

Chapter 4

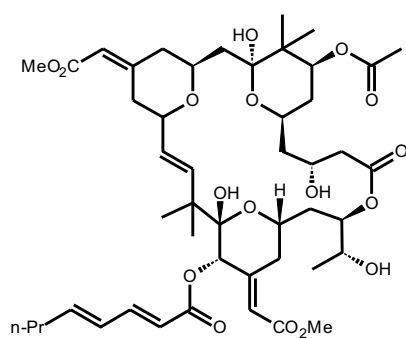
STUDIES TOWARDS THE SYNTHESIS OF AMATHAMIDES AND THEIR ANTIMICROBIAL ACTIVITY

I. Introduction

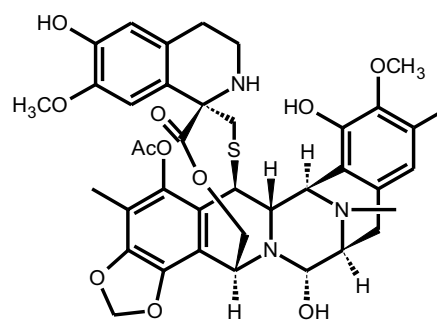
i) General

During past 30 to 40 years, numerous novel compounds have been isolated from marine organisms and many of these have been reported to exhibit various biological activities, some of which are of interest from the point of view of potential drug development. Despite, over 22000 compounds isolated from marine organisms and the biological activities attributed to many of them, those that have either been marketed or are under development are very few (Blunt et al., 2011). There are probably several reasons why only few compounds originating from marine plants and animals have been developed as drugs. Much of the work undertaken in the 1960-1970s and probably the early 1980s was driven by an interest in the chemistry of new compounds rather than their biological activities. Another issue was to tackle with the supply of MNPs, which has been major limiting factor for further pharmaceutical development. Often, a metabolite occurs in trace amounts in an organism, and a steady source of supply from wild harvest cannot provide enough of the target compound for preclinical studies. In general, the natural abundance of the source organisms do not support production based on wild harvest. Unless, there is a feasible alternative to harvesting, promising chemicals will remain undeveloped.

The total synthesis or perhaps semi-synthesis in some cases has been the one of the best solutions to avail these substances in sufficient amount for further development (Cuevas et al., 2000). Moreover, the considerable interest in the synthesis of marine natural products is developed from the fact that many marine natural products have unique structures not found in terrestrial metabolites and so their syntheses represent a challenge for the organic chemists. The synthesis of some important marine natural products those are under clinical evaluation for e.g. bryostatins 1 (**1**) (Evans et al., 1999), or in the market as potential drug for e.g. ecteinascidin 743 (**2**) (Endo et al., 2002) have been achieved. Herein, we intend to synthesize amthamides, the secondary metabolites of a bryozoan, *Amathia wilsoni* and assess their antimicrobial activity. The synthetic studies towards amthamides along with literature review on amthamides and related compounds from bryozoan genus *Amathia* and the ecology and biological activity of amthamides is presented in this chapter.



Bryostatin 1 (1)

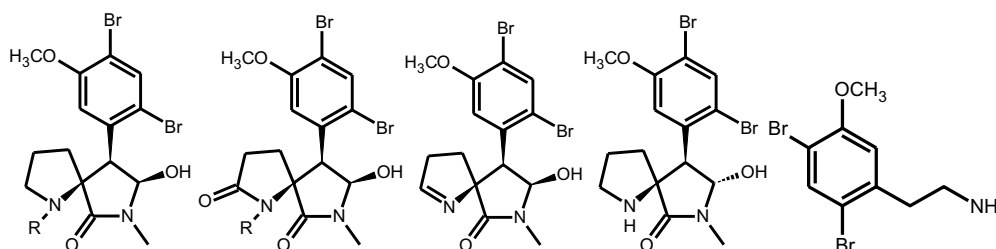


Ecteinascidin 743 (2)

ii) Amathamides and related metabolites from bryozoan genus Amathia

Bryozoans, also known as seamats or sea mosses, are found in both freshwater and marine environments. It is interesting to note that as reviewed by Sharp et al., (2007), the metabolites so far reported from bryozoans are exclusively from marine habitats. A bryozoan of genus *Amathia* is a member of phylum Stenolaemata, order Cyclostomata, family Vesiculariidae.

A species, *Amathia wilsoni* Kirkpatrick, a reasonably common foliose bryozoan from Tasmanian coastal waters, has been shown to contain a series of brominated alkaloids, amathamides A-F (**3-8**) (Blackman and Matthews, 1985; Blackman, and Green, 1987) and another group of biosynthetically related alkaloids, amathaspiramides A-F (**9-14**) (Morris and Prinsep, 1999). The isolation of 2-(2,4-dibromo-5-methoxyphenyl)ethanamine (**15**) from this species suggest that amathamides and amathaspiramides are biosynthesized from phenylalanine by a series of aromatic substitution reactions and a decarboxylation giving the amines of the type **15**, which then reacts with a proline derivative (Blackman and Fu, 1989).



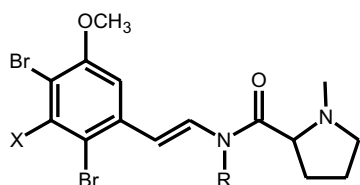
9 R = CH₃
11 R = H

10 R = CH₃
12 R = H

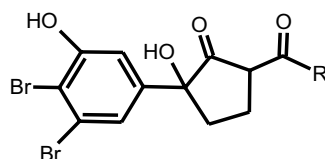
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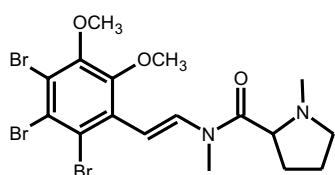
15



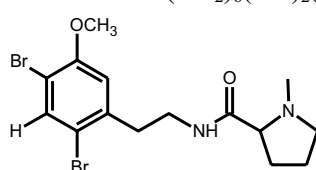
- 3** R = H, X = H
5 R = H, X = Br
7 R = H, X = H
4 R = H, X = H *cis* geometry
8 R = H, X = Br *cis* geometry



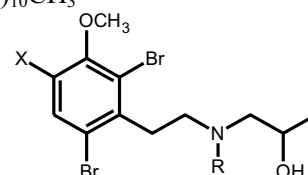
- 17** R = (CH₂)₁₂CH₃
18 R = (CH₂)₇(CH)₂(CH₂)₅CH₃
19 R = (CH₂)₁₄CH₃
20 R = (CH₂)₇(CH)₂(CH₂)₇CH₃
21 R = (CH₂)₁₆CH₃
22 R = (CH₂)₆(CH)₂(CH₂)₁₀CH₃



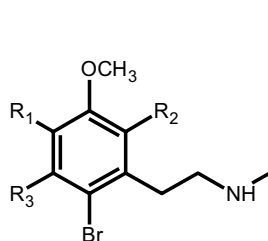
Amathamide G (**16**)



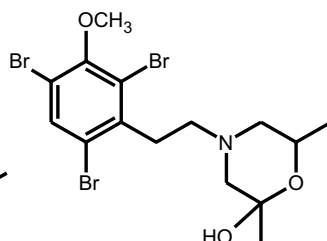
Amathamide E (**6**)



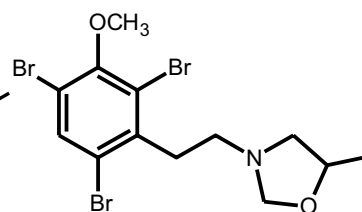
- 23** X = Br, R = CH₃
24 X = H R = CH₃
25 X = Br R = H



- 28** R₁ = R₂ = Br R₃ = H
29 R₁ = R₃ = H R₂ = Br
30 R₁ = R₃ = Br R₂ = OCH₃



27



26

Amathamide G (**16**), a new member of amathamide series was isolated from another species *Amathia convoluta*, (Narkowicz et al., 2002) which also produces related metabolites that includes convolutamides A–F (**17–22**) (Zhang et al., 2002), convolutamines A–H (**23–30**) (Narkowicz, et al., 2002; Zhang et al., 1994b; Kamano et al., 1999), convolutamydines A–E (Kamano et al., 1999; Zhang et al., 1995; Kamano et al., 1995), convolutindole A, and volutamides A–E (Montanari et al., 1996). Interestingly, the species *Amathia convoluta* has been also shown to produce a chemically different class of compounds called bryostatins (Petit et al., 1985), although their production by *A. convoluta* cannot be confirmed due to the presence of another bryozoan *Bugula neritina* growing epiphytically on the sampled colonies.

The bryostatins are a group of compounds usually associated with *Bugula neritina* of which, bryostatin 1 (**1**) is at advance stage of clinical evaluation as anticancer drug. Another species in the same genus, *Amathia alternata*, is known to produce antibacterial brominated dipeptides alternamides A–D (Lee et al., 1997)

iii) Ecology and biological activity of amathamides

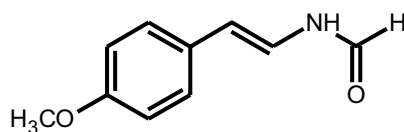
A. wilsoni has been the subject of extensive ecological studies. The amathamide content of *A. wilsoni* varies between sampling sites, but individual sites remain constant over the time, exhibiting no seasonal variation or individual colony variation (Blackman, and Green, 1987). In addition, the distribution of the amathamides within individual colonies decreases from the colony tip (9%) to the base, with the basal areas being totally devoid of metabolites (Walls et al., 1991). Although, the function of such a gradient is unknown, Walls et al., (1991) have suggested that new growth would be more susceptible to predation and larval settlement, therefore, enhanced chemical defence in these areas could reduce grazing and fouling, although high metabolite content in one area does not necessarily indicate that metabolites are produced in these areas. However, Walls et al., (1995) suggest that the surface-associated bacteria may be responsible for the production of the amathamides. High bromine levels on the bryozoan surface, but not in the cells, indicate that the amathamides are present on the surface but are not found in the cells. The surface bromine distribution correlates with the presence of surface bacteria, suggesting that production is associated with these bacteria. Although, in previous studies it had been reported that there were differing amounts of amathamides from *A. wilsoni* collected in different Tasmanian sites, none had recorded an absence of amathamides. However, a study of *A. wilsoni* from New Zealand reported the presence of amathaspiramides A–E **99-13**) (biogenetically modified amathamides) but no amathamides (Morris and Prinsep, 1999). The authors of this study suggest that the different chemical profiles reflect genetic variability or differing environmental conditions between the populations studied which contradicts the opinion of Narcowicz et al., (2002) who suggested that the difference in chemical profiles of the closely related species, *A. convoluta*, could be the result of bacterial symbionts

producing the products associated with the bryozoan. There is currently no conclusive evidence as to which of these hypotheses is most likely to be a true representation of the situation.

None of the amathamides have been reported for any biological activities, but extract containing amathamide G (**16**) has been shown to be nematocidal (Narcowicz et al., 2002). The predicted ecological functions of these amathamides as discussed above and biosynthetically related amathaspiramides found in the New Zealand *A. wilsoni*, of which A and E found to exhibit moderate cytotoxicity to cancer cell lines and also mild antimicrobial activity implies that amathamides could be potential antimicrobial agents.

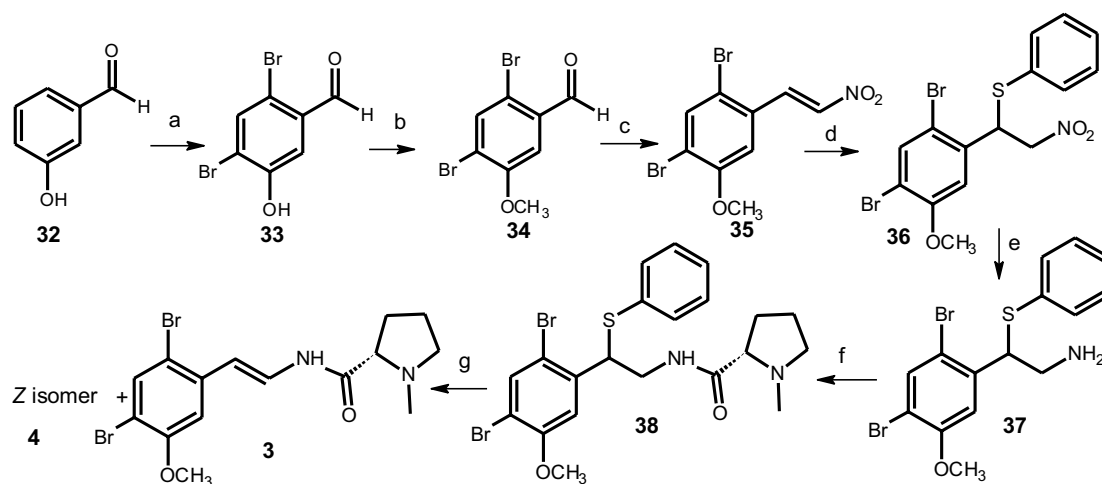
iv) Previous synthetic studies on amathamides

Amathamides are enamides of a styrylamine and proline derivative. Only one synthetic route is known towards the synthesis of amthamides. This method uses the methodology used for the synthesis of tuberine (**31**), an enamide of styrylamine and formic acid. Tuberine (**31**) was synthesized from a nitrostyrene prepared from 3-methoxybenzaldehyde (Somanathan et al., 1996). The ene functionality of nitrostyrene was protected using thiophenol and nitro group was reduced to amine with Zn/HCl. The resulting amine was coupled with acetic formic anhydride and thiophenol was removed by oxidation with NaIO₄ and elimination with a base to get **31**.



Tuberine (**31**)

Riding on the similar strategy Osuna et al., (2002) prepared amathamides A (**3**) and B (**4**) by coupling *N*-methylproline (**39**) with a suitable amine derivative as shown in **Scheme 4.1**.



Reagents and conditions: (a) CHCl_3 , Br_2 , 92%; (b) CH_3I , DMF, K_2CO_3 , 98%; (c) CH_3NO_2 , AcOH, AcONH_4 , 50%; (d) PhSH, Base, CH_2Cl_2 , 95%; (e) SmI_2 , THF, 65%; (f) DCC, DMAP, HOBT, **39**, 33%; (g) i. NaIO_4 , MeOH; ii. K_2CO_3 , Toluene, reflux, **3** 23%, **4** 1.15%.

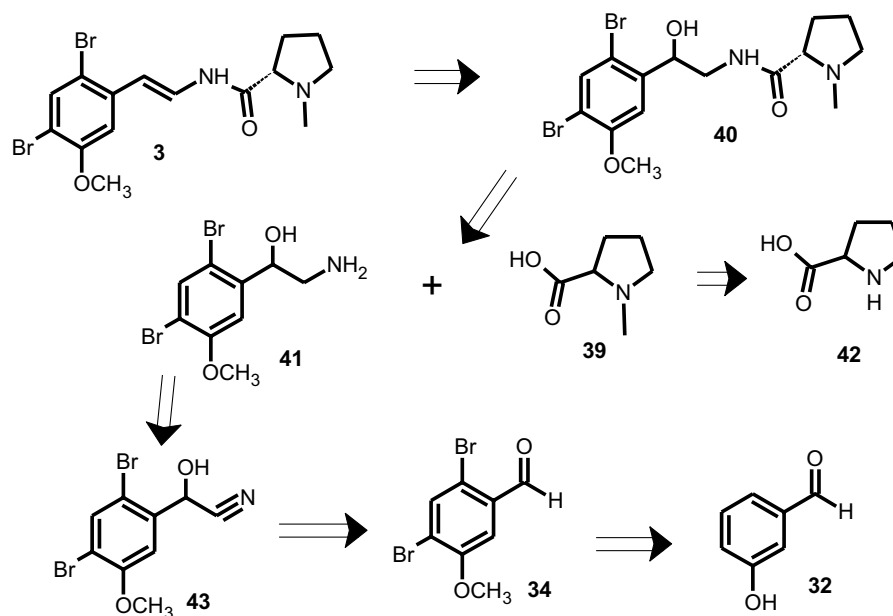
Scheme 4.1: Synthesis of amathamides A (3) and B (4)

II. Results and Discussion

i) Synthetic studies

Biological activity of amathamide alkaloids has not been well studied, but related metabolites have shown to possess nematocidal, antifungal, and antibacterial activity against few strains, which is discussed in our earlier sections. Their ecological roles suggest that these metabolites could be potentially antimicrobial. This prompted us in finding a new pathway for the synthesis of amathamides and their analogs for discovery of new antimicrobial agents.

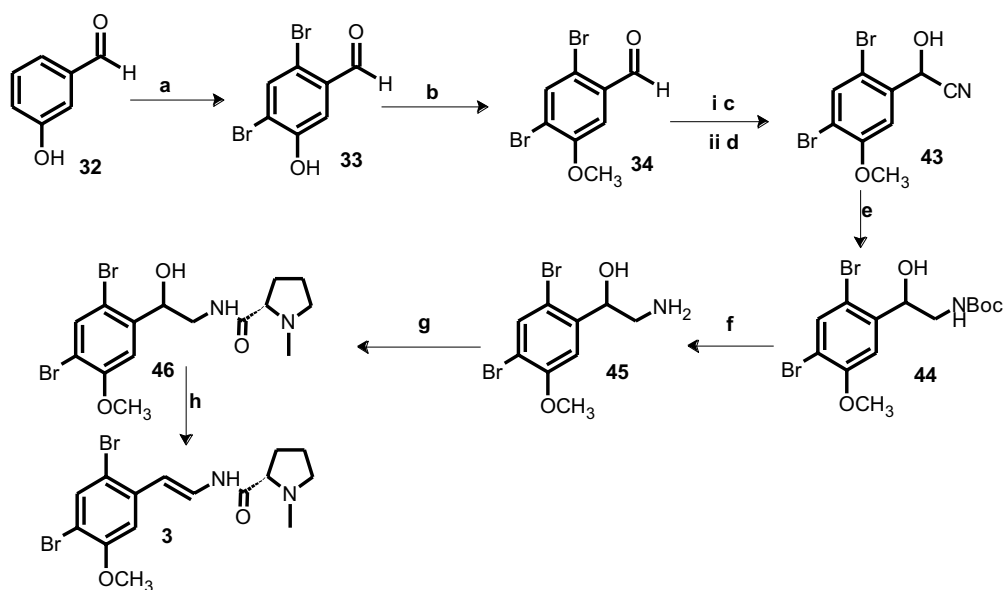
On retrosynthetic analysis, we considered the key precursor is to prepare amathamide alcohol, which can be eventually dehydrated to obtain amathamide A (**Scheme 4.2**). The key precursor thus can be obtained by coupling of 2-(2,4-dibromo-5-methoxyphenyl)-1-aminoethan-2-ol (**41**) (a β -amino alcohol) and *N*-methyl proline (**39**). The β -amino alcohol (**41**) can be prepared by reduction of a cyanohydrin (**43**) obtained from corresponding benzaldehyde (**34**).



Scheme 4.2: Retrosynthetic analysis of amathamide A (3)

Accordingly, the synthetic scheme was designed as shown **scheme 4.3**. Thus, 2,4-dibromo-5-methoxybenzaldehyde (**34**) was prepared in two steps from commercially available 3-hydroxybenzaldehyde (**32**). 3-hydroxybenzaldehyde (**32**) was dibrominated with two moles of bromine in acetic acid to give solid compound (**33**), in 91% yield which was methylated using methyl iodide and anhyd. K_2CO_3 in DMF to give **34**. Compound (**34**) was obtained as solid in 98% yield and has been characterized using spectroscopic methods.

Once we had in our hand, the required benzaldehyde derivative (**34**), the next task was to prepare cyanohydrin (**43**) from it. The simplest and primitive way of preparing cyanohydrin is to react an aldehyde with HCN. HCN is generated *in situ* from cyanide salt such as NaCN, KCN, etc by treatment with a mineral acid. It is not safe to use HCN or its salts owing to its toxic effects that are fatal. Moreover, the yields of these reactions are poor and also direct synthesis from aldehyde/ketone and HCN may be difficult owing to inherent thermodynamic instability of the aldehyde/ketone derivatives. Several alternatives to prepare cyanohydrins, which avoid using directly HCN or its salts, have been developed. They mainly involve process of trialkylcyanosilylation and then hydrolysis leading to cyanohydrin.



Reagents and conditions: (a) Br₂, CHCl₃, 3days, rt, stir, 93% (b) CH₃I, DMF, K₂CO₃, 4h, rt stir, 98% (c) Me₃SiCN, cat. LiCl/THF, rt, stir, 1h (d) 1N HCl/MeOH-aq, 1h, rt 96% (e) NaBH₄, cat NiCl₂·H₂O, Boc₂O, MeOH 0° C, 30 min and rt, 2 h., 78% (f) 6N, HCl, reflux 3h, 76% (g) **39**, oxyma, DIPC, DMF, rt, 30 min, 92% (h) CuCl-DCC, DMF, 24 h, 50° C.

Scheme 4.3: Synthesis of Amthamide A (3)

A process developed by Cabirol et al., (2008) uses NaCN and TMSCN to give cyanotrimethylsilylhydriens of ketones in 60-99% yield in 30 min at rt. Kim et al., (2004) has shown the preparation of cyanotrimethylsilylhydriens using *N*-methylmorpholine-*N*-oxide as catalyst in 91-99% yield in 5-20 h at rt, whereas Lewis acid catalysis using ZnI₂ in CH₂Cl₂ yields 90% cyanotrimethylsilylhydriens of benzophenone (Talley and Gassman, 1990). The above three procedures have not been used earlier for the preparation of cyanotrimethylsilylhydriens of aldehydes.

Even though, ZnI₂ was reported to be the catalyst for cyanosilylation for ketones (Talley and Gassman 1990), we used the same to prepare cyanohydriens of aromatic benzaldehydes, assuming that aldehydes being more reactive than ketones should also easily react, but to our disappointment we recovered our starting material **34** after the reaction. Therefore, we thought of using a method specifically used to prepare cyanotrimethylsilylhydriens of aldehydes.

There are several procedures for preparations of cyanotrimethylsilylhydriens of aldehydes and some are preferably used to obtain the product in enantiopure form. Those used for chiral synthesis include dinuclear chiral (Salen) titanium complex

catalysis (Belokon et al., 2000), chiral oxazaborolidinium ion catalysis (Ryu and Corey, 2004), (DHQ)₂PHAL catalysis (Denmark and Chung, 2006) and vanadium(V) salen complex catalysis (Khan et al., 2006).

There are at least four methods in the literature used to prepare cyanotrimethylsilylhydriens in racemic form. They are

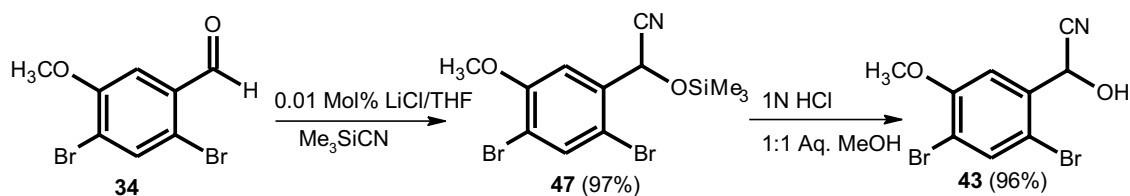
1. A method developed by Suzuki et al., (2006) gives over 93% of yield in 30-180 min catalyzed by nitrogen-heterocyclic carbenes (NHCs) in THF. If chiral NHCs are used then optically pure compounds can also be obtained.

2. A Lewis base catalysis using triethylamine (TEA) or *N,N*-(dimethylamino)pyridine (DMAP) in acetonitrile have also been used to obtain cyanotrimethylsilylhydriens. Yields of >90% have been achieved on aromatic aldehydes using DMAP (Denmark and Chung, 2006)

3. Another method makes use of NbF₅ catalysis in solvent free condition (Kim and Rajgopal, 2007). Yields of 80-99% can be obtained in 10-30 min at rt depending upon the type of aldehyde.

4. Yet another method to obtain cyanotrimethylsilylhydriens is by using LiCl catalysis in solvent free condition at room temperature (Kurono et al., 2005). The reaction is completed in 10-30 min at rt with quantitative yields.

NHCs and NbF₅ were not readily available to us, so we did not opt for methods 1 and 3. Thus, the reaction was carried out using DMAP as per the reported method 2, but again no conversion of 2,4-dibrom-5-methoxybenzaldehyde was observed. Therefore, we opted for 4th method *i.e.* LiCl catalysis. The reaction in presence of 0.01 mol % of LiCl in solvent free condition showed complete conversion of starting material in 15 min, monitored by TLC. After stirring the reaction mixture for another half an hour it was concentrated under vacuum to remove any traces of TMSCN and THF left in the reaction mixture to obtain 2-(2,4-dibromo-5-methoxyphenyl)-2-trimethylsilyloxyacetonitrile (**47**) in 97% yield. This product without characterization was used for further reaction, but a similar compound 2-(2-bromo-5-methoxyphenyl)-2-trimethylsilyloxyacetonitrile (**50**) has been isolated and characterized with NMR data.



Scheme 4.4: Preparation of a cyanohydrins through cyanosilylation of an aldehyde

Compound **47** was then hydrolysed to give corresponding cyanohydrin, 2-(2,4-dibromo-5-methoxyphenyl)-2-hydroxyacetonitrile (**43**) in 1N HCl in aqueous MeOH (**Scheme 4.4**). The product was extracted in CHCl_3 to give buff colour solid in 96% yield. In its IR spectrum it showed a very weak absorption for cyano at 2250 cm^{-1} , and strong absorption for hydroxyl at 3350 cm^{-1} . No Peak in the aldehydic region was observed. Usually, cyano compounds show very strong absorption in IR for cyanide stretching, but it has been observed that certain cyanohydrins either show very weak or no absorption in IR for cyano group.

In NMR spectra (**Fig 4.1a** and **b**) it showed two aromatic methines at δ_{H} 7.76 (s, 1H) δ_{C} 136.7 (d, C-5) and δ_{H} 7.23 (s, 1H) and δ_{C} 111.2 (d, C-8). The cyanide group was evident from a singlet at δ_{C} 117.6 (C-1) and benzylic hydroxymethine from the signal at δ_{H} 5.76 (br s, 1H), 3.67 (br s, OH) and δ_{C} 62.6, (d, C-2).

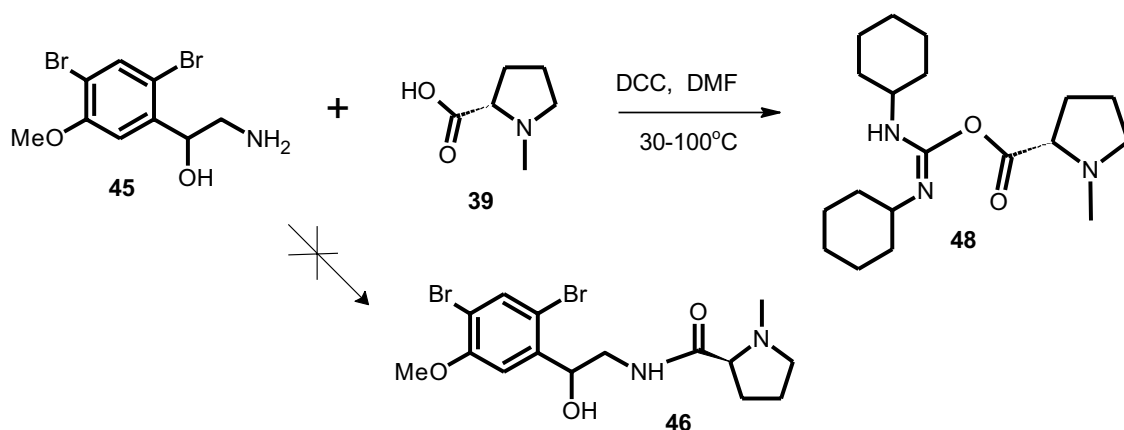
The next step in the scheme was to reduce cyanohydrin to β -amino alcohol without hydrogenolysis of benzylic hydroxyl. In reduction of nitriles, the yields are low due to formation of some unwanted dimeric products resulting from intermediates. Through literature survey we found that NiCl_2 catalysis of sodium borohydride reduction is one of the best ways of selectively reducing nitriles to *N*-protected amines (Caddick et al., 2003). Only catalytic amount of NiCl_2 is necessary to carry out the reaction and the active species is $\text{Ni}(\text{BH}_4)_2$. Accordingly, the reaction was carried out on **43** with Boc_2O as protecting agent. At the end of the reaction the solvent (MeOH) was evaporated under vacuum, the product was extracted in EtOAc and washed with sat. bicarbonate solution. After evaporation of EtOAc, a viscous liquid compound (**44**) was obtained in 76% yield, which was characterized using NMR (**Fig 4.2a** and **b**). Proton NMR showed signals at δ_{H} 5.03, br t for benzylic hydroxyl methine (C-3) coupled to a vinylic methylene at δ_{H} 3.45, ddd (α -HC-2, $J = 15.0, 6.1, 1.8\text{ Hz}$) and

3.36, m (β -HC-2). The three equivalent methyls of Boc group were observed at δ_{H} 1.45, s.

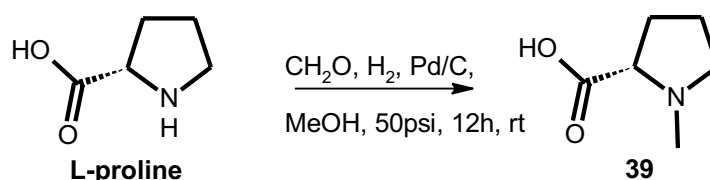
The next task was to deprotect the amine (**43**) by removing Boc group, which was done by refluxing the protected amine in 6N HCl for 3 h. On completion of reaction, fine drops of *tert*-butanol were seen floating on reaction mixture, which were extracted in ethyl acetate. Aqueous portion was basified with dil. NaOH and the free amine was extracted in CHCl_3 . After evaporation of the solvent the amine, 2-(2,4-dibromo-5-methoxyphenyl)-1-aminoethan-2-ol (**45**) was obtained as brown solid in 63% yield. In NMR (**Fig 4.3a** and **b**) spectra it showed pattern of signals similar to **43** except that the signals for Boc group disappeared.

The next task was to prepare amathamide A alcohol (**46**), the immediate precursor to amathamide A, which requires formation of amide bond between the amine (**45**) and *L-N*-methyl proline (**39**).

Formation of amide bond by coupling an amine with a carboxylic acid is one of the most important processes in organic chemistry and the same is required in syntheses of several biologically important peptides. A lot of attention has been given to the development of efficient coupling reagents that give high yields in less reaction time with prevention of racemization. For the formation amide bond, a carboxyl group needs to be activated to react with an amine nucleophile. The simplest way in which carboxylic acid is converted into an amide is by converting carboxylic acid into a more reactive acyl chloride and reacting it with an amine. But this methodology has limitations due to several reasons such as high reactivity and non-selectivity of acyl chlorides towards nucleophiles. Therefore, one of the most preferred strategies is carbodiimide approach of activation of carboxyl in amide formation. The carbodiimide alone either gives poor yields or no yields, as also observed in our coupling reaction of **45** with (*S*)-*N*-methyl-*L*-proline (**39**) (**Scheme 4.5**). *N*-methyl *L*-proline (**39**) was prepared by reacting *L*-proline with aq. formaldehyde and reducing it *in situ* with palladized charcoal in MeOH in quantitative yield as shown in **scheme 4.6** (Han et al., 2005).



Scheme 4.5: Coupling of an amine with *N*-methyl proline using DCC

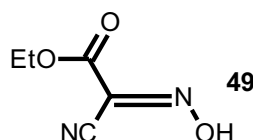


Scheme 4.6: Synthesis of *N*-methyl-L-proline

The reaction of an amine (**45**) with proline derivative (**39**) using dicyclohexyl carbodiimide (DCC) saw no attack of **45** on the activated adduct (**48**) (**Scheme 4.5**). After the reaction, the adduct **48** was isolated and characterized by NMR spectroscopy. In ESI-MS, it showed pseudomolecular ions $[M+H]^+$ at m/z 336 $[M+Na]^+$ at m/z 358 $[M+K]^+$ at m/z 374. Its ^{13}C NMR showed a signal at δ_C 173 for ester carbonyl and showed a signal at δ_C 153 for the sp^2 carbon attached to the two nitrogen atoms and an oxygen atom. The signals for the other thirteen carbon atoms were observed in the region δ_C 20-70 accounting for two non equivalent cyclohexane rings and *N*-methyl proline ring.

Poor yield or no reaction are not the only problem with carbodiimide approach, but it also bring racemization, hence an additive, which can enhance yield and suppress racemization is also used in the coupling. Of these compounds, the most intensively used display a benzotriazole core: 1-hydroxybenzotriazole (HOBt), probably the most common reagent found in a peptide synthesis laboratory, whereas later the use of the

more powerful 1-hydroxy-7-azabenzotriazole (HOAt) (Carpino, 1993) and, more recently, 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) has been reported (Azev et al., 1976). Other coupling strategies, such as the combination of base and stand-alone coupling reagents, such as immonium (HATU, HBTU/TBTU, and HCTU/TCTU) (El-Faham and Albericio 2008), or phosphonium salts (PyAOP, PyBOP, and PyClock) have also been enhanced by the use of these additives. However, a report of a potentially explosive character of HOBt and its related additives have recently led to the finding of an alternative reagents, such as oxyma (**49**) (Funosas et al., 2009) and uronium salts (the adducts of oxyma/isonitrosoMeldrum's acid and morpholonium-based immonium moiety) such as COMU and HTMU as a superior and safer coupling reagent for amide formation (El-Faham et al., 2010).



The reported synthesis of **3** (Scheme 4.1) makes use of DCC/HOBt/DMAP for coupling of their amine with **39**, which gave them only 33% yield of coupling product (**38**) after 18 h. Considering the low yield of coupling using HOBt and also due to its explosive nature we opted for **49** as a coupling reagent. In contrast to the reported reaction, the coupling between **45** with **39** using DIPC/oxyma fetched us the amide (amathamide A alcohol) (**46**) in 92% yield in just 1 h. The amide from the reaction mixture was isolated by washing the acidified reaction mixture with EtOAc. The acidic aqueous part was then basified with dil NaOH and extracted in EtOAc. Upon evaporation of EtOAc, **46** was obtained as colourless crystalline needles. The compound has been characterized using spectroscopic methods.

Table 4.1 : NMR data of amathamide alcohols 46 and 60

Position	46		60	
	δ_C , mult	δ_H , mult	δ_C , mult	δ_H , mult., J(Hz)
2	56.46 t (56.42)	3.05 m, 2.17 m	54.6 t	
3	24.3 t (24.2)	1.75 m, 1.57 m	20.0 t	
4	31.1 t (31.0)	2.17 m, 1.76 m	28.8 t (28.9)	
5	68.5 d	2.95 m	67.1	
6	178.0 s (177.9)		174.0 s (173.9)	
8	41.6 t (41.7)	3.64 m, 3.57 m	42.7 t (42.4)	3.43, s
9	73.9 d (77.9)	5.07 br s	69.3 d (69.2)	
10	141.5 s (141.4)		140.7 s (140.6)	7.54, d, (8.7)
11	111.0 s		110.2 s (110.1)	6.85, d, (8.7)
12	136.1d (135.9)	7.72, s	131.4 d	
13	111.4 s		112.0 d (111.9)	
14	155.5 s (155.3)		157.9 s (157.8)	6.40, d (1.8)
15	111.3 d	7.23 s	113.1 d (112.9)	
16	45.9 q, (45.8)	2.38 s	39.7 q	
17	56.6 q (56.5)	3.89 s	53.0 q	6.49, d, (8.1)

* The values given parenthesis are observed due C-9 epimer

The ESI-TOF-MS (**Fig 4.4d**) of **46** showed the presence of the required product containing two bromine atoms by giving triplets of pseudomolecular ions $[M+H]^+$ at m/z at 435/437/437 and $[M+Na]^+$ 457/459/461, corresponding to average molecular weight of 436 as required for **3**. In the MS/MS spectrum (**Fig 4.4e**) the base peak occurred at m/z 84, which is characteristic of *N*-methylpyrrolidines, also observed for natural amathamides (Blackman and Green, 1985). Its IR spectrum (**Fig 4.4a**) showed conjugated amide carbonyl absorption at 1666 cm^{-1} , a hydroxyl and amide N-H absorption centred at 3340 cm^{-1} and various C-H str peaks in the region $2790\text{-}3050\text{ cm}^{-1}$. In ^1H NMR (**Fig 4.4b**) it showed peaks in the region for two aromatic protons as singlets at δ_H 7.72 and 7.23. A benzylic hydroxymethine at δ_H 5.07 (br s); methoxy at δ_H 3.89, s; and two multiplets of diastereotopic protons of methylene at δ_H 3.64 and 3.57 for the amine part of the compound. The signals for proline part were observed as δ_H 3.04 (m, 1H), 2.92, (m, 1H), 2.35 (s, 3H), 2.44 (m 2H), 1.76 (m, 2H) and 1.57 (m, 1H). The ^{13}C NMR (**Fig 4.4c**) spectrum showed either peaks in duplicate or larger peaks (merging of two peaks) indicating it to be a mixture of epimers at C-9. The amino alcohol (**45**) used in the reaction was racemic, while the *L*-proline

derivative (**39**) used was chiral, therefore the reaction between them produced a mixture of epimers (at C-9) of **46**. The formation of mixture of epimers is not concern to us as the epimeric chiral centre (C-9) would be destroyed in the dehydration step leading to single stereoisomer. The NMR data of **46** and **60** are tabulated in **table no. 4.1**

The final step in our synthesis was to dehydrate **46** to give **3**. In the literature, methods of eliminations of alcohols using carbamates (Atkinson et al., 1981), acetates (DePuy and King, 1960) and xanthates (O'Connor and Nace, 1953) are reported. These methods give good yield, but require high temperatures and long reaction times. A pseudourea-mediated dehydration of tertiary and benzylic alcohols using CuCl-DCC reported by Majetich et al., (1998) requires comparatively lower temperatures. We chose this method for dehydration of our compound **46**. Accordingly, the reaction was carried out, and although, disappearance of the **46** was observed as monitored by TLC, to our disappointment mixture of products, inseparable from by-product dicyclohexyl urea (DCU) was obtained. The formation of product is inferred from the triplet (due to two bromine atoms) of the pseudomolecular ions observed at $[M+H]^+$ m/z at 417/419/421 in the ratio 1:2:1 in its ESI-MS(**Fig 4.5a**). The ^1H NMR(**Fig 4.5b**) spectrum showed multiple signals due to the mixture of compounds dominated by the signal in the region δ_{H} 1-2 due cyclohexyls of DCC.

The effort to dehydrate the **46** by replacing DCC with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) is in progress (Sai et al., 2003). The by-product 1-ethyl-3-(3-dimethylaminopropyl) urea formed from EDC is unlike DCU is fairly soluble in water, thus can be easily separated from the main product.

Once we had achieved the synthesis of key intermediate amathamide A alcohol (**46**), the next task was to synthesize its analogues for screening against human pathogens for antimicrobial activity. In a time period, we were able to prepare three more analogous amathamide alcohols, **55**, **60** and **65** from three different starting materials, 3-methoxybenzaldehyde (**51**), 2-bromo-4-methoxybenzaldehyde (**56**) and 3-bromo-4-methoxybenzaldehyde (**61**) using methodology followed for amathamide A alcohol

(46). The yields of each step in the synthesis of the different analogues were comparable to the corresponding synthetic step of **3**.

3-Methoxybenzaldehyde (**51**) was prepared by methylation using $\text{CH}_3\text{I}/\text{K}_2\text{CO}_3$ in acetone in 98% yield. 2-bromo-5-methoxybenzaldehyde (**56**) was made available by bromination of **51** using Br_2 and NaOAc in AcOH, and in same way 3-bromo-4-methoxybenzaldehyde (**61**) was prepared from commercially available *p*-anisaldehyde. Benzaldehydes **51**, **56** and **61** were used to synthesize the amathamide alcohols **55**, **60** and **65** respectively. The structures of products of each step and their yields are tabulated in **table 4.2**

In the synthesis of amathamide alcohol (**60**) the intermediate silyloxy cyanohydrin compound (**50**) prepared from 2-bromo-5-methoxybenzaldehyde (**50**) was the only compound in its series which was characterized using NMR data while all other derivatives were used for further reaction without purification. In IR spectrum, **50** showed no signal for any aldehydic carbonyl which confirms the conversion of aldehyde, but also no signal for a cyanide group was observed. It has been observed that in all our cyanohydrins cyanide stretching is either weak or not observed. The NMR (**Fig 4.9**) spectrum of **50** showed the benzylic methine at δ_{H} 5.79, s, δ_{C} 63.1, d cyano carbon signal at δ_{C} 11.8.9, s a highly deshielded methyls were observed at δ_{H} 0.27, δ_{C} -0.34.

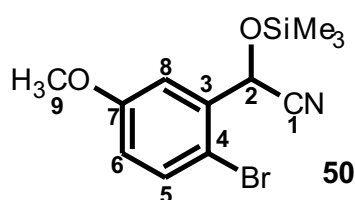


Table 4.2: Products of each synthetic step of amathamide alcohol and their yields

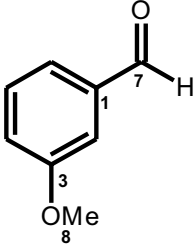
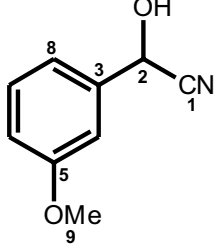
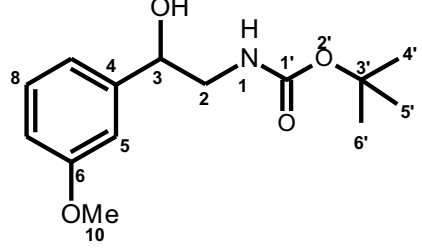
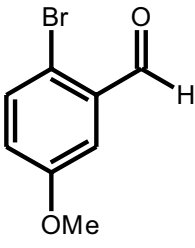
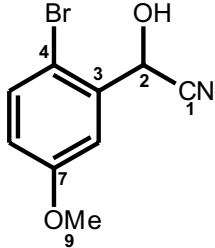
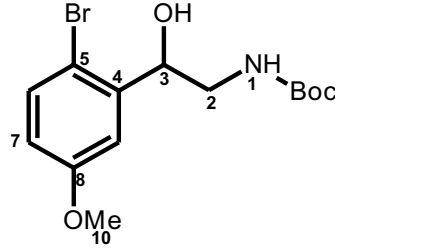
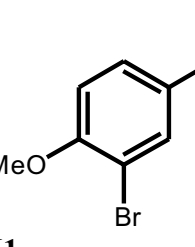
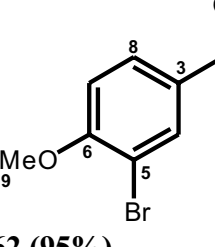
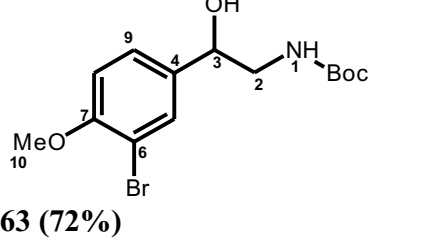
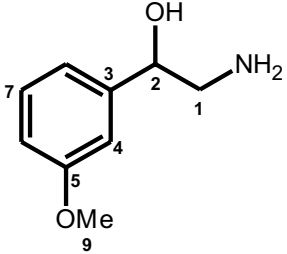
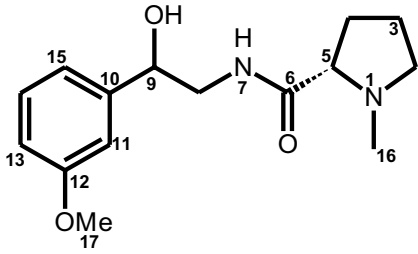
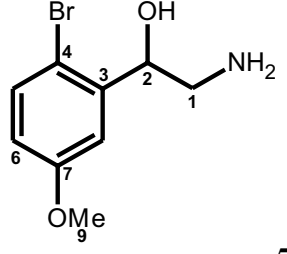
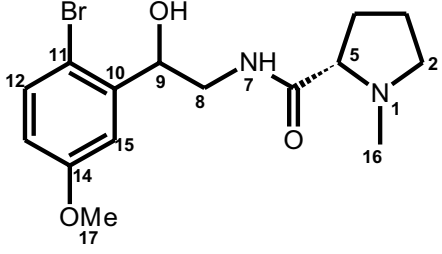
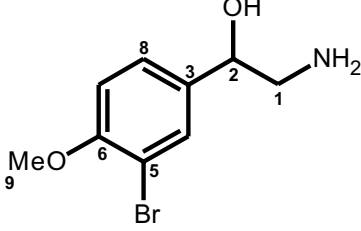
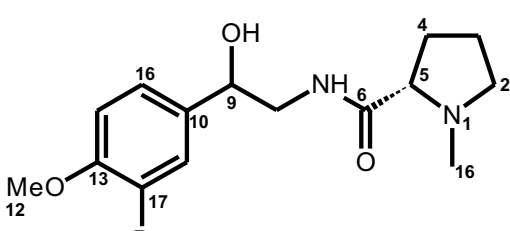
Sr.No.	Aldehyde	Cyanohydrin	N-Boc amino alcohol
1	 <p>51</p>	 <p>52 (93%)</p>	 <p>53 (71%)</p>
2	 <p>56</p>	 <p>57 (94%)</p>	 <p>58 (69%)</p>
3	 <p>61</p>	 <p>62 (95%)</p>	 <p>63 (72%)</p>

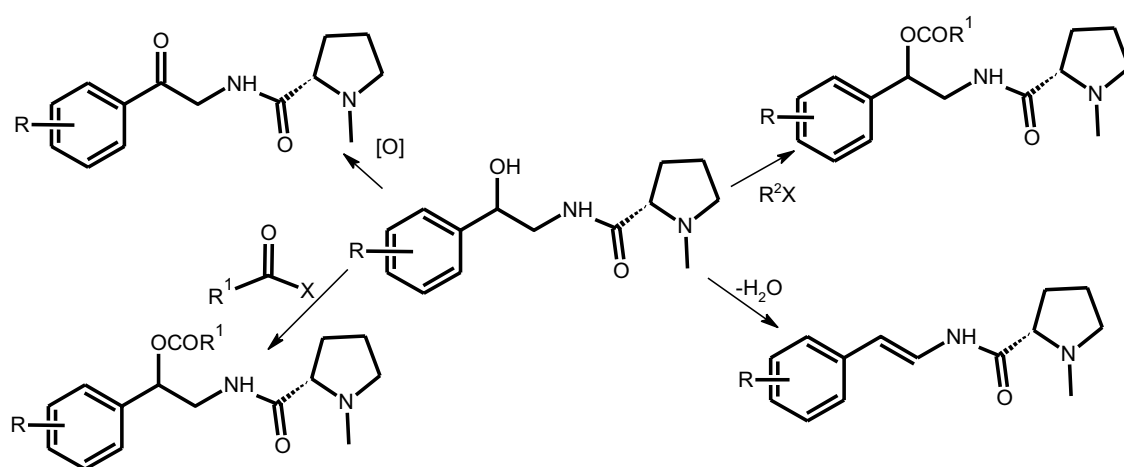
Table 4.2 continues..

Sr. No	Amino alcohol	Amathamide alcohol
1.	 <p style="text-align: right;">54 (62%)</p>	 <p style="text-align: right;">55 (85%)</p>
2.	 <p style="text-align: right;">59 (82%)</p>	 <p style="text-align: right;">60 (89%)</p>
3.	 <p style="text-align: right;">64 (60%)</p>	 <p style="text-align: right;">65 (90%)</p>

Our method towards the synthesis of amathamides is second only to Osuna et al., (2002). A comparison drawn between our synthetic method (**scheme 4.3**) and the reported method (**scheme 4.1**) indicates that our methodology of preparing amathamides through cyanohydrins instead of nitrostyrenes requires a step less, as our route do not require a step to protect the styryl double bond prior to reduction. Moreover, the yield of key step of our method that is preparation of cyanohydrins in were between 92-98%, whereas the yield of corresponding step of preparation of nitro styrene in literature method are 44-50%. An important step in the both the synthetic method is coupling of an amine and proline derivative. The DCC/HOBt

coupling methodology has given Osuna et al., (2002) the yields of 30-33%, while the use of oxyma/DIPC in similar coupling by us has yielded of 88-95% of amides.

Additionally, our synthetic scheme of amathamides through amathamide alcohols represents diversity oriented approach towards amathamides and their analogues as depicted in the scheme below (**scheme 4.7**). The amathamide alcohols in addition to precursor to amathamides also can be treated with various carboxylic acids/alkyl halides to obtain esters/ethers or can be oxidized to ketones, which themselves can give several more derivatives.



Scheme 4.7: Diversity oriented synthesis of amathamide alcohols

ii) Biological activity

Our main aim was to discover potent antimicrobial agents through synthesis of various analogues of amathamides. It has been discussed in one of the above sections that amathamides could be potential antibacterial agent. The four synthesized amathamide alcohols were tested against 14 clinical pathogens. The pathogens include 9 bacterial strains of which two were multidrug resistant strains and five fungal strains. The name of the strains and disease caused by them is listed **table 4.3**. Unfortunately, none of our synthesized amathamide alcohols (**46**, **55**, **60**, and **65**) showed activity at concentration of 100 µg/disc in a disc diffusion assay.

Table 4.3: details of pathogens used ion antimicrobial activity

Name of the pathogens	Disease caused	Code
<i>Bacterial Strains</i>		
<i>Escherichia coli</i>	Neonative meningitis	B1
<i>Pseudomonas aeruginosa</i>	Urinary tract infection	B2
<i>Staphylococcus aureus</i>	Skin infection	B3
<i>Salmonella typhi</i>	Typhoid	B4
<i>Shigella flexineri</i>	Gastrointestinal infection	B5
<i>Klebsiella sp</i>	Urinary tract infection	B6
<i>Vibrio cholera</i>	Cholera	B7
<i>Multi Drug Resistant Strains</i>		
<i>Acinetobacter sp.</i>	Urinary tract infection	D1
<i>Methicillin resistant staphylococcus aureus (MSRA)</i>	Skin infection	D2
<i>Fungal Strains</i>		
<i>Aspergillus fumigates</i>	Skin infection	F1
<i>Rhodotorulla sp</i>	Skin infection	F2
<i>Candida albicans</i>	Candiasis	F3
<i>Aspergillus niger</i>	Skin infection	F4
<i>Cryptococcus neoformans</i>	Skin infection	F5

III. Experimental

i) Bromination of aromatic aldehydes

a) 2-Bromo-5-methoxybenzaldehyde (56)

To a solution of 3-methoxybenzaldehyde (2g, 14.7 mmol), and NaOAc (2.4g, 17.6 mmol) in AcOH (20 ml) was added Br₂ (0.91ml, 17.6 mmol) in AcOH (5 ml) at 0-5°C over 30 min. The reaction mixture was stirred at room temperature for a day. Reaction was quenched by addition of saturated NaHSO₃ to decompose excess Br₂. Further reaction mixture was diluted with chilled DW (60 ml) and extracted in CHCl₃ (30 ml x 3). CHCl₃ layer was washed with DW, dried over anhyd Na₂SO₄ and solvent evaporated to get crude solid mixture. Crude mixture was purified over Si gel using EtOAc-petroleum ether (2%-10% v/v) as mobile phase. Elution with 2% EtOAc in PE gave **56** (1.8g, 58%) and 4% EtOAc in PE gave 4-bromo-3-methoxybenzaldehyde (0.82g, 26%).

2-Bromo-5-methoxybenzaldehyde (**56**) crystalline solid; IR (KBr) 3074, 3006, 2979, 2943, 2875, 2844, 2746, 1681, 1639, 1596, 1571, 1463, 1244, 1197, 1058, 933, 821, 867, 821, 755 cm^{-1} .

b) 2,4-Dibromo-5-hydroxybenzaldehyde (33)

To a solution of 3-hydroxybenzaldehyde (40.9 mmol, 5.00g) in CHCl_3 (60ml) was added solution of bromine (81.7mmol, 4.2ml) in CHCl_3 (20 ml) over 30 min at 0-5°C. The resulting solution was stirred at rt for 3 days. The excess bromine was removed by addition of a chilled saturated solution of sodium sulphite (20 ml). Chilled DW (50 ml) was added to the reaction mixture and the organic phase was separated. The aqueous portion was washed with CHCl_3 (30 ml x 2) and the combined organic phase was washed with water and dried over anhyd. Na_2SO_4 . Removal of solvent gave a solid (**33**) which was recrystallized from hexane/ CHCl_3 to give (10.3 g, 91%) light brown solid; mp 134–5°C; IR (KBr) 3379, 3072, 2896, 1687, 1531, 1440, 1292, 1176, 1002, 968, 866, 792 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ_{H} 10.23 (s, 1H, H-7), 7.80 (s, 1H, H-3), 6.33 (s, 1H, H-6) ppm; MS (EI) m/z (%) 278 (M^+ , 15), 63 (100).

c) 3-Bromo-4-methoxybenzaldehyde (61)

To a solution of 4-methoxybenzaldehyde (1.0 g, 7.35 mmol), and NaOAc (1.2g 8.8 mmol) in AcOH (15 ml) was added Br_2 (0.46 ml, 8.8 mmol) in AcOH (5 ml) at 0-5°C over 30 min. The reaction mixture was stirred at room temperature for a day. Reaction was quenched by addition of saturated NaHSO_3 to decompose excess Br_2 . reaction mixture was diluted with chilled DW (100 ml) and extracted in CHCl_3 (30 ml x 3). CHCl_3 layer was washed with DW, dried over anhyd. Na_2SO_4 and solvent evaporated to get orange solid, which was recrystallized from MeOH-water to give light brown needles (1.40g 89%). ^1H NMR (300 MHz, CDCl_3) δ_{H} 9.81 (s, 1H, H-7), 8.09 (d, $J = 1.9$, 1H, H-2), 7.83 (d, $J = 8.4$, 1.9, 1H, H-6), 7.02 (d, $J = 8.4$, 1H, H-5), 3.99 (s, 1H, H-9) ppm;

ii) General procedure for methylation of phenols

To a solution of phenol (1 mmol) and K_2CO_3 (0.165g, 1.2 mmol) in dried DMF (5 ml) or dried acetone (5 ml) was added methyl iodide (1.1 mmol, 0.068 ml) and the mixture was stirred for 4-6 h at rt. The reaction mixture was quenched with water (50ml) and the ppt/emulsion was either filtered or extracted in $CHCl_3$ (30 ml x 3). After extraction, solvent was evaporated to obtain methoxy derivative in pure form.

a) 3-Methoxybenzaldehyde (51)

Reactants and reagents used were 3-hydroxybenzaldehyde (6.0g, 49.18 mmol), K_2CO_3 (8.15g, 59.01 mmol), acetone (150 ml). **51** was obtained as yellowish liquid, yield (6.28, 94%).; IR (KBr) 3066, 3004, 2960, 2837, 2731, 1701, 1683, 1593, 1558, 1490, 1458, 1263, 1047, 792, 738 cm^{-1} . 1H NMR (300 MHz, $CDCl_3$) δ_H 7.16 (m, 1H), 6.90 (br s, 7.8 Hz, 1H) 6.81 (dd, $J=7.8Hz, 2.1 Hz$) 3.67, (s, 3H).

c) 2,4-Dibromo-5-methoxybenzaldehyde (34)

Reactants and reagents used were **33** (1.0 g, 3.40 mmol), K_2CO_3 (0.96 g), DMF (40 ml). **34** was obtained as crystalline needles, light brown yield (0.79, 96%), mp 110-111°C.; IR (KBr) 3010, 2993, 1679, 1575 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$) 10.25 (s, 1H), 7.85 (s, 1H), 7.40 (s, 1H), 3.95 (s, 3H).

iii) General procedure for cyanotrimethylsilylation of aldehydes

LiCl (130.3 mg, 3.07 mmol) and THF (10 ml) were placed in a round bottom flask, sonicated for 10 min and this mixture was used as a catalyst stock solution. An aldehyde (100.0 mmol) and $(CH_3)_3SiCN$ (115 mmol) were placed in a 50 ml two necked flask, and the mixture was stirred at 20° C. The catalyst solution (33 μ l, 10.1 μ mol) was added to the mixture, and the reaction proceeded exothermically, which was kept in control by keeping reaction mixture under water bath. Reaction mixture stirred for 1-2 h and traces of THF and excess TMSCN was removed under vacuum to obtain trimethylsilyl cyanohydrin.

Caution: TMSCN must be used in a well-ventilated hood due to its high toxicity.

a) 2-(3-methoxyphenyl)-2-trimethylsilyloxyacetonitrile

Reagents and reactants used were **51** (500 mg, 3.67 mmol), TMSCN (539.28 μ l, 4.04 mmol) and catalyst solution (1.19 μ l, 0.37 μ mol). This product was obtained as thick liquid (846.69 mg 98%) and used for further reaction without characterization.

*b) 2-(2-bromo-5-methoxyphenyl)-2-trimethylsilyloxyacetonitrile (**50**)*

Reagents and reactants used were **56** (500 mg, 2.32 mmol), TMSCN (341 μ l, 2.55 mmol) and catalyst solution (0.755 μ l, 0.23 μ mol); **50** was obtained as brownish thick liquid (715.63 mg, 98%); IR (KBr) 3004, 2960, 2902, 2839, 1595, 1575, 1473, 1257, 1163, 1099, 956, 867, 756 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3 , δ_{H} ppm) 7.46 (d, $J = 8.7\text{Hz}$, 1H), 7.26, (br s, 1H), 6.83 (d, $J = 8.7\text{Hz}$, 1H), 3.84 (s, 3H), 0.20 (s, 9H), ^{13}C NMR (75 MHz, CDCl_3 , δ_{C} ppm) 159.4, 136.2, 133.5, 118.1, 116.8, 113.8, 111.6, 63.1, 55.5, -0.34.

*c) 2-(2,4-dibromo-5-methoxyphenyl)-2-trimethylsilyloxyacetonitrile (**47**)*

Reagents and reactants used were **34** (1.0 g, 3.4 mmol), TMSCN(0.499 ml, 3.74 mmol) and catalyst solution (1.11 μ l 0.34 μ mol). This product was obtained as thick liquid (1.30 g, 69 mg 97%) and used for further reaction without characterization.

d) 2-(3-bromo-4-methoxyphenyl)-2-trimethylsilyloxyacetonitrile

Reagents and reactants used were **61** (500 mg, 2.32 mmol), TMSCN (341 μ l, 2.55 mmol) and catalyst solution (0.755 μ l, 0.23 μ mol). This product was obtained as thick liquid (716.03 mg, 97%) and used for further reaction without characterization.

iv) General procedure to prepare cyanohydrins by hydrolysis of trimethylsilyl cyanohydrins

To a trimethylsilyl cyanohydrin (1 mmol) was added solution of hydrochloric acid (0.5 mmol) in aq-MeOH (1:1 v/v, 5 ml) and stirred at rt for 1-2 h. Reaction mixture was concentrated under vacuum to remove MeOH. DW (5 ml) was added to the reaction mixture and extracted with CHCl_3 (3 ml x 3) The organic layer was washed with DW, dried over anhyd Na_2SO_4 and evaporated under vacuum to get cyanohydrin

a) 2-(3-methoxyphenyl)-2-hydroxyacetonitrile (52)

Reagents and reactants used were *2-(3-methoxyphenyl)-2-trimethylsilyloxyacetonitrile* (864 mg, 3.67 mmol), 0.2 N HCl in aq-MeOH (20 ml, 1:1 v/v); product was obtained as reddish-brown liquid, (557.36 mg, 93%), ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.16 (dd, J = 8.1, 7.9 Hz, 1H), 6.94, (brd, J = 7.9, 1H), 6.90 (br s, 1H), 6.81 (dd, J = 8.1, 2.1Hz, 1H). 5.33 (s, 1H), 4.38 (brs, OH), 3.67 (s, 3H),

b) 2-(2-bromo-5-methoxyphenyl)-2-hydroxyacetonitrile (57)

Reagents and reactants used were **50** (730 mg, 2.32 mmol), 0.2 N HCl in aq-MeOH (13 ml, 1:1 v/v) low melting colourless solid (528.25 mg, 94%), IR (KBr) 3400, 3014, 2939, 2906, 2839, 2250, 1595, 1475, 1288, 1163, 1053, 956, 860, 813 , ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.39 (d, J = 9 Hz, 1H, H-5), 7.16, (br s, 1H, H-8), 6.75 (dd, J = 9.0, 3.0Hz, 1H, H-6), 5.70 (d, J = 6.0 Hz, 1H, H-2), 3.74 (s, 1H); ¹³C NMR (75 MHz, CDCl₃, δ ppm), 159.4 (s, C-7), 135.2 (s, C-3), 133.8 (d, C-5), 118.0 (s, C-1), 117.1 (d, C-6 or C-8) 113 (d, C-6 or C-8), 112 (s, C-4), 62.8 (d, C-2), 55.8 (q, C-9).

c) 2-(2,4-dibromo-5-methoxyphenyl)-2-hydroxyacetonitrile (43)

Reagents and reactants used were **47** (1.34g, 3.40 mmol), HCl in aq-MeOH (18.5 ml, 1:1, v/v), product was obtained as low solid (1.04 g, 95%) IR (KBr) 3400, 3016, 2942, 2829, 2250, 1597, 1475, 1167, 1053, 956, 864, 813; ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.86 (s, 1H, H-5), 7.24, (s, 1H, H-8), 5.70 (br s, 1H, H-2), 3.93 (s, 1H); ¹³C NMR (75 MHz, CDCl₃, δ ppm), 156.1 (s, C-7), 136.7 (s, C-5), 134.4 (d, C-3), 1187.6 (s, C-1), 114.0 (s, C-4 or C-6) 112.2 (s, C-4 or C-6), 111.2 (d, C-8), 62.6 (d, C-2), 56.6 (q, C-9).

d) 2-(3-bromo-4-methoxyphenyl) -2-hydroxyacetonitrile (62)

Reagents and reactants used were *2-(3-bromo-4-methoxyphenyl)-2-trimethylsilyloxyacetonitrile* (730 mg, 2.32 mmol), 0.2N HCl in aq-MeOH (13 ml). Product was obtained as low melting colourless solid (534.48 mg, 95%); ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.51 (d, J = 2.1 Hz, 1H, C-4), 7.24, (dd, J = 8.7, 2.1 Hz 1H, C-8), 6.64 (d, J = 9.0Hz, 1H, C-9), 5.31 (s, 1H, H-2). 4.35 (br s, OH), 3.75 (s, 3H, H-9); ¹³C NMR (75 MHz, CDCl₃, δ ppm), 156.7 (s, C-9), 131.6 (d, C-8 or C-4), 128.6

(s, C-3), 127.2 (d, C-8 or C-4), 118.9 (s, C-1) 112.1 (d, C-7) 112.0 (s, C-5), 62.2 (d, C-2), 56.8 (q, C-9).

v) General procedure for reduction of cyanohydrins to N-Boc-2-(3-aryl)-1-aminoethan-2-ol

To a stirred solution of a cyanohydrin (2 mmol) in dry methanol (15 ml), cooled to 0°C, were added Boc₂O (873 mg, 4.0 mmol) and NiCl₂·6H₂O (48 mg, 0.2 mmol). NaBH₄ (530 mg, 14.0 mmol) was then added in small portions over 30 min. The reaction was exothermic and effervescent. The resulting reaction mixture containing a finely divided black precipitate was allowed to warm to the room temperature and left to stir for a further 2-3 h, before solvent evaporation under vacuum. The residue was dissolved in EtOAc (50 ml) and washed with saturated aq.NaHCO₃ (2 x 50 ml). The organic layer was dried over anhyd Na₂SO₄ and the solvent was removed in vacuum to obtain to yield....

a) N-Boc-2-(3-methoxyphenyl)-1-aminoethan-2-ol (53)

Reagents and reactants used were **52** (1.72 g, 10.55 mmol), NiCl₂·6H₂O (259 mg, 1.09 mmol), NaBH₄ (2.79 g, 73.75 mmol) and Boc₂O (4.843 ml, 21.1 mmol) and MeOH (80 ml); **53** was obtained as viscous liquid (2.00 g, 71%); ESI MS [M+H]⁺ at m/z 168.1494, [M+Na]⁺ at m/z 190.1824. ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.08 (t, J = 8.1 Hz, 1H, H-7) 6.76 (m, 2H), 6.65 (br d, J = 7.2 Hz), 5.19 (br s, 1H, H-2), 4.70, (br d, OH), 3.62, (s, H-9), 3.26 (m, 1H, βH-1), 3.037 (m, 1H αH-1), 1.28 (s, 9H, H-4', H-5', H-6'); ¹³C NMR (75 MHz, CDCl₃, δ ppm) 159.6 (s, C-2'), 156.5 (s, C-5), 143.7 (s, C-2), 129.3 (d), 118.2 (d), 112.9 (d), 111.2 (d), 79.6 (s, C-4'), 73.3 (d, C-2), 55.0 (q, C-9), 48.1 (t, C-1), 28.1 (q, C-4', C-5', C-6')

b) N-Boc-2-(2-bromo-5-methoxyphenyl)-1-aminoethan-2-ol (58)

Reagents and reactants used were **57** (500 mg, 2.07 mmol), NiCl₂·6H₂O (48.97 mg, 0.21 mmol), NaBH₄ (547.1 mg, 14.45 mmol) and Boc₂O (0.95 ml, 4.13 mmol) and MeOH (15.5 ml). **58** was obtained as viscous liquid viscous liquid (493.69 mg, 69%); ESIMS [M+H]⁺ at m/z 346/348, [M+Na]⁺ at m/z 368/370 [M+K]⁺ at m/z 384/386,

[2M+H]⁺ 713/715/717; MS/MS at m/z 346 328, 272,254, 228,193,175,149,134,106; ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.27 (d, J = 8.7, Hz, 1H, H-5), 7.08 (d, J = 3 Hz 1H, H-8), 6.60 (dd, J = 8.7, 3.0Hz, 1H, H-6), 5.25 (br s, OH), 4.95 (br d, 1H, H-2), 3.69 (s, 3H, H-9), 3.40 (m, 1H, βH-1), 3.22 (m, 1H, αH-1), 1.34 (s, 9H, H-5', H-6',H-7'); ¹³C NMR (75 MHz, CDCl₃, δ ppm) 158.7 (s, C-7), 157.3 (s, C-2'), 141.6 (s, C-3), 132.8 (d, C-5), 114.9 (d, C-6 or C-8), 112.9 (d, C-6 or C-8), 111.7 (s, C-4) 79.71 (s, C-4') 72.9 (d, C-2) 55.1 (q, C-9) 46.2 (t, C-1) 28.1 (q, C-5', C-6' C-7')

c) *N-Boc-2-(2,4-Dibromo-5-methoxyphenyl)-1-aminoethan-2-ol (44)*

Reagents and reactants used were **43** (800 mg, 2.49 mmol), NiCl₂.6H₂O (59 mg, 0.249 mmol), NaBH₄ (660 mg, 17.84 mmol) and Boc₂O (1.14 ml, 4.98 mmol) and MeOH (18.7 ml). **44** was obtained as viscous liquid (804.98 mg, 76%). ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.61 (s, 1H, H-6), 7.15 (s, 1H, H-9), 5.04 (m, 1H, H-3), 3.86 (s, 3H, H-10), 3.45 (ddd, J = 15.0, 6.1, 1.8 Hz, 1H, βH-2), 3.31 (m, 1H, αH-2), 1.40 (s, 9H, H-5', H-6',H-7') ¹³C NMR (75 MHz, CDCl₃, δ ppm) 155.8 (s, C-8), 155.5 (s, C-1'), 141.1 (s, C-4), 133.0 (d, C-6), 111.8 (s, C-5 or C-7), 111.5 (s, C-5 or C-7), 111.5 (d, C-9), 80.3 (s, C-3'), 73.4 (d, C-3), 56.3 (q, C-10), 39.9 (t, C-2), 28.2 (q, C-5', C-6' C-7').

d) *N-Boc-2-(3-bromo-4-methoxyphenyl)-1-aminoethan-2-ol (63)*

Reagents and reactants used were **62** (500 mg, 2.07 mmol), NiCl₂.6H₂O (48.97 mg, 0.21 mmol), NaBH₄ (547.1 mg, 14.45 mmol) and Boc₂O (0.95 ml, 4.13 mmol) and MeOH (15.5 ml) **63** was obtained as viscous liquid (515.15 mg, 72%). ESIMS [M+H]⁺ at m/z 346.2061/348.2863, [M+Na]⁺ at m/z 368.843/370.2843 [M+K]⁺ at m/z 384.2766/386.7776, [2M+H]⁺ 713.6253/715.6278/717.6295; ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.51 (br s, 1H, H-5), 7.21 (d, 1H, J = 8.4 Hz, H-9), 6.83 (d, J = 8.4 Hz, 1H, H-8), 5.05 (t, 1H, H-2), 4.55 (d, 1H, OH), 3.85 (s, 3H, H-10), 3.36 (m, 1H, βH-2), 3.17 (m, 1H, αH-2), 1.41 (s, 9H, H-5', H-6',H-7').

vi) Procedure for deprotection of N-Boc-2-(3-aryl)-1-aminoethan-2-ol (54, 45, and 64)

To an *N*-Boc amine (10 mmol) was added 6N HCl (100 ml) and refluxed for 3 h. Reaction mixture was cooled and washed with EtOAc (3 x 30 ml). The aqueous portion was basified with liq NH₃, extracted with EtOAc (3 x 30 ml) and dried over anhyd Na₂SO₄. Upon evaporation of solvent an amine was obtained.

a) 2-(3-methoxyphenyl)-1-aminoethan-2-ol (54)

Reagents and reactants used were **53** (1.0 g, 3.74 mmol), 6 N HCl (37.5 ml); **54** was obtained as solid (387.35, 62%); IR (KBr) 3404, 2929, 2839, 1558, 1413, 1261, 1159, 1045, 786, 700 cm⁻¹; ESIMS [M+H]⁺ at m/z 168.1494, [M+Na]⁺ at m/z 190.1824; ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.11 (t, J = 8.1 Hz, 1H, H-7), 6.82 (m, 2H), 6.69 (d, J = 7.5 Hz), 4.82 (br s, H-2), 3.65 (s, 3H, H-9), 3.00 (m, 1H, βH-1), 2.91 (m, 1H αH-1); ¹³C NMR (75 MHz, CDCl₃, δ ppm), 159.6 (s, C-5), 142.8 (s, C-3), 129.5 (d), 118.0 (d), 13.1 (9d), 111.5 (d), 70.3 (d, C-2), 55.0 (q, C-9), 46.6 (t, C-6).

b) 2-(2-bromo-5-methoxyphenyl)-1-aminoethan-2-ol (59)

To the **58** (500 mg, 1.45 mmol) in RB flask was added 1:1 TFA/CH₂Cl₂ (10 ml) and stirred for 2 h and the solvent and the TFA was removed under vacuum. The resultant residue was dissolved and CHCl₃ (50 ml) and was washed with water (20 ml x 3). The organic layer was dried over anhyd. Na₂SO₄ and solvent evaporated to solid compound (291.50 mg, 82%), ¹H NMR (300 MHz, CDCl₃, δ ppm) 7.38 (d, J = 8.7 Hz, 1H, H-5), 7.14 (d, J = 2.7 Hz, 1H, H-8), 6.69 (dd, J = 8.4, 2.7 Hz, 1H, H-6), 4.92 (dd, J = 7.5, 3.9 Hz, 1H, H-2), 3.80 (s, 3H, H-9), 3.10 (d, J = 12.6 Hz, 1H, βH-1), 2.69 (dd, J = 12.6, 7.5 Hz, αH-1).

c) 2-(2,4-Dibromo-5-methoxyphenyl)-1-aminoethan-2-ol (45)

Reagents and reactants used were **44** (1.1g, 2.59 mmol), 6 N HCl (26.0 ml). **45** was obtained as solid (529.94, 63%); NMR data recorded on HCl salt. ¹H NMR (300 MHz, D₂O, δ ppm) 7.75 (s, 1H, H-5), 7.18 (s, 1H, H-8), 5.18 (dd, 1H, H-2), 3.84 (s, 3H, H-9), 3.29 (dd, 1H, βH-1), 3.06 (dd, 1H, αH-1). ¹³C NMR (75 MHz, D₂O, δ ppm)

158.9 (s, C-7), 139.4 (s, C-3), 136.2 (d, C-5), 111.8 (s, C-6 or C-4), 111.4 (s, C-6 or C-4), 111.1 (d, C-8), 68.7 (d, C-2), 56.5 (q, C-9), 43.7 (t, C-1).

d) 2-(3-bromo-4-methoxyphenyl)-1-aminoethan-2-ol(64)

Reagents and reactants used were **63** (700 mg, 2.02 mmol), 6 N HCl (21.0 ml) **64** was obtained as solid (298.61 mg, 60%). IR (KBr), 3354, 2929, 2841, 1598, 1496, 1460, 1259, 1053, 1016, 900, 815 cm^{-1} . ^{13}C NMR (75 MHz, CDCl_3 , δ ppm) 155.2 (s, C-7), 135.8 (s, C-3), 130.9 (d, C-4 or C-8), 126.4 (d, C-4 or C-8), 111.7 (d, C-7), 96.4 (s, C-5), 77.8 (d, C-2), 54.5 (q, C-9), 49.1 (t, C-1)

vii) General procedure for preparation of amathamide alcohol by coupling 2-Aryl-1-aminoethan-2-ol with N-methyl-L-proline (39)

A solution of oxyma (21.9 mg, 0.154 mmol), diisopropyl carbodiimide (DIC) (39.0 mg, 47.94 μl , 0.31 mmol), **39** (19.87 mg, 0.154 mmol) in dry DMF (5 ml) was stirred at rt. for 2 min (preactivation). To this was added solution of 2-aryl-1-aminoethan-2-ol (0.154 mmol) in DMF (1 ml), stirred at rt. for 30-60 min and monitored with TLC. On completion of reaction, the reaction mixture was added to cold 1N HCl (30 ml) and extracted with EtOAc (10 ml x 3). The aqueous portion was basified with liq. NH_3 and extracted with EtOAc (15 ml x 3). This EtOAc portion was washed with water and dried over anhyd. Na_2SO_4 . Upon evaporation of the solvent a sticky solid was obtained which was purified on flash Si gel column by eluting with gradients of MeOH- CHCl_3 to give crystalline solid.

a) 2(S)-N-[2-(5-methoxyphenyl)-2-hydroxyethenyl]-1-methyl-2-pyrrolinecarboxamide (55)

Reagents and reactants used were oxyma (30.25 mg, 0.209 mmol), diisopropyl carbodiimide (DIC) (52.9 mg, 64.90 μl , 0.419 mmol), **39** (27.07 mg, 0.209 mmol), **54** (35.0 mg, 0.209 mmol) and DMF (6.8 ml); **55** was obtained as colourless crystalline needles (49.52 mg, 85%);

b) 2(S)-N-[2-(2-Bromo-5-methoxyphenyl)-2-hydroxyethenyl]-1-methyl-2-pyrrolinecarboxamide (60)

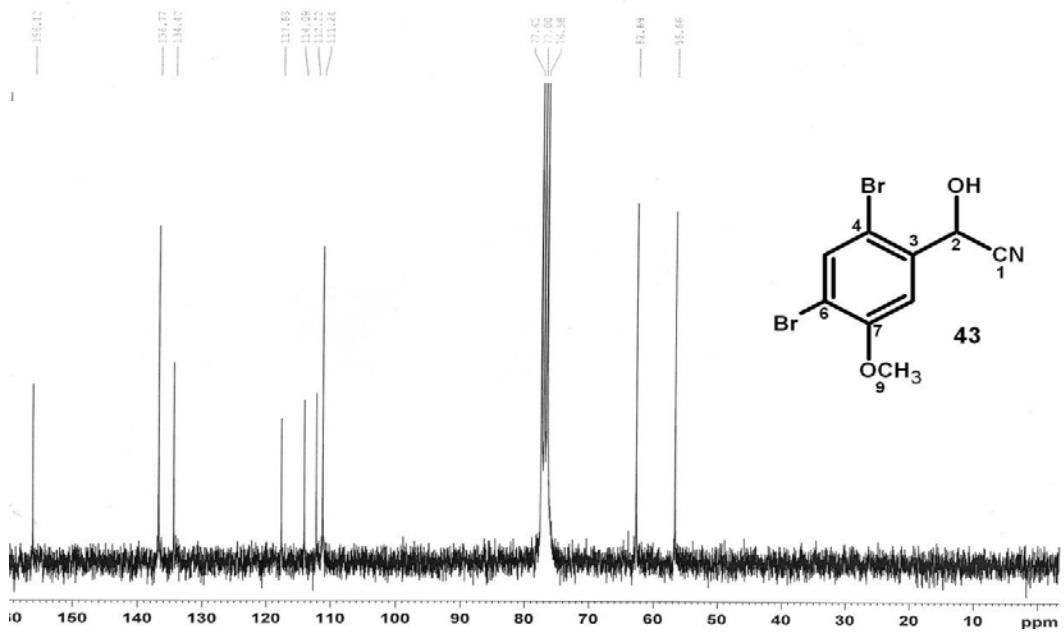


Fig 4.1b: ¹³C NMR of 43

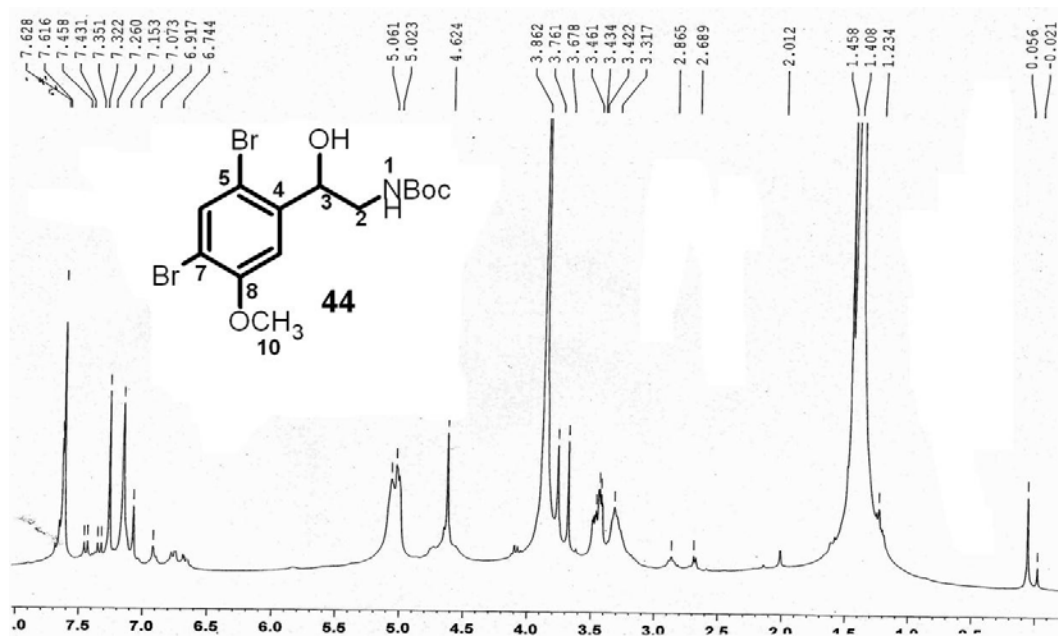


Fig4.2a: ¹H NMR of 44

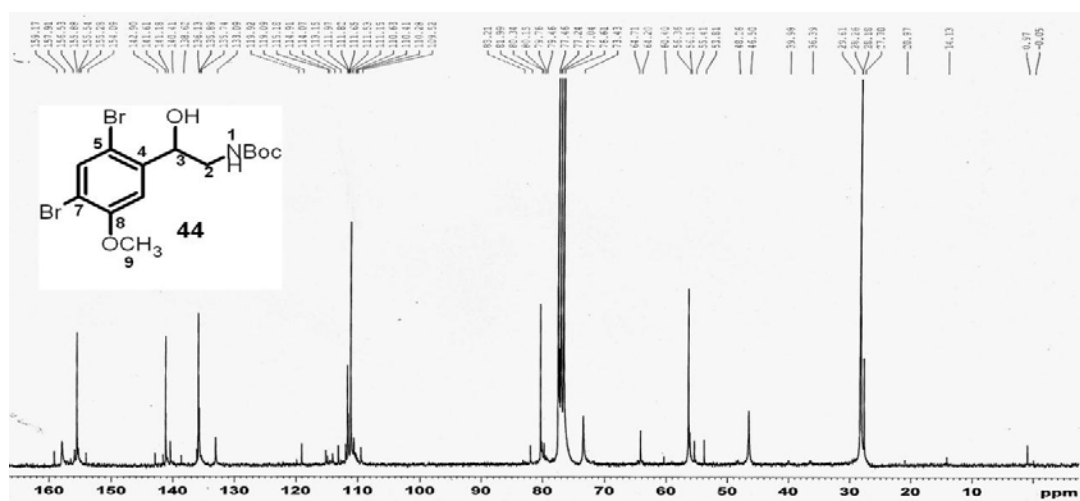


Fig 4.2b ¹³C NMR of 44

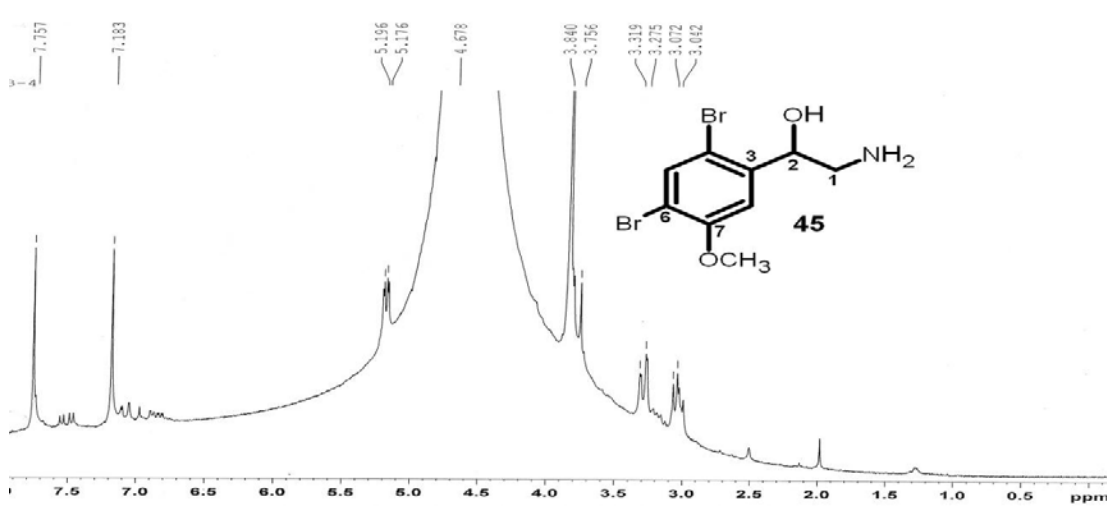


Fig 4.3a ¹H NMR of 45

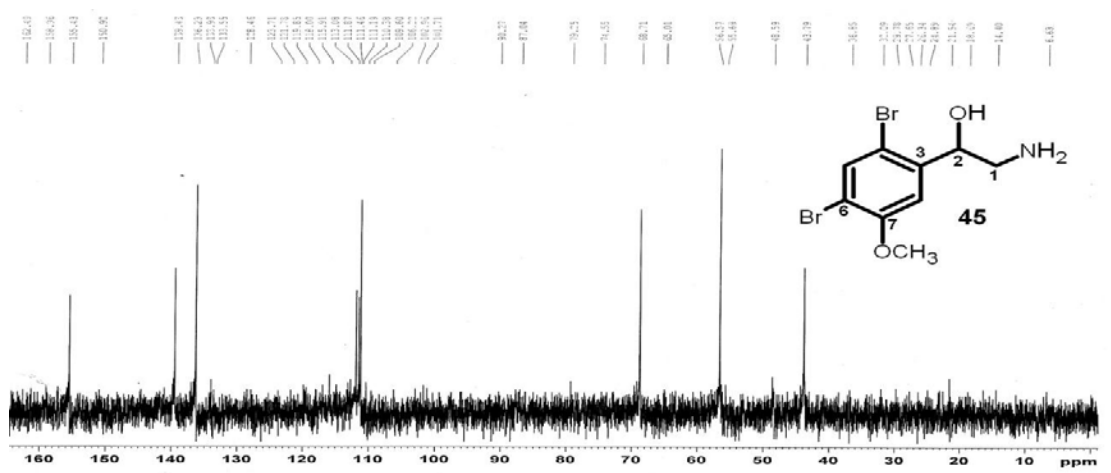


Fig 4.3 b ¹³C NMR of 45

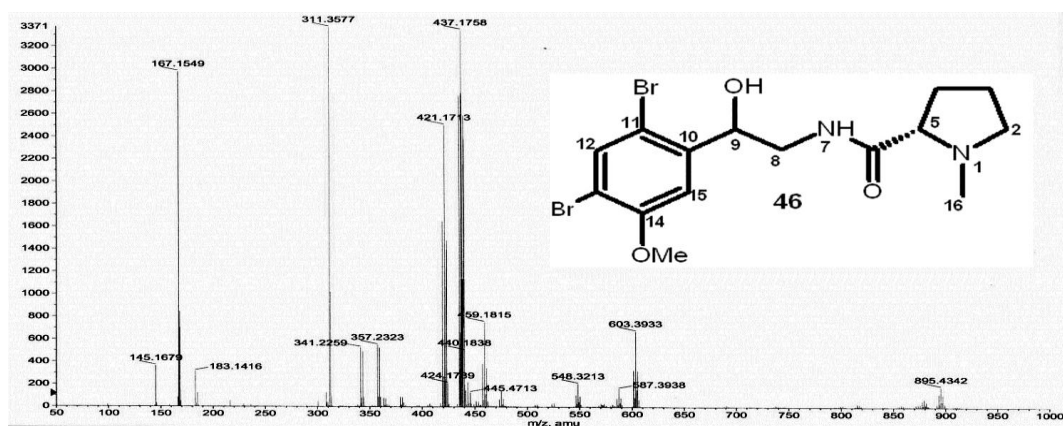


Fig 4.4d: ESITOFMS of 46

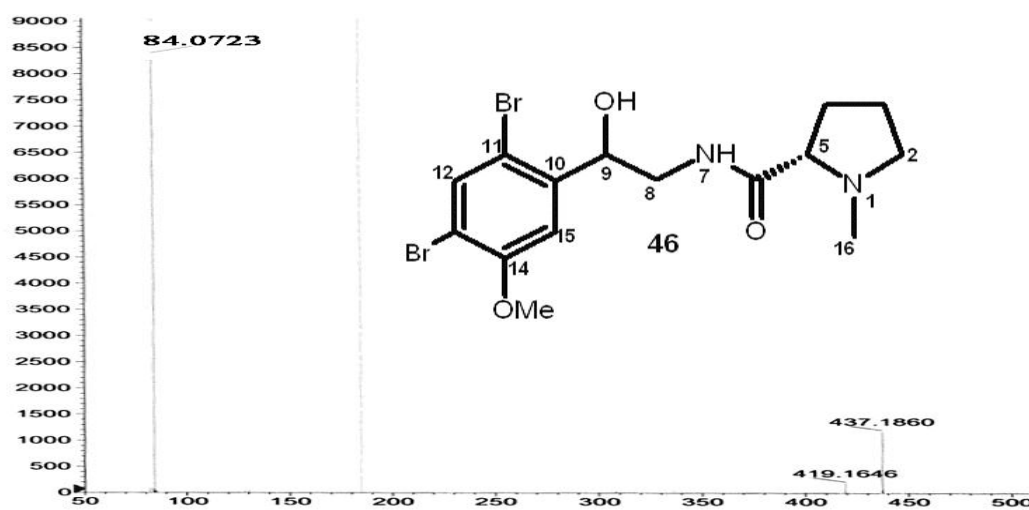


Fig 4.4e: MS/MS at m/z 437 of 46

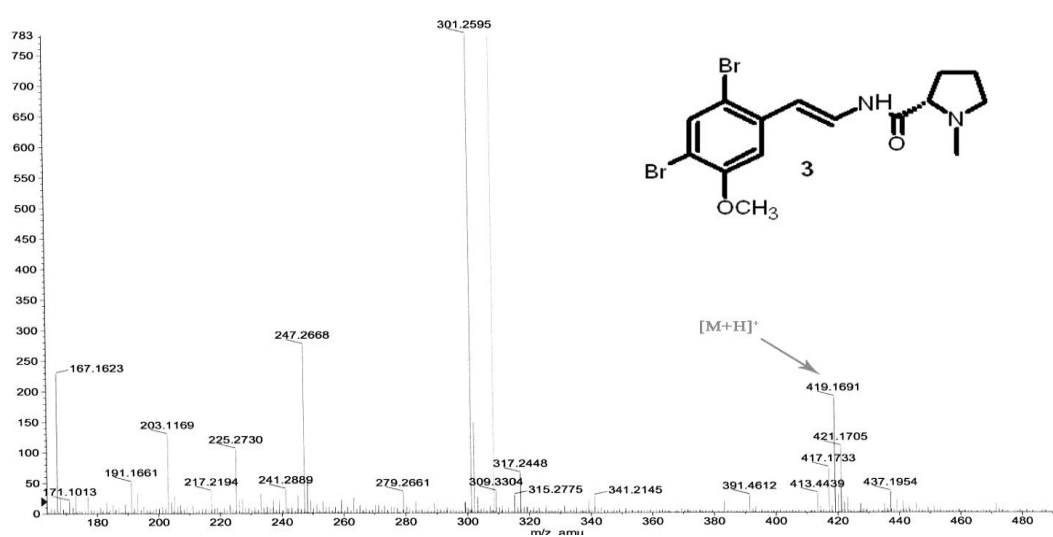


Fig 4.5a: ESITOFMS of amamthamide A (3)

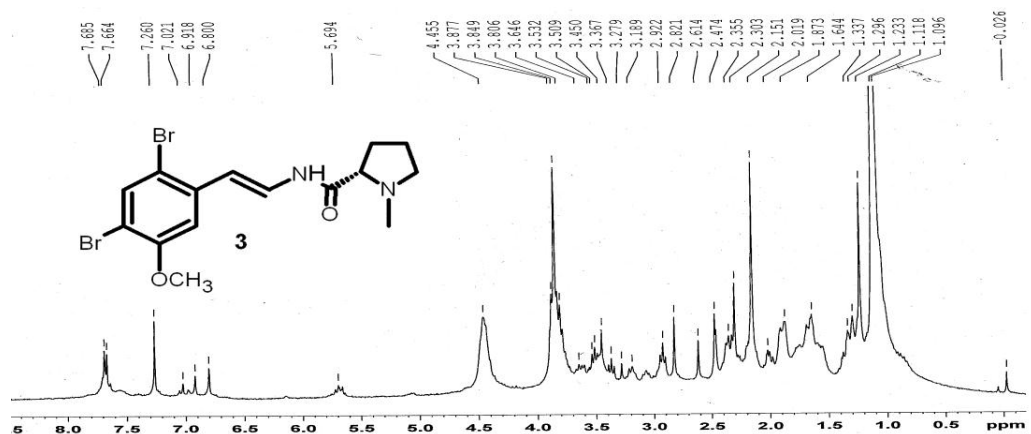


Fig 4.5b ¹H NMR of mixture containing amathamide A (3)

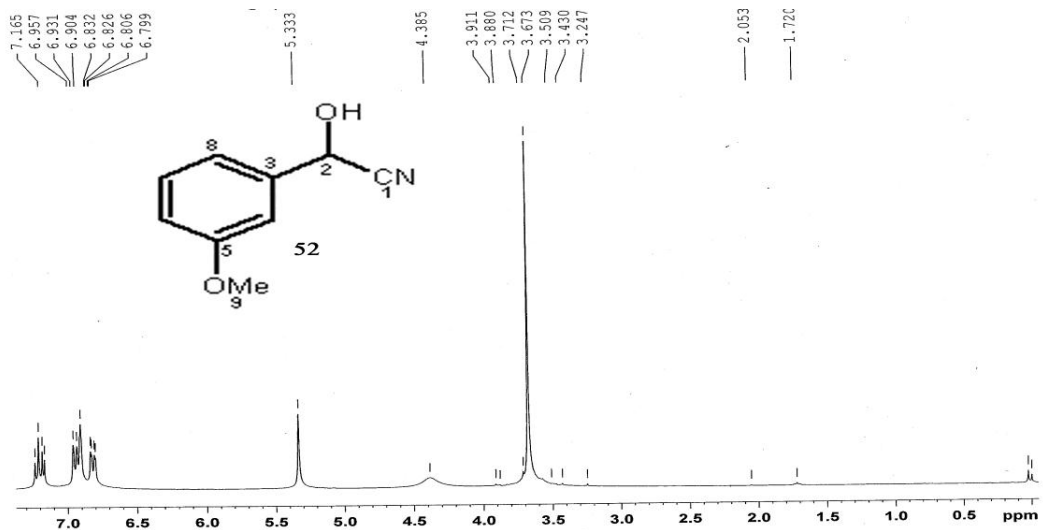


Fig 4.6: ¹H NMR of 52

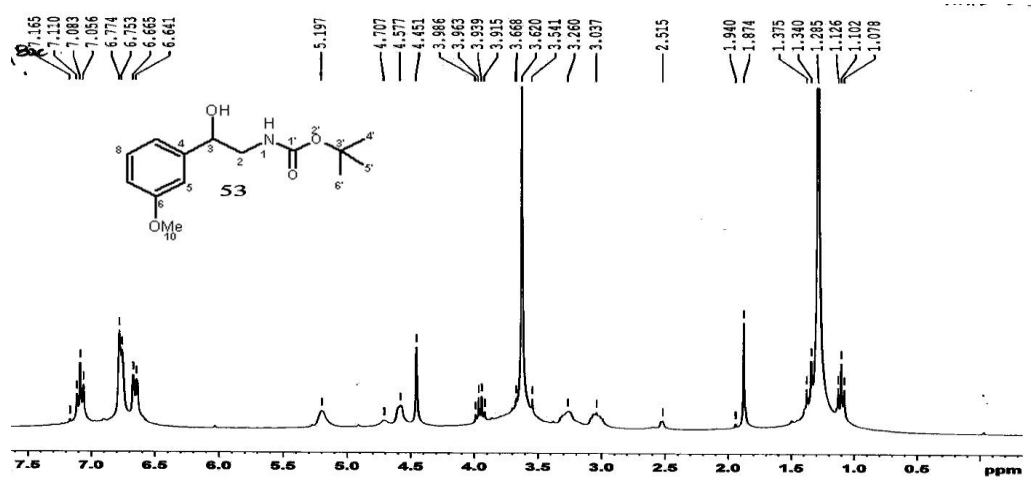
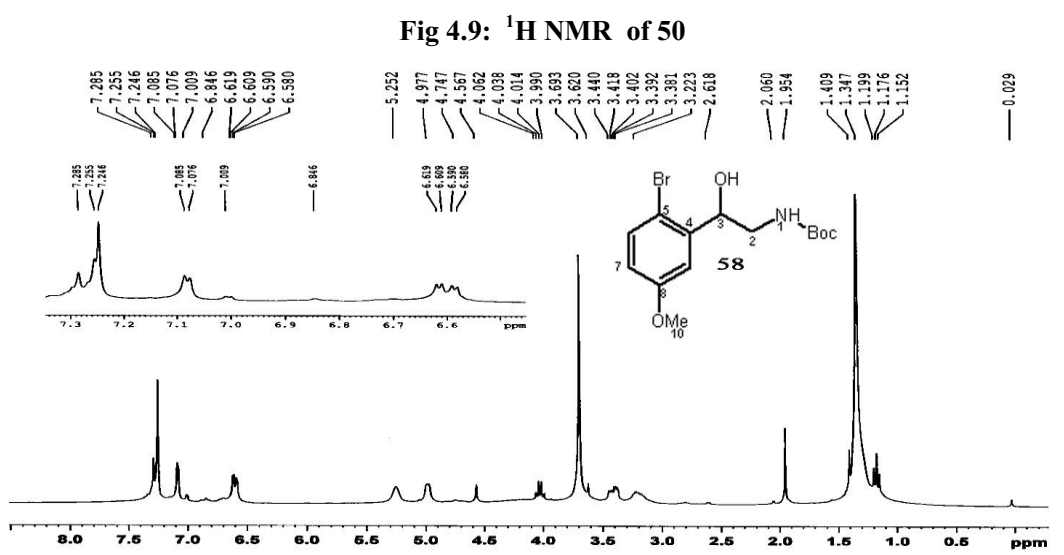
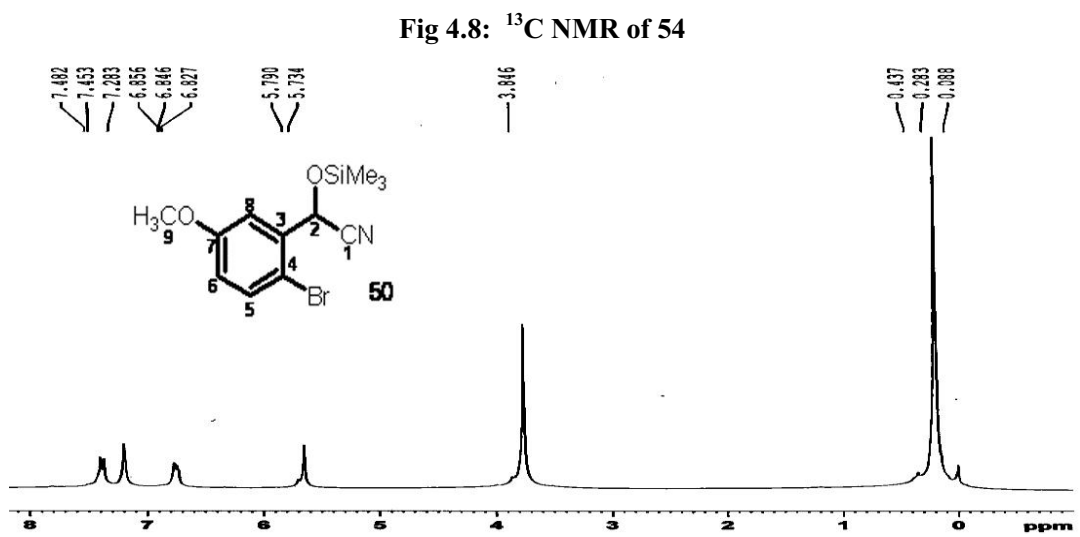
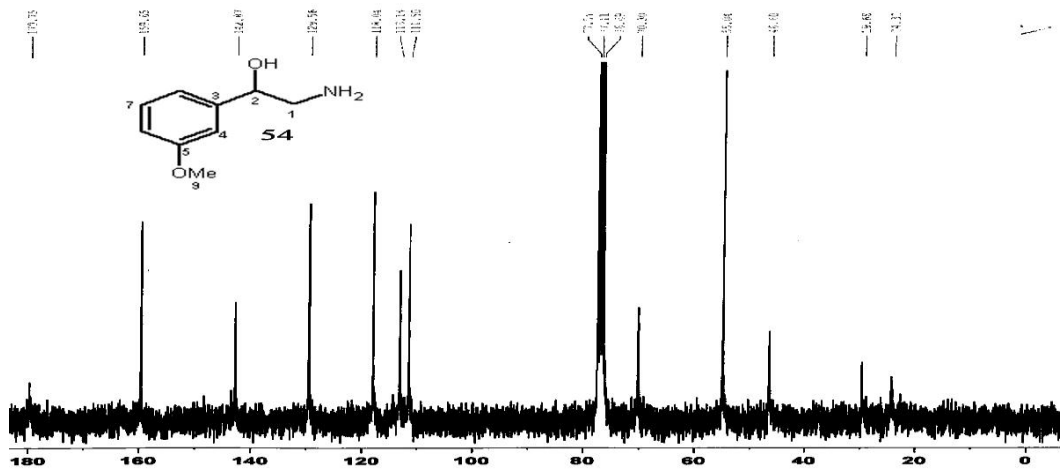


Fig 4.7: ¹H NMR of 53



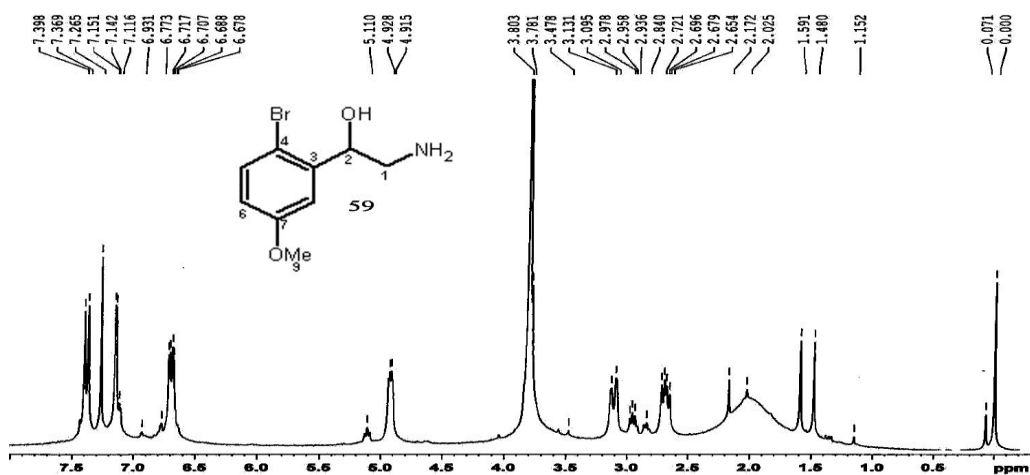


Fig 4.11: ¹H NMR of 59

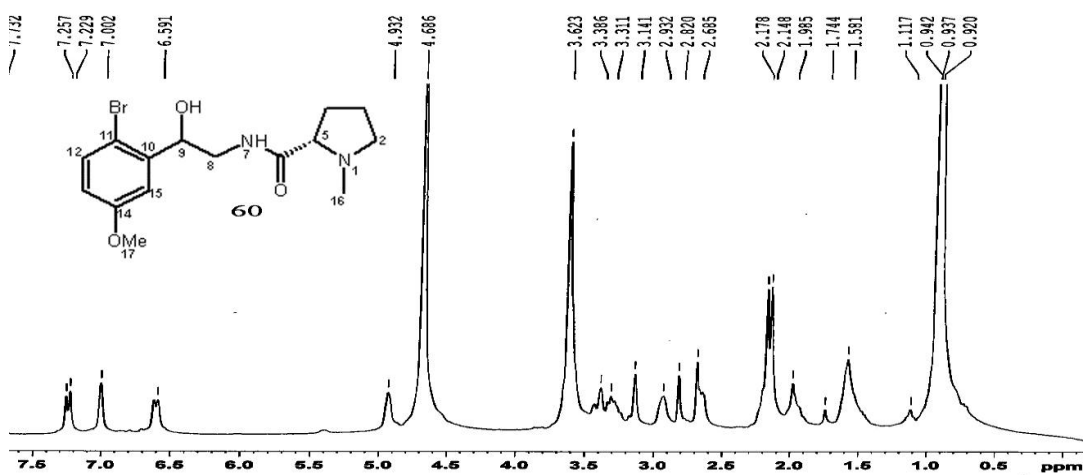


Fig 4.12: ¹H NMR of 60

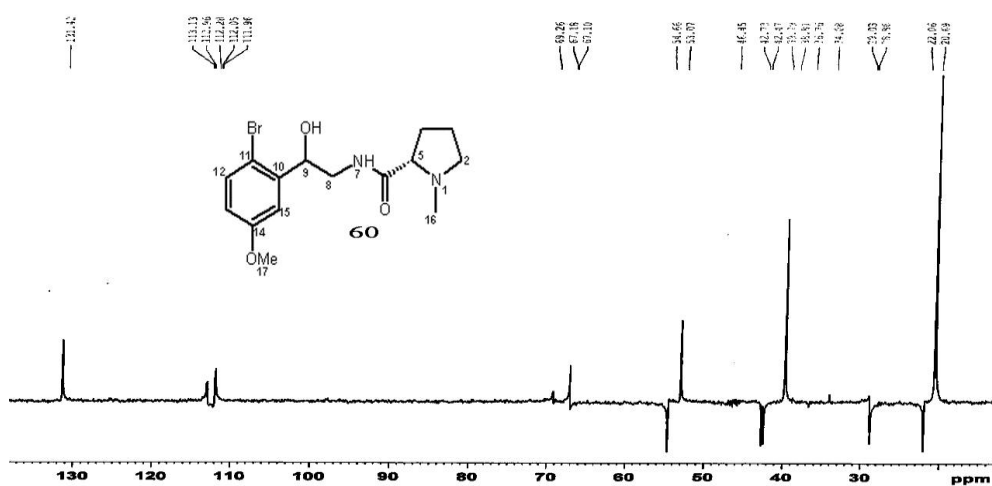


Fig 4.13: DEPT NMR of 60

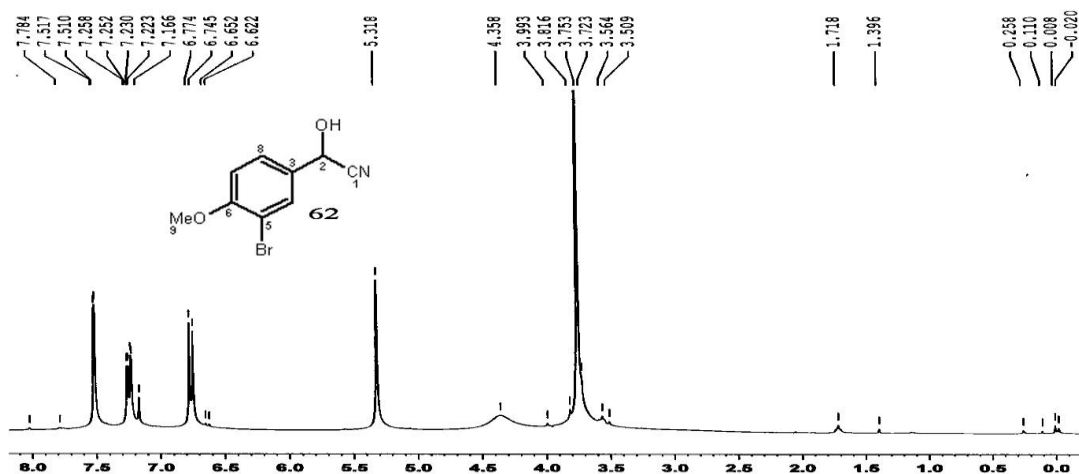


Fig 4.14: ¹H NMR of 62

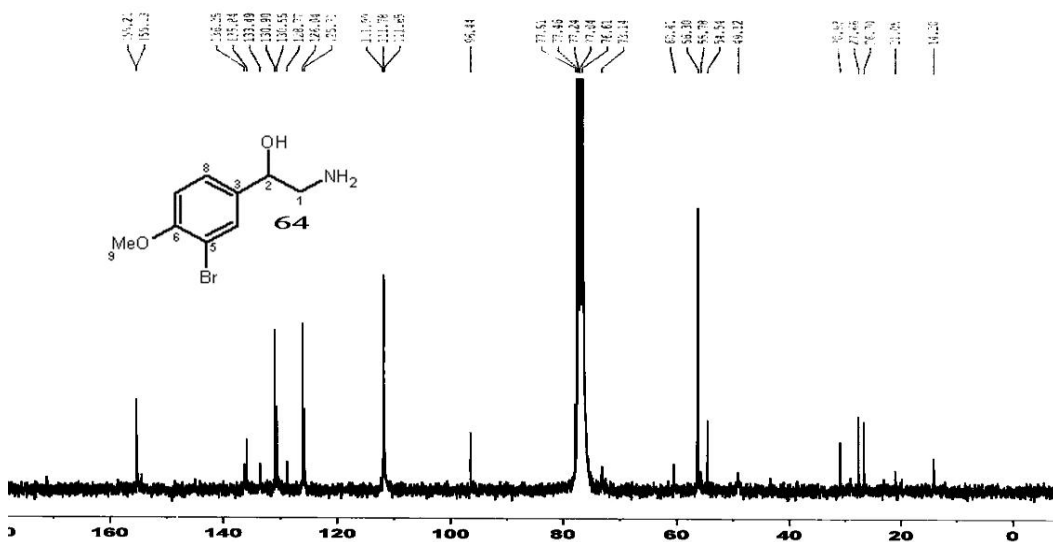


Fig 4.15: ¹³C NMR of 64

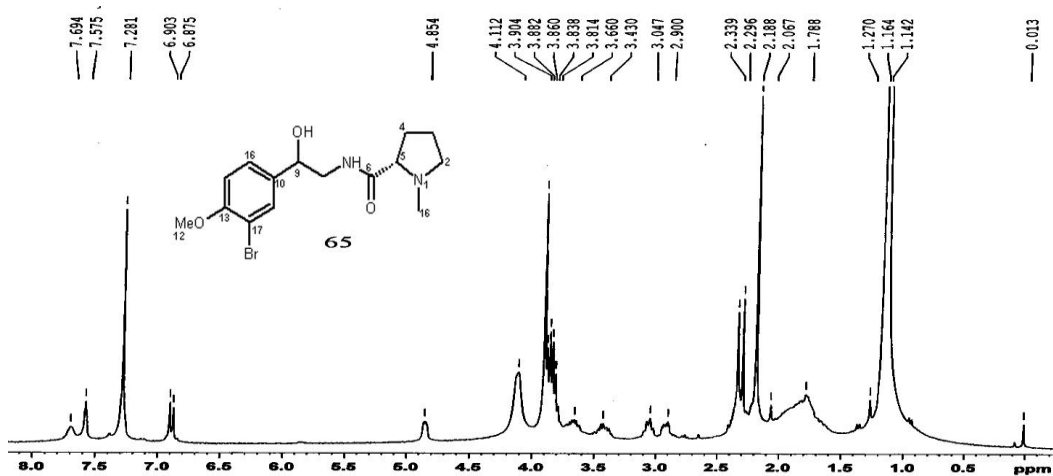


Fig 4.16: ¹H NMR of 65

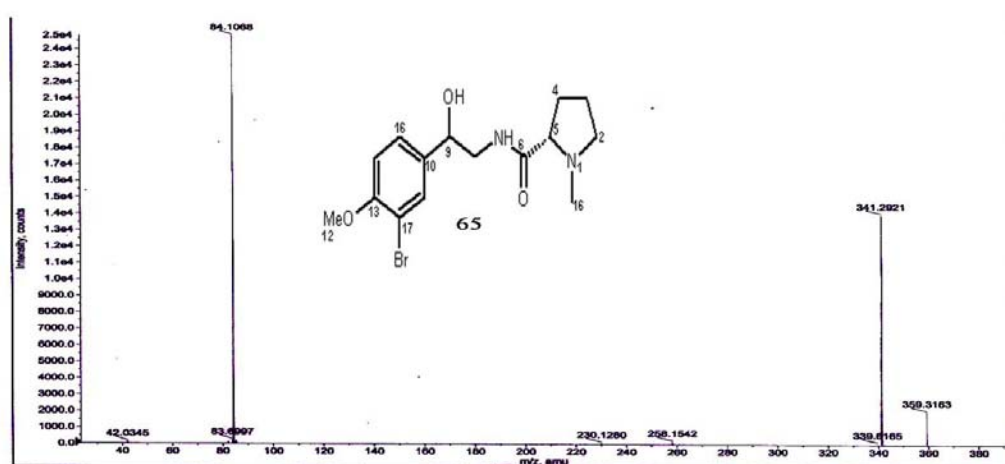


Fig 4.17: MS/MS of 65 at m/z 359

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SUMMARY

SUMMARY

The thesis entitled “Studies on some Important Secondary Metabolites from Marine Organisms” is divided into four chapters, and describes the efforts directed towards the discovery of bioactive metabolites from marine-derived *Aspergillus* species of fungi, marine invertebrates such as sponge *Oceanapia* and soft coral *Lobophytum crassum*, and diversity oriented synthetic route towards the synthesis of bryozoan metabolites, amathamides.

Chapter 1: A review of literature published on the secondary metabolites of marine-derived fungi during the first half of 2010 has been presented with current status on marine mycochemistry.

Chapter 2 section 2.1: Seven epiphytic fungi were isolated from pneumatophores of mangrove *Acanthus illicifolius*. High throughput screening revealed *Aspergillus cervinus* and *Aspergillus ornatus* to have anticancer activity and *Aspergillus flavipes* to have anti-inflammatory activity. The chemical investigation of the fungus *A. flavipes* led to the isolation one new compound, (+) 4-(1'-propenyl) – *trans*-cyclopentane-1,2-diol (**FM-5**) and four known compounds, butyrolactone I (**FM-1**), butyrolactone II (**FM-2**), (+) terrein (**FM-3**), and *meso*-4 β -(1'-propenyl)cyclopentane-1 β ,2 β -diol (**FM-4**). **FM-1**, **FM-2** and **FM-4** have been isolated for the first time from marine derived source and also for the first time from *A. flavipes*. The structures of the metabolites were mainly elucidated using spectroscopic techniques those included 1D, 2D NMR and MS. **FM-1** and **FM-3** exhibited strong anti-inflammatory activity. A review of the metabolites of *A. flavipes*, which includes 13 structures, has been also presented in the beginning of this section.

Section 2.2: Two new aromatic butenolides, aspernolides A (**FM-6**) and B (**FM-7**) along with the known metabolites, butyrolactone I (**FM-1**), terrein (**FM-3**) and physcion (**FM-8**) were isolated from the fermentation broth of a soft coral derived fungus *Aspergillus terreus*. The structures of these metabolites were assigned on the basis of detailed spectroscopic analysis. The absolute stereochemistry of **FM-6** and **FM-7** was established by their preparation from the known butenolide **FM-1**. When tested, **FM-6** exhibited mild cytotoxicity against cancer cell lines. These

type of butenolides are previously known only from terrestrial *A. terreus* isolates. Some of the important metabolites from *A. terreus* with reference to their structure and bioactivity have also been reviewed in this section.

Section 2.3: A marine sponge derived fungus *Aspergillus aculeatus* produced diastereomers of secalonic acids, one of these was characterized to be secalonic acid D (**FM-17**) from its NMR and XRD data. **FM-17** showed antifungal activity against *Candida albicans*, superior to fluconazole, a drug currently in use. The section is initiated with a review of literature on *A. aculeatus* and its metabolites, and brief introduction to secalonic acids and their biosynthesis.

Section 2.4: The section depicts the closeness of two marine-derived fungi *A. flavipes* and *A. terreus* by comparison of their metabolites and phylogenetic analysis. Using electrospray ionization-tandem mass spectrometry (ESI-MS/MS) the metabolites were characterized from the crude extracts (without purification) of fungi *A. flavipes* and *A. terreus*. Both the fungi displayed similar metabolic profile. Butyrolactone I (**FM-1**), butyrolactone III (**FM-9**) and terrein (**FM-3**) were the common metabolites to both the fungi whereas, derivatives of butyrolactone I, such as butyrolactone sulfates, butyrolactone carboxylic acid have been detected only in the extract of *A. flavipes*. The phylogenetic analyses revealed that *A. terreus* and *A. flavipes* have 99% and 98% similarity to the several *A. terreus* isolates. The section also presents the discussion on biosynthesis of butyrolactones, aspulvinones and asterriquinones.

Section 2.5: Culture of *A. terreus* grown in medium containing different seawater concentration was analysed for butyrolactone I production which revealed that butyrolactone I production in *A. terreus* is optimum at 50% conc of sea water, whereas negligible amount or no butyrolactone I is produced at 100% sea water concentration. This study proves that butyrolactone I production in *A. terreus* is function of sea water concentration of culture medium.

Chapter 3 Section 3.1: From the antibacterial extract of sponge *Oceanapia* sp. three known compound mimosamycin and xestospongins C and D were purified, while several known xestospongins were detected to be present from the ESI-MS spectrum. The three isolated metabolites are known sponge metabolites but not from *Oceanapia* sp. Sponge *Oceanapia* is relatively less known for its metabolites

and majority of the metabolites known from it are alkaloids, which are presented in a short review.

Section 3.2: A diterpene, cembrenoid 1, campesterol, cholesterol, and three polyhydroxy sterols have been isolated from a soft coral *Lobophytum crassum* and their structures were mainly elucidated by comparison of their spectroscopic data (IR and NMR) with the literature records.

Chapter 4: Amathamide alkaloids are the enamides of stryl amines and *N*-methyl prolines. They are the metabolites of bryzoan *Amathia wilsoni* and are not well studied for biological activity. We have synthesized amathamide alkaloid A and four amathamide alcohols using a new diversity oriented approach, which involves preparation of β -hydroxy- β -arylethamine by reduction of cyanohydrin and then coupling of β -hydroxy- β -arylethamine with *N*-methyl proline using oxyma as coupling agent to yield amthamide alkaloids. One such amathamide alkaloid was dehydrated using DCC-CuCl₂ to give amathamide A. This synthesis is second synthetic route for amathamides and superior to the previous known method.