

Studies on Some Important Secondary Metabolites from Marine Organisms

A thesis submitted to
Goa University

For the degree of
Doctor of Philosophy
(In Chemistry)

By
Mr. Rajesh Ramnath Parvatkar
M. Sc.



**Bio-Organic Chemistry Laboratory
National Institute of Oceanography
Dona Paula Goa 403 004, India**



**Department of Chemistry
Goa University
Goa 403 206, India**

June 2011



Dr. Chandrakant G. Naik
Emeritus scientist
Bio-Organic Chemistry Group
National Institute of Oceanography
Dona Paula Goa 403 004 India



Dr. Shrivallabh P. Kamat
Professor
Department of Chemistry
Goa University, Taleigao Plateau
Goa 403 206 India

CERTIFICATE

Certified that the work incorporated in the thesis entitled "**Studies on Some Important Secondary Metabolites from Marine Organisms**" submitted by **Mr. Rajesh Ramnath Parvatkar**, was carried out by the candidate under our supervision and the same has not been submitted elsewhere for the award of a degree.

Dr. Chandrakant G. Naik
(Guide)

Dr. Shrivallabh P. Kamat
(Co-Guide)

June 2011

Declaration

As required under the University ordinance 0.19.8 (vi), I hereby declared that the work embodied in the thesis entitled "**Studies on Some Important Secondary Metabolites from Marine Organisms**" is the result of investigation carried out by me under the guidance of Dr. Chandrakant G. Naik (NIO) and Prof. Shrivallabh P. Kamat (Goa University) and it has not previously formed basis for any other titles. In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Rajesh R. Parvatkar

(Research Student)

June 2011

ACKNOWLEDGEMENTS

With pleasure and deep sense of respect, I express my heartfelt and sincere gratitude to all the people who have directly or indirectly helped me in various capacities in the successful completion of the thesis.

*I take the opportunity to express my gratefulness to **Dr. Chandrakant G. Naik**, my guide and mentor for my PhD work. Words are not sufficient to express my respect for his valuable guidance and constant encouragement throughout the course of my research work. It is with great pride, I submit this work as his student.*

*I would also wish to thank my co-guide, **Dr. Shrivallabh Kamat**, Professor, Goa University for his guidance, valuable comments and suggestions.*

*Words would not suffice to express my deep sense of indebtedness to retired professor of Goa University, **Prof. S. K. Paknikar** who has been the great source of inspiration since my postgraduate studies and throughout the PhD work, and **Dr. Solimabi Wahidullah**, NIO for constant support and care, filled with endurance and enthusiasm throughout my study.*

*I acknowledge Professor my subject expert, **Dr. V. P. Kamat** (Registrar, Goa University), for giving valuable advice and suggestions throughout my work.*

*I offer my sincere thanks to the **Dr. E. Desa**, former Director and **Dr. S. Shetye**, Director of National Institute of Oceanography for giving me an opportunity to work in their institute.*

I also acknowledge the present and former Vice-chancellors of Goa University for allowing me to work for Ph D degree in their institute.

*I am pleased to express my thanks to **Dr. Lisette DeSouza**, Head of Bio-Organic Chemistry Group for providing facilities and assistance required to carry out the research work.*

*I am also very much thankful to Prof. **A. V. Salker** (Head, Department of Chemistry, Goa University), Prof. **J. B. Fernandes** (Ex-Head and Ex-Dean, and Prof. **K. S. Rane** (Ex-Head) for providing all the necessary facilities to carry out my research work.*

*I gratefully appreciate the members of the Bio-organic Chemistry group, Dr. **Supriya Tilvi**, Dr. **Prabha Devi**, Dr. **Narsinh Thakur**, Dr. **Sarjit Singh** and also the former group member, Dr. **P. S. Parmeswaran** for sparing their precious time to help me out in my PhD work.*

*I am equally thankful to Dr. **Santosh G. Tilve**, Dr. **B. R. Srinivassan** and other professors and non-teaching staff of Goa University for their kind support.*

*I thank Dr. **P. D. Mishra** and group, Piramal Life Sciences Limited, Mumbai, for HPLC analysis, recording NMR and biological screening and Dr. **Badbade**, and his Group, NCL, Pune for XRD facility.*

*Financial support in the form of Research Fellowship offered by **University Grant Commission** (UGC, New Delhi) and **Ministry of Earth Sciences** (MoES, New Delhi), which allowed me to pursue my full time research is gratefully acknowledged.*

All the support staffs of NIO, including HRM, Administration, Finance and Accounts, ITG, DTP, and Stores and Purchase have been extremely helpful and I thank all of them. I also acknowledge the Librarian and the staff members of Library, NIO and Goa University for their constant help.

*Special thanks to the other staff members of Bio-organic Chemistry group, Mr. **Bhobe**, Mr. **Uday** and Mr. **Sasi** for their support and cooperation.*

I would be failing in my duty, if I don't express my gratefulness to all my friends and lab mates for their help and support during the entire period of my thesis work. During my tenure, many of them joined, left and some stayed throughout. Their untiring company suggestions and memories are unforgettable and will be cherished in my whole life. Their constant encouragement always boosted my morale enabling me to give my

investigation the present shape and making my stay enjoyable over here. To name few are Ammar, Divya, Mahesh, Celina, Deepak, Sweta, Deepa, Snehal, Samiya, Imran, Savia, Cheryl, Tonima, Janak, Sucharita, Stacey, Marissa, Rahul, Mayuri, Darshana, Ankush (all from NIO), and Sulaksha, Jose, Vinod, Prakash, Rupesh, Aashish, Shrikant, Puzy, Santosh, Reshma, Prachi, Soniya, Rathan, Rohan, Satish, Chinmaya, Priyanka, Lactina (all from Goa University).

I am at loss of words to express my thanks and love to my mother and sister who have always been my pillar of strength throughout my life. Without their love it would not have been possible for me to be where I am at present.

I thank my father and grandmother, both of them passed away at the start of my research career, but I will never forget that they were the one who taught me to read and write the first word "SHRI GANESHA" in my life, This thesis is especially dedicated to my late father as it was his dream to see me climbing up and higher in field of science and education.

I close with thanks to "BABA SWAMI" who has granted me all the graces to fulfill this work and who supported and blessed me by his power and mercy.

Rajesh

Dedicated
To My Late
Father



CONTENTS

| | Page No. |
|---|-----------------|
| General Remarks | i |
| Abbreviations | ii-iii |
| List of Publications | iv-v |
| | |
| Introduction | 1-12 |
| | |
| Chapter 1 - Marine-derived fungi : A Source of biologically potent and novel chemicals -A review | 13-39 |
| | |
| I. Introduction | |
| <i>i) General</i> | 13-13 |
| <i>ii) Marine habitats and associated fungi as bioactive resource</i> | 13-14 |
| <i>iii) Bioactive metabolite symbioses: fungi as an alternative for the supply of natural products.</i> | 14-15 |
| <i>iv) Biology and ecology of marine fungi</i> | 15-16 |
| <i>v) Historical perspective</i> | 16-17 |
| <i>vi) Current status of marine mycochemistry</i> | 17-19 |
| | |
| II. Metabolites of marine derived fungi reported in first half of 2010 | |
| <i>i) Metabolites from <i>Aspergillus</i> sp.</i> | 19-22 |
| <i>ii) Metabolites from <i>Penicillium</i> sp.</i> | 22-24 |
| <i>iii) Metabolites from <i>Trichoderma</i> sp.</i> | 24-25 |
| <i>iv) Metabolites from <i>Chaetomium</i> sp.</i> | 25-26 |
| <i>v) Metabolites from <i>Phomopsis</i> sp.</i> | 26-27 |
| <i>vi) Metabolites from <i>Phoma</i> sp.</i> | 27-27 |
| <i>vii) Metabolites from <i>Fusarium</i> sp</i> | 28-28 |
| <i>viii) Metabolites from other species</i> | 28-32 |
| <i>ix) Metabolites from Unidentified species</i> | 33-33 |
| <i>x) Concluding remarks</i> | 34-34 |
| | |
| References | 34-39 |
| | |
| Chapter 2 - Secondary Metabolites from three different marine-derived <i>Aspergillus</i> species | 40-154 |
| | |
| I) Introduction | |
| <i>i) General</i> | 40-40 |
| <i>ii) Role of genus <i>Aspergillus</i> in human economy</i> | 40-41 |
| <i>iii) Scope of our study</i> | 41-42 |
| | |
| Section 2.1.: Bioactive secondary metabolites from the mangrove-derived fungus <i>Aspergillus flavipes</i> | |

| | |
|---|-------|
| I. Review of Literature on metabolites from <i>Aspergillus flavipes</i> | 43-47 |
| II. Results and Discussion | |
| <i>i) Isolation and cultivation mangrove associated fungi</i> | 47-48 |
| <i>ii) Purification of metabolites</i> | 48-50 |
| <i>iii) Structure elucidation of metabolites</i> | 50-66 |
| <i>iv) Biogenesis of FM-1 to FM-5</i> | 66-66 |
| <i>v) Biological properties of the metabolites: Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) activity</i> | 67-68 |
| <i>vi) Structure-activity relationship</i> | 68-69 |
| III. Experimental | 69-72 |

Section 2.2: Bioactive secondary metabolites from the soft coral associated fungus *Aspergillus terreus*

| | |
|---|-------|
| I. Bioactive metabolites from <i>Aspergillus terreus</i>- a review | 73-80 |
| II. Results and discussion | |
| <i>i) Biological materials</i> | 81-81 |
| <i>ii) Purification of metabolites</i> | 82-83 |
| <i>iii) Structure elucidation of metabolites</i> | 83-94 |
| <i>v) Biological activity</i> | 94-95 |
| III. Experimental | 96-98 |

Section 2.3: Antifungal secalonic acid D from the marine sponge associated fungus *Aspergillus aculeatus*

| | |
|--|---------|
| I. Introduction | 99-99 |
| II. Bioactive metabolites from <i>Aspergillus terreus</i>- a review | 100-102 |
| III. Secalonic acids and their biosynthesis | 102-106 |
| IV. Results and discussion | |
| <i>i) Biological materials</i> | 106-106 |
| <i>ii) Purification of metabolites</i> | 106-107 |
| <i>iii) Structure elucidation of FM-16</i> | 107-112 |
| <i>iv) Antifungal activity</i> | 113-113 |
| V. Experimental | 114-115 |

Section 2.4 Comparison and Identification of metabolites from the fungi *Aspergillus flavipes* and *Aspergillus terreus* using tandem mass spectrometry

| | |
|---|---------|
| I. Introduction | 116-117 |
| II. Introduction to electrospray ionization tandem mass spectrometry (ESI-MS/MS) | 117-120 |

| | |
|--|---------|
| III. Biosynthesis of aryl pyruvate dimers in <i>Aspergillus terreus</i> | 120-123 |
| IV. Results and discussion | |
| <i>i) Positive ESI-MS/MS analysis</i> | 124-134 |
| <i>ii) Negative ESI-MS/MS analysis</i> | 134-137 |
| <i>iii) Phylogenetic analysis</i> | 137-140 |
| <i>iv) Concluding remarks</i> | 140-140 |
| V. Experimentals | 140-141 |
| Section 2.5 Effect of seawater concentration on the production of butyrolactone I in <i>Aspergillus terreus</i> | |
| I. Introduction | 142-143 |
| II. Results and discussion | |
| <i>i) Cultivation of fungus</i> | 143-144 |
| <i>ii) Analysis of the extracts on UPLC</i> | 144-148 |
| III. Experimental | 148-149 |
| References | 149-154 |
| | |
| Chapter 3. Chemical investigation of marine invertebrates <i>Oceanapia sp.</i> and <i>Lobophytum crassum</i> | 155-182 |
| I. Introduction | |
| <i>i) General</i> | 155-155 |
| <i>ii) Natural products from marine invertebrates as source of lead compounds</i> | 156-157 |
| Section 3.1 Secondary Metabolites from marine sponge <i>Oceanapia sp</i> | |
| I. Introduction to marine sponges | 158-158 |
| II. Metabolite of marine sponge genus <i>Oceanapia</i> | |
| <i>i) Alkaloids</i> | 159-160 |
| <i>ii) Non-alkaloid</i> | 160-161 |
| III. Results and discussion | |
| <i>i) Biological material</i> | 161-161 |
| <i>ii) Extraction and purification</i> | 162-162 |
| <i>iii) Structure elucidation of metabolites</i> | 162-167 |
| <i>iv) Biological activity</i> | 167-167 |
| IV. Experimental | 167-168 |
| Section 3.2: Secondary metabolites from soft coral <i>Lobophytum crassum</i> | |

| | |
|---|---------|
| I. Introduction to soft corals and their metabolites | 169-169 |
| II. Results and discussion | |
| <i>i) Biological material, extraction and purification of metabolites</i> | 169-171 |
| <i>ii) Structure elucidation of purified compounds</i> | 171-178 |
| III. Experimental | 178-180 |
| References | 180-182 |
| | |
| Chapter 4. Studies towards the synthesis of amathamides and their antimicrobial activity | 183-222 |
| I. Introduction | |
| <i>i) General</i> | 183-184 |
| <i>ii) Amathamides and related metabolites from bryozoan genus Amathia</i> | 184-186 |
| <i>iii) Ecology and biological activity of amathamides</i> | 186-187 |
| <i>iv) Previous synthetic studies on amathamides</i> | 187-188 |
| II. Results and Discussion | |
| <i>i) Synthetic studies</i> | 188-201 |
| <i>ii) Biological activity</i> | 201-202 |
| III. Experimental | 202-211 |
| IV. Spectra | 211-220 |
| References | 220-222 |
| Summary | 223-225 |
| Publications | |

GENERAL REMARKS

- 1) The compound numbers, figure numbers, scheme numbers and reference numbers given in each chapter refer to that particular chapter only.
- 2) All melting points and boiling points were recorded using electronic Melting point machine and are uncorrected.
- 3) Commercial reagents were used without further purification.
- 4) All solvents were distilled prior to use and then dried using standard procedures.
- 5) Petroleum ether refers to the hydrocarbon fraction collected in the boiling range 60 – 80° C.
- 6) All reagents were prepared using literature methods.
- 7) For normal and flash silica gel chromatography silica gel (60 – 120 mesh size) and (230-400 mesh size) were used.
- 8) SephadexLH-20 (Pharmacia) was used gel permeation column chromatography.
- 9) Thin layer chromatography (TLC) was carried out on Precoated kieselgel 60 F₂₅₄ (Merck)
- 10) The UV and IR spectra were recorded on Shimadzu UV-2401 PC and Shimadzu FTIR-8201 PC spectrometers.
- 11) UPLC was performed on Waters ACQUITY machine.
- 12) NMR (¹H, ¹³C, DEPT, HSQC and HMBC) data were obtained on Bruker Avance 300 and Bruker Avance 500 spectrometer with TMS as internal standard.
- 13) EI-MS and HRESITOFMS were recorded on Shimadzu 2010 and QSTARXL MS/MS, Applied Biosystems, Switzerland.
- 14) Optical rotations were measured on optical polarimeter ADP220 (Bellingham + Stanley Ltd)

General Abbreviations and Symbols

| | |
|-----------|---------------------------|
| anhyd | Anhydrous |
| aq | Aqueous |
| atm | Atmospheric |
| cat | Catalytic |
| °C | Degree Celcius |
| Conc | Concentrated |
| dil | Dilute |
| DW | Distilled water |
| E | Eentegen (opposite) |
| et al | Et alia (and others) |
| Fig | Figure |
| g | Gram |
| h | Hour |
| lit | Literature |
| <i>m</i> | Meta |
| mg | Milligram |
| min | Minute |
| ml | Milliliter |
| mmol | Millimole |
| MNP(s) | Marine natural product(s) |
| Mp / mp | Melting point |
| MW | Molecular weight |
| NP(s) | Natural product(s) |
| <i>o</i> | Ortho |
| <i>p</i> | Para |
| PDA | Potato dextrose agar |
| PDB | Potato dextrose broth |
| psi | Pounds per square inch |
| R | Rectus |
| RT / r.t. | Room temperature |
| S | Sinister |
| Sat | Saturated |

| | |
|------|---------------------------|
| sec | Second |
| Temp | Temperature |
| TLC | Thin layer chromatography |
| SW | Sea-water |
| Z | Zusammen (together) |

Spectroscopic abbreviations and Symbols

| | |
|---------------------|---|
| br s | Broad singlet |
| CDCl ₃ | Deuterated chloroform |
| CID | Collision induced dissociation |
| cm ⁻¹ | unit of wavenumber |
| COSY | Correlation spectroscopy |
| d | Doublet |
| δ | Delta (Chemical shift in ppm) |
| DEPT | Distortionless Enhancement by Polarization Transfer |
| DMSO-d ₆ | Deuterated dimethyl sulfoxide |
| HMQC | Heteronuclear multiple quantum coherence |
| HMBC | Heteronuclear multiple bond correlation |
| Hz | Hertz |
| IR | Infrared |
| <i>J</i> | Coupling constant |
| m | Multiplet |
| MHz | MegaHertz |
| MS | Mass spectrometry |
| NMR | Nuclear magnetic resonance |
| NOESY | Nuclear overhauser effect spectroscopy |
| ν _{max} | Frequency maximum |
| ppm | Parts per million |
| q | Quartet |
| s | Singlet |
| t | Triplet |
| UV | Ultra violet |
| XRD | X-Ray diffractometry |

LIST OF PUBLICATIONS

1. **Parvatkar, R.R.**, DeSouza, C., Tripathi, A., Naik, C.G.; Aspernolide A and B, butenolides from a marine derived fungus *Aspergillus terreus*. *Phytochemistry*: 70(1); 2009; 128-132 (**IF 3.15**)
2. **Parvatkar, R. R.**, Mendon, M., Almeida A., Devi, P., Naik. C. G., Anti-inflammatory agents from mangrove-derived fungus *Aspergillus flavipes* (ready for submission)
3. Verma, A. K., Raghukumar C., **Parvatkar R.** and Naik, C. G. (2012) A Rapid Two-Step Bioremediation of the Anthraquinone dye, Reactive Blue 4 by a Marine-derived Fungus. *Water, Air, and Soil Pollution* (**IF 1.765**) (article in press, DOI 10.1007/s11270-012-1127-3).
4. Thakur, N. L., Salvi, J., Naik, D., **Parvatkar, R. R.**, Meena, R. M., Naik, C. G., Beta-lactamase mediated antibiotic resistance in marine sponge-associated bacteria: A selected metabolomic study of ampicillin (Submitted to “*Journal of Chemical Ecology*” **IF 2.486**)

CONFERENCE/SYMPOSIUM PUBLICATIONS

1. **Rajesh R Parvatkar**, Chandrakant G. Naik. Comparison of Closely Related Fungi *Aspergillus flavipes* and *Aspergillus terreus* Using Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS). *7th Junior National Organic Symposium Trust (JNOST) Conference for Research Scholars at IISER Mohali* (December 15-18, **2011**). (Poster Presentation)
2. Celina D'Souza; **Rajesh R. Parvatkar**; Prabhadevi; Naik, C.G. Mangrove associated fungi and bioactive metabolites from *A. flavipes*. National conference on *Marine Biology to Marine Biotechnology* 18-20 January **2008**, Ruparel College, Mumbai. (Oral Presentation)
3. **Parvatkar R. R.**, D'Souza C., Prabha D. and Naik C. G. Butenolides and cyclopentane diols from marine derived fungus *Aspergillus flavipes*:

Structure elucidation using advanced spectroscopic techniques. *RSC West India Section Ph.D. Students Symposium 2007*, Goa (Oral Presentation).

4. Celina Rodrigues, Tonima Kamat, **Rajesh Parvatkar**, Ashootosh Tripathi, Naik, C.G. Antibacterial Potential of Marine Associated Fungi. National Seminar on *Prospecting fungal diversity in Tropical India, it's conservation and applications in biotechnology*, April **2006**, Agharkar Research Institute. Pune, India

Introduction

Introduction

I. Natural products

Natural products (NPs) in simplest term are the chemical compounds, produced by living organisms. The cells of living organisms can be considered as micro chemical reactors producing large number of chemical compounds through metabolic reactions. These compounds can be categorized into primary and secondary metabolites. Primary metabolites such as proteins, carbohydrates, etc. are required as nutrients and source of energy for the organisms. The secondary metabolites, which may be categorized into different chemical classes depending on their biosynthetic origin such as terpenes, polyketides, alkaloids, etc. do not play a direct role in different functions within the organism, but have prominent role to play in an ecosystem.

NPs unlike synthetic chemicals have low toxicity, complete biodegradability, availability from renewable sources and some cases low cost. It is because of these reasons that health care products and environmentally acceptable agricultural compounds are mainly natural products or are derived by modification of natural product leads. Moreover, nature provides compounds having unique and diverse chemical structures with potential biological properties, which are beyond the reach of human imagination. For the past many centuries man has been using these NPs or the extracts of the organisms (especially plants) containing these NPs for human benefit. Today agriculture along with health care industry has been the prime area of application of NPs, but it was mainly the treatment of diseases with the aid of NPs or more specifically extract containing these NPs was the main use of NPs in the ancient time.

II. Evolution of natural product science

Historically, the use of NPs as medicinal agents presumably predates the earliest recorded history as the earliest humans used various, but specific plants (terrestrial) to treat an illness. In India, Rishis or Vaidyas (doctors), who treated the diseases using

herbs, passed the knowledge orally to their disciples. It was only between 1,400 and 1,000 BC that ayurveda got a written reference material. Ebers papyrus, written in about 1550 B.C., includes many of the plants used in Egyptian medicine. In the late 1700s, studies like William Withering's *An Account of the Foxglove, and its Medicinal Uses* (1785) began to appear.

The effectiveness of natural products in mitigating illnesses has inspired pharmaceutical scientists to search for new directions in drug discovery. In 1928, the accidental, but the most important discovery of antibiotic penicillin from *Penicillium notatum* by A. Fleming revolutionized medicinal sciences. This discovery diverted the attention of the researchers from plants, towards microorganisms as new source of drugs, and many new antibiotics resulted from such studies in mid 1900s. The transition from fortuitous discovery to systematic screening through validation in experimental models has taken place since the 1930s. High-throughput screening (HTS) and high-throughput chemistry technologies developed in the last two or three decades have significantly improved the speed, scale and quality of this process. Many important natural products, like anticancer agent, taxol from Pacific yew tree, *Taxus brevifolia* in 1967 (Goodman, 2001), a cholesterol lowering agent, lovastatin from *A. terreus* in 1970 were discovered (Alberts et al 1990) from terrestrial sources.

With ineffectiveness of some drugs in severe diseases, development of bacterial resistance to the antibiotics in use, emergence of deadly infections and diseases (Swine flu, SARS, Cancer, AIDS, etc) and limitations of the terrestrial resources, a need was felt for search of new resources and potent drugs. The oceans which were untouched until 1960 were recognized to be an alternative and undoubtedly the final frontier to be explored for the much needed source of NPs that could lead to the potent drugs.

III. Marine Biosphere (Oceans): The final frontier of bioactive natural products.

Over the past three decades or so, marine organisms have been recognized as an untapped resource for novel bioactive compounds. The oceans cover more than 70% of the earth's surface, and contain more than 300,000 described species of plants and

animals. Yet until relatively recently, chemists were confirmed landlubbers and until about 1960, no one even thought of the ocean. The ocean environment is massively complex, consisting of extreme variations in pressure, salinity, temperature, and biological habitats. The marine environment ranges from nutrient-rich regions to nutritionally sparse locations, where only a few organisms can survive. The surface and internal spaces of the sessile marine organisms provide a unique micro-habitat, and several symbiotic microorganisms are reported to occupy sometimes up to 40% of their tissue volume. These microorganisms are proven to be highly specific in their relationship with filter-feeding organisms like sponges, soft corals, ascidians and marine plants. The unique metabolic and physiological functions resulting due to various stresses in marine environment offer a potential for the production of novel enzymes and bioactive metabolites having novel chemistry by these organisms for potential exploitation.

The fact that the marine organisms have overcome, and adapted to all sorts of environments, and these creatures are constantly under tremendous selection pressure, including nutrition, space competition, predation, surface fouling and reproduction, is the opportunity for the researchers, to search for the potential drugs from the sea.

IV. Marine natural product chemistry

i) Opportunities and challenges

Natural product chemistry represents enormous opportunity for the drug development, and therefore drug discovery has been the prime aim for the scientist to have diverted their attention towards marine natural products (Chin et al., 2006). Before a drug comes into market, its discovery goes through various phases of scientific studies which require several years. The scientists from various disciplines such as biology, chemistry, pharmacy, medicine and interdisciplinary sciences like biotechnology, biophysics, biochemistry and medicinal chemistry are engaged in the process. The process of drug discovery goes through mainly following phases; drug target identification, target validation, lead compounds identification, and its

optimization and lastly preclinical and clinical evaluation. It is the lead identification and optimization stages, where chemists with mainly organic chemistry knowledge, are involved in the job. The process involves extraction of the compounds from an organism, their purification using various chemical and chromatographic techniques, followed by structural elucidation using chemical methods and spectroscopic analysis like IR, NMR, MS. etc. Furthermore efficient synthesis and structural modification of the NPs are needed to facilitate the further processes.

As the process of drug discovery being a multidisciplinary, technical deficiencies of one discipline has been the limitations to the development of the other, which has hampered the discovery of drugs from the ocean. Moreover, men fear the ocean as it is considered very hostile and inhospitable place to deal with. Initial obstacles of collection of organisms living beneath and on sediments below the ocean were overcome by development of SCUBA diving. The difficulties of retrieving a sustained, reliable harvest of marine organisms; leading to insufficient quantities of extracts and NPs was the main concern for natural product chemists. As the availability and reproducibility of sample was limited, natural product chemists did not have the luxury of working with large amount of the extracts, for the purification of NPs from the complex mixture, to allow the completion of chemical structure analysis with the available underdeveloped technologies.

Organic chemistry itself is evolved from the study of NPs (terrestrial origin). Earlier large quantities of plant material were used to prepare the extract, and then further purification using solvent partition and chromatographic techniques. The structural elucidation would follow various degradative chemical reactions for determining elemental composition, molecular weight, carbon framework, functional groups and stereochemistry. For example elemental composition would be determined by estimation of CO_2 , H_2O and N_2 produced by pyrolysis of the sample and then the molecular formula hence, molecular weight would be determined. To test the presence of a functional group, specific chemical reactions would be carried out. For example, carbonyl functionality would be tested by reaction with 2,4-dinitrophenylhydrazine or hydroxyl amine. A carbon-carbon double bond would be tested by KMnO_4 or Br_2 -Acetic acid. Stereochemistry would be determined by

degradation or conversion of the compound to a chiral compound, whose chirality was known. These analysis would take decades to characterize the structure after its purification. The synthesis would follow this cumbersome process for the confirmation of the structure. Nevertheless, these processes has led to the discovery of so many important reactions in organic chemistry. Glucose, the most important chemical as source of energy for living being and cholesterol almost found in all the organisms were assigned the structure after anylyses that took decades together.

It would have been virtually impossible for the chemist to characterize the NPs from the seas, if the same methodologies had to be followed over last three decades. Fortunately, continuous parallel development in the purification methologies, such as flash chromatography, GC, HPLC, and very recently UPLC, SEP box and the spectroscopic methods such as UV-VIS, IR, MS and NMR, chemists were able to characterize the chemical structures with fewer evidences from classical chemical methods. Especially the discovery of the NMR in 1950 is considered to be the most wonderful discovery for the natural product chemistry. The advent and commercial availability of instrument with broadband excitation and fourier transform methods has been the boost for sensitivity and speed in the analyses. Today, a spectrum can be recorded on sample quantities down to submilligram level. These advances in the instrumentaion techniques coupled with discoveries of two dimentional NMR correaltion methods such as COSY, HMBC, NOESY etc, have enabled chemist to assign complete structure even without the use of XRD. A relatively small number of marine plants, animals and microbes have already yielded more than 22,000 novel chemicals (Blunt et al, 2011), but majority of marine chemical biodiversity still remains to be fully realized.

ii) Current status

The technological advances over the past half century have seen the identification of several NPs from marine sources as well the minor constituents of terrestrial sources, which remained uninvestigated. As per the MarineLit database more than 22000 new compounds have been reported from the marine sources. These compounds have been

derived from 3,700 species from 2,255 genera belonging to 40 phyla (Blunt et al, 2007). The number of metabolites reported annually is increasing every year. In the 1960s decade, which marked the beginning of the marine natural product chemistry the number of new MNPs discovered were just over hundred (**fig 1**). The next decade (1970s) saw sharp rise in discovery of new MNPs and over 350 compounds were reported in this decade. A significant increase in the discovery of new MNPs was also observed in the subsequent decade. The marine natural product chemistry blossomed in 1990s and the last two decades have seen the discovery of nearly 14000 new MNPs accounting for 60% of total new MNPs discovered. More than 1000 new MNPs have been discovered in each 2008 and 2009 calendar years.

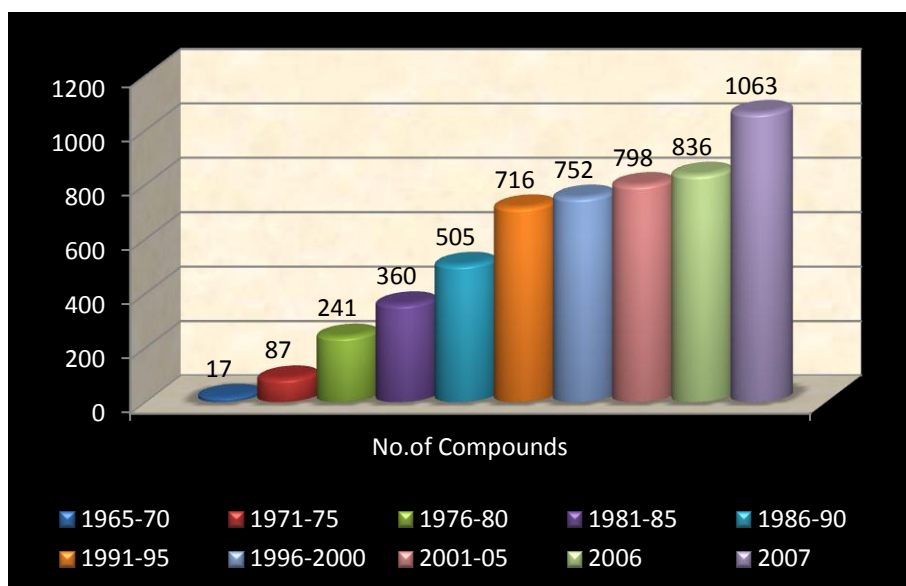


Fig 1: Annual output of marine natural products, 1965–2007. The average output/year for the period 1965 to 2005, in five-year periods, has been plotted with that for 2006 and 2007

The output by geographic region of collection of sources examined shows that seas around Japan, western Pacific region, Caribbean Sea and China Sea have been the major contributors to new MNPs for 1965-2007 as shown in the **fig 2**. Not far behind them are Mediterranean Sea, Indian Ocean and seas around Australia. However, the comparison of this data with average annual distribution for 2001–2007 generates a snapshot of the differential changes in output by region that has taken place over the years. In the recent years China Sea has emerged as highly significant source of new

MNPs. The discovery of new compounds from China Sea from 2001-2007 has risen to around 230 per year from 50 compounds per year for 1965-2007, which is far ahead from other geographical regions. Nearly 75 new compounds on an average have been discovered from the seas around India and ranks 4th behind China Sea, western pacific (100 compounds per year) and Caribbean Sea (80 compound per year) for the period 2001-2007 (Blunt et al., 2009).

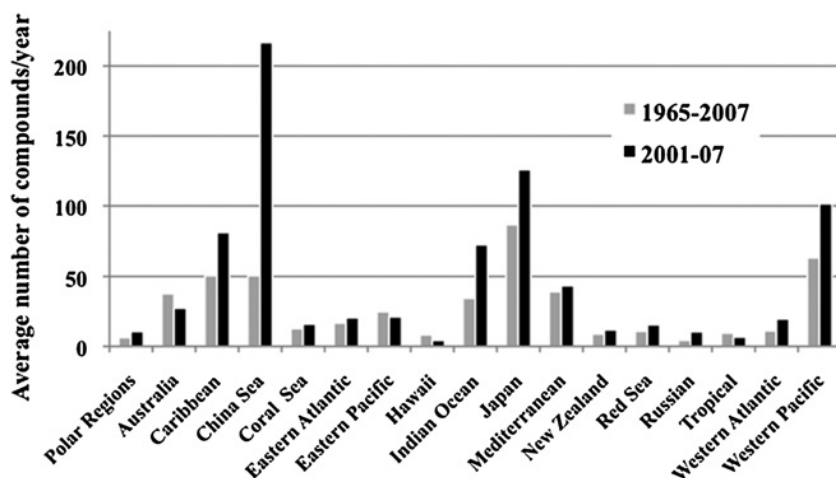


Fig. 2 Trends in geographic distribution of collection sources. For clarity the Polar Regions include Antarctic and Arctic; North Sea and Baltic Sea records are included with the Eastern Atlantic; Caspian Sea and Black Sea records have been added to the Russian region.

Of the total new MNPs discovered 5% of them belong to bacteria (prokaryotes), while 95% are eukaryota in origin (Blunt et al., 2008). It is noteworthy that just three Phyla, Porifera, Cnidaria and Chromophyta, constitute 60% of the species examined, and that if a further five Phyla are added (Mollusca, Rhodophyta, Chordata, Cyanobacteria, Echinodermata), nearly 94% of all of the species recorded are accounted for (**fig 3**). The remaining 6% are scattered across another 14 Phyla. As in the recent years the marine natural product science has been bioactivity driven rather than chemistry, the sessile benthic organisms, mainly invertebrates such as sponges, ascidians, bryozoans etc attracted maximum attention.

Marine Natural Products Chemistry is essentially a child of the 1970's that developed rapidly during the 1980's and matured in the two last decades. Several compound

have entered into the clinical trials and few of them have entered the market as well (Newman and Cragg, 2004, Jimenez et al., 2009; Faulkner, 2000).

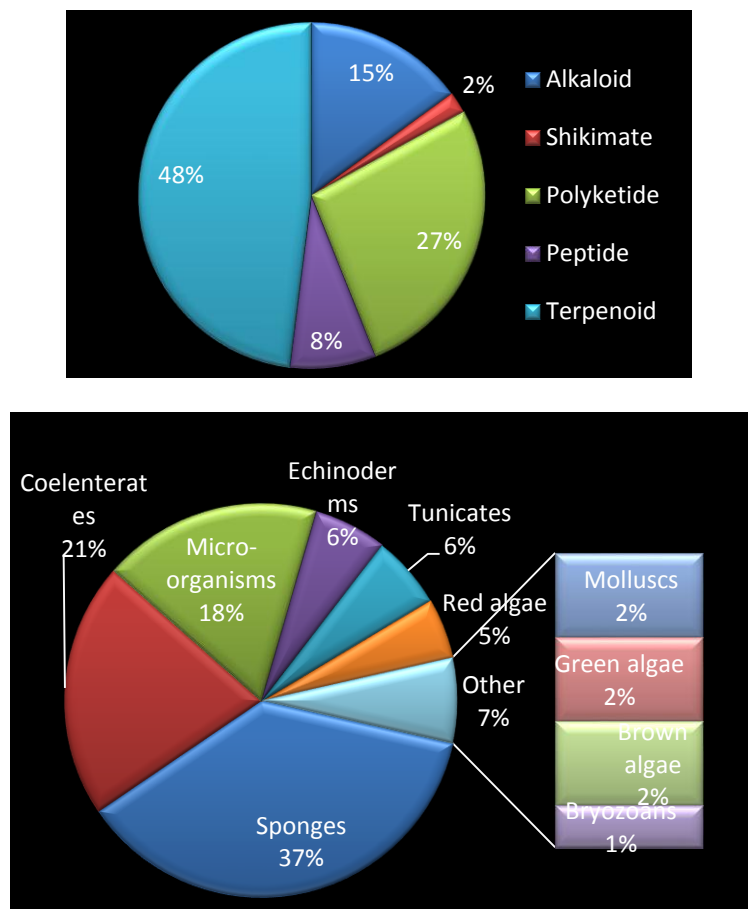


Fig 3: New marine natural products reported in 2002 divided according to phyla (1) and biosynthetic origin (2)

iii) Future of MNP science

It was a big question whether marine natural products will play a major role in drug discovery in the future, when marine natural product research was in the beginning or even perhaps before the two decades (Grabbly et al., 2000). The steady arithmetic rate of discovery of new natural products is increasing since 1965, but recent few years have not seen any increase in the discovery of natural products. It might be that marine natural product discovery research have attained its peak, as last three years have seen nearly or over 1000 metabolites being discovered per year. It appears that trend will continue over next several years at least due to shift towards unexplored

microorganisms. There is any doubt left that discovery of natural products from macro-organisms is going to slow down as many of them have been explored but their associated fauna and flora represents huge biodiversity for exploration. Microbial diversity constitutes an infinite pool of novel chemistry, making up a valuable source for innovative biotechnology. So far we have only scratched the surface of it. The most recent estimates suggest that by now we only know approx. 5% of the total species of fungi and may be as little as 0.1 % of the bacteria. Among the ones already described, only a small fraction has been examined for metabolite profile (Lange, 1996; Debbab et al., 2010). If the previous era saw change from terrestrial natural products to marine natural products, the coming era will turn out to be a change from marine macroorganisms to marine micro-organisms natural products.

V. Marine Natural product chemistry at National institute of Oceanography (NIO), Goa

A group of scientist at Bio-organic Chemistry Laboratory, NIO is engaged on a major project “Drugs from the sea” and several other related projects funded by various Indian government organization and a project “Marex” funded by European commission involving European institutes besides NIO. The group also has collaborations with CNR Institute of Biomolecular Chemistry, Italy; Piramal Life Sciences Limited, Mumbai and these programs uses different strategies for discovery and development of MNPs.

The MNP research at NIO has resulted in isolation of several novel and known bioactive molecules from marine resources obtained from seas around India. The novel compounds discovered by the research group at NIO include antitumor alkaloid jorumycin from *Joruna funebris* (Fontana et al., 2000), caulerpenyne from alga *Caulerpa* sp. (Fontana et al., 1999), kahalalide R and S from green alga *Bryopsis* sp (Tilvi et al., 2007), γ -pyrone propionates from molluscs *Placobranchus ocellatus* (Manzo et al., 2005), peroxyacetic acid C and D (Fontana et al., 1999), bromotyrosine alkaloids from sponge *Psammaphysilla purpurea* (Tilvi et al., 2004),

ceramide from sponge *Haliclona* sp. (Tilvi et al., 2005), armatols A-F and glycerolipids from red alga *Chondria armata* (Al-Fadhli et al., 2006; Ciavatta et al., 2001); pregnenes from octocoral *Carijoa* sp (Ciavatta et al., 2004) and several other known compounds from these macroorganisms.

The microbial bioactive products those have been discovered include mainly known compounds cyclosporine and terrein from fungus *A. terreus*; kojic acid and cyclopiazinic acid from fungus *A. sulphereus*; auroglocinnsa and anthraquinone derivatives from fungus *Eurotium* sp.; 4,4'-oxybis[3-phenylpropionic acid] from bacterium *Bacillus licheniformis*. A novel antibiotic, cyclic peptide PM181104 from a bacterium *Kocuria* sp. associated with a marine sponge having potent antibacterial activity has been patented (Patent application number: 20090076094).

A strong research interest in the field of organic chemistry made me to join the bioorganic chemistry group to pursue research career in the field of marine natural product chemistry. During the course of my research work, I was involved in isolation, structure elucidation and bioactive properties of the metabolites from marine-derived fungi of genus *Aspergillus*; marine invertebrates, a sponge and a soft coral; and synthesis of amathamide alkaloids, the metabolites of a bryozoan. The investigation carried out is the subject of present thesis. The thesis is divided into four chapters.

Chapter 1 contains a review of literature on the metabolites of marine fungi with special reference to metabolites isolated in year 2010

The content of **Chapter 2** forms chemical investigation of three marine derived fungi. It has been sub-divided into five sections

In sections 2.1, 2.2 and 2.3 isolation, structure elucidation and bioactivity of metabolites of the fungi *A. flavipes*, *A. terreus*, and *A. aculeatus* respectively has been described.

Section 2.4 deals with comparison of metabolic profile of the two fungi *A. flavipes* and *A. terreus* using electrospray ionization tandem mass spectrometry (ESI-MS/MS).

Section 2.5 describes the effect of seawater concentration on production of an important metabolite, butyrolactone I from *A. terreus*.

Chapter 3 presents the investigation carried on chemical constituents of two marine invertebrates and it is divided into two sections.

Section 3.1 describes the isolation and characterization of metabolites from antibacterial extract of rarely investigated a marine sponge *Oceanapia* sp.

Section 3.2 describes the metabolites isolated from a soft coral *Lobophytum crassum*

In **Chapter 4**, the studies carried out toward the synthesis of amathamides and their biological activity is reported.

References

- Al-Fadhli, A., Wahidullah, S., DeSouza, L., 2006. *Glycobiology*. 16 (10), 902-915.
- Blunt, J. W., Copp, B. R., Hu, W., Munro, M. H. G., Northcote, P. T., Prinsep, M. R., 2009. *Nat. Prod. Rep.*, 26, 170–244.
- Blunt, J. W., Copp, B. R., Hu, W., Munro, M. H. G., Northcote, P. T., Prinsep, M. R., 2011. *Nat. Prod. Rep.*, 28, 196-268.
- Blunt, J. W., Copp, B. R., Hu, W., Munro, M. H. G., Northcote, P. T., Prinsep, M. R., 2007. *Nat. Prod. Rep.*, 24, 31-86.
- Blunt, J. W., Copp, B. R., Munro, M. H. G., Northcote, P. T., Prinsep, M. R., 2008. *Nat. Prod. Rep.*, 25, 35–94.
- Ciavatta, M. L., Gresa, M. P. L., Manzo, E., Gavagnin, M., Wahidullah, S., Cimino, G., 2004. *Tetrahedron Lett.*, 45(41), 7745-7748.
- Ciavatta, M. L., Wahidullah, S., DeSouza, L., Scognamiglio, G., Cimino, G., 2001. *Tetrahedron*. 57(3), 617-623.
- Fontana, A., Cavaliere, P., Wahidullah, S., Naik, C. G., Cimino, G., 2000. *Tetrahedron*, 56; 7305-7308.

- Fontana, A., Ciavatta, M. L., Mollo, E., Naik, C. G., Wahidullah, S., DeSouza, L., Cimino, G., 1999. *J. Nat. Prod.*, 62(6), 931-933.
- Tilvi, S., Naik, C. G., 2007. *J. Mass. Spectrom.*, 42(1), 70-80.
- Tilvi, S., Majik, M., Naik, C. G., 2005, *Eur. J. Mass Spectrom.*, 11(3) 345-351.
- Tilvi, S., Rodrigues, C., Naik, C. G., Parameswaran, P. S.; Wahidullah, S., *Tetrahedron*, 2004, 60(45), 10207-10215.
- Chin Y.-W., Balunas M. J., Chai H. B., Kinghorn, A. D., 2006. *The AAPS Journal*, 8 (2) E239-E253.
- Debbab, A. Aly, A. H., Lin, W. H., Proksch, P., 2010. *Microbial Biotech.*, 3(5), 544-563.
- Faulkner, D. J., 2000. *Nat. Prod. Rep.*, 17, 1-6.
- Newman, D. J., Cragg, G. M., 2004. *J. Nat. Prod.*, 67, 1216-1238.
- Goodman, J., Walsh, V., 2001. *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug*. Cambridge University Press. Pg. 17.
- Grabley, S., Thiericke, R., Sattler, I., 2000. *In: Ernst Schering Research Foundation Workshop 32: The role of natural products in drug discovery*, Springer, Berlin, 217-252.
- Jimenez, J. T., Sturdikova, M., Sturdika, E., 2009. *Acta Chimica Slovaca*, 2(2), 63-74.
- Lange, L., 1996. *Pure & Appl. Chem.*, 68(3), 745-748.
- Prabha, D., Wahidullah, S., Rodrigues, C., DeSouza, L., 2010. *Mar. Drugs*, 8(4), 1203-1212.
- Manzo, E., Ciavatta, M. L., Gavagnin, M., Mollo, E., Wahidullah, S., Cimino, G., 2005. *Tetrahedron Lett.*, 46(3), 465-468.