Isolation, Purification and Characterization of Natural Products from Marine Pseudomonas species

26th Oct 2015

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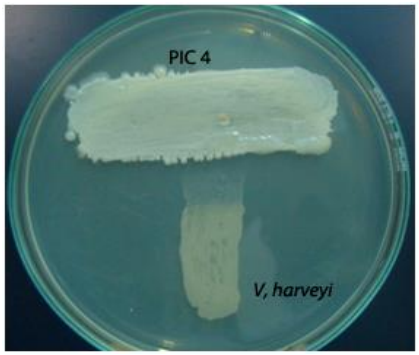

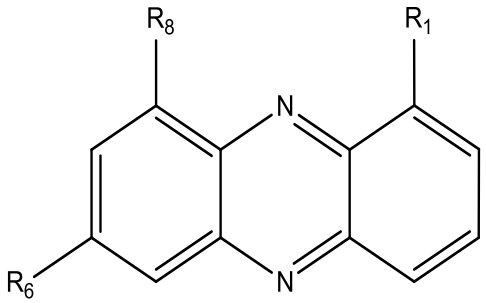

Introduction

- Microbial metabolites are an important source of pharmacologically active compounds such as alkaloids, polyenes, macrolides, peptides, and terpenoids, polyketides.
- Phenazines are heterocyclic pigmented compounds produced by the species of the bacterial genera, Brevibacterium, Burkholderia and Pseudomonas and also by the actinomycetes genus, Streptomyces.
- More than 50 naturally occurring phenazines have been described and as many as ten different phenazine derivatives can occur simultaneously in one organism.
- Microbial alkaloids like Phenazines are a large family of N-containing tricyclic molecules with antibiotic, antitumor, and antiparasitic activity.
- Among the Pseudomonas genus, strains of *P. aeruginosa*, *P. chlororaphis*, and *P. fluorescens* are the most prominent producers of phenazines.

Materials and Methods

Isolation of bacteria from marine sediments

↓

Screening of bioactive strains (antibiotic and anticancer activity)

↓

Fermentation

↓

Isolation and purification of bioactive compounds/Bioactive guided purification

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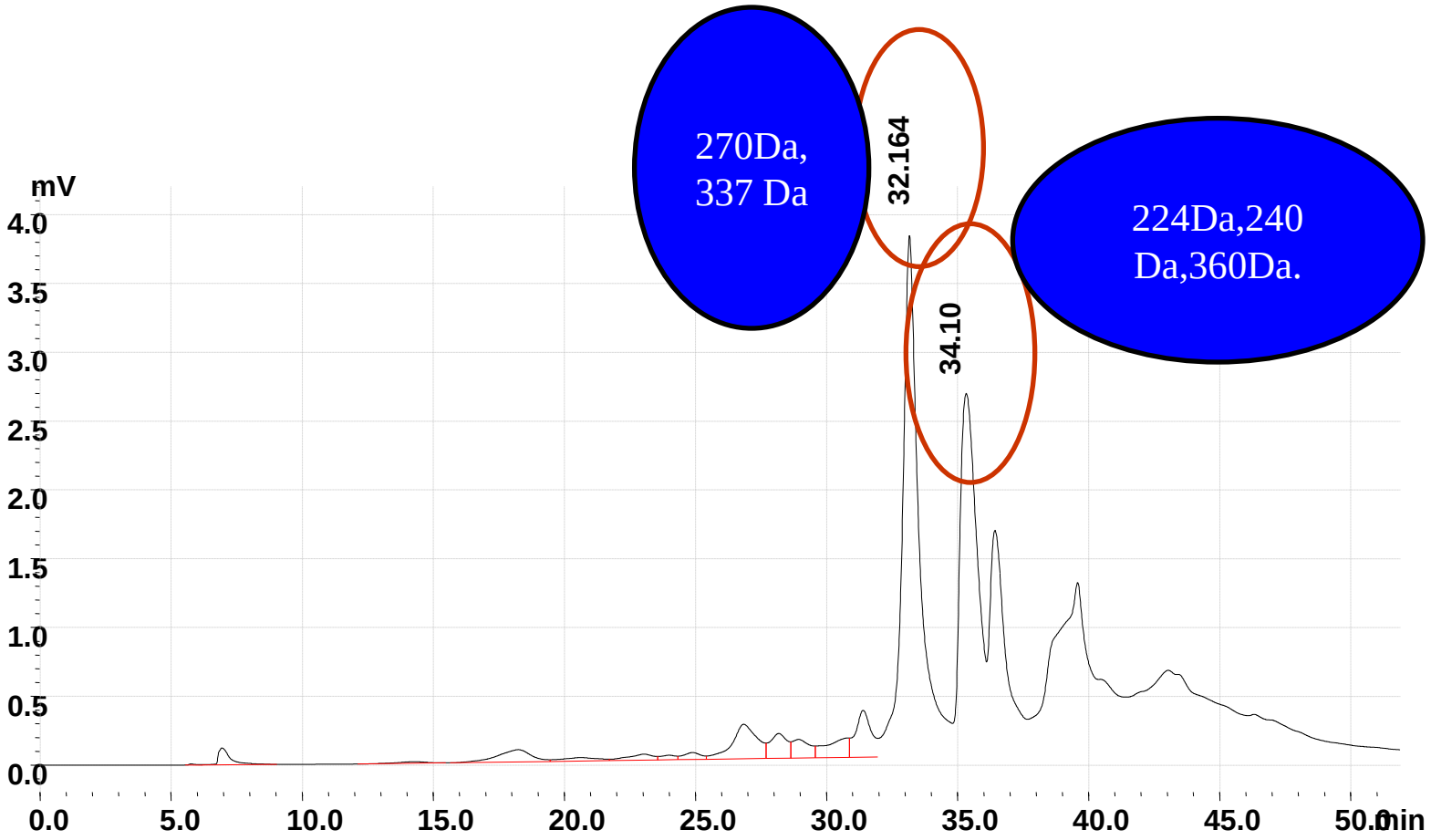
Chemical characterization by UV, IR, Mass, NMR,X-ray crystallography

Marine bacterial isolates

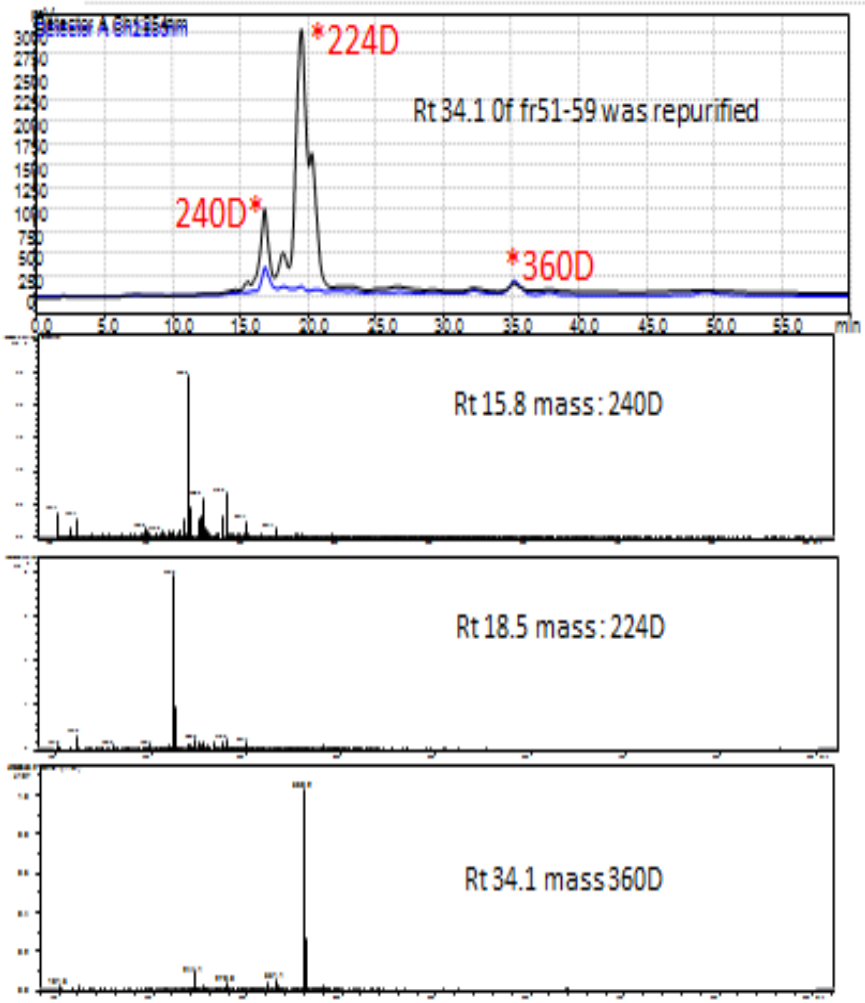
Column chromatography

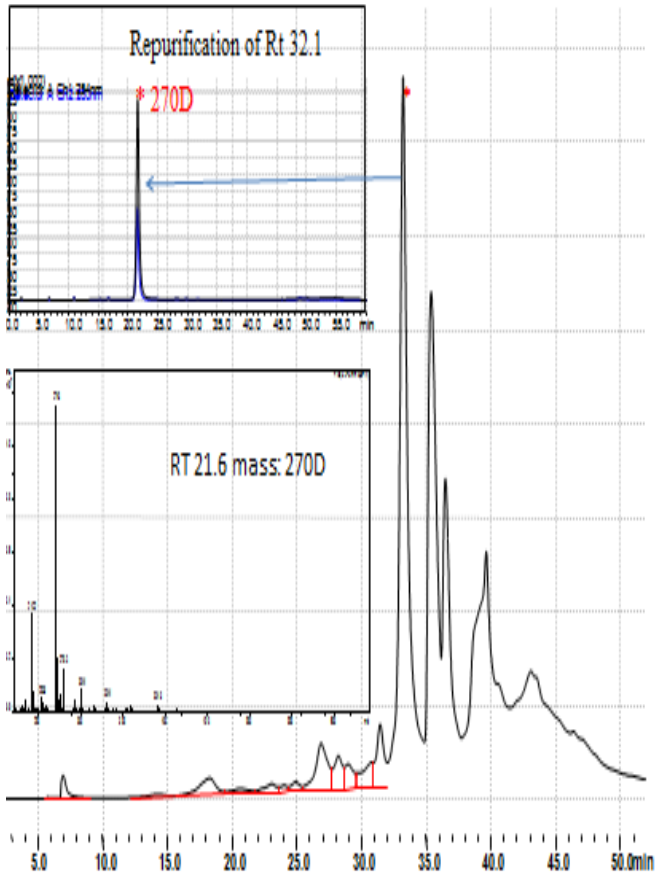
HPLC trace of compounds by using semi prep C18 column

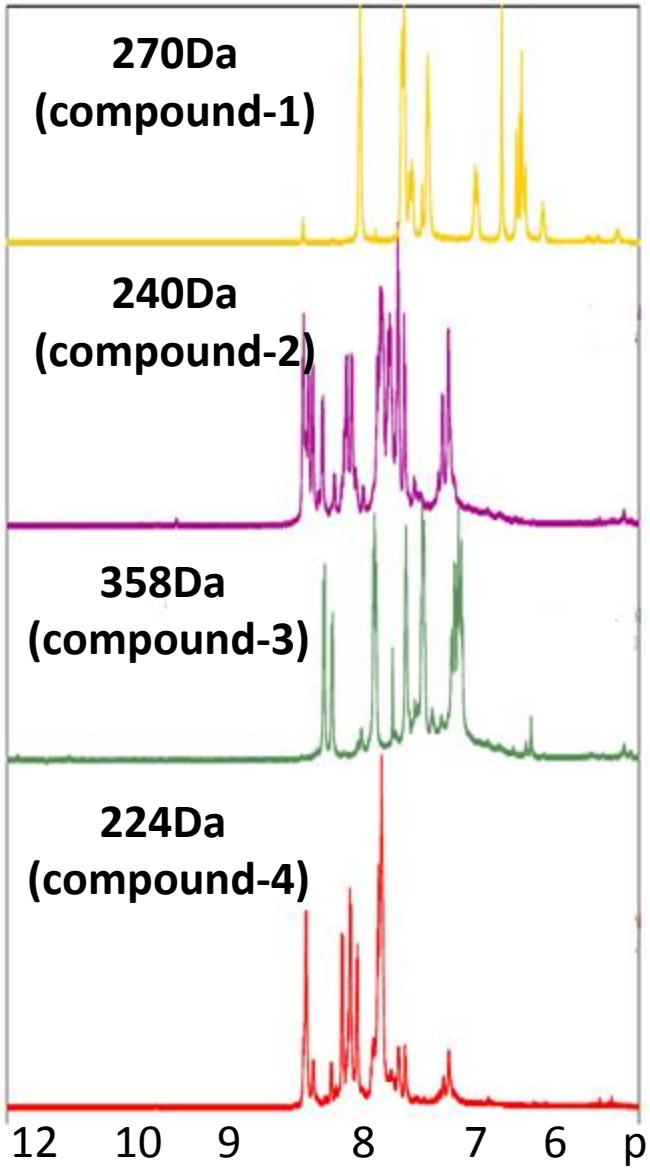


Results and Discussion:

HPLC Trace by using analytical column C18 and ESI-MS data of Compounds 1 to 4







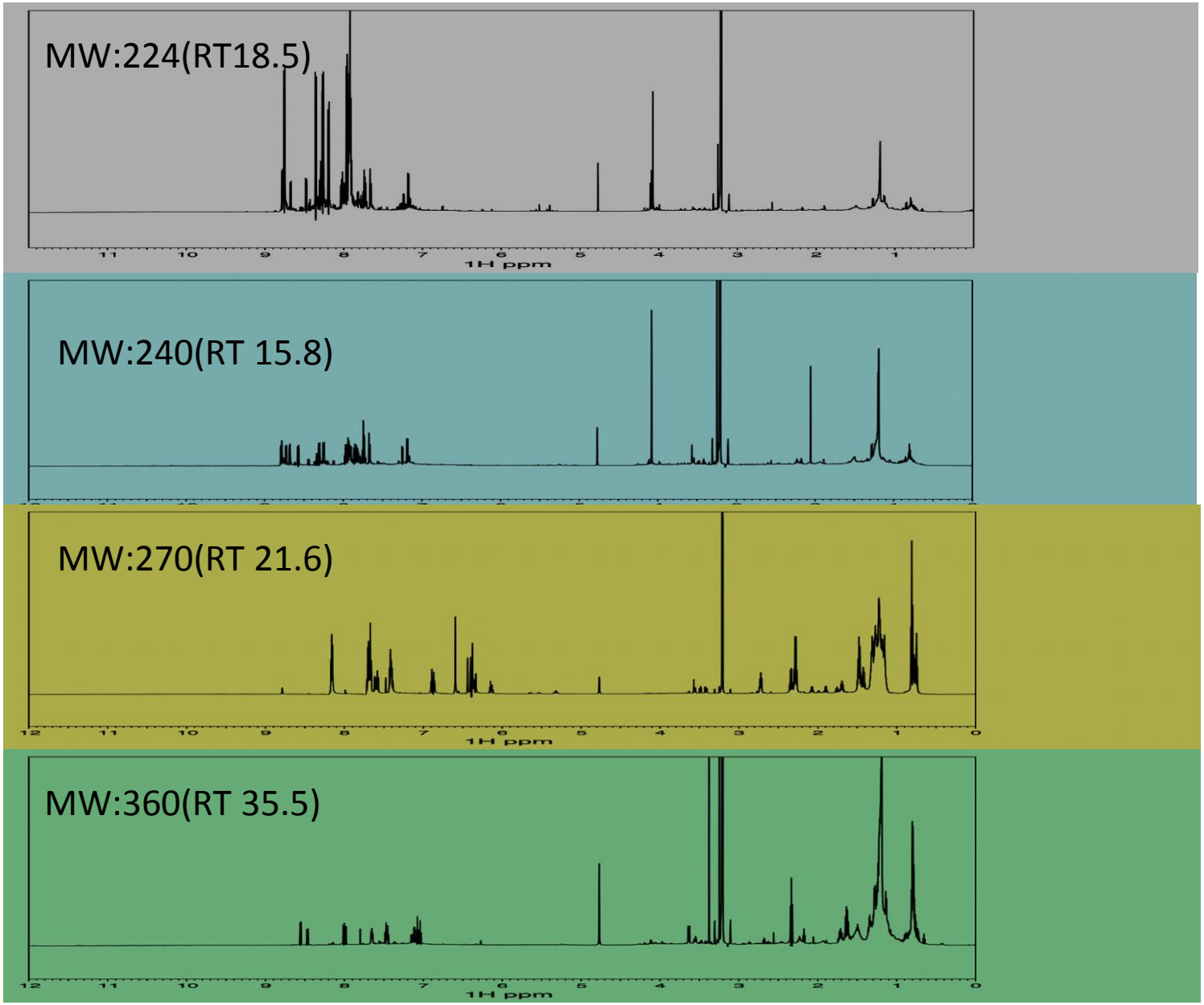
^{1}H NMR spectra Compounds (1-4) aromatic region

- The ^{1}H NMR spectrum indicated that the aromatic signals represent two sets of aromatic rings.

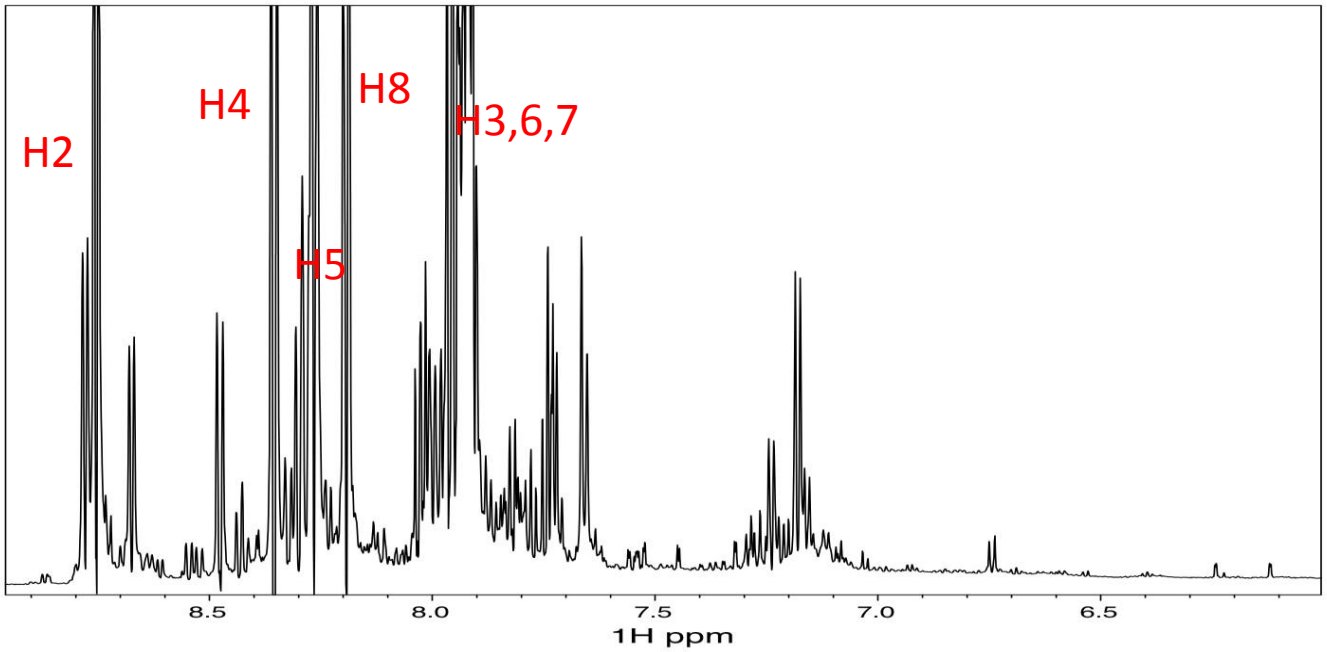
- UV, IR and 1D-NMR spectra revealed that the compounds belongs to phenazines.

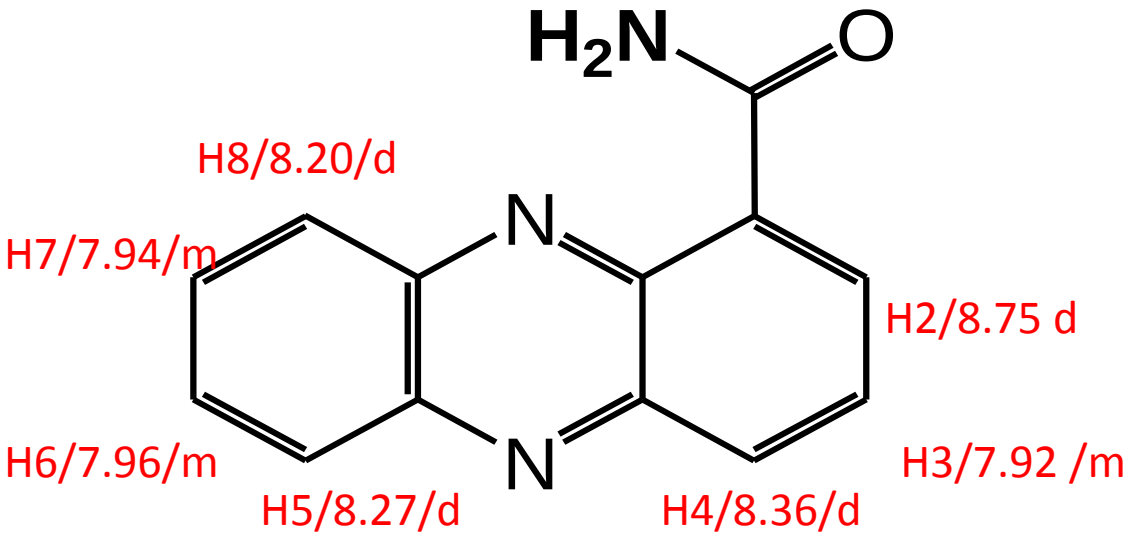
(The strong absorption band at 248, 370 nm is indicative of aromatic groups. The IR bands at 3369 cm-1(NH group), 2963, 2924 and 2853 cm-1 (aromatic C–H),1649 cm-1 ($CONH_2$))

^{1}H proton NMR of compounds 1to 4



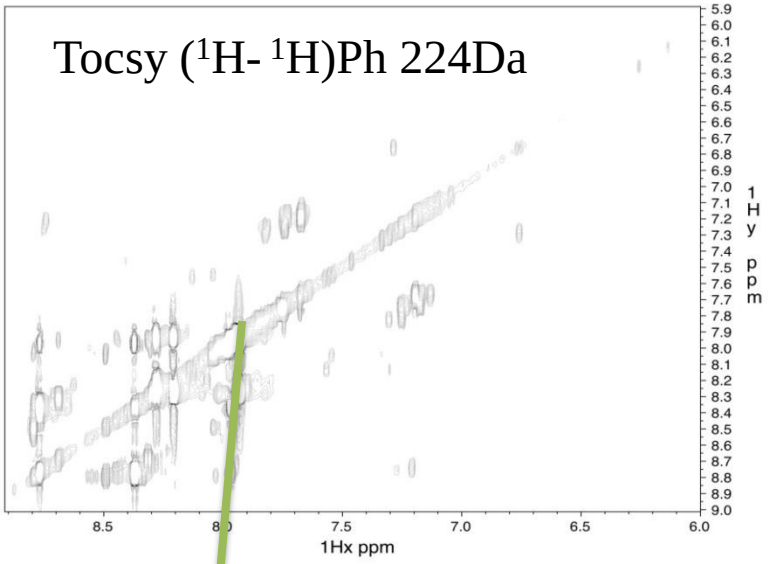
^{1}H Proton NMR aromatic region Ph 224Da

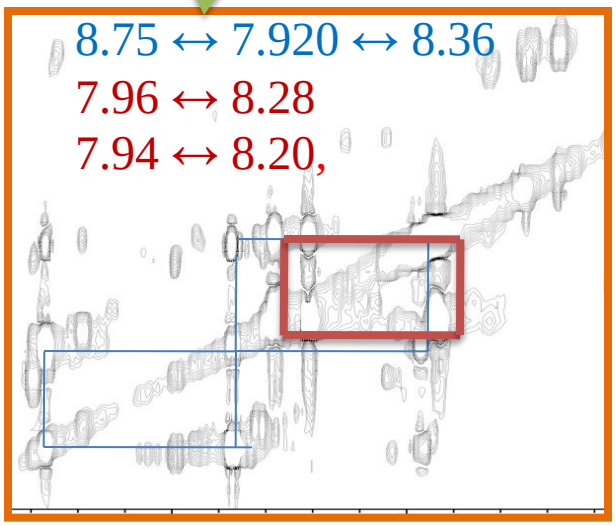


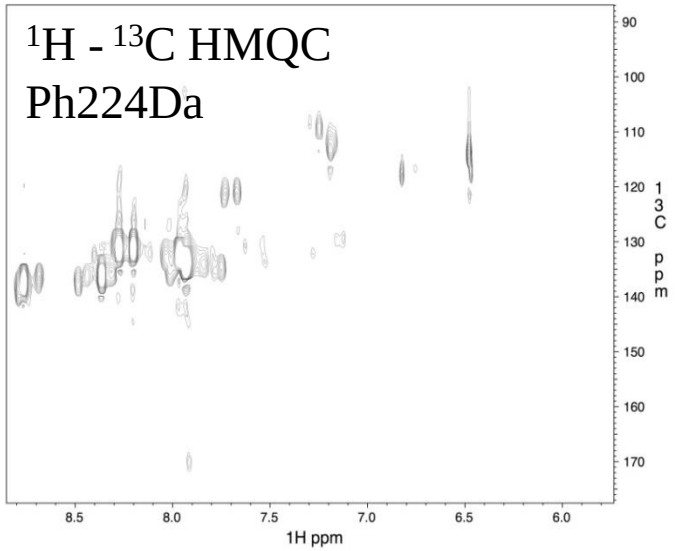


10.5 = $CONH_2$

2D NMR spectra of compound-1



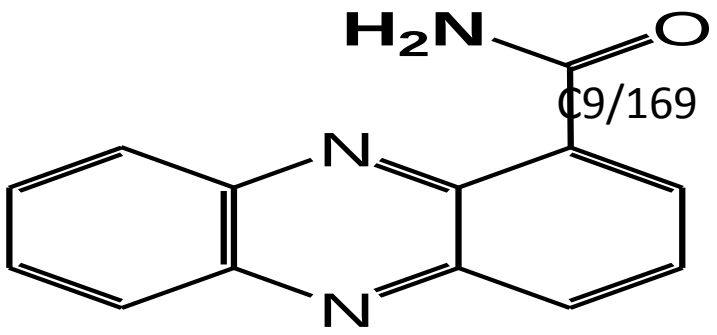




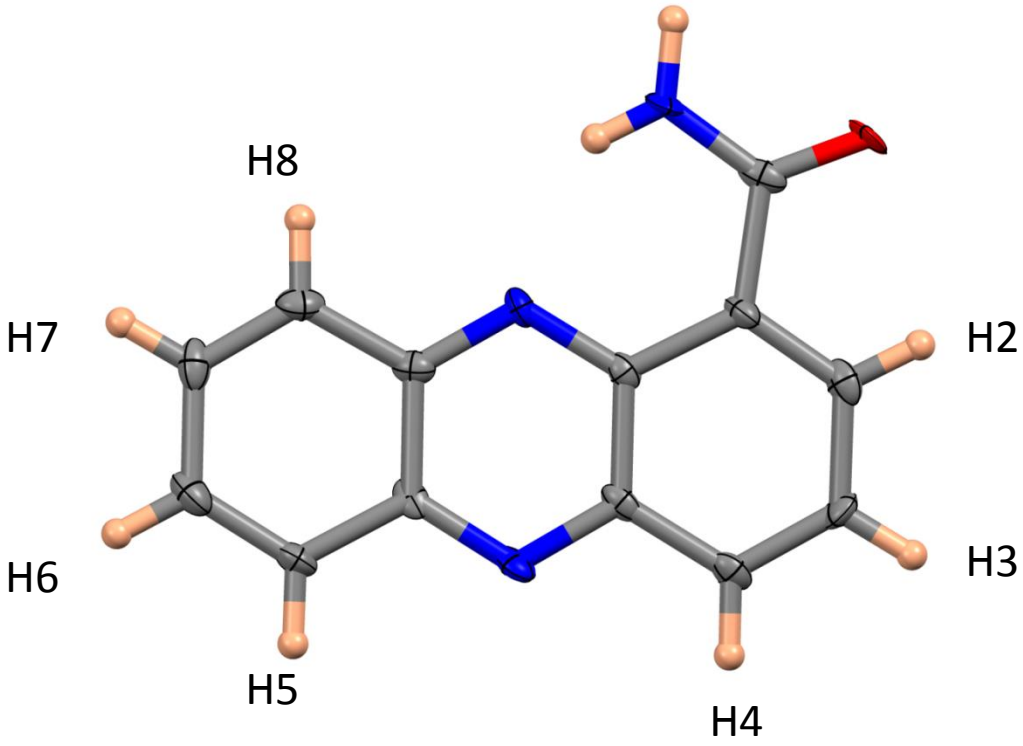
HMQC:
8.75/137, 7.92/133,8.36/136,
8.27/131, 7.96/132,7.98/132,
8.20/130

NMR Data for Compound 1: MW=224 Da

Position	δH (*J* in Hz)	δC	HMBC
1.	-	130.1	-
2.	8.89(d,1H,7)	137.6	C1,C3,C9
3.	8.04(m,1H)	134.2	C1,C2, C4
4.	8.48(d,1H,7)	136.4	C2,C3,C4a, C4b
4a.	-	140.1	-
4b.	-	140.1	-
5.	8.40(d,1H,14)	127.8	C6,C8b
6.	8.08(m,1H)	131.9	C5,C8,C8a,C8b
7.	8.08(m,1H)	131.9	C5,C8,C8a,C8b
8.	8.32(d,1H,14)	131.0	C7,C8a
8a.	-	143.1	-
8b.	-	143.1	-
9.	-	169.5	-

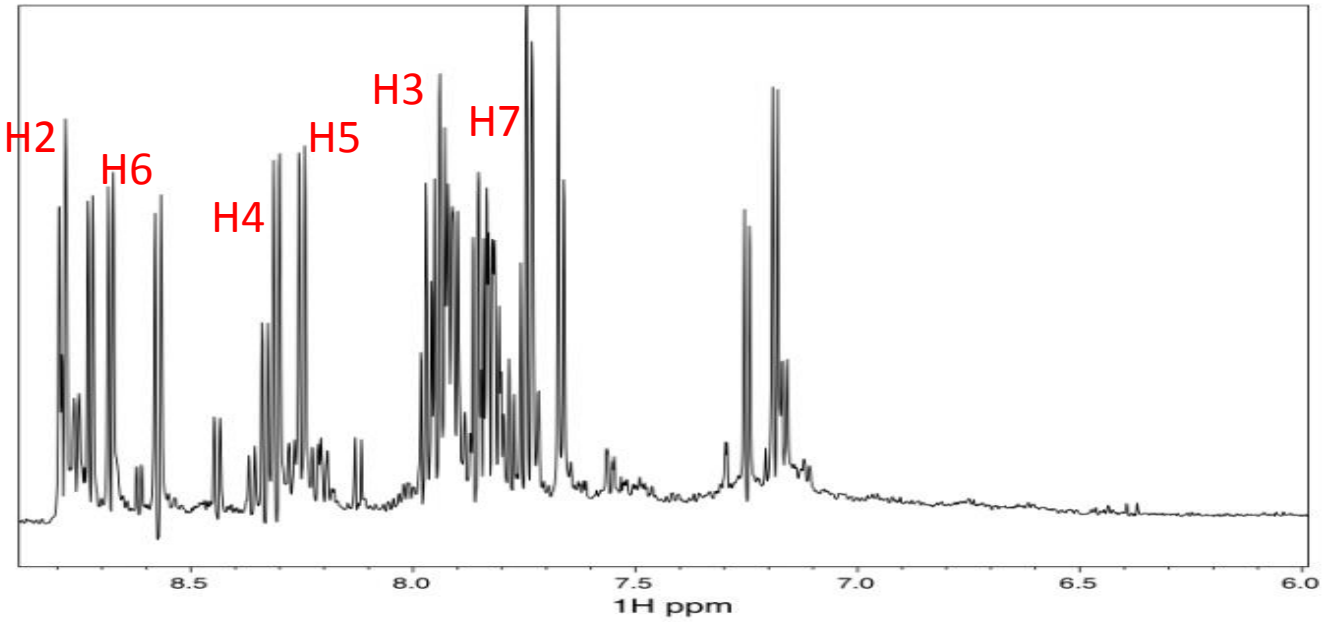


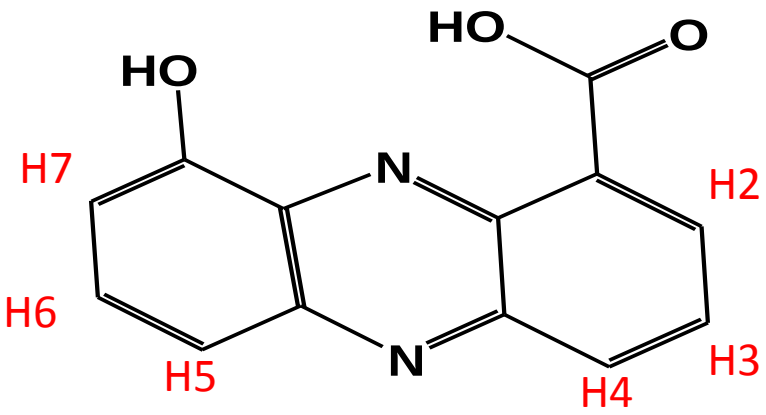
Crystal structure of compound-1



Phenazine-1-carboxamide

^{1}H Proton NMR aromatic region Ph 240Da

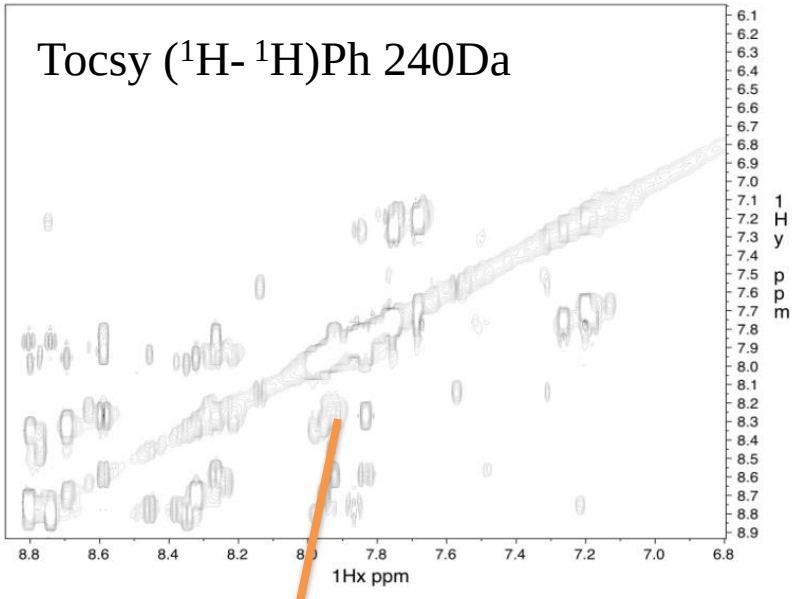


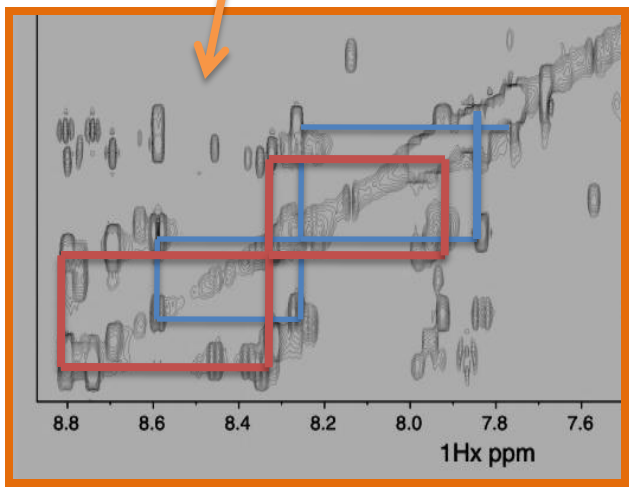


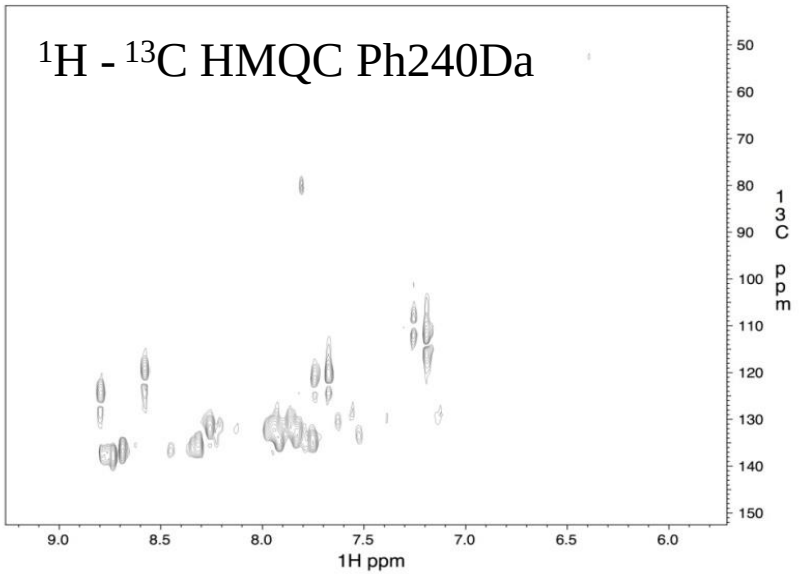
10.5 = COOH,
4.9= OH

1 Hydroxy Phenazine Carboxylic acid

2D NMR spectra of compound-2







HMQC:
8.79/137.5,
7.93/132.1,
8.34/135.4,
8.27/131.5,
8.68/136.5
7.83/131.3,

TOCSY:

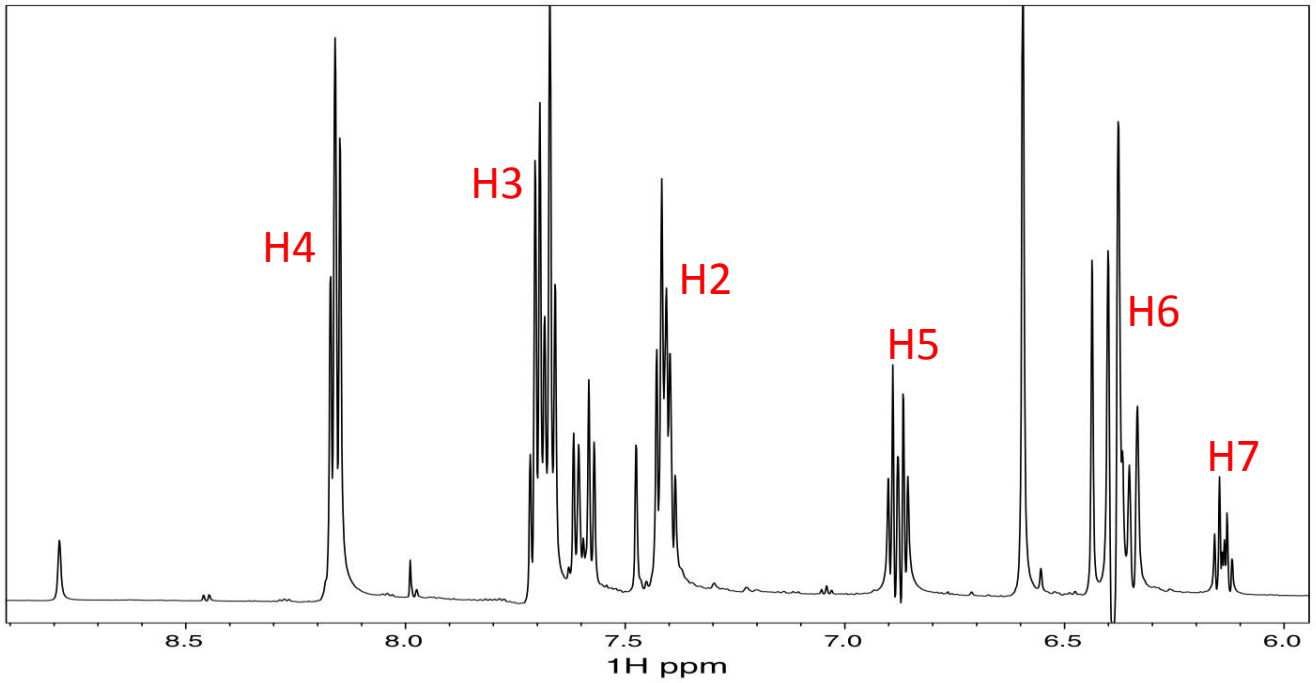
7.93 ↔ 8.33 ↔ 8.79

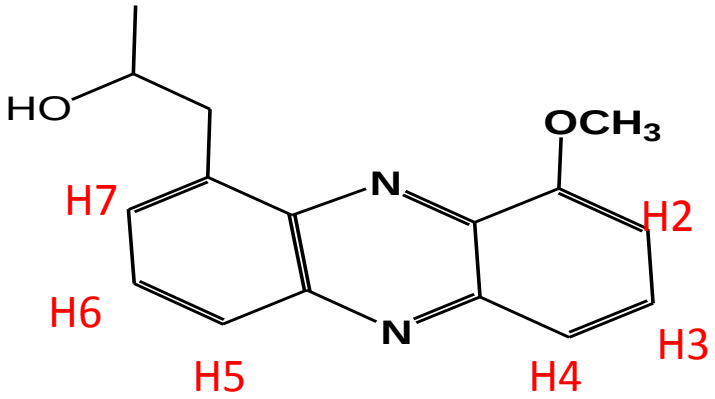
7.83 ↔ 8.26↔ 8.68

NMR Data for Compound 2: MW=240 Da

Position	δH (*J* in Hz)	δC	HMBC
1.	-	130.1	-
2.	8.81(d,1H,7)	134.1	C1,C9
3.	8.08(m,1H)	128.3	C1,C2, C4,C4a
4.	8.43(d,1H,7)	131.1	C2,C3,C4a, C4b
4a.	-	140.1	-
4b.	-	140.1	-
5.	7.80(m,1H)	120.3	-
6.	-	150.1	-
7.	7.32(d,1H,7)	112.2	C5,C6,C8,C8a
8.	7.80(m,1H)	130.1	C6,C7,C8a,C8b
8a.	-	143.1	-
8b.	-	143.1	-
9.	-	169.5	-
6-OH	4.91	-	-

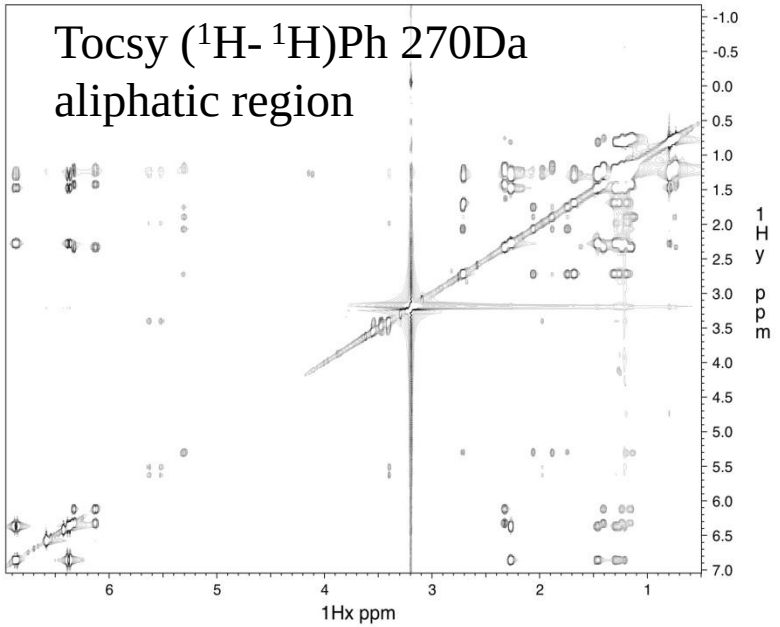
^{1}H Proton NMR aromatic region Ph 270Da

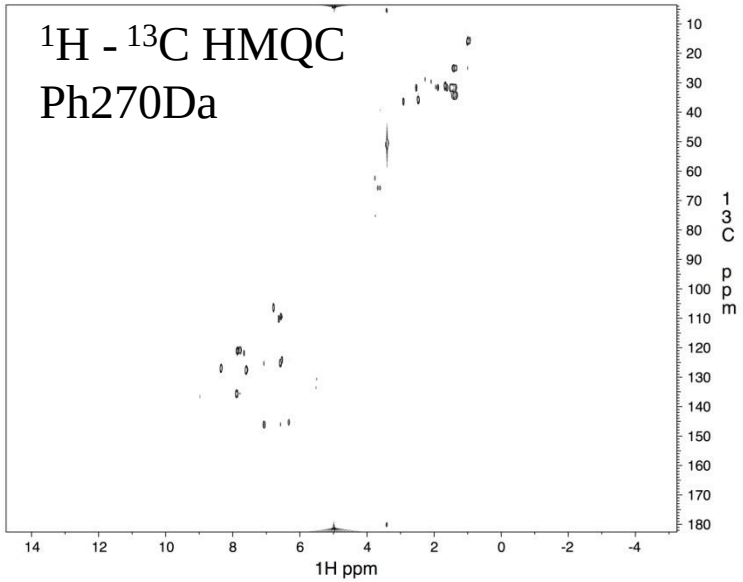


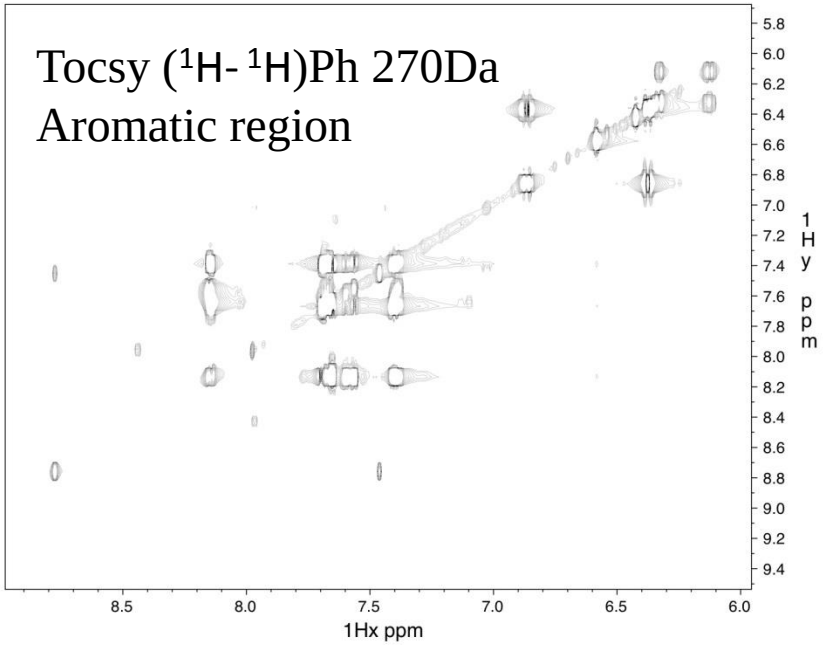


Phenazine 1-methoxy 8(2-hydroxy) propyl

2D NMR spectra of compound-3







TOCSY:

1.28 ↔1.46 ↔2.28 ↔6.13 ↔6.31 ↔6.85.

7.38 ↔ 7.68 ↔8.14.

HMQC:

7.38/125, 7.68/127, 8.14/133, 1.28/24, 1.46/31, 2.24/29, 6.13/123, 6.31/125, 6.85/127.

NMR Data for Compound 3: MW=270 Da.

Position	δH (*J* in Hz)	δC	HMBC
1.	-	132.1	-
2.	8.28(d,1H,7)	124.1	C1,C3,C4a
3.	7.54(m,1H)	125.3	C1,C2,
4.	7.80(d,1H,7)	118.1	C4,C4a,C4b
4a.	-	140.1	C3,C4b
4b.	-	140.1	-
5.	6.99(d,1H,7)	122.3	-
6.	6.52(d,1H,7)	122.1	C6,C7,C8b
7.	6.51(dd,1H,7,21)	122.2	C5,C8,C8a,C8b
8.	-	143.1	C5,C8,C8a,C8b
8a.	-	140.1	-
8b.	-	140.1	-
9.	2.42(t,2H,7)	38.5	-
10.	1.64(m,1H)	32.5	C7,C8,C8a,C10
11.	1.36(t,3H,7)	28.2	C8,C9
12.	4.89	59.76	C9,C10
2-OH.	2.84	-	-
			-

Antimicrobial activities of extract from fermentation broth of Ps.aeruginosa

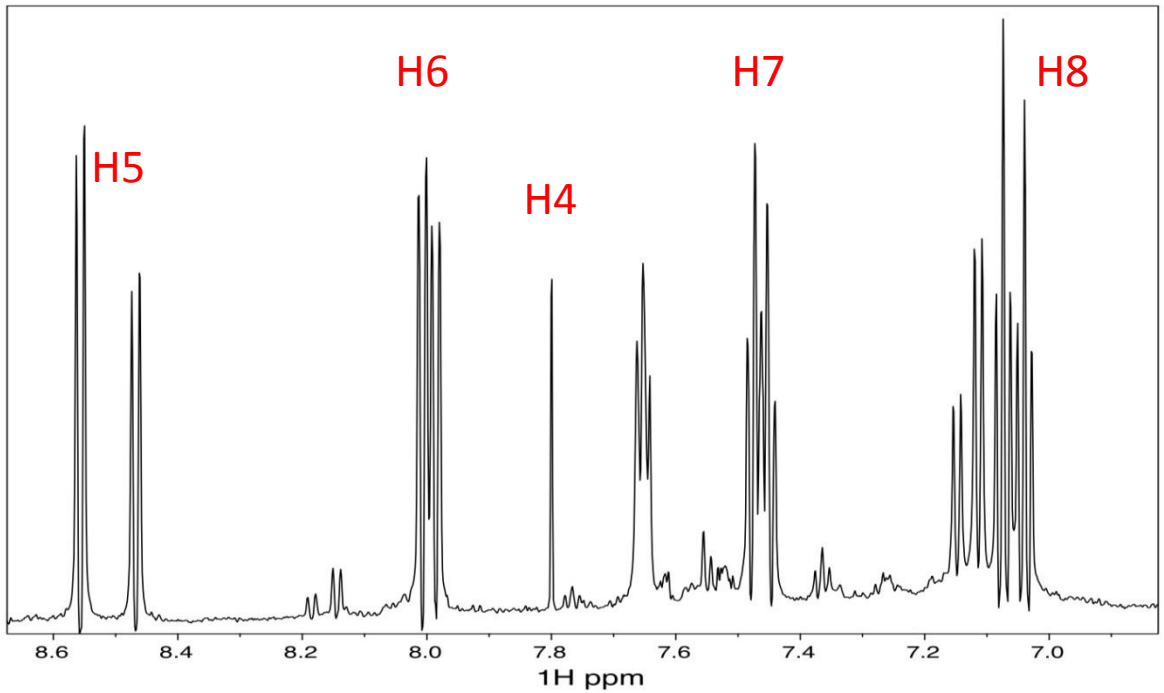
Microorgnaism	Inhibition zone diameter in cm
Bactria:	
B.subtilis	2.3
B.pumilis	2.2
S.aureus	1.9
E.coli	2.0
A.hydrophila	3.3
V.harveyi	3.5
Fungi:	
A.oryzae	1.5
A.niger	1.2
C.albicans	1.6

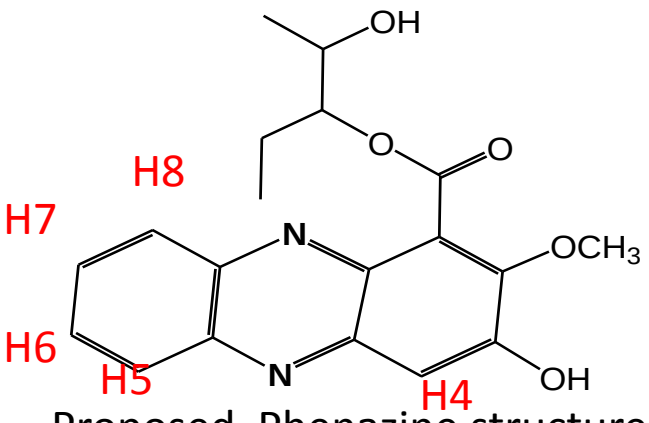
Antimicrobial activities of HPLC fractions

Microorgnaism	Inhibition zone diameter in cm
Bactria:	
B.subtilis	2.8
B.pumilis	2.8
S.aureus	2.3
E.coli	2.4
Fungi:	
A.oryzae	1.8
A.niger	1.6
C.albicans	2.0

	IC 50 value (ng/ml)		
Cell line	Compound 1 (Mw: 224Da)	Compound 2 (Mw: 240Da)	Compound 3 (Mw: 270Da)
Mouse macrophage RAW 264.7	100	50	100
Human neuro blastoma SH-SY5Y	30	40	70
Human lung cancerous A549	50	80	100
Human liver cancer HEPG2	50	50	70

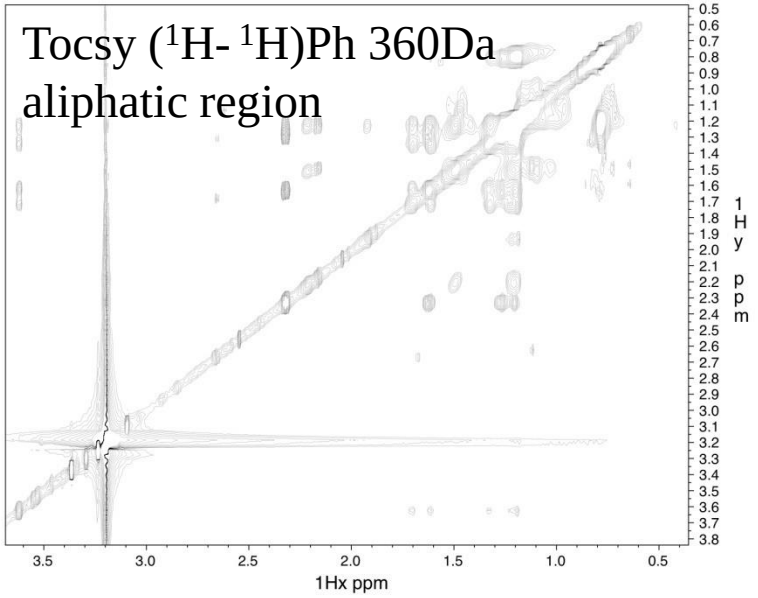
^{1}H Proton NMR aromatic region Ph 360Da

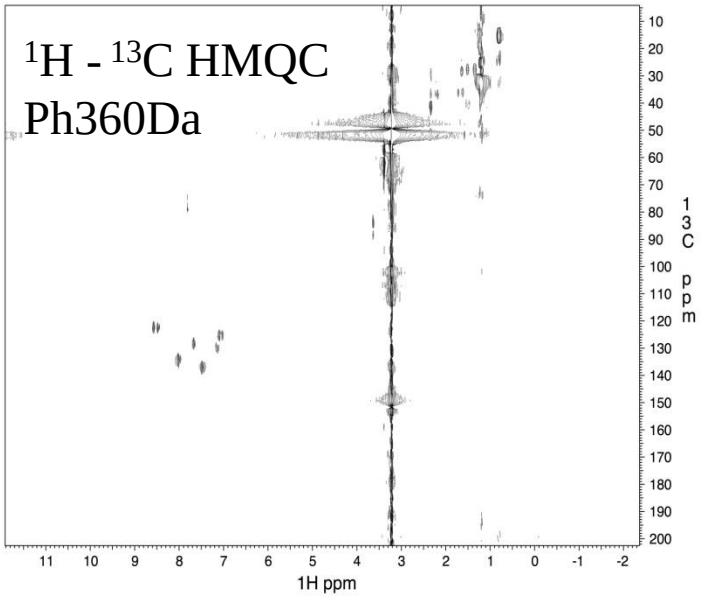


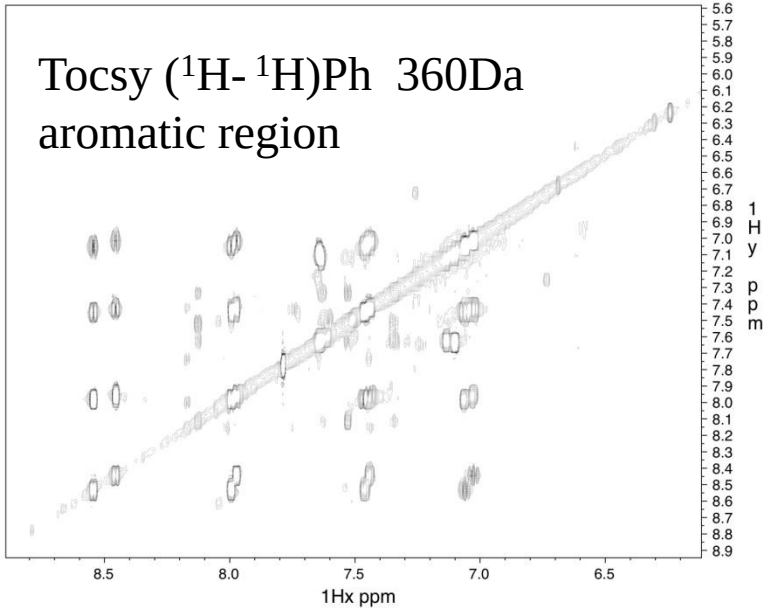


Proposed Phenazine structure

2D NMR spectra of compound-4







Tocsy:

1.28 ↔1.64 ↔2.31 ↔3.61.

1.64 ↔3.61 .

1.28 ↔3.61 .

7.05 ↔ 7.44 ↔7.97 ↔8.45.

HMQC:

7.05/125.3, 7.44/136.9, 7.97/134.3, 8.45/122.5, 1.28/31.8, 1.64/28.1, 2.31/41.4, 3.62/85.13.

Conclusion

- The exploitation of the marine environment has been successful in recent years in the search for structurally unusual and biologically highly active natural products.

- In this study 4 Phenazine derivatives have been isolated from marine Pseudomonas species and deposited in Genbank with accession no. ***KJ558373.***

- Purified compounds were characterized by UV-Vis, IR and NMR spectroscopy and by Mass spectrometry and showed broad spectrum of activity.

- Structural characterization of Ph224, 240 , 270Da has been accomplished and 360 Da under tentative proposal.

- 2D-NMR spectra revealed the tentatively molecular formula of four compounds - 270 Da ($C_{14}N_2H_8O_4$), 240 Da ($C_{13}N_2H_8O_3$), 223 Da ($C_{13}N_3H_9O$), and 360 Da ($C_{17}N_2H_{12}O_7$)

References:

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THANK YOU!