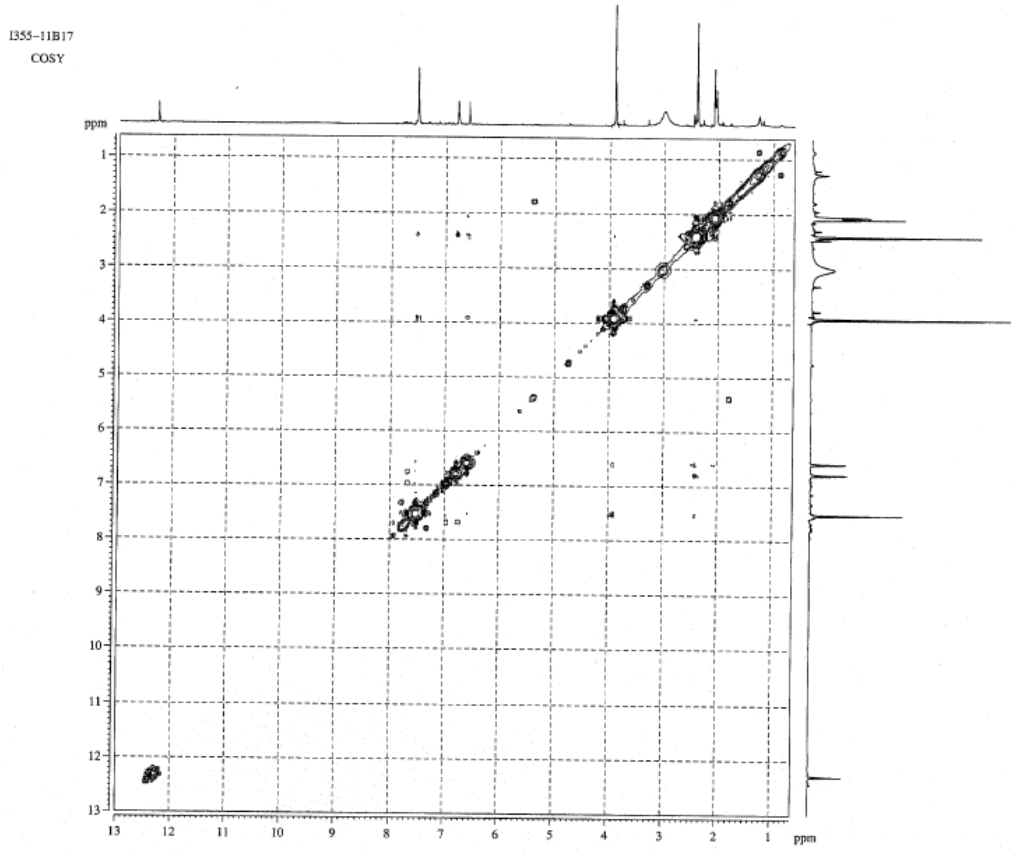

Figure 110 ^{1}H-^{1}H COSY spectrum of compound **9**

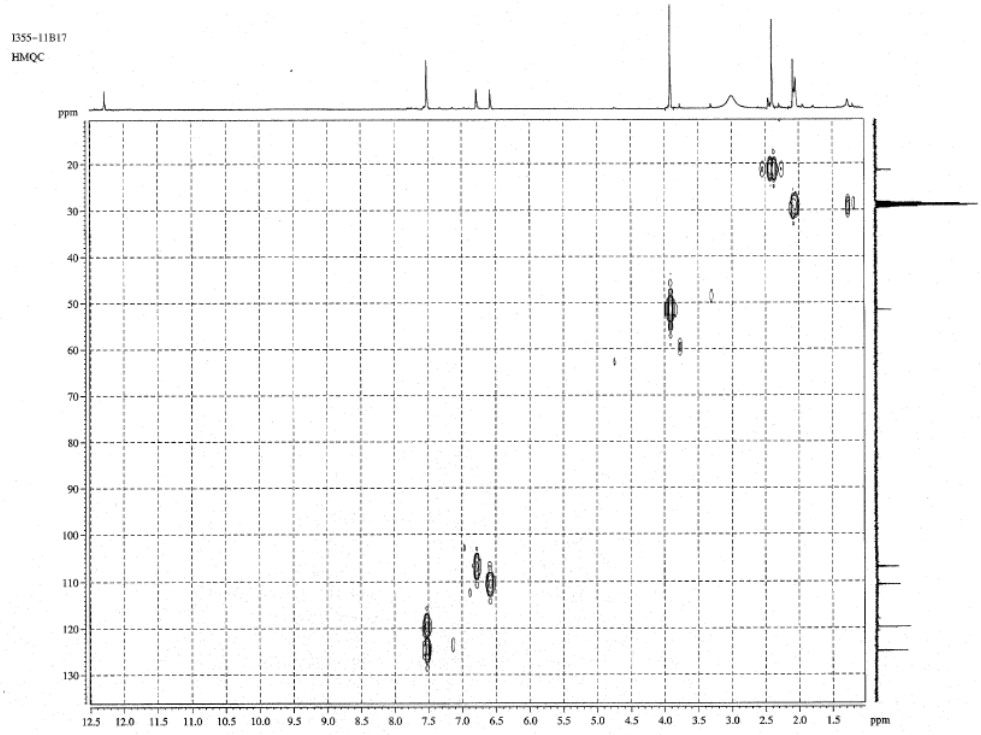

Figure 111 HMQC spectrum of compound **9**

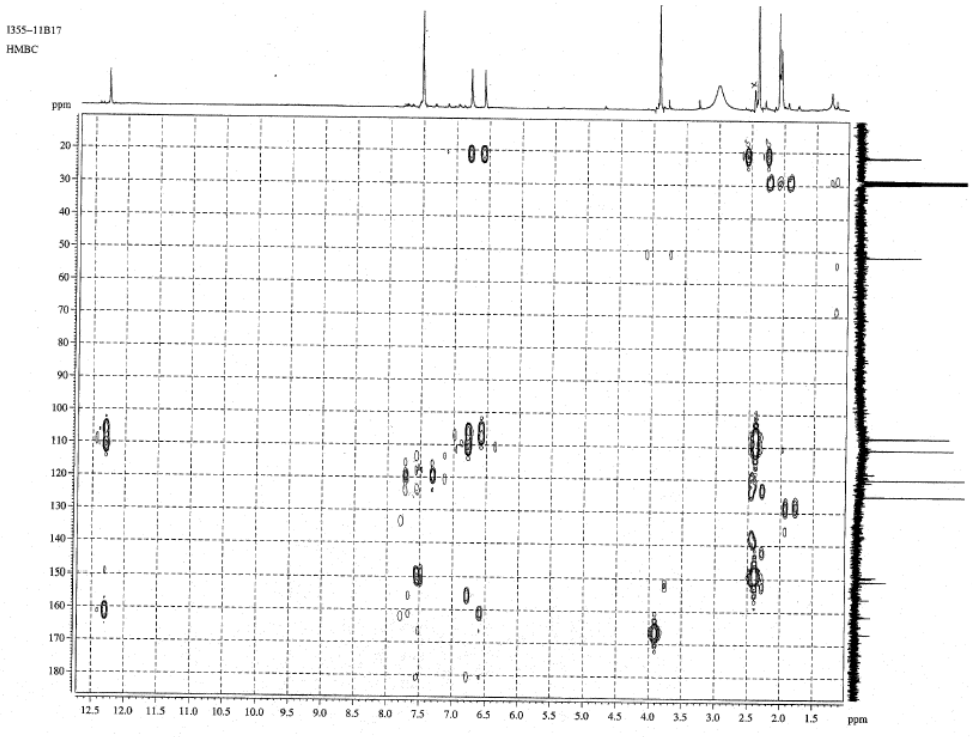

Figure 112 Long range ^{1}H-^{13}C NMR correlations (HMBC spectrum) of compound **9**

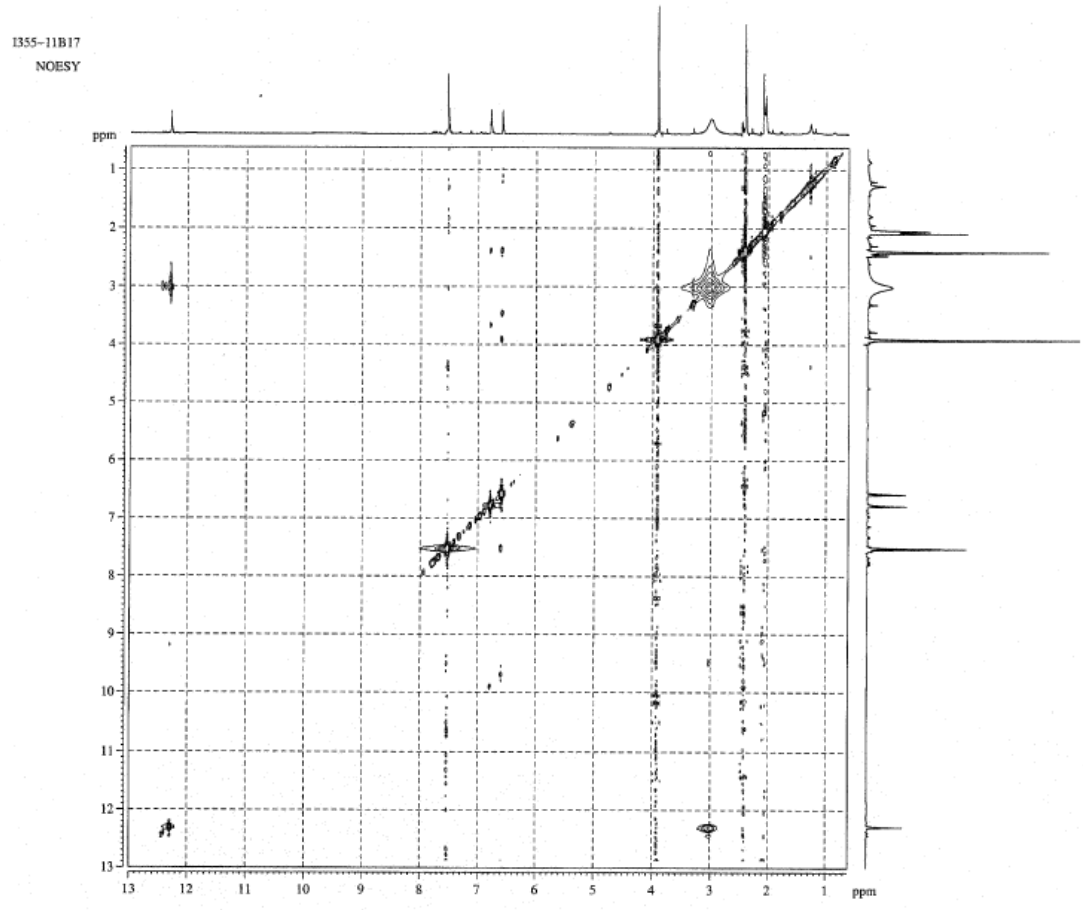

Figure 113 NOESY spectrum of compound 9

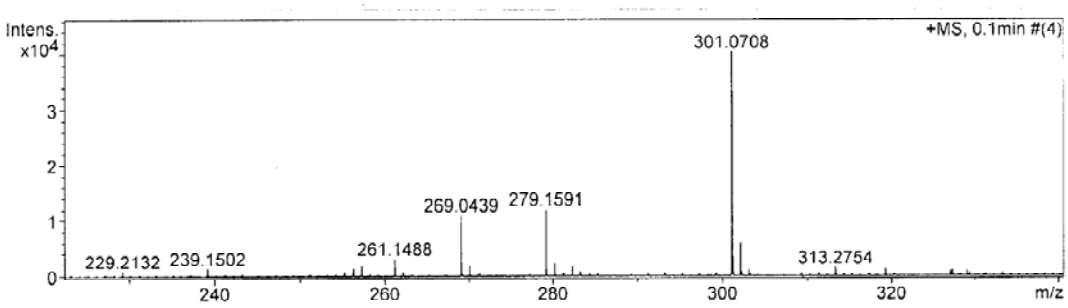

Figure 114 Mass spectrum of compound 9

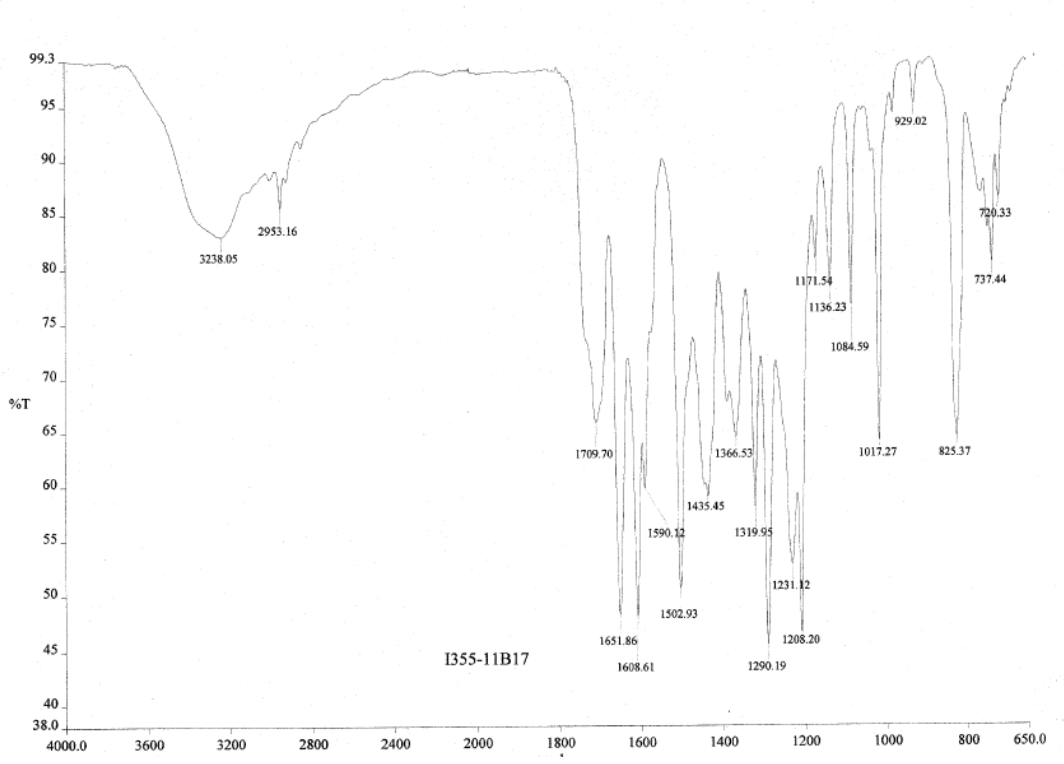

Figure 115 IR spectrum of compound 9

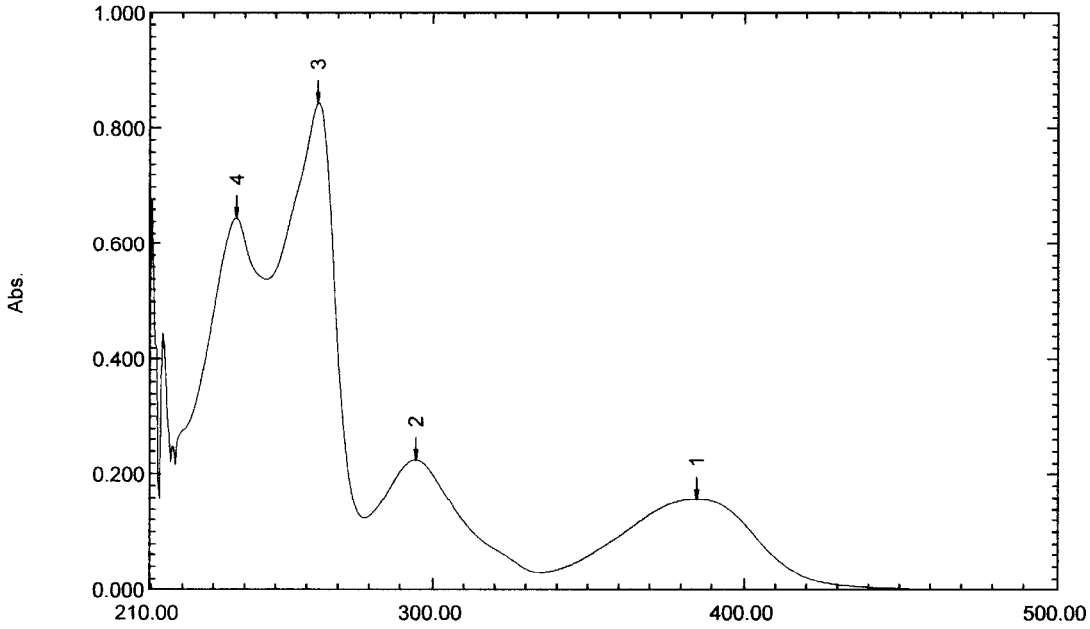

Figure 116 UV spectrum of compound 9

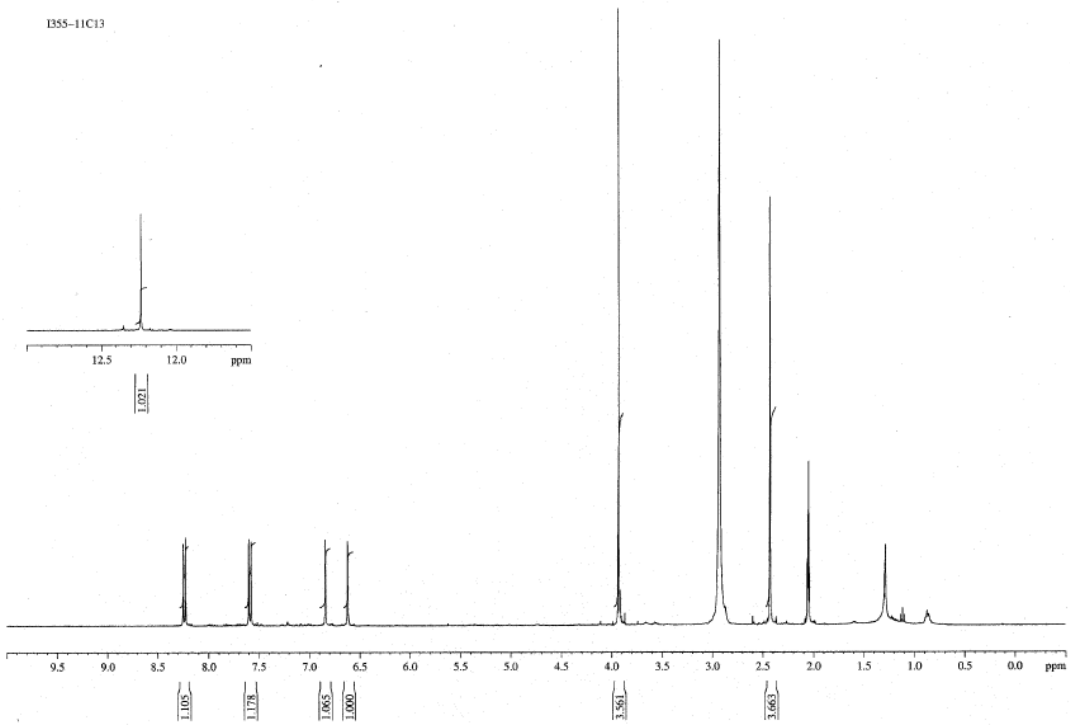

Figure 117 400 MHz ^{1}H NMR (acetone-d_6) spectrum of compound **10**

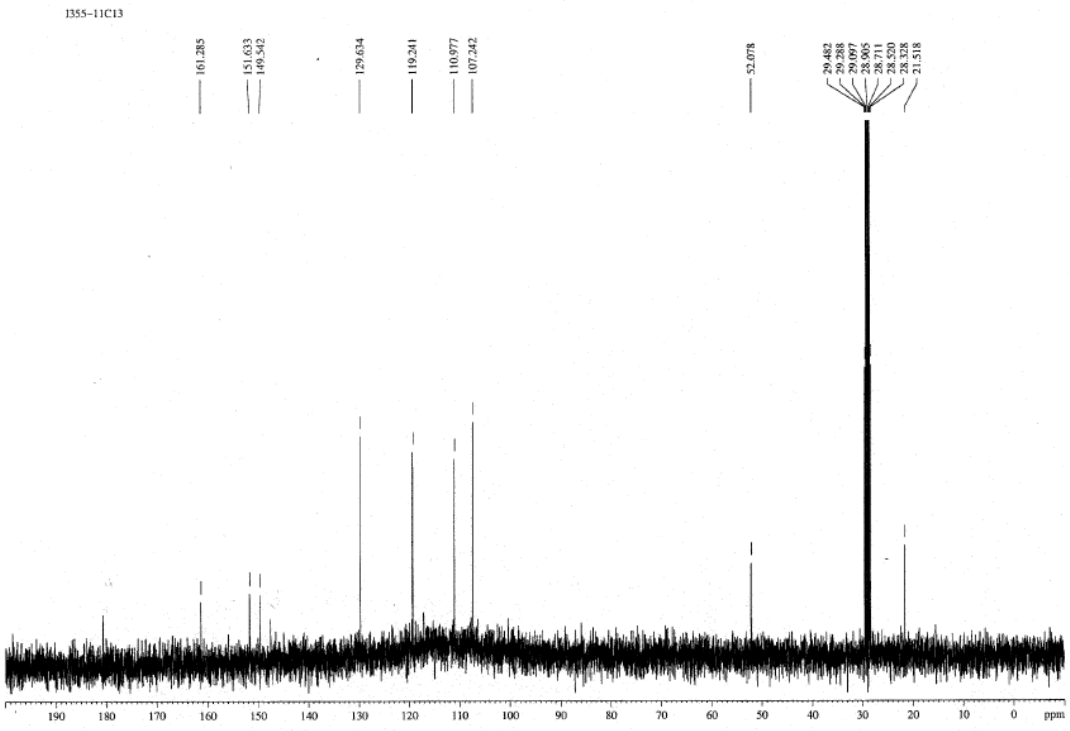

Figure 118 ^{13}C NMR (acetone-d_6) spectrum of compound **10**

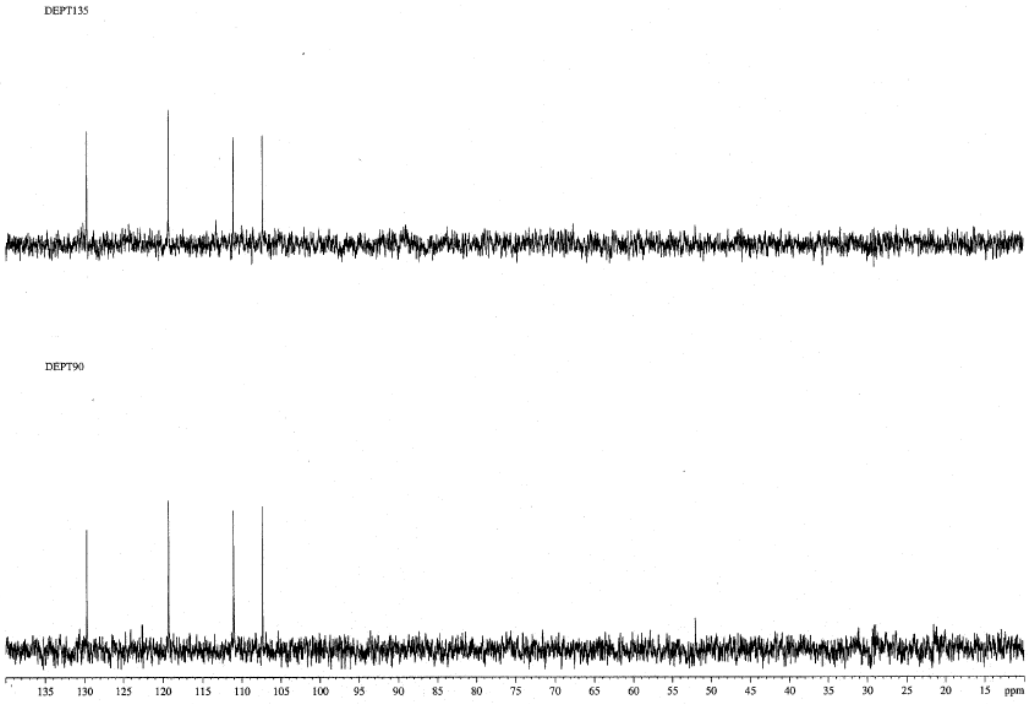

Figure 119 DEPT 90 and 135 spectra of compound **10**

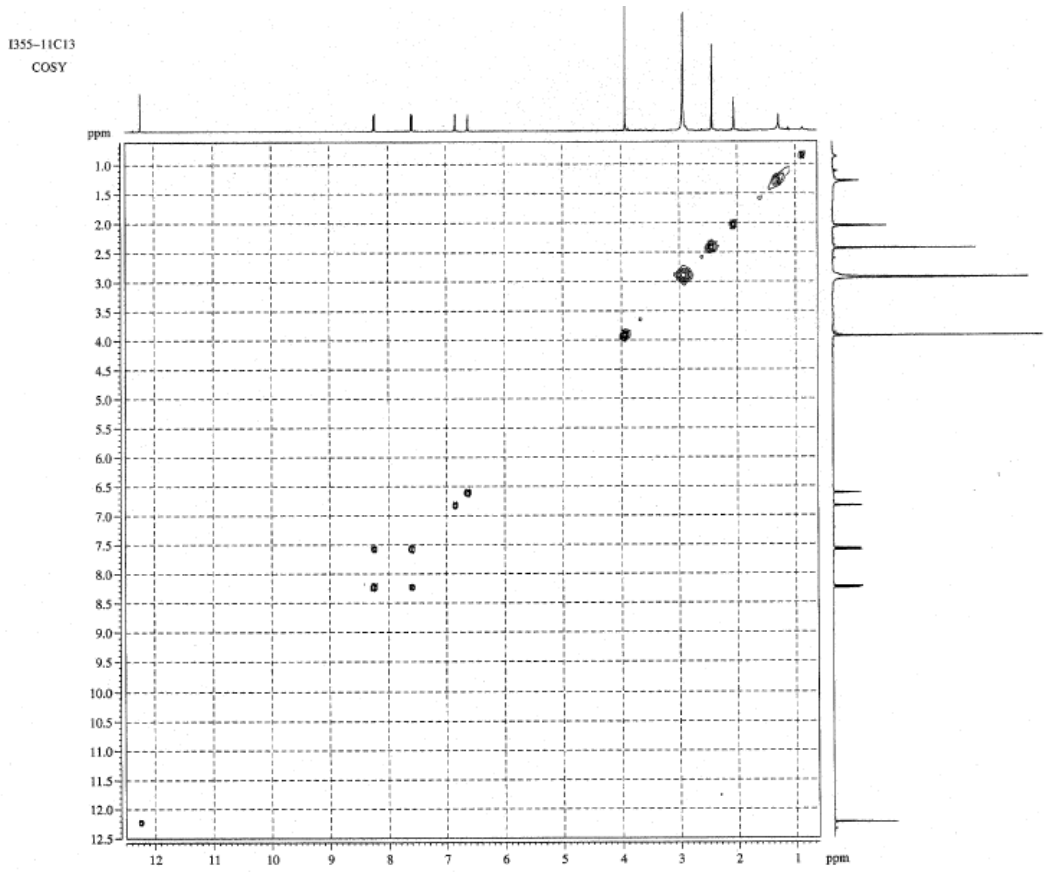

Figure 120 ^{1}H-^{1}H COSY spectrum of compound **10**

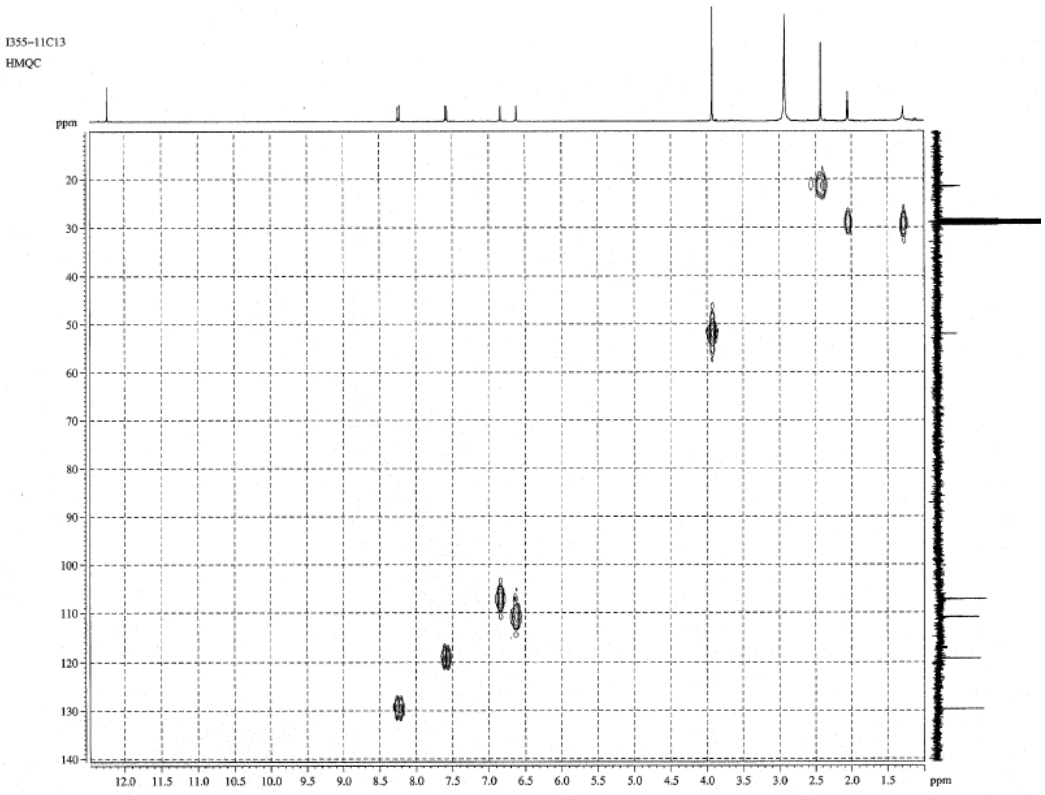

Figure 121 HMQC spectrum of compound **10**

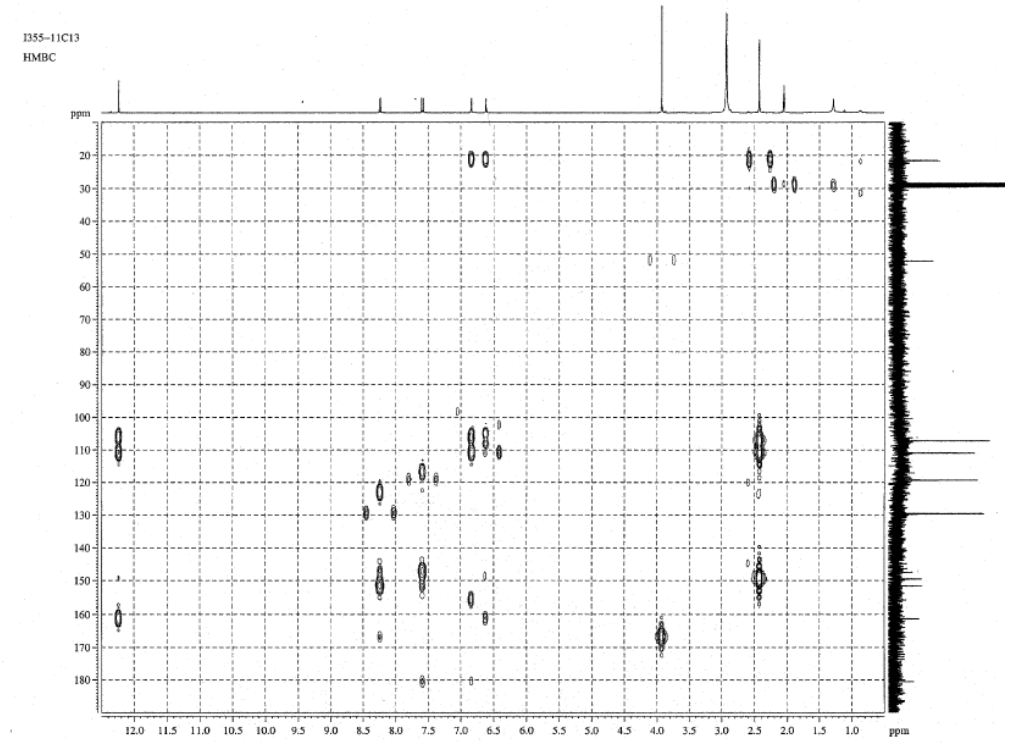

Figure 122 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound **10**

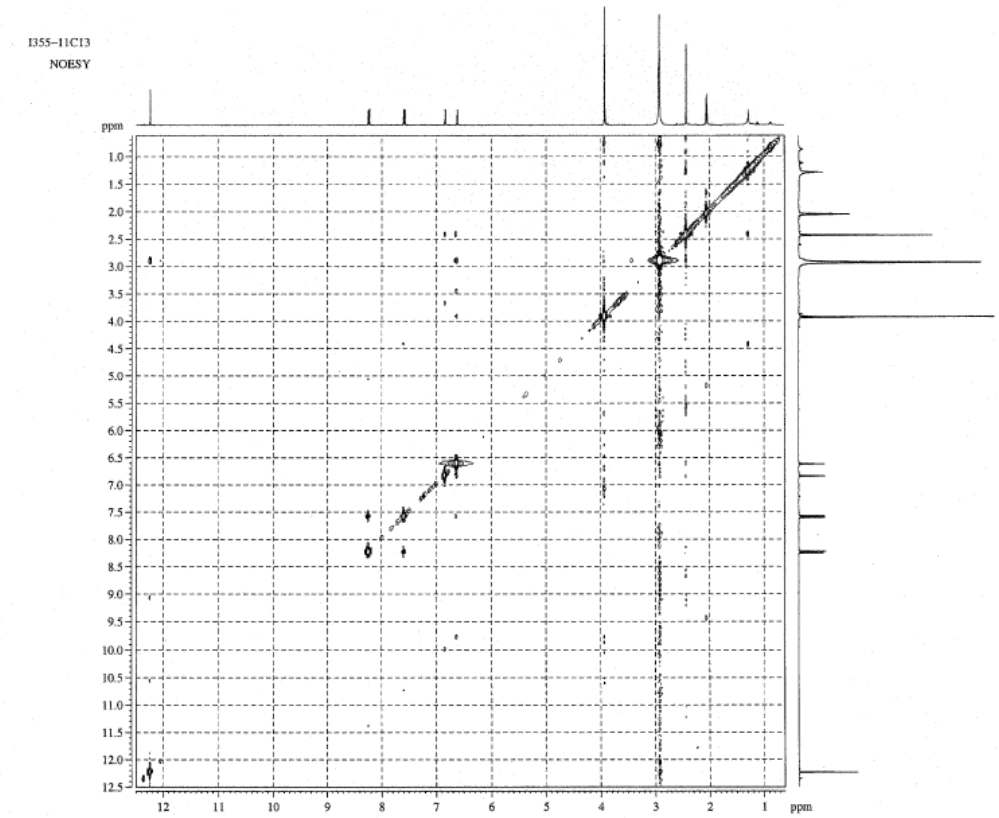

Figure 123 NOESY spectrum of compound **10**

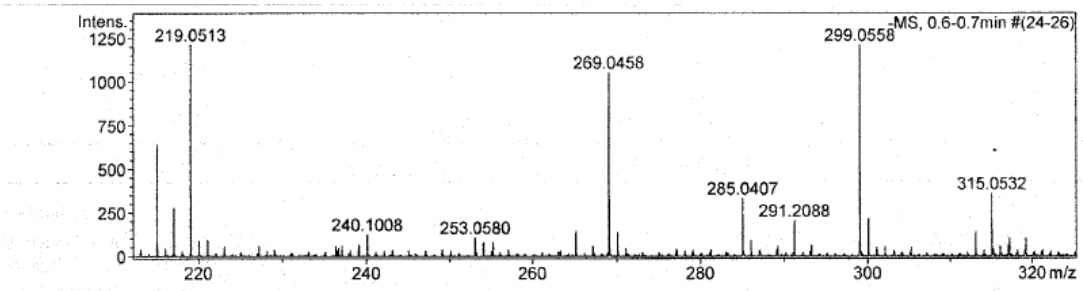

Figure 124 Mass spectrum of compound **10**

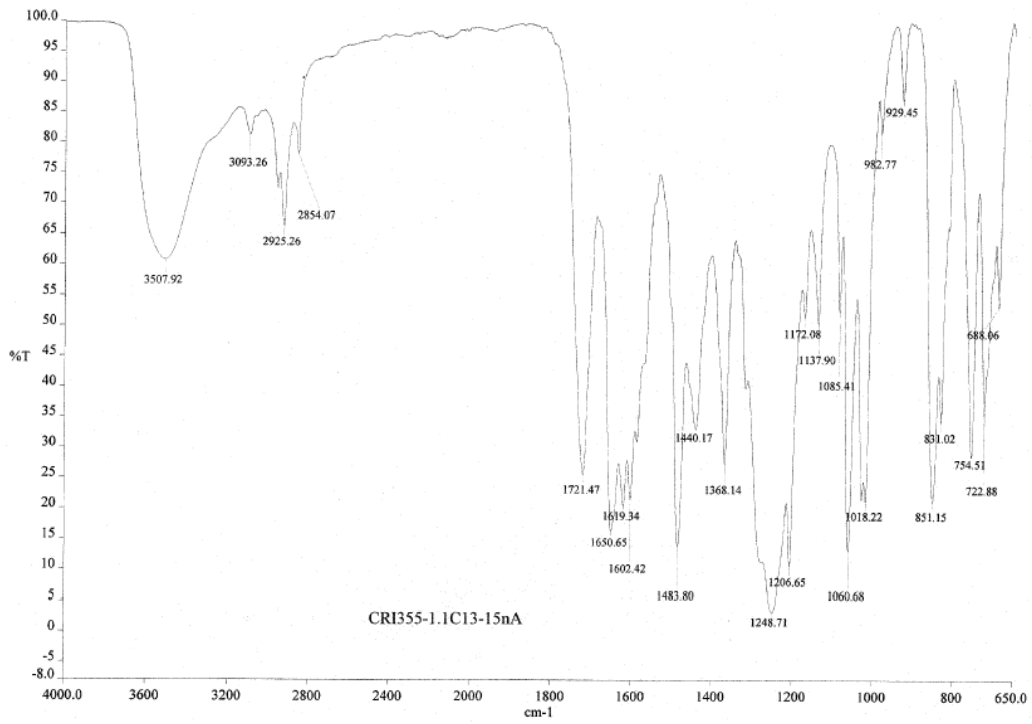

Figure 125 IR spectrum of compound 10

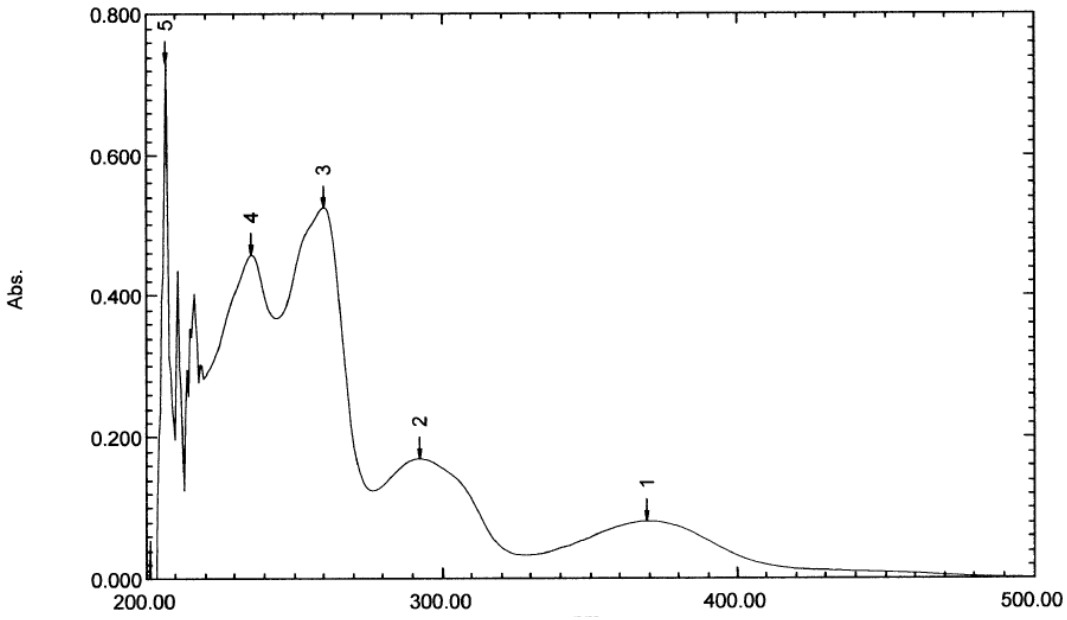

Figure 126 UV spectrum of compound 10

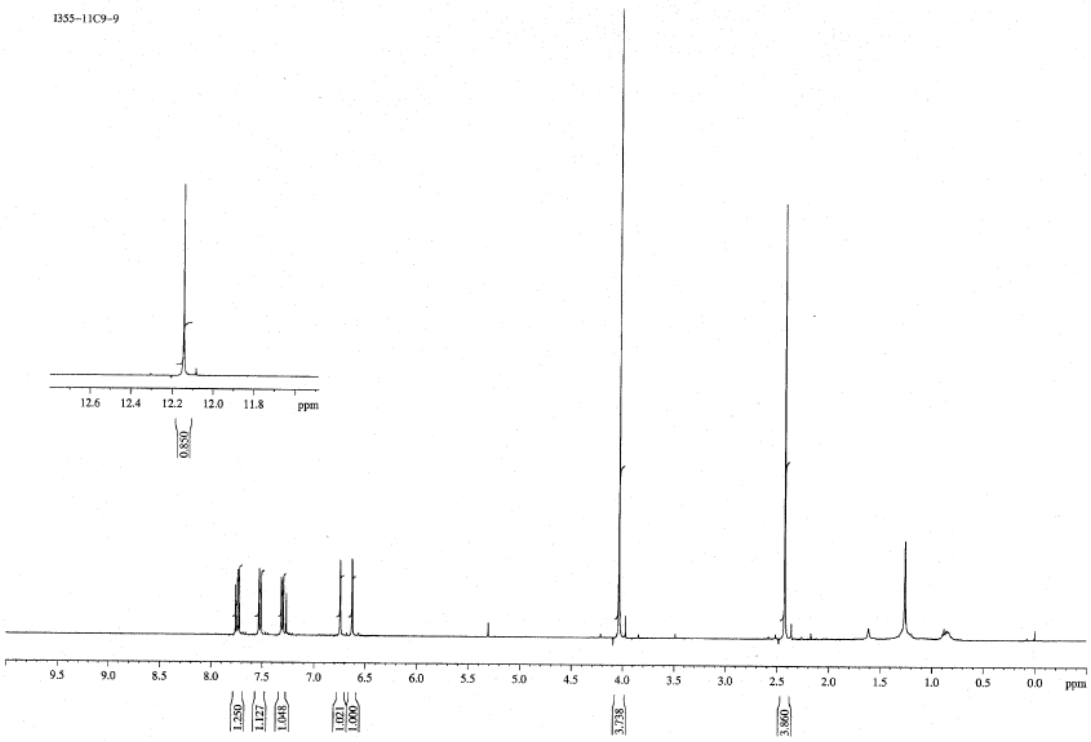

Figure 127 400 MHz ^{1}H NMR (acetone-d_6) spectrum of compound **11**

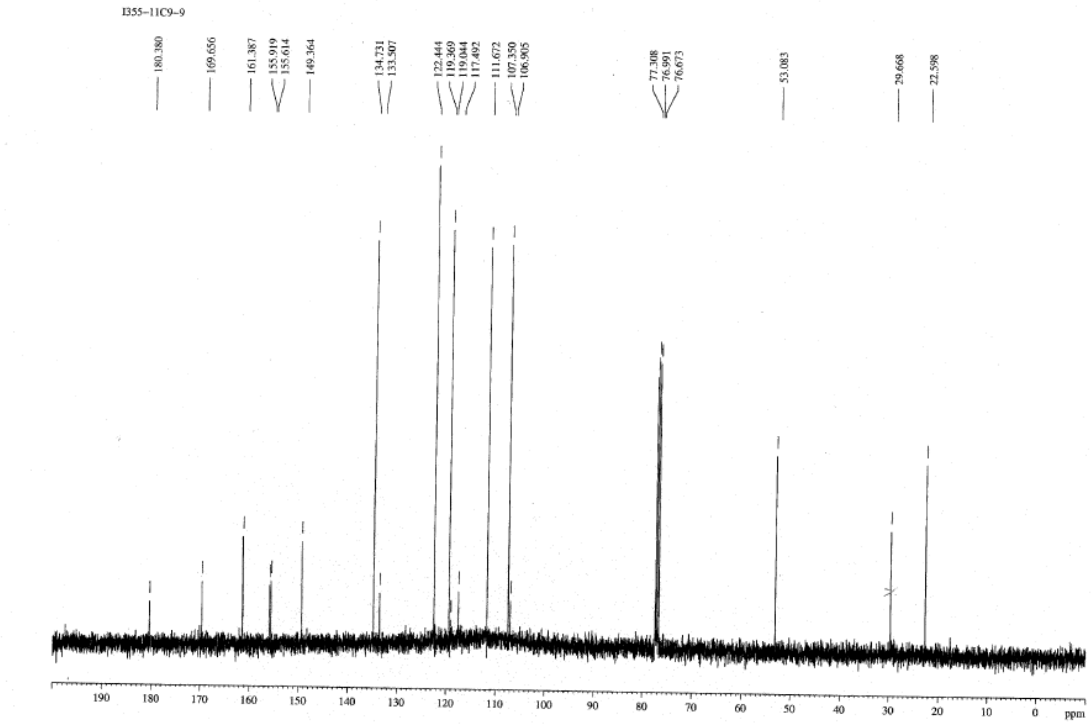

Figure 128 ^{13}C NMR (acetone-d_6) spectrum of compound **11**

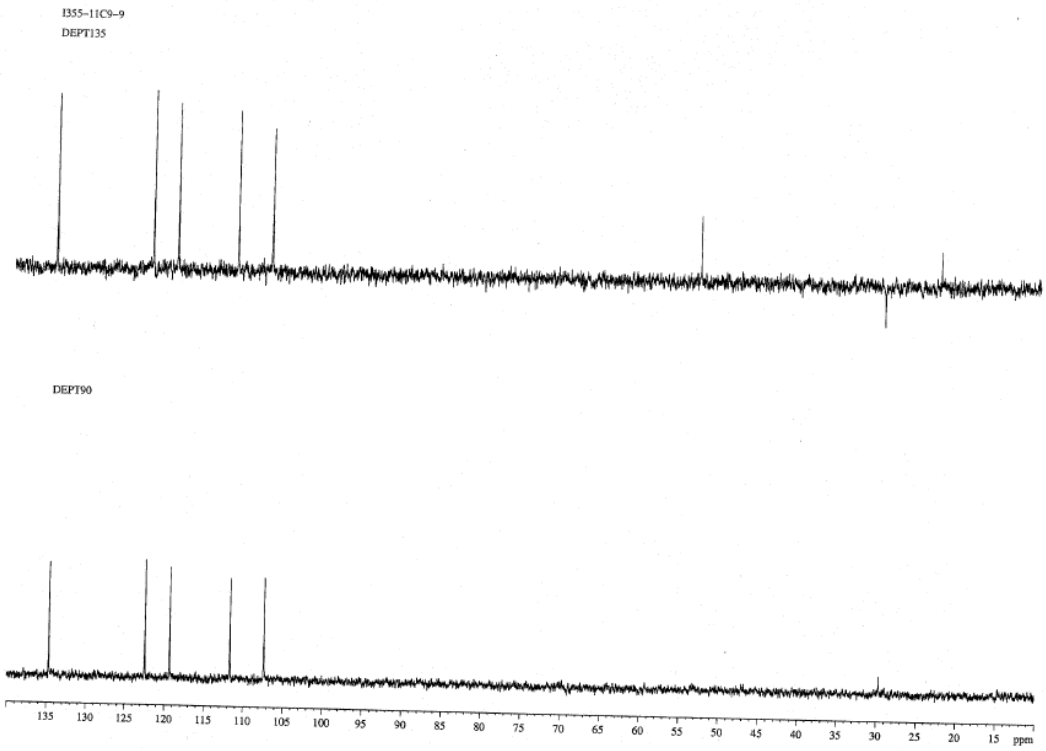

Figure 129 DEPT 90 and 135 spectra of compound **11**

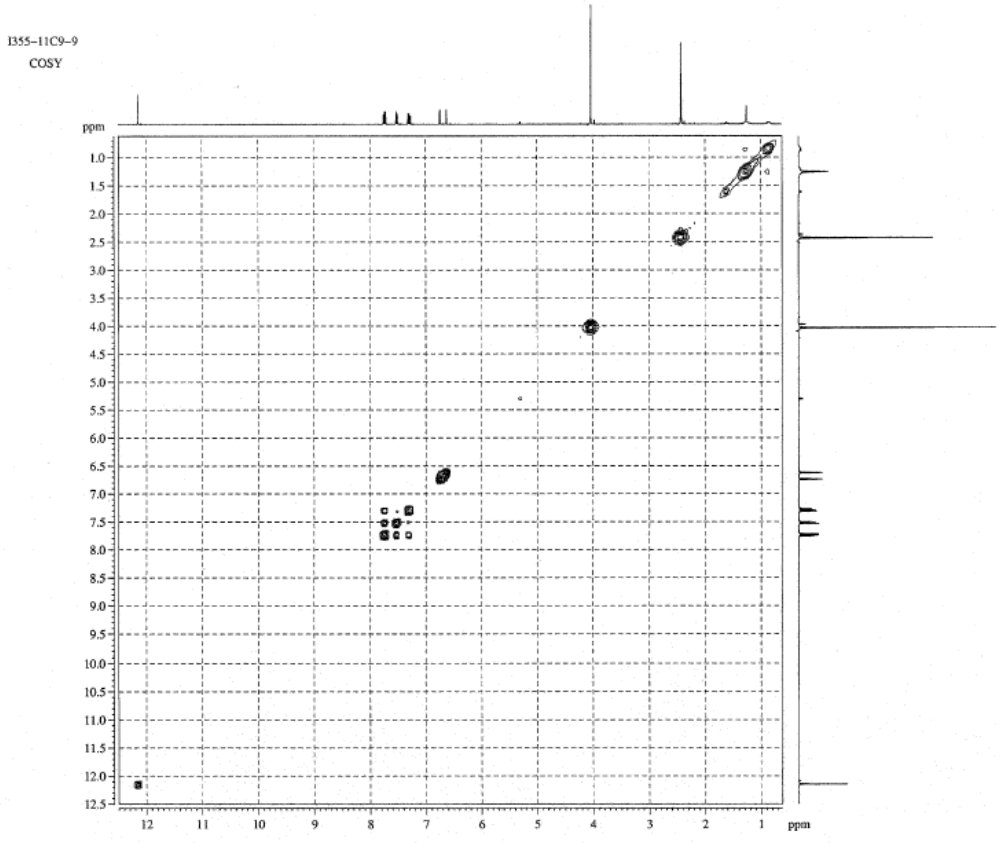

Figure 130 ^{1}H-^{1}H COSY spectrum of compound **11**

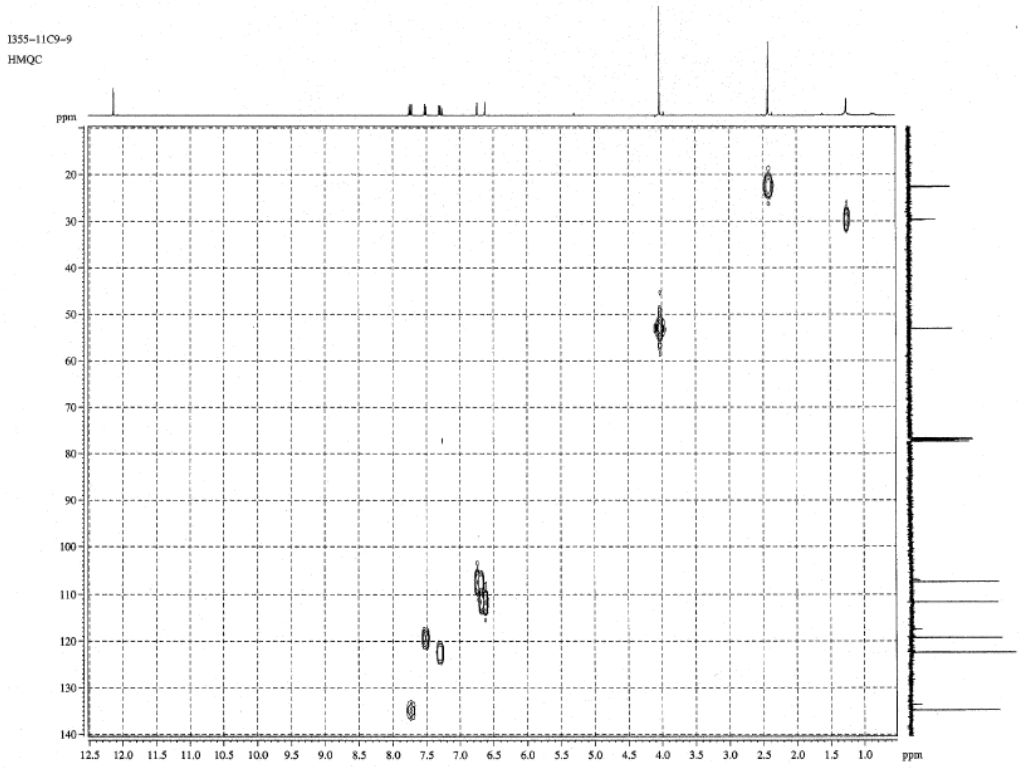

Figure 131 HMQC spectrum of compound **11**

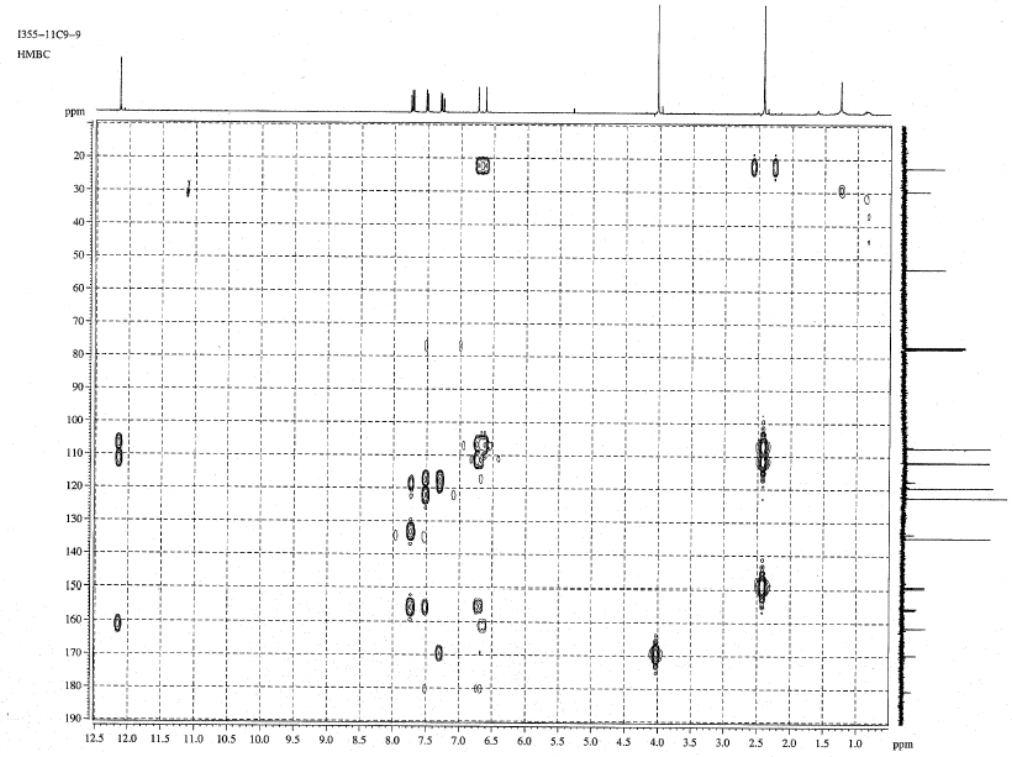

Figure 132 Long range ^{1}H-^{13}C correlations (HMBC spectrum) of compound **11**

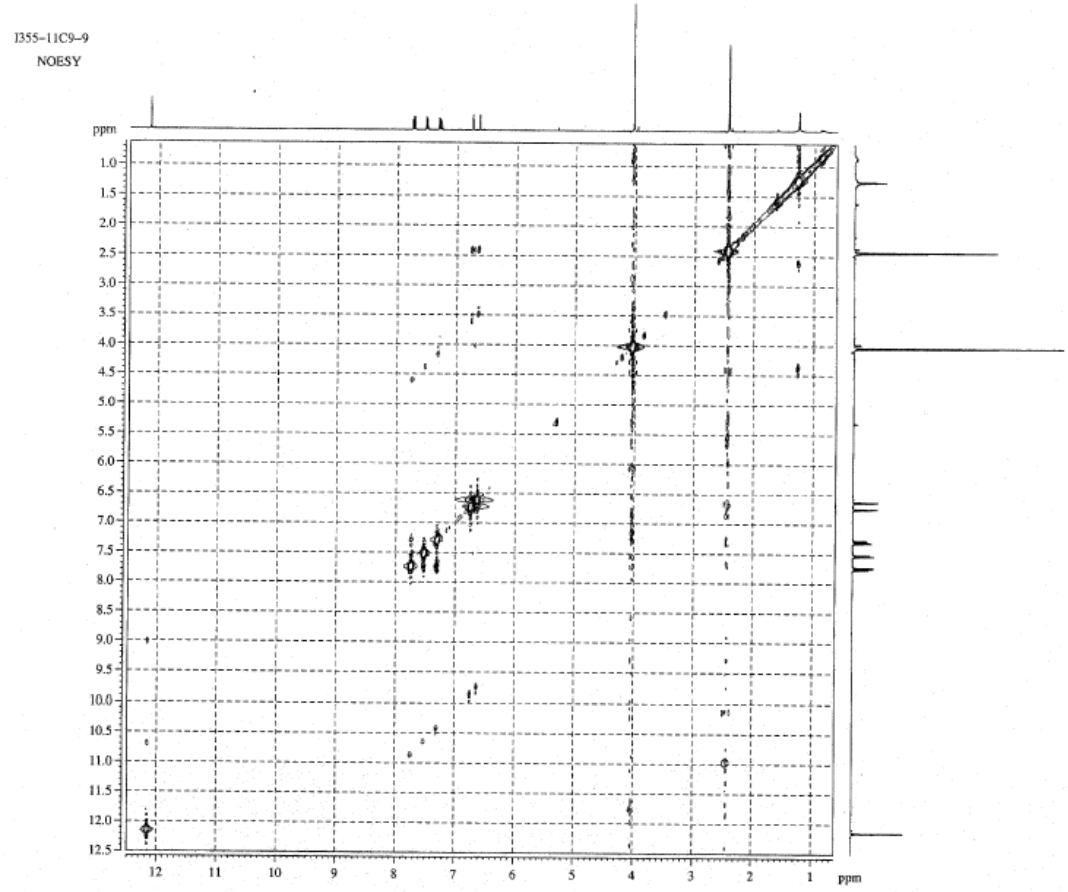

Figure 133 NOESY spectrum of compound **11**

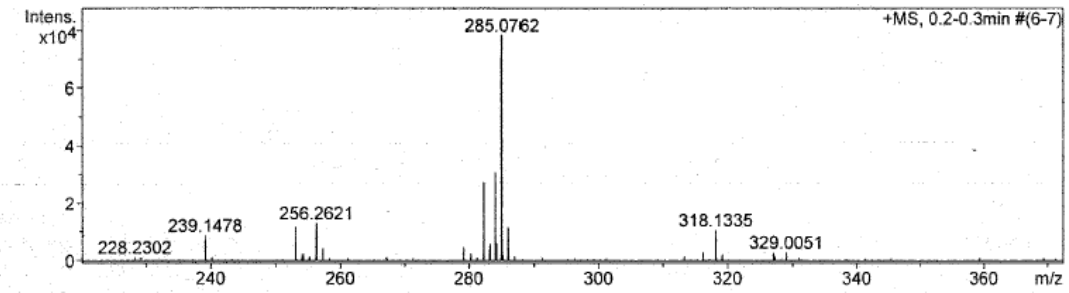

Figure 134 Mass spectrum of compound **11**

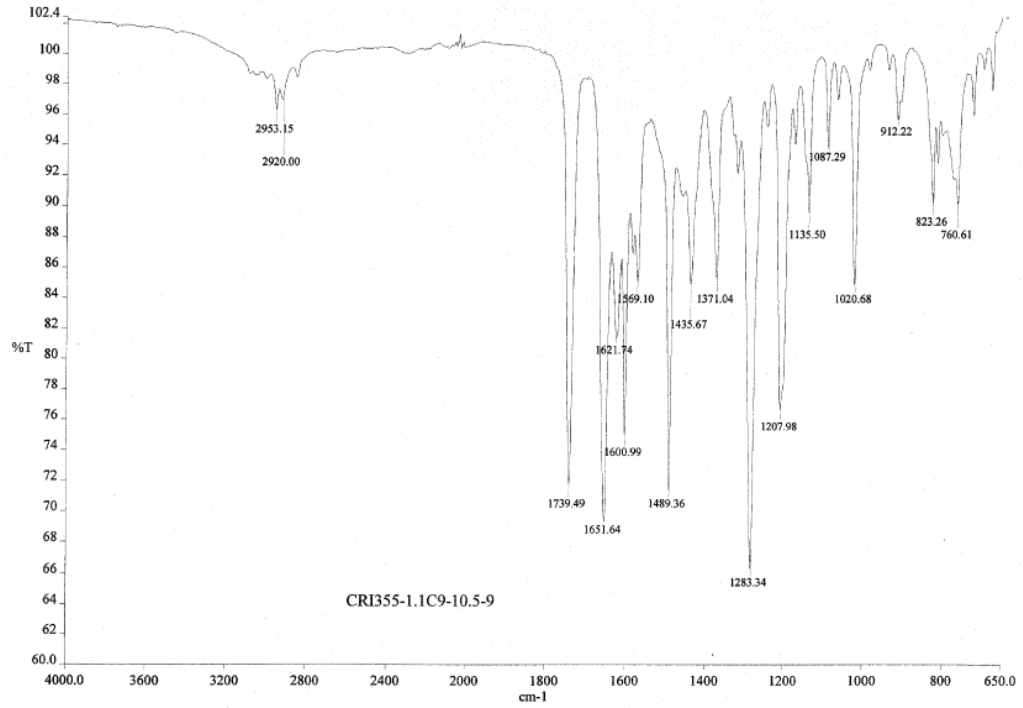

Figure 135 IR spectrum of compound 11

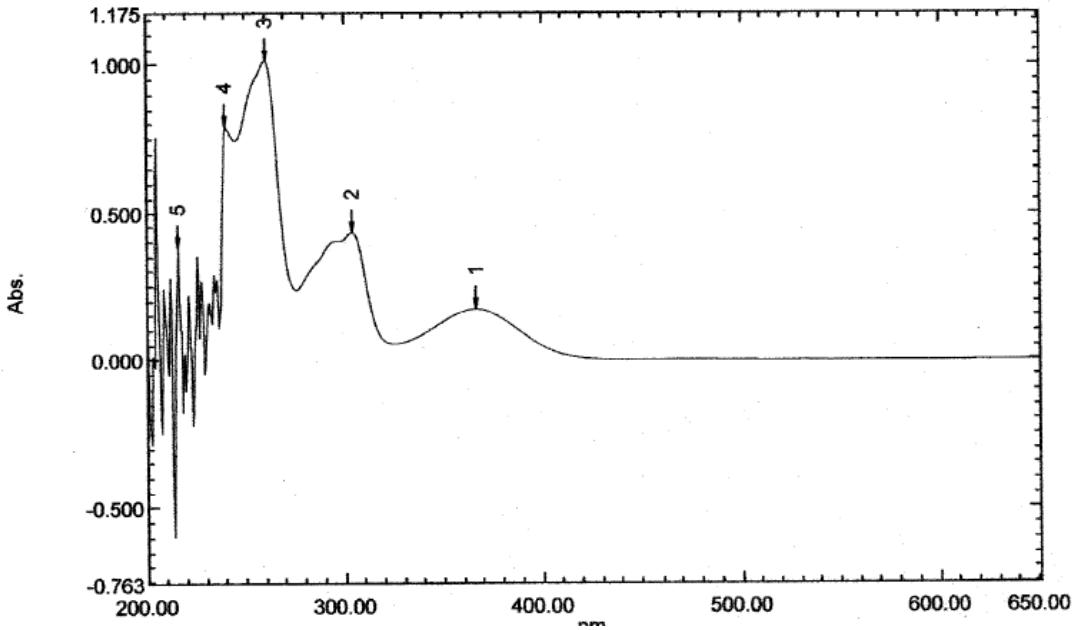

Figure 136 UV spectrum of compound 11

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