Synopsis

The thesis entitled "**Isolation of Myrrhanones A, B & C from *Commiphora mukul* Gum Resin and their Chemical Transformations for Anticancer and Anti-inflammatory Agents**" is presented in four chapters.

Chapter 1: Chemical Investigation of *Commiphora mukul*: Isolation and Biological Activity of Myrrhanones A, B & C

This chapter consists of two parts: Section A and B. Section A gives brief introduction about the role of natural products in drug discovery and the chemical and biological potential of gum resin of *Commiphora mukul*. Section B deals with the present work on systematic chemical investigation of *Commiphora mukul* with special emphasis on myrrh-terpenoids and their biological activities.

Section A

Natural products are prolific sources of diverse secondary metabolites with potent biological activities. They are the most productive sources of lead molecules for developing clinically useful drugs for human health disorders. Nature has been cornerstones of drug development with 70% drugs in the market are based on natural products. Natural product based drugs have been derived from various sources such as plants, marine organisms, animals and microorganisms. Ever since the first isolation of pharmacologically active pure morphine from a plant source *Papaver somniferum* in 1805 by Friedrich Serturner, there is a continuous stream of biologically active natural products being isolated from various natural sources. Natural products research continues to explore a variety of lead structures, which may be used as templates in the development of new drugs for the prevention of human diseases.

Commiphora (or *Balsamodendron) mukul* Hook, a small tree belongs to the family Burseraceae, found growing especially in the dry regions of the India, Pakistan and Bangladesh. The gum resin of *Commiphora mukul* (*ver*. Guggul) is used widely in Indian Ayurvedic system of medicine to treat inflammation, obesity and lipid disorders. The resinous exudate of this plant was found to exhibit a wide range of biological activities including anti-inflammatory, anticancer, antibacterial, anti-mycobacterial, rheumatoid arthritis. The chemistry of guggul is very interesting and found to elaborate a wide range of secondary metabolites such as terpenoids, steroids, cembrenoids and lignans. Among them, myrrh-terpenoids are prominent components and are structurally related to

polypodane type bicyclic triterpene framework. So far, few research groups have reported the isolation of myrrhanones A, B, C from guggul gum with very little information on their biological potential and their chemical modifications. Myrrh-terpenoids are good lead structures and can serve as useful natural scaffolds and there exists tremendous scope to synthesize a range of New Chemical Entities (NCEs). Therefore, as part of our continuous research endeavours, the detailed and systematic phytochemical investigation and pharmacological studies on the gum resin of *Commiphora mukul* has now been taken up.

Section B

In the present work, the gum resin of *Commiphora mukul* was procured through Baidyanath Ayurvedic Pharmacy, Patna, India. The gum resin was powdered and exhaustively extracted with various polar solvents using soxhlet apparatus. As the n-hexane extract showed well resolved patterns on TLC, it was taken up for extensive column chromatographic separations and isolated seven pure compounds (**I-VII,** Figure 1). The structures of the isolated compounds were elucidated by their spectroscopic data (^{1}H & ^{13}C NMR, Mass and IR) as cholest-4-en-3-one (**I**, 0.024%), myrrhanone C (**II**, 0.49%), myrrhanone A acetate (**III**, 0.26%), Z-guggulsterone (**IV**, 0.40%), E-guggulsterone (**V**, 0.36%), myrrhanone A (**VI**, 0.53%) and myrrhanone B (**VII**, 0.58%). While compounds **I**, **IV** and **V** are found with steroidal framework, compounds **II, III, VI** and **VII** are polypodane type bicyclic triterpenes. Compounds **IV** and **V** are well known guggulsterones and considerable chemical and biological studies have been done on these metabolites. Compounds **II, VI** and **VII** are myrrh-triterpenes and have very interesting structural features with a common bicyclic core structure and a triene side chain. These three compounds however, differ in their side chain terminal functionality. In case of compound **II,** it has a methyl, whereas in compounds **VI** and **VII**, the terminal side chain is substituted with a hydroxyl or carboxylic acid group. Literature search reveals that these myrrh-triterpenes are totally unexplored in both chemical and biological point of views. As triterpenes are known to exhibit significant anticancer and anti-inflammatory activities, the three myrrh-triterpenes were evaluated to see their potentiality in the aforesaid activities. The biological results are very interesting and revealed that compounds **II** and **VI** showed significant anticancer activity. In contrast,

compound **VI** displayed good anti-inflammatory activity. Hence, their unique and intriguing structures as well as their biological results are more encouraged for further chemical modification.

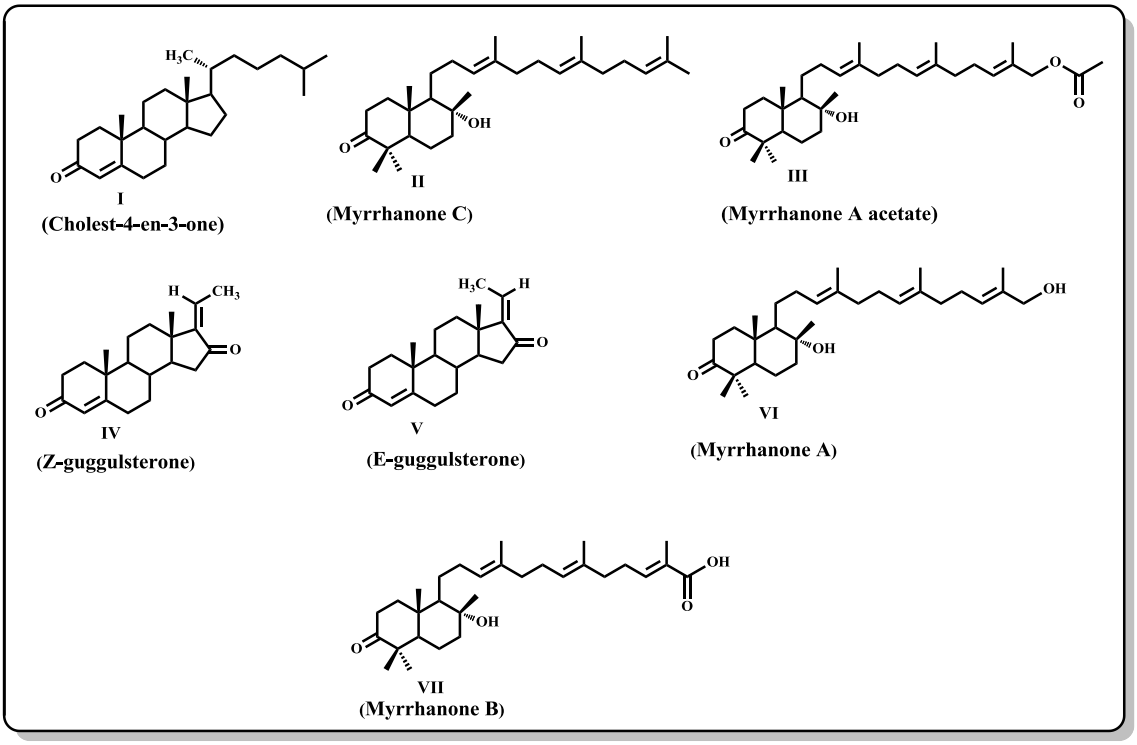
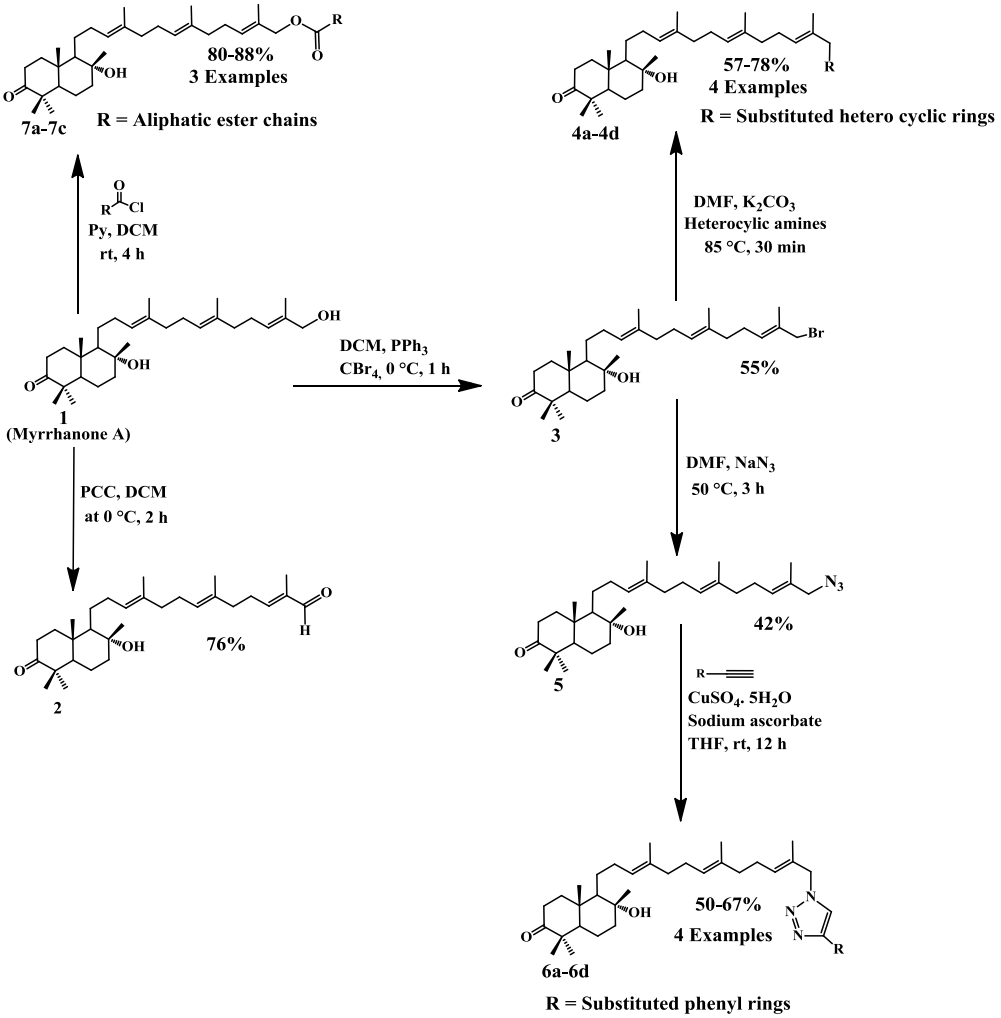


Figure 1: Isolated Compounds from Gum Resin of *Commiphora mukul*

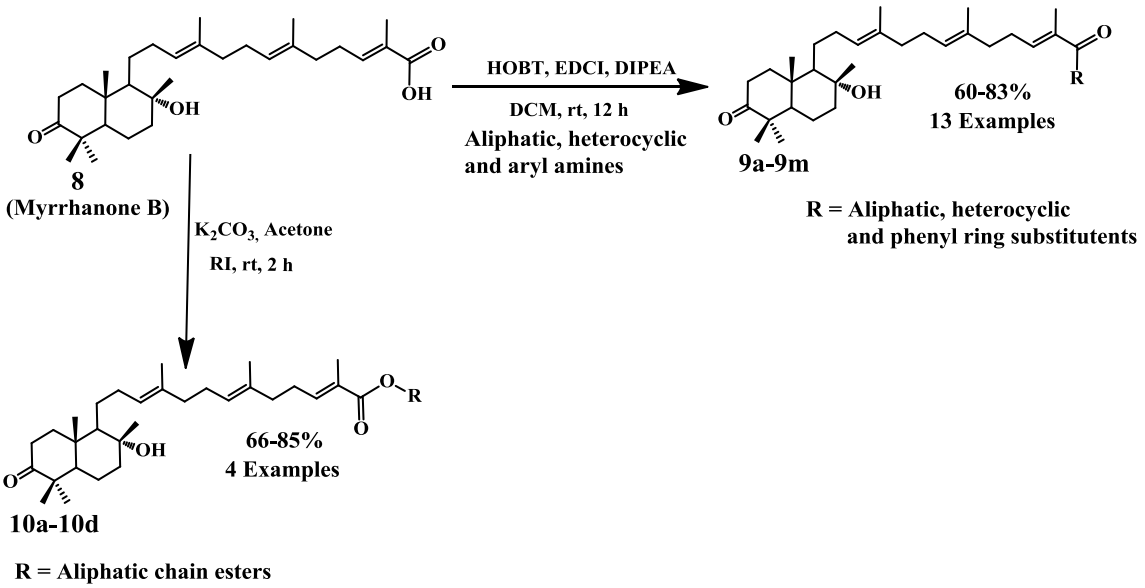
Chapter 2: Synthesis and Biological Evaluation of Some Novel Side Chain Modified Analogues of Myrrhanones A and B

This chapter incorporates the structural modifications of the side chains of myrrhanones A and B and biological evaluation of the synthesized analogues. The present work is aimed to synthesize a series of analogues such as amides, esters, amines and triazoles by using hydroxyl and carboxylic acid functionalities of myrrhanone A and myrrhanone B to understand the key functionalities required for exhibits potent anticancer and anti-inflammatory activities (Schemes 1 and 2). The newly synthesized analogues were evaluated for their anticancer activity against a panel of five human cancer cell lines namely DU145 (Prostate), HT-29 (Colon), MCF-7 (Breast), Hela (Cervical) and U87MG (Glioblastoma) along with a normal lung cell line (L132) by using MTT assay. The results revealed that most of the synthesized analogues exhibited moderate to very good

activity against the tested cancer cell lines. Among the tested compounds, **4a, 4b, 9d** and **9i** showed most promising activity with IC_{50} values of 7.74, 4.65, 5.48 and 6.63 μM respectively. In addition, these analogues were also screened for their anti-inflammatory activity against TNF-α and IL-1β. The results demonstrated that the compounds **9c** and **9g** are the most potent ones against TNF-α and found to exhibit almost identical inhibitory activity [IC_{50} (μM): 10.02, 10.53]. Surprisingly, compound **2** with an aldehyde functional group at terminal side chain displayed enhanced anti-inflammatory activity against both TNF-α (IC_{50}: 9.39 μM) and IL-1β (IC_{50}: 12.15 μM) compared to the myrrhanone A (**1**) and myrrhanone B (**8**). Detailed analysis of the biological activity data and the structural features of the synthesized analogues revealed some very interesting structure-activity relationship (SAR).

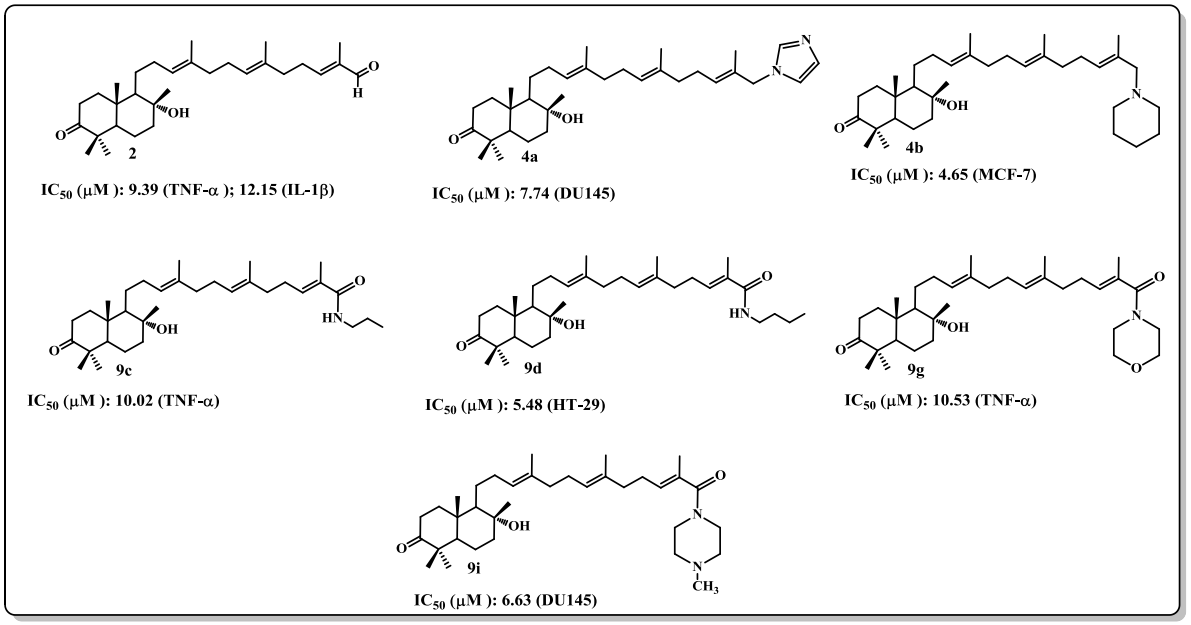


Scheme: 1



Scheme: 2

Potent Molecules of Myrrhanones A & B Analogues

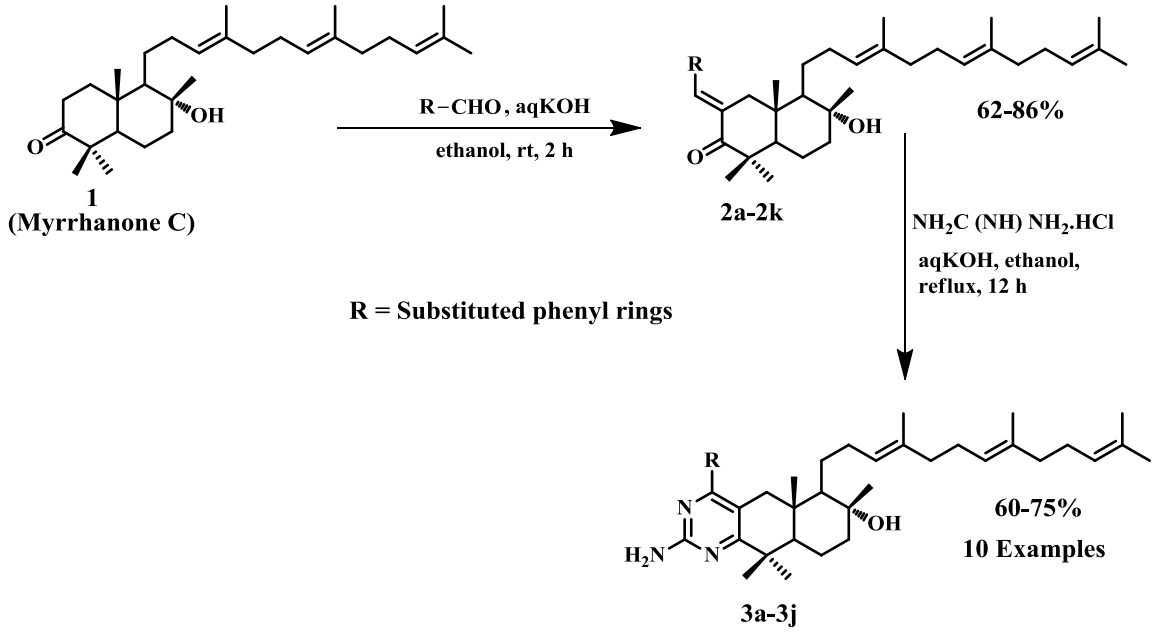


Chapter 3: Synthesis and Biological Evaluation of Some Novel Ring A Fused Pyrimidine Hybrids of Myrrhanones A and C

This chapter is divided into two sections, A and B: Section A describes the synthesis and anticancer activity of myrrhanone C hybrids, whereas section B describes the synthesis and anti-inflammatory activity of myrrhanone A hybrids.

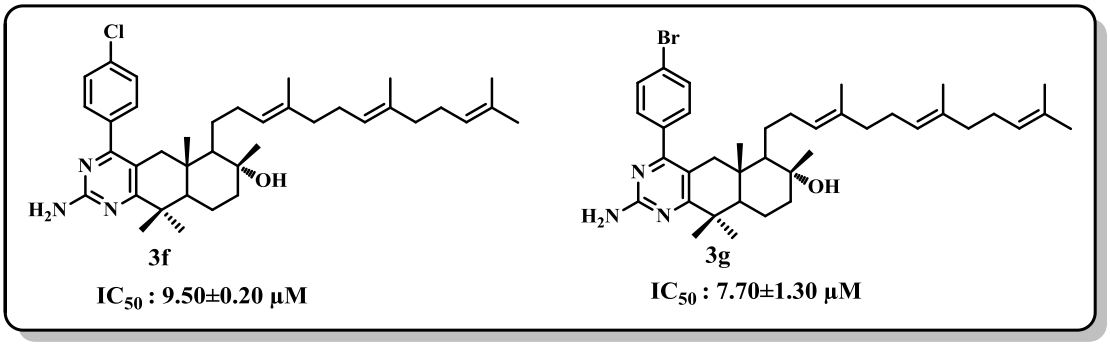
Section A

This section describes the synthesis of pyrimidine hybrids of myrrhanone C and studied their anticancer potential against a panel of six cancer cell lines including A549 (Lung), Hela (Cervical), MCF-7 (Breast), ACHN (Renal), Colo-205 (Colon) and B-16 (Mouse melanoma) by employing MTT assay. The screening results revealed that the compounds, 4 chloro pyrimidine hybrid (**3f**) and 4 bromo pyrimidine hybrid (**3g**) exhibited potent anticancer activity against A-549 with IC_{50} values 9.50±0.20 and 7.70±1.30 µM respectively, which is 5 and 6 times higher than the parent myrrhanone C (**1**) [IC_{50}: 44.90±2.70 µM].



Scheme: 3

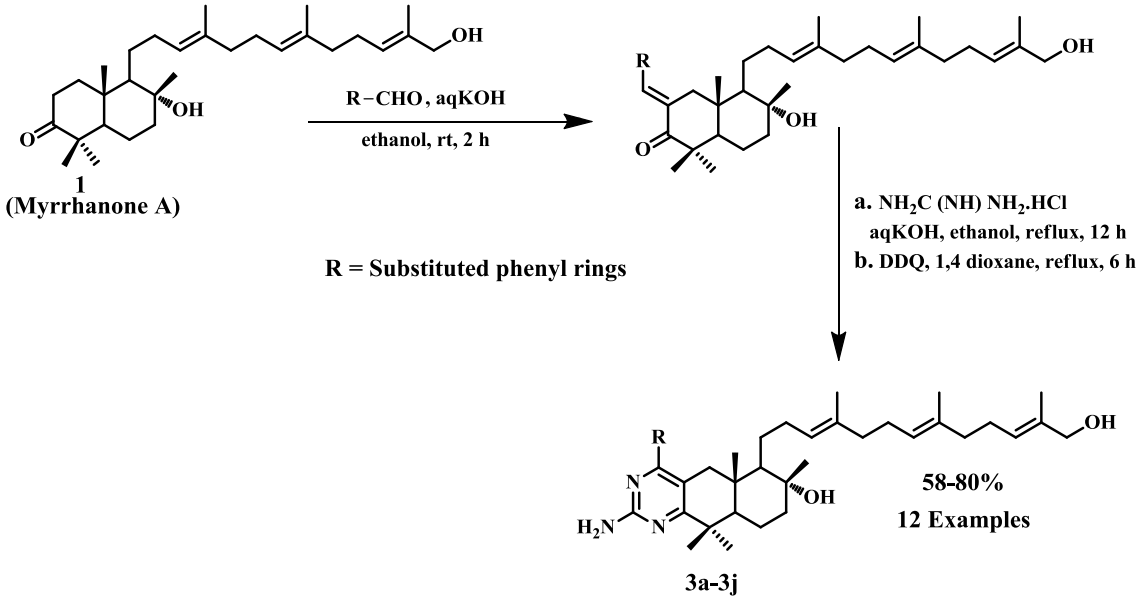
Potent Molecules of Myrrhanone C Hybrids



Results published in *Mol Divers.* 2015, *19*, 745-757

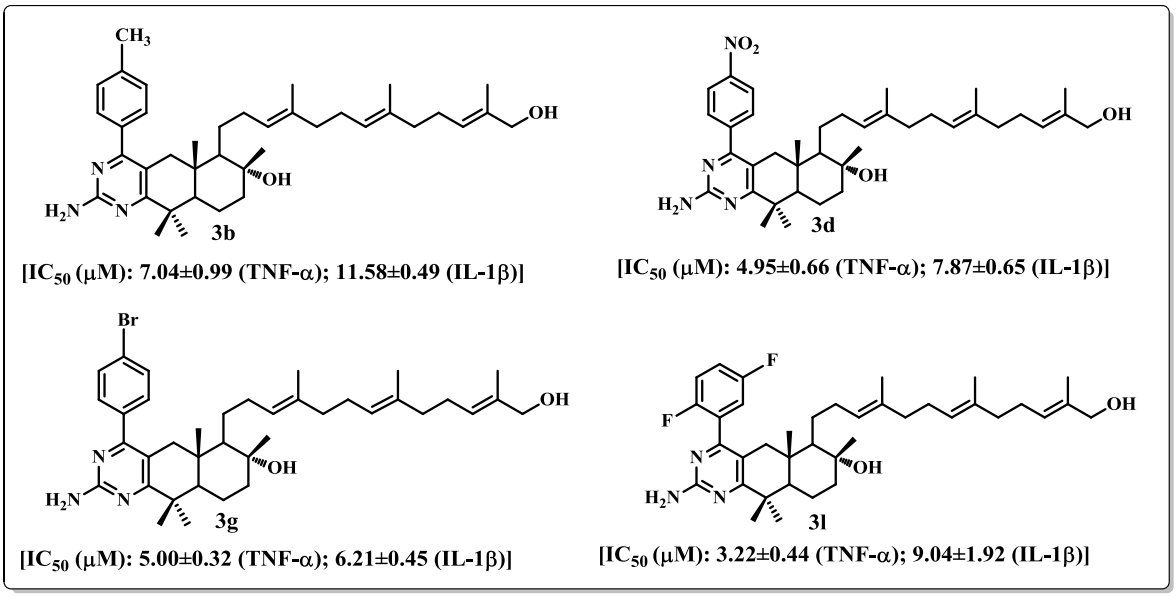
Section B

This section incorporates the synthesis of a series of novel pyrimidine hybrids of myrrhanone A and evaluated for their anti-inflammatory activity. The biological results revealed that compounds **3b**, **3d**, **3g** and **3l** were found to possess highly potent anti-inflammatory activity against TNF-α with IC_{50} 7.04±0.99, 4.95±0.66, 5.00±0.32 and 3.22±0.44 µM respectively, which is 3~6 times more active than the parent myrrhanone A (**1**) [IC_{50}: 20.87±3.01 µM]. Similarly, compounds **3b**, **3d**, **3g** and **3l** showed enhanced activity against IL-1β with IC_{50} 11.58±0.49, 7.87±0.65, 6.21±0.45 and 9.04±1.92 µM respectively.



Scheme: 4

Potent Molecules of Myrrhanone A Hybrids

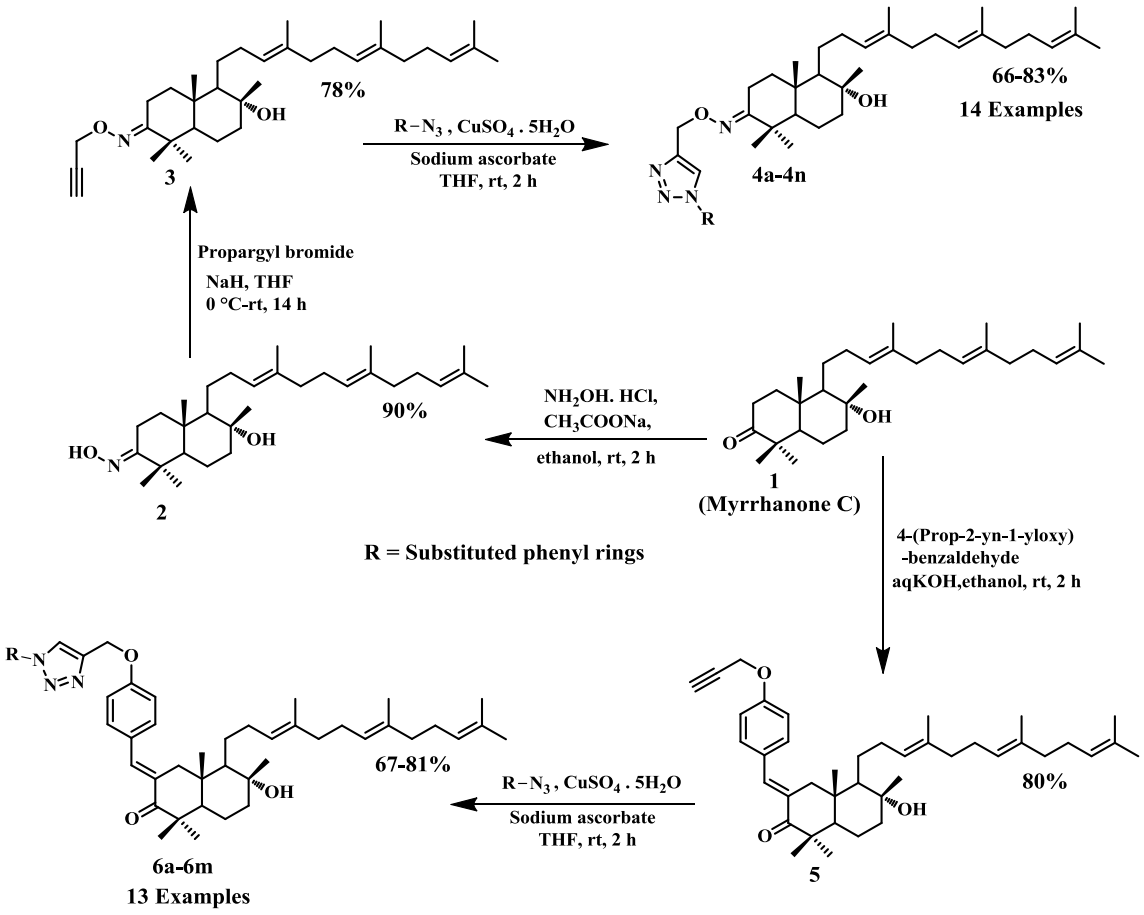


Chapter 4: Synthesis and Biological Evaluation of Some Novel Side Chain and Ring A Substituted Triazoles of Myrrhanones B and C

This chapter is divided into two sections, A and B. Section A describes the synthesis and anticancer activity of some novel myrrhanone C based ring A triazoles, whereas section B describes the synthesis and anticancer and anti-inflammatory activities of some novel myrrhanone B based triazoles.

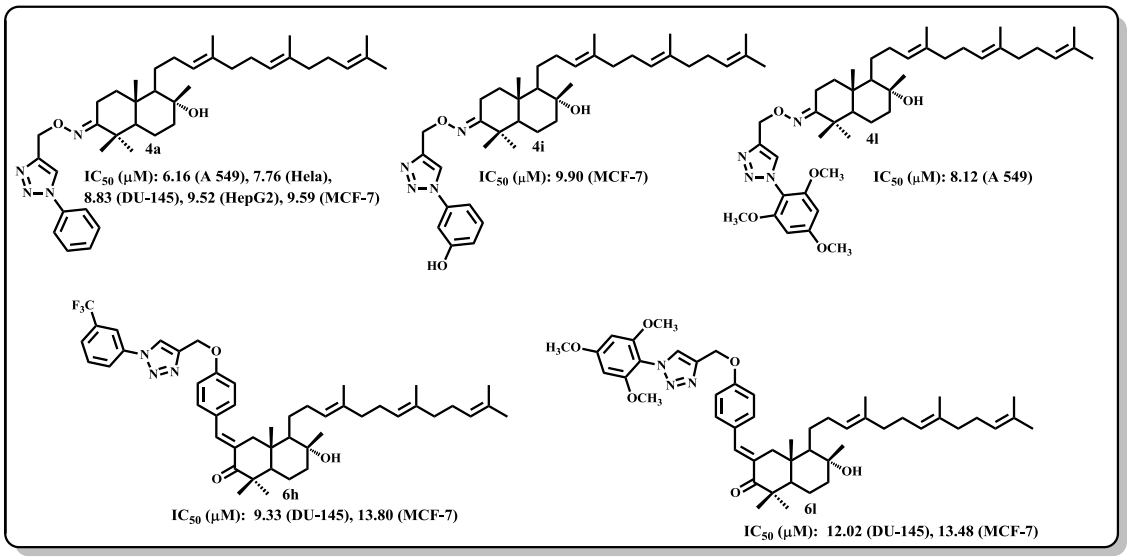
Section A

In this section, the synthesis of two series [oxime (1) and benzylidene (2)] of novel 1,2,3-triazole hybrids of myrrhanone C. All the synthesized compounds of series 1 (**4a-4n**) and series 2 (**6a-6m**) were evaluated for their anticancer activity against five human cancer cell lines such as A549 (Lung), Hela (Cervical), MCF-7 (Breast), DU-145 (Prostate) HepG2 (Liver) by employing MTT assay. The results revealed that compound **4a** exhibited significant inhibitory activity (IC_{50}: 6.16 µM) against A549 cell line, which is 2 fold higher than that of parent myrrhanone C (**1**). The other potent molecules in this series are **4l** (A549 - IC_{50}: 8.12 µM) and **4i** (MCF 7 - IC_{50}: 9.90 µM). Among the series (**6a-6m)** compounds **6h** showed higher activity against DU-145 (IC_{50}: 9.3 µM), whereas, compound **6l** showed maximum activity against on DU-145 (IC_{50}: 12.02 µM).



Scheme: 5

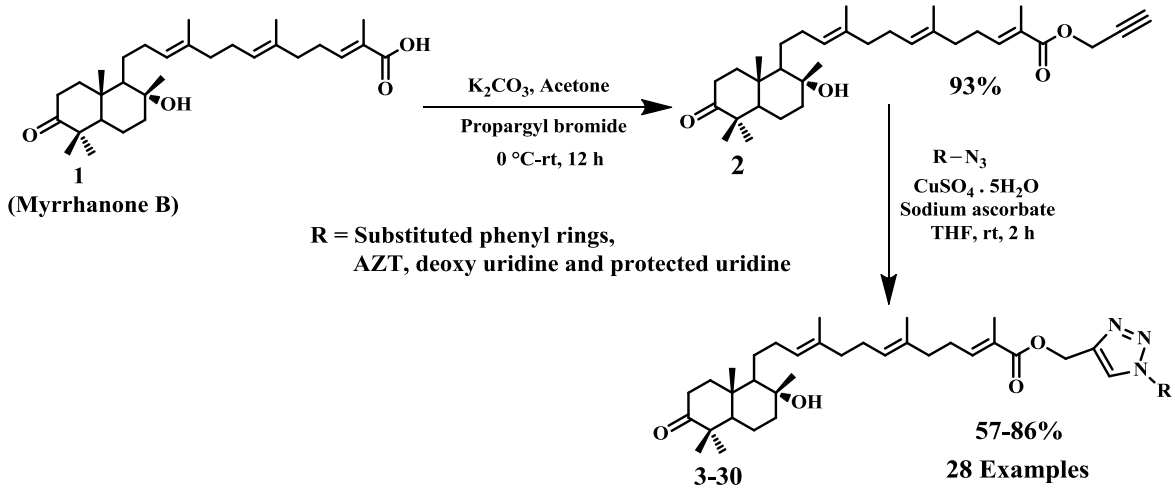
Potent Molecules of Myrrhanone C Hybrids



Results published in *Eur. J. Med. Chem*. 2016, *114*, 293-307

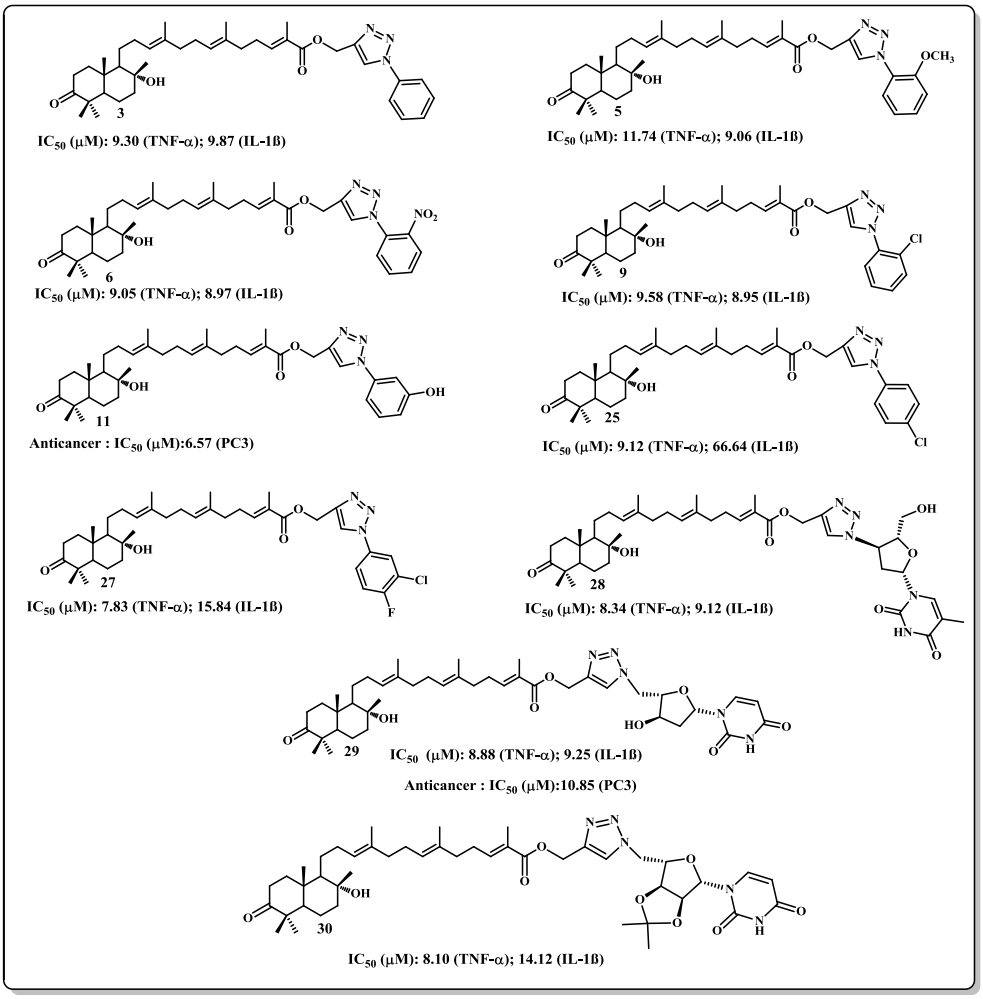
Section B

This section incorporates the synthesis of a series of novel 1,2,3-triazole hybrids of myrrhanone B. The cytotoxic activity of synthesized compounds along with the parent molecule was screened against a panel of seven human cancer cell lines including, A549 (Lung), DU145 (Prostate), MDAMB-231 (Breast), SiHa (Cervical), U87MG (Glioblastoma), PC3 (Prostate) and HT-29 (Colon) along with a normal Lung cell line (L132) by employing MTT assay. The IC_{50} values showed that the triazole (**11**) with a meta hydroxy phenyl ring and triazole (**29**) with a deoxy-uridine moiety exhibited promising inhibitory activities against PC3 cell line with IC_{50} values of 6.57 and 10.85 μM respectively. These compounds were also tested for their anti-inflammatory activity and results indicated that some of the synthesized compounds displayed promising inhibitory activity. Among the tested compounds, **3, 5, 6, 9, 25, 27, 28, 29** and **30** exhibited enhanced inhibitory activity against TNF-α and IL-1β with IC_{50} values in the range of 7.83±0.95-66.64±1.71 μM.



Scheme: 6

Potent Molecules of Myrrhanone B Hybrids



Conclusion

In conclusion, the thesis incorporates the isolation of myrrhanones A, B & C from *Commiphora mukul* gum resin and their chemical transformations to synthesize some novel and diverse chemical structures. In total 106 analogues were synthesized and characterized by their spectroscopic data. The synthesized analogues were evaluated for anticancer and anti-inflammatory activities. The biological results demonstrated that 21 compounds showed potent anti-inflammatory activity against TNF-α and IL-1β. Among these, pyrimidine hybrid of myrrhanone A (Scheme 4: **3l**) found to be the most potent one [IC_{50} (μM): 3.22 (TNF-α)]. Whereas, the anticancer results revealed that the 17 analogues showed potent inhibitory activity against selected human cancer cell lines. Among these, piperidine analogue of myrrhanone A (Scheme 1: **4b**) found to be the most potent one [IC_{50} (μM): 4.65 (MCF-7)].