

The thesis entitled “**Synthetic Studies of Macrolides: Stagonolide-G, Hygrocin-A & Synthesis and Bioevaluation of Nitrogen Containing Heterocyclic Compounds**” has been divided into two parts, **Part A** and **Part B**, and each part is further divided into chapters. Each chapter is further sub-divided into the following sections: Introduction, Present Work, Results and discussion, Experimental, References and Spectroscopic data.

PART A: Deals with the synthesis of macrolide natural products-Stagonolide-G and Hygrocin-A, and is divided into **Chapter I** and **Chapter II**.

Chapter I: Stereoselective Synthesis of Stagonolide-G from D-Mannitol

This chapter deals with the introduction of natural products viz., macrolides, nonenolides and summarizes some of the important approaches towards the stagonolides and stereoselective synthesis of desired stagonolide-G from D-mannitol.

Macrolides, particularly lactones with medium-sized rings (8–10 membered), have continued to attract the attention of both biologists and chemists during recent years, due to their interesting biological properties and scarce availability. Ten membered ring lactones nonenolides, stagonolides B-I (**1-8**) and modiolide A (**9**) are the recent examples (**Figure 1**) which have been isolated in both liquid and solid cultures of *Stagonospora cirsi* Davis, a fungal pathogen isolated from *Cirsium arvense*. Herein, a simple and efficient approach for the total synthesis of stagonolide-G is described from the cheap and easily available starting material, D-mannitol.

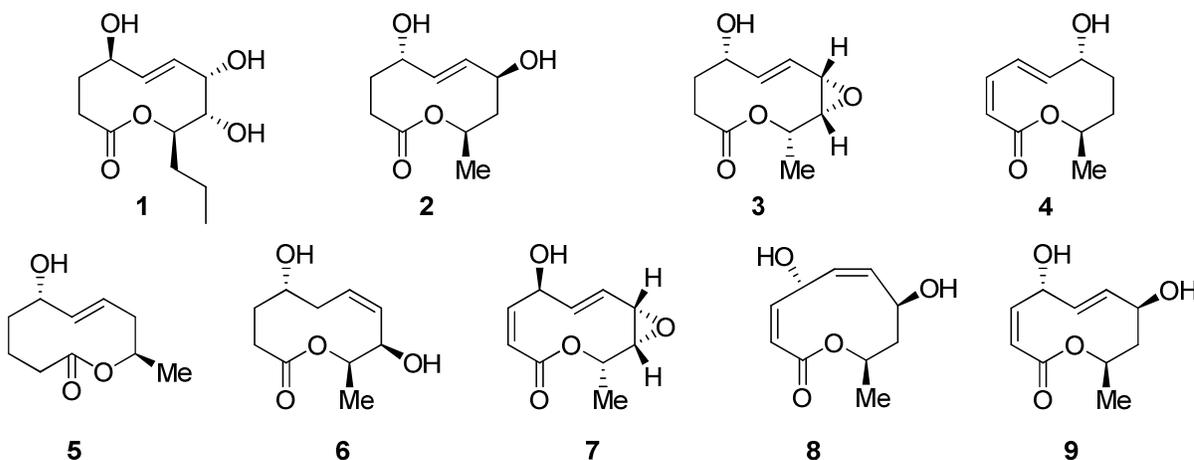
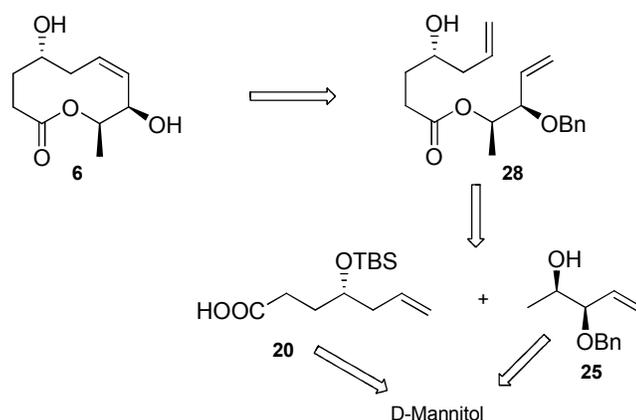


Figure 1: Phytotoxic nonenolides

Retrosynthetic analysis is a technique for solving problems in the planning of organic synthesis with disconnection approach. The retrosynthetic strategy for stagonolide-G is depicted in **Scheme 1**. The retrosynthetic analysis revealed that **6** could be prepared efficiently by a RCM protocol from bis-olefin **28** which in turn could be prepared by Yamaguchi esterification of acid **20** and vinyl alcohol **25**. Both the fragments **20** and **25** could be envisaged from cyclohexylidene-D-glyceraldehyde obtained easily from D-mannitol.

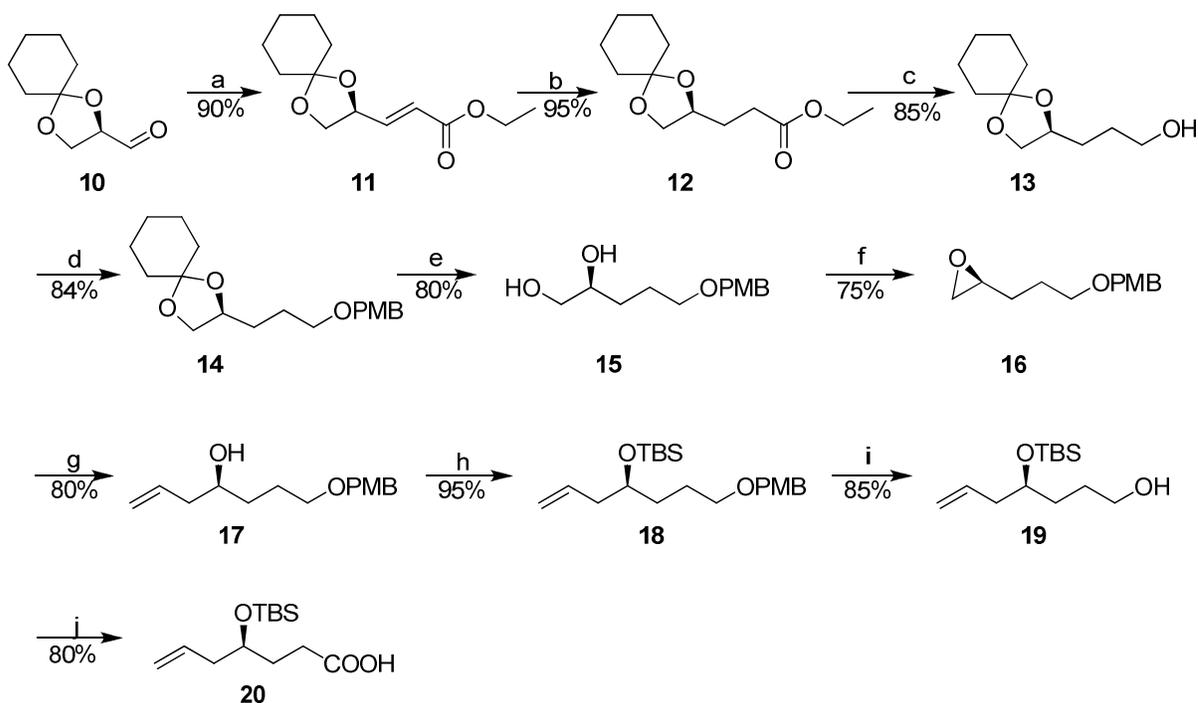


Scheme 1: Retrosynthetic strategy of Stagonolide-G

Synthesis of Acid fragment:

Accordingly, our synthesis began with 2,3-*O*-cyclohexylidene-D-glyceraldehyde **10** which was readily obtained from D-mannitol, subjected to C₂-Wittig olefination under Masamune-Roush conditions to get unsaturated ester **11**. Hydrogenation of **11** with Pd/C, followed by LAH reduction afforded alcohol **13** in high yield. Protection of the alcohol using PMBCl and sodium hydride produced the corresponding ether **14**, which, after subsequent cyclohexylidene deprotection with 1N HCl gave the diol **15**. Selective tosylation of diol **15** with tosyl chloride using triethylamine and a catalytic amount of Bu₂SnO at 0 °C, followed by treatment with K₂CO₃ in MeOH, provided epoxide **16**. The crucial regioselective ring-opening of the epoxide to allyl alcohol **17** was achieved by treatment of **16** with vinylmagnesium bromide and CuI at -20 °C. Silylation of allylic alcohol **17** with TBSOTf/2,6-lutidine afforded the corresponding ether **18**, which was subjected to selective PMB deprotection with DDQ in CH₂Cl₂-H₂O to give **19**. Finally, oxidation of alcohol **19**

either with TEMPO/Bis(acetoxy)iodobenzene (BAIB) or PDC/DMF provided the desired acid fragment **20** in 80% yield (**Scheme 2**).

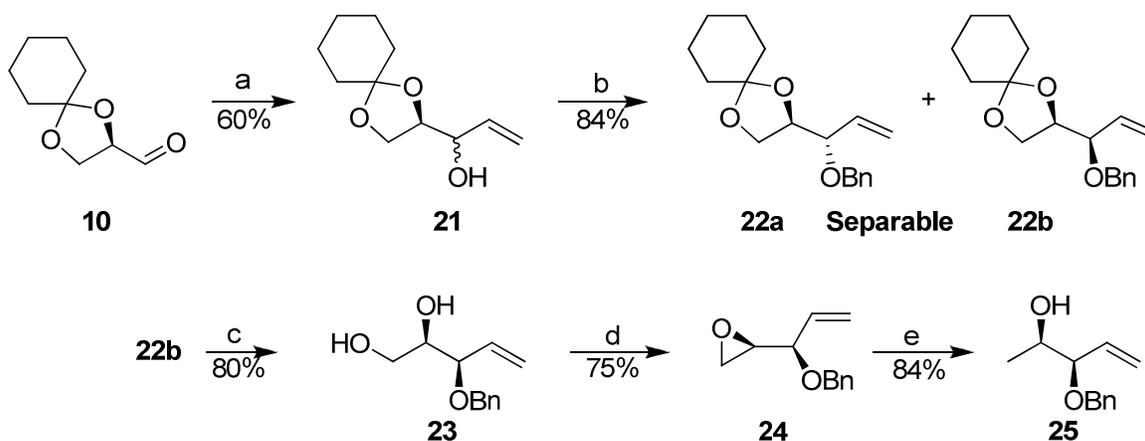


Scheme 2: Reagents and conditions: (a) $(\text{OEt})_2\text{P}(\text{O})\text{CH}_2\text{COOEt}$, LiCl, DIPEA, CH_3CN , rt; (b) Pd/C, H_2 , EtOAc, rt; (c) LiAlH_4 , THF, 0°C -rt; (d) NaH, PMBCl, dry DMF, 0°C -rt; (e) 1N HCl, CH_3CN , rt; (f) i) TsCl, NEt_3 , $n\text{Bu}_2\text{SnO}$, DCM, 0°C -rt; ii) K_2CO_3 , MeOH, 0°C -rt; (g) $\text{CH}_2=\text{CH-MgBr}$, CuI, dry THF, -20°C ; (h) TBDMSOTf, 2,6-lutidine, DCM, 0°C ; (i) DDQ, DCM/ H_2O (10:1), 0°C -rt; (j) TEMPO, BAIB, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1), rt or PDC, DMF, rt

Synthesis of Alcohol fragment:

The synthesis of alcohol fragment **25** is summarized in **Scheme 3**. In this regard, 2,3-*O*-cyclohexylidene-D-glyceraldehyde was treated with vinylmagnesium bromide to give **21** as an almost inseparable 1:1 diastereomeric mixture (from GC-MS analysis); the free hydroxyl group in the mixture of **21** was protected as the corresponding ethers by treatment with benzyl bromide to give compounds **22a** and **22b**, which were separated by silica gel column chromatography. For the assignment of *syn* and *anti* configurations, a small amount of the two compounds **22a/22b** were subjected to cyclohexylidene deprotection separately and the resulting *syn* and *anti* alcohols were compared with the reported spectral and rotation values. Moreover, the *anti* isomer was subjected to debenylation, followed by Mitsunobu reaction to furnish the desired *syn* product. Cyclohexylidene cleavage of the *syn* compound **22b** led to the formation of diol **23**. Subsequent selective tosylation of the primary hydroxyl group **23** with tosyl chloride, triethylamine and a catalytic amount of Bu_2SnO produced the

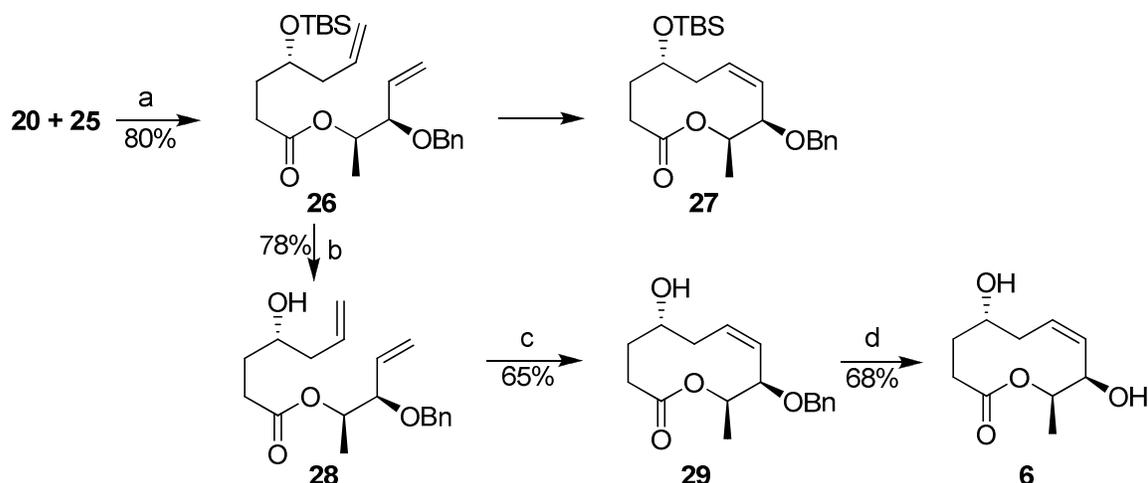
corresponding tosylate, which, on treatment with K_2CO_3 in MeOH, gave epoxide **24**. Finally, epoxide opening with LAH in dry THF afforded alcohol fragment **25** in 84% yield.



Scheme 3: Reagents and conditions: a) $CH_2=CH-MgBr$, dry THF, $0\text{ }^\circ\text{C}$; b) $BnBr$, NaH , $0\text{ }^\circ\text{C}$ -rt-reflux; c) $1N\ HCl$, CH_3CN , rt; d) (i) $p-TsCl$, NEt_3 , nBu_2SnO , rt; (ii) anhyd K_2CO_3 , $0\text{ }^\circ\text{C}$ -rt e) $LiAlH_4$, dry THF, $0\text{ }^\circ\text{C}$ -rt

Condensation of fragments **20** and **25** was achieved under Yamaguchi conditions to furnish bis-olefinic ester **26** in 80% yield. An initial attempt for the typical ring closing metathesis of **26** appeared to be problematic.

After numerous conditions were tried with Grubbs I and Grubbs II catalysts in a range of traditional solvents for metathesis (DCM, benzene, or toluene), the final outcome was the same in all the cases. Either inseparable mixtures of various compounds (along with a small amount of **27**) were obtained or the starting material had decomposed during the course of the reaction. Gratifyingly, after desilylation of **26** under neutral conditions with HF-Pyridine and subsequent olefin metathesis using Grubbs II catalyst in refluxing anhydrous DCM under high dilution conditions, furnished desired lactone **29** in *Z* form. The δ values at 5.4–5.7 ppm in the crude 1H NMR spectrum with a coupling constant of $J_{H-6,H-7} = 11.3\text{ Hz}$, allowed the *Z*-stereochemistry to be assigned for **29**. Finally, deprotection of the benzyl moiety with sodium and liquid ammonia under Birch conditions afforded the target molecule **6** (Scheme 4).



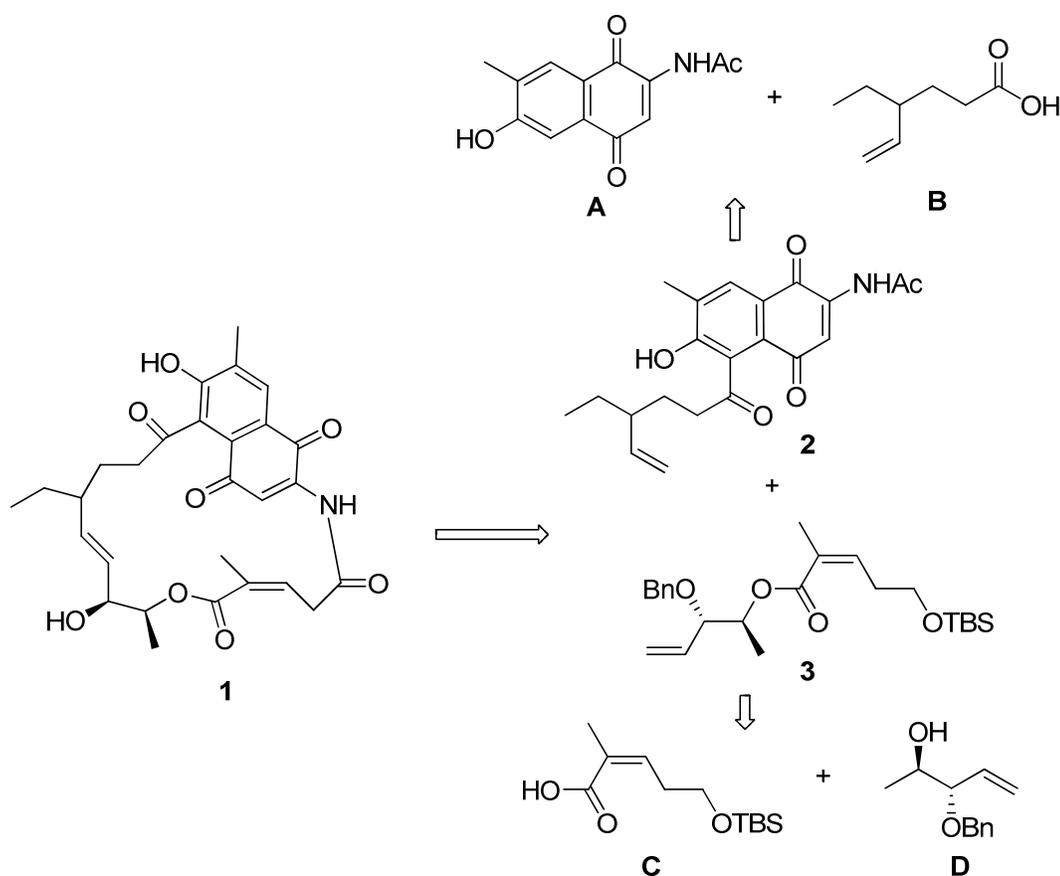
Scheme 4: Reagents and conditions: (a) 2,4,6-trichlorobenzoyl chloride, NEt_3 , dry THF, rt, 2 h, DMAP, toluene, rt; (b) HF-Pyridine complex, dry THF, 0°C -rt; (c) Grubbs' 2nd generation catalyst, dry DCM, reflux; (d) Na, liq. NH_3 , dry THF, -78°C

A simple, convenient and economic route for the stereoselective synthesis of stagonolide-G was developed by employing D-mannitol as a chiral template. This protocol involves the use of a Grignard reaction and RCM as key steps. Both the olefinic acid and olefinic alcohol moieties were constructed using the same chiral pool material, D-mannitol.

Chapter II: Attempted synthesis of ansamycin macrolide, Hygrocin-A

This chapter deals with the introduction to ansamycins, some of the earlier approaches for the synthesis of ansamycin class of natural products and studies towards the synthesis of hygrocin-A.

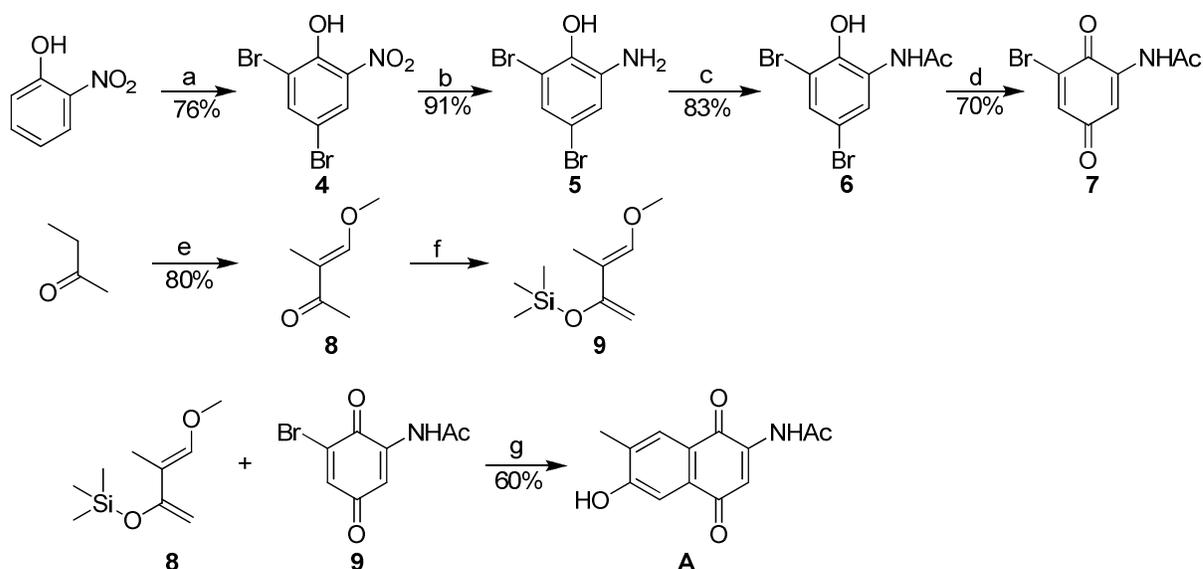
Hygrocin-A (1), a naphthoquinone macrolide was isolated from the fermentation broth of *Streptomyces hygroscopicus* by Ping Cai and co-workers in 2005. Well known antimicrobial agents such as streptovaricins, naphthomycins and rifamycins all belong to this group, and also hygrocin-A ansamycin displaying antimicrobial activity against *Streptococcus pneumonia*, *Aspergillus fumigatus* and *Aspergillus niger* organisms with MIC values 16, 16 and 32 $\mu\text{g}/\text{mL}$ respectively. The molecule consists of three stereogenic centres and a Z-trisubstituted olefin, so it became an attractive target molecule for the total synthesis. We targeted the synthesis in a highly convergent and economic route.



Scheme 1: Retrosynthetic strategy of Hygrocin-A

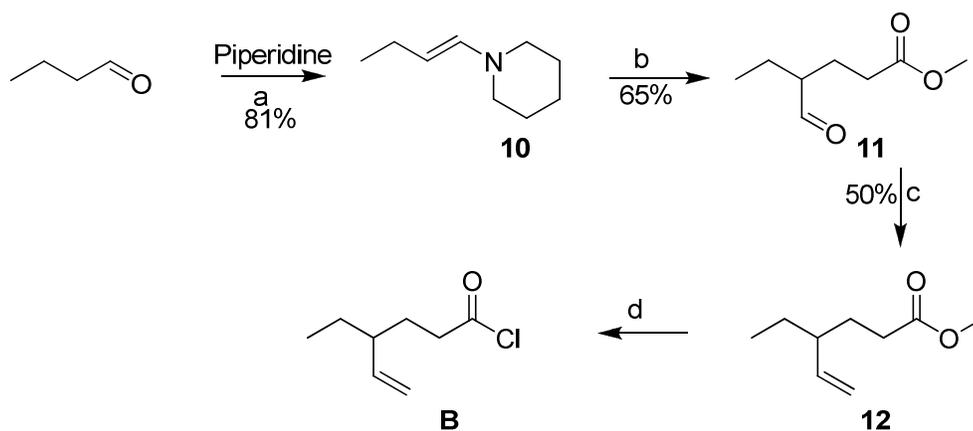
The retrosynthetic strategy of **1** is depicted as in **Scheme 1**. The retrosynthetic analysis revealed that compound **1** could be prepared efficiently by RCM protocol and subsequent coupling reaction of **2** & **3**, which in turn intermediate **2** could be prepared by the acylation of fragment **A** with **B** through Fries rearrangement. Intermediate **3** could be envisaged from the esterification of acid **C** and alcohol **D**. Thus in the present strategy naphthoquinone moiety is achieved through Diels-Alder reaction.

The fragment **A** was synthesized as in **Scheme 2**. The naphthoquinone moiety was attained using a neat Diels-Alder reaction between Danishefsky type diene (**9**) and 2-acetamido-6-bromobenzoquinone (**7**) as dienophile. The requisite diene was prepared from 2-butanone and methyl formate to give enone **8** which on treatment with LDA and TMSCl to afford diene **9**. The Diels-Alder reaction underwent smoothly in anhydrous benzene at room temperature with good yield.



Scheme 2: Reagents and conditions: a) Br_2 , AcOH, rt; b) Fe, HCl, 100 °C; c) $\text{Ac}_2\text{O}/\text{AcOH}$, aq. NaHCO_3 , rt; d) CrO_3 , AcOH, 0 °C; e) (i) NaH, HCO_2Me , THF, 0 °C-rt; (ii) Me_2SO_4 , DMF: H_2O , 50-55 °C; f) anhyd ZnCl_2 , NEt_3 , TMSCl, dry C_6H_6 , rt or LDA, TMSCl, -78 °C-rt; g) dry C_6H_6 , 2% HCl, MeOH, rt

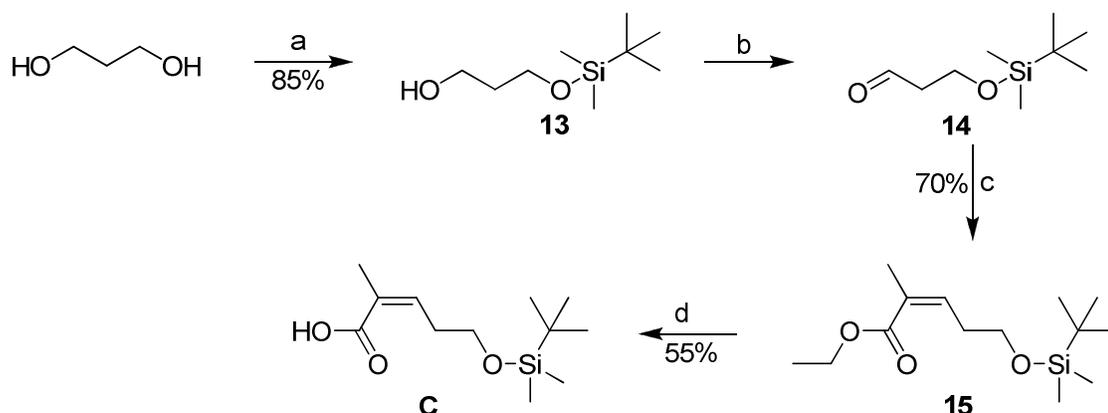
For the synthesis of fragment B, butanal & piperidine are taken as starting materials. Enamine **10** was obtained by the reaction of butanal and piperidine using K_2CO_3 as base and subsequent methyl acrylate treatment followed by hydrolysis afforded 4-formyl ester **11**. C1-Wittig homologation of ester **11** and subsequent saponification provided B (**Scheme 3**).



Scheme 3: Reagents and conditions: a) K_2CO_3 , 0 °C; b) (i) $\text{CH}_2=\text{CH}-\text{CO}_2\text{Me}$, dry CH_3CN , 0 °C-rt-reflux; (ii) AcOH/ H_2O , reflux; c) $\text{PPh}_3^+\text{CH}_3\text{Br}^-$, $n\text{BuLi}$, dry THF, 0 °C-rt; d) (i) LiOH, THF/ H_2O , rt; (ii) $(\text{COCl})_2$, dry CH_2Cl_2 , 0 °C-rt

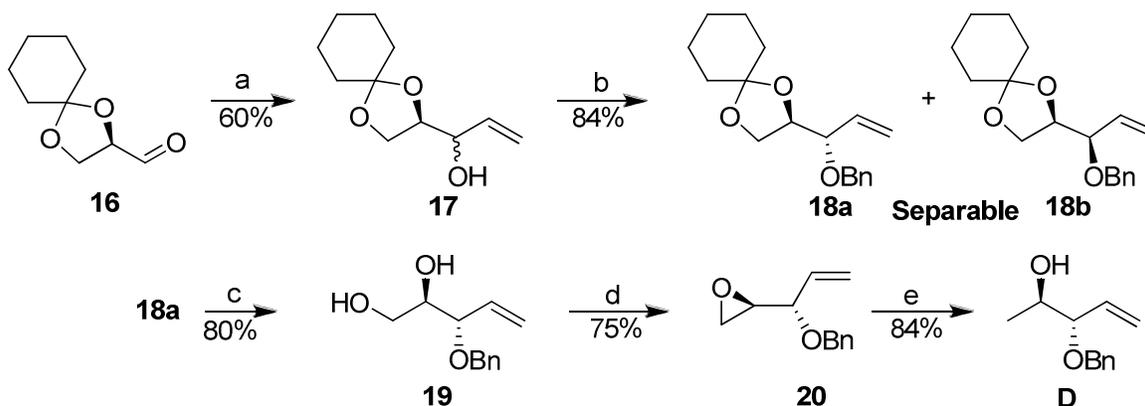
For the synthesis of Fragment C, 1,3-propanediol was subjected to monoprotection using TBSCl and imidazole. The monoprotected alcohol **13** was oxidized to corresponding aldehyde **14** using PCC, the obtained aldehyde on treatment with ethyl 2-(di-*o*-

tolylphosphono)propanoate under modified HWE reaction mentioned as Ando's protocol furnished *Z*-trisubstituted olefin **15**. The diastereoselectivity (*E* & *Z* isomers ratio) was determined from crude ^1H NMR and GC-MS analysis. From the analysis, the ratio was revealed as 85:15 in favour of *Z*-isomer. The *Z*-isomer was separated by chromatography, and saponification of the ester afforded required acid **C** (**Scheme 4**).



Scheme 4: Reagents and conditions: a) TBDMSCl, Imidazole, dry THF, rt; b) PCC, dry DCM, rt; c) (*o*-cresol) $_2$ P(O)CH(CH $_3$)COOEt, NaH, dry THF, 0 $^{\circ}\text{C}$ to -78 $^{\circ}\text{C}$ to rt; d) 2N aq. NaOH, MeOH, rt

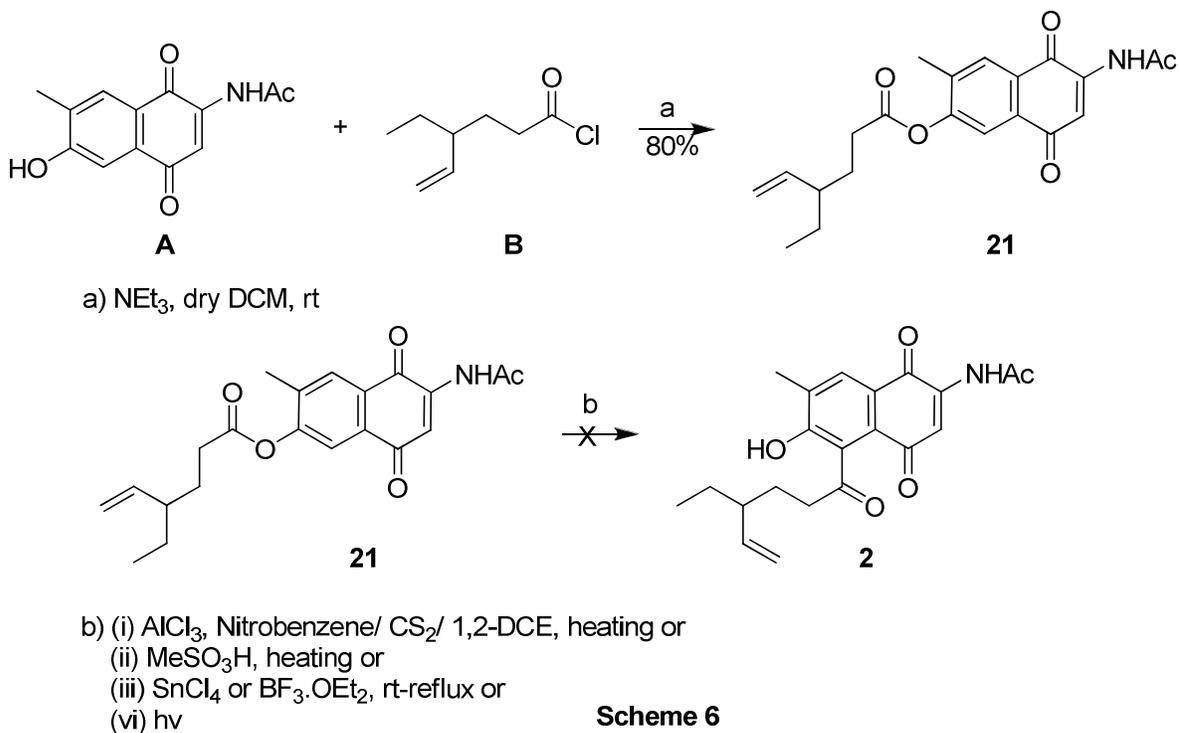
The synthesis of alcohol fragment D was summarized in **Scheme 5**. Accordingly, 2,3-cyclohexylidene-(*R*)-(+)-glyceraldehyde **16** obtained from *D*-mannitol was treated with vinylmagnesium bromide to give **17**, and was treated with benzyl bromide to give compounds **18a** & **18b**, which were separated by column chromatography (as discussed in Chapter I).



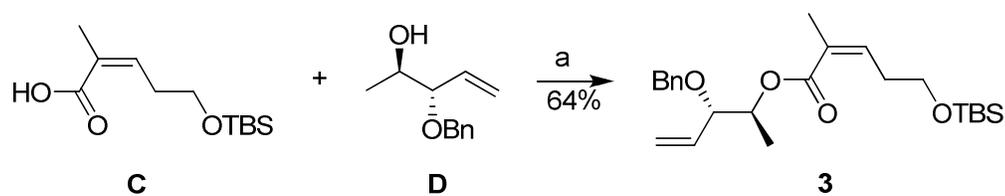
Scheme 5: Reagents and conditions: a) $\text{CH}_2=\text{CH-MgBr}$, dry THF, 0 $^{\circ}\text{C}$; b) BnBr, NaH, 0 $^{\circ}\text{C}$ -rt-reflux; c) 1N HCl, CH_3CN , rt; d) (i) *p*-TsCl, NEt_3 , *n*Bu $_2$ SnO, rt; (ii) anhyd K_2CO_3 , 0 $^{\circ}\text{C}$ -rt e) LiAlH_4 , dry THF, 0 $^{\circ}\text{C}$ -rt

Cyclohexylidene cleavage of compound **18a** led to the diol **19**, the spectral characteristic data of diol were in perfect agreement with the reported spectral data. Selective tosylation of primary hydroxyl group with tosyl chloride, triethyl amine and catalytic amount of Bu_2SnO produced corresponding tosylate, which on treatment with K_2CO_3 in MeOH gave epoxide **20**. Finally, epoxide opening with LAH in dry THF afforded alcohol fragment D in 86% yield. The products are completely characterized by spectroscopic data.

The two fragments are coupled together by treatment of acid chloride **B** on phenol **A** to get the quinone ester **21**. The obtained ester **21** was subjected to Fries rearrangement under various conditions like heating in presence of $\text{AlCl}_3/\text{Nitrobenzene}$ or $\text{AlCl}_3/\text{CS}_2$ or $\text{AlCl}_3/1,2\text{-DCE}$ or using other Lewis acids SnCl_4 or $\text{BF}_3\cdot\text{OEt}_2$, requisite rearranged product **2** was not formed. In all the attempts, starting material had decomposed during the course of the reaction or giving other side products. The same observation was found on methanesulfonic acid (bronsted acid) treatment or under photochemical irradiation in benzene (**Scheme 6**).



On the other hand, the required ester **3** was obtained under standard Mitsunobu conditions (PPh_3 , DIAD) by the treatment of TBS acid **C** with allyl alcohol **D** (**Scheme 7**).



a) PPh₃, DIAD, dry THF, 0 °C-rt

Scheme 7

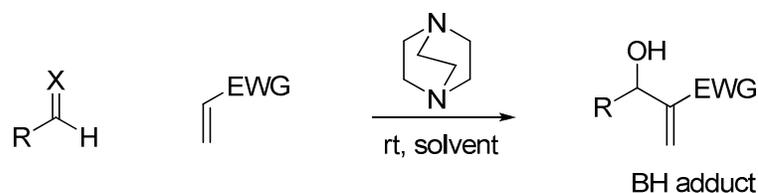
Having met with no success in our effort to obtain the rearranged product, the coupling of the two fragments was incomplete to accomplish the synthesis of Hygrocin-A. Efforts are still continuing for the total synthesis of Hygrocin-A, which has no synthetic reports till now.

PART B: Deals with the synthesis of various heterocyclic compounds and their bioevaluation, and is divided into **Chapter III**, **Chapter IV** and **Chapter V**.

Chapter III: *Synthesis and anticancer activity of tetrazole derivatives from Baylis-Hillman allyl amines.*

This chapter dealt with the introduction to Baylis-Hillman reaction and their applications in cycloaddition reactions and describes the synthesis of new tetrazole derivatives from Baylis-Hillman allyl amines. Furthermore, the synthesized compounds are evaluated for anticancer activity on five cell lines. For the hit compound, DNA binding affinity also studied, assayed by UV-Vis absorption and fluorescence spectroscopic methods.

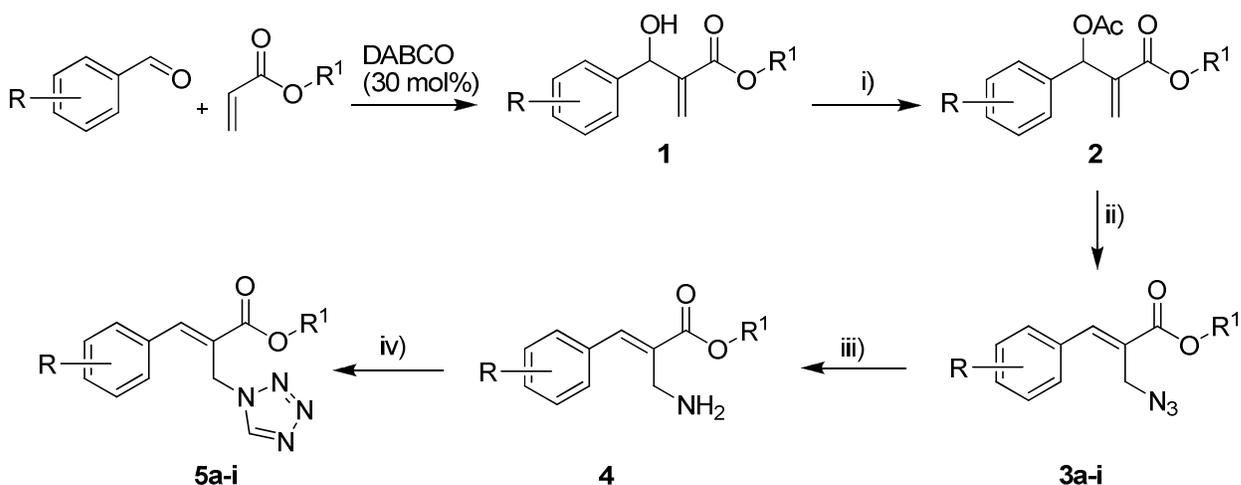
The carbon-carbon bond formation and the functional group transformations are the fundamental reactions for the construction of a molecular framework and hence represent a forefront of research in organic chemistry. Baylis-Hillman reaction is a good example for carbon-carbon bond forming reaction between activated alkenes and aldehydes in the presence of tertiary amine base catalysts, provides a valuable multifunctional compounds are called Baylis-Hillman adducts (**Scheme 1**). Recently a considerable attention was focused on the Baylis-Hillman reaction because, various applications have grown tremendously from the Baylis-Hillman adducts and are a good synthons for making of other important compounds by employing simple alterations.



X = O, NTs
 R = alkyl, aryl, heteroaryl
 EWG = electron withdrawing groups; COR, CN,
 COOR, PO(OEt)₂, SO₂Ph, SO₃Ph, SOPh

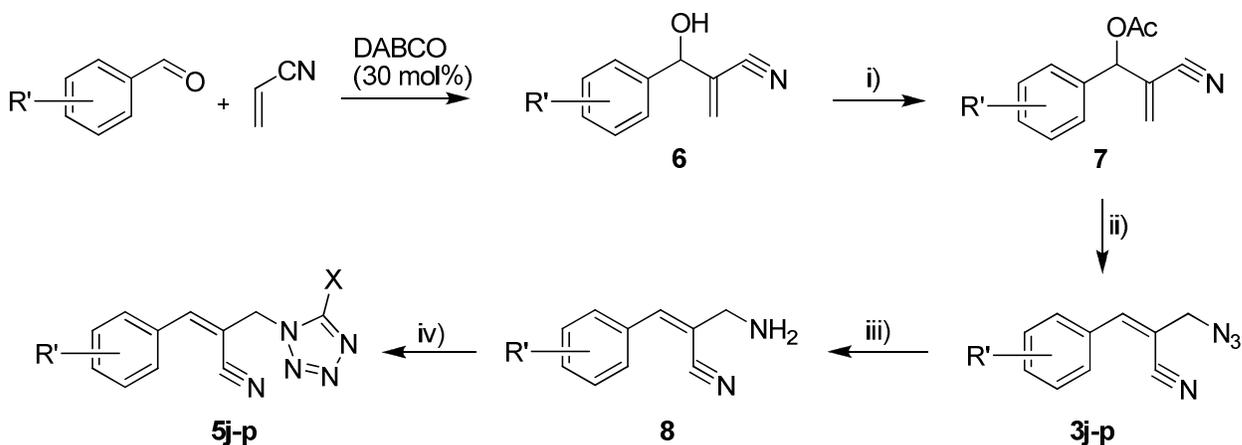
Scheme 1

Heterocyclic compounds have received special attention due to their broad range of biological activities, and many of pharmaceuticals and agrochemical are heterocycles. Tetrazoles and their derivatives possess diverse range of biological activities such as antiviral, antibacterial, antifungal, anti-allergic, anticonvulsant, anti-inflammatory, anticancer and as angiotensin-II inhibitors. In drug design, tetrazoles are regarded as an isoster for the carboxylate group. In continuation of our interest on the applications of Baylis-Hillman chemistry, the efficient synthesis of new tetrazoles from Baylis-Hillman allyl amines is described. These compounds are evaluated for *in vitro* anticancer activity against five cell lines.



Scheme 2: Reagents and conditions. (i) CH₃COCl, Pyridine, DCM, 0 °C-rt; (ii) NaN₃, DMSO, rt; (iii) Zn, NH₄Cl, EtOH/H₂O (3:1), rt; (iv) HC(OEt)₃, NaN₃, AcOH, 90 °C

R=H, R¹=Me (**5a**); R=*p*-Cl, R¹=Et (**5b**); R=*p*-Me, R¹=Me (**5c**); R=*p*-F, R¹=Me (**5d**); R=*p*-Br, R¹=Et (**5e**); R=*p*-NO₂, R¹=Me (**5f**); R=*p*-OMe, R¹=Me (**5g**); R=*o*-NO₂, R¹=Me (**5h**); R=*o*-Br, R¹=Et (**5i**)



Scheme 3: Reagents and conditions. (i) CH_3COCl , Pyridine, DCM, $0\text{ }^\circ\text{C}$ -rt; (ii) NaN_3 , DMSO, rt; (iii) Zn , NH_4Cl , $\text{EtOH}/\text{H}_2\text{O}$ (3:1), rt; (iv) $\text{HC}(\text{OEt})_3$ or $\text{CH}_3\text{C}(\text{OEt})_3$, NaN_3 , AcOH , $90\text{ }^\circ\text{C}$

$\text{R}'=\text{H}$, $\text{X}=\text{H}$ (**5j**); $\text{R}'=p\text{-OMe}$, $\text{X}=\text{H}$ (**5k**); $\text{R}'=p\text{-CF}_3$, $\text{X}=\text{H}$ (**5l**); $\text{R}'=p\text{-Me}$, $\text{X}=\text{H}$ (**5m**); $\text{R}'=\text{Nap}$, $\text{X}=\text{H}$ (**5n**); $\text{R}'=\text{Nap}$, $\text{X}=\text{Me}$ (**5o**); $\text{R}'=p\text{-F}$, $\text{X}=\text{Me}$ (**5p**)

The efficient preparation of new 1-substituted tetrazoles from allyl amines of the Baylis-Hillman adducts is described as shown in the **Scheme 2 & 3**. Synthesis of allyl amines is achieved in a convenient manner from respective azides derived from acetates of Baylis-Hillman adducts using $\text{Zn}/\text{NH}_4\text{Cl}$ (**Scheme 2 & 3**). The allyl amines obtained are directly used for the protocol of targeted tetrazoles. The amines are treated with triethyl orthoformate and sodium azide in the presence of acetic acid to get the dipolar cycloaddition product, 1-substituted 1H-1,2,3,4-tetrazole. 1,5-Disubstituted tetrazoles are obtained while using triethyl orthoacetate instead of triethyl orthoformate. Stereochemistry of obtained tetrazoles having ester functionality is predicted as *E* and nitrile functionality as *Z*-configuration. Following their reports and according to ^1H NMR data of prepared azides, we finally envisaged that the stereochemistry of ester containing functional groups is *E* and nitrile containing functional groups is *Z*. The configuration of double bond and structure is also confirmed by NOESY experiments and single X-ray crystallographic data for the compounds **5h** and **5m**.

The *in vitro* cytotoxicity of tetrazole derivatives were determined in selected human cancer cell lines of liver carcinoma (Hep-G2), lung adenocarcinoma (A-549), breast (MDA-MB-231), prostate carcinoma (DU-145), neuroblastoma (SK-N-SH) origin. We evaluated the IC_{50} in vitro growth inhibitory values of the compounds under study by means of the

sulforhodamine B (SRB) colorimetric assay. The IC₅₀ values of all the 16 compounds are listed in the **Table 1**.

Table 1. IC₅₀ values of Tetrazole Compounds in μM Conc. against various cell lines

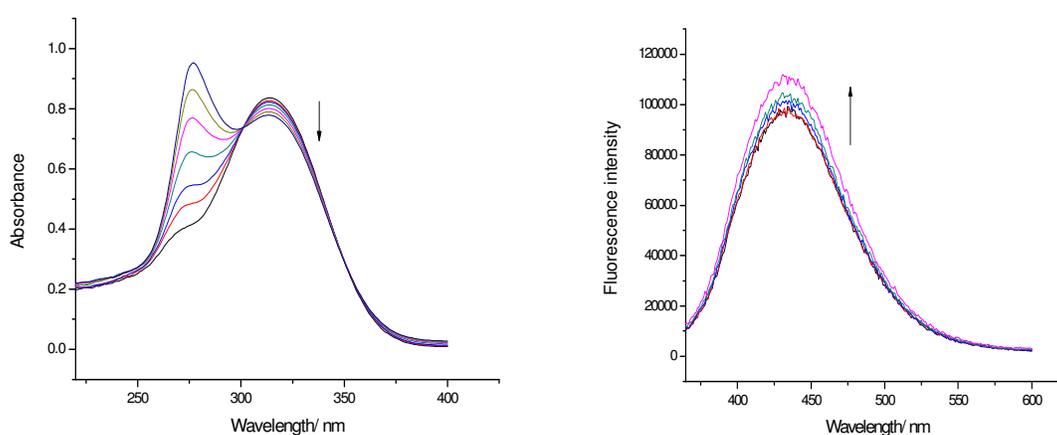
Compound	Cell lines (IC ₅₀ values; $\mu\text{M} \pm \text{SD}^{\text{a}}$)				
	Hep G2	A549	MDA-MB-231	DU145	SK-N-SH
5a	10.73 \pm 1.2	7.91 \pm 0.94	16.35 \pm 1.8	9.02 \pm 1.14	31.6 \pm 5.43
5b	3.0 \pm 0.34	3.18 \pm 0.43	23.9 \pm 2.21	8.44 \pm 0.76	28.2 \pm 3.5
5c	10.73 \pm 1.2	16.03 \pm 2.1	62.64 \pm 1.4	8.42 \pm 0.93	71.67 \pm 1.3
5d	12.07 \pm 1.25	6.9 \pm 0.59	15.9 \pm 1.3	8.57 \pm 0.82	18.33 \pm 2.4
5e	4.89 \pm 0.6	4.78 \pm 0.64	18.94 \pm 2.04	5.5 \pm 0.64	27.34 \pm 3.37
5f	2.07 \pm 0.41	2.84 \pm 0.9	9.14 \pm 1.26	5.5 \pm 0.84	22.26 \pm 1.9
5g	6.65 \pm 0.87	3.83 \pm 1.2	21.03 \pm 2.41	5.6 \pm 0.48	7.9 \pm 1.3
5h	4.33 \pm 0.65	6.88 \pm 0.84	43.67 \pm 5.2	10.18 \pm 1.23	19.82 \pm 1.7
5i	3.33 \pm 0.42	5.94 \pm 0.49	14.78 \pm 1.84	7.79 \pm 0.87	16.26 \pm 2.1
5j	5.83 \pm 0.43	9.99 \pm 1.32	18.35 \pm 2.1	9.45 \pm 1.3	23.26 \pm 3.02
5k	27.39 \pm 3.7	23.79 \pm 2.86	20.3 \pm 1.9	19.41 \pm 2.37	64.2 \pm 2.1
5l	1.65 \pm 0.42	3.51 \pm 0.58	8.7 \pm 1.05	5.92 \pm 0.54	11.79 \pm 1.54
5m	6.56 \pm 0.82	7.95 \pm 0.93	25.65 \pm 2.38	7.3 \pm 0.85	20.12 \pm 2.7
5n	7.11 \pm 0.85	5.7 \pm 0.63	31.4 \pm 3.42	7.36 \pm 0.91	16.86 \pm 1.95
5o	2.60 \pm 0.61	2.95 \pm 0.52	14.1 \pm 1.62	5.76 \pm 0.68	13.7 \pm 1.5
5p	7.29 \pm 0.94	6.4 \pm 0.82	28.76 \pm 3.2	7.19 \pm 0.62	20.16 \pm 2.8
Nocodazole	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Podophyllotoxin	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

^aStandard deviation; All experiments were independently performed three times

The data in **Table 1** also reveal that out of 16 compounds under study, the compounds **5b**, **5f**, **5l** and **5o** displayed the highest *in vitro* anticancer activity on both liver carcinoma (Hep G2) and lung adenocarcinoma (A 549) cell lines. Compounds **5e**, **5f**, **5g** and **5o**

displayed significant activity against prostate (DU 145) cell line. The substituents having *p*-trifluoromethyl, *p*-chloro and *p*-nitro on the phenyl ring and 1,5-disubstituted tetrazole moiety displayed more activity compared to other compounds. All the compounds have shown moderate to good anticancer activity on both breast and neuronal cell lines.

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. Hence, DNA binding studies for the hit compound **5o** assayed by UV-Vis absorption and fluorescence spectroscopic methods. From the results of the experiments carried out indicate the compound interacting with the ct DNA (**Figure 1**).



UV-vis absorption spectra of tetrazole
[DNA] = 0, 10, 20, 40, 60, 80, 100 μ M

Fluorescence emission spectra
[DNA] = 0, 50, 100, 150, 200 μ M
after excitation at λ_{exc} 315 nm

Figure 1. DNA binding affinity of tetrazole **5o**

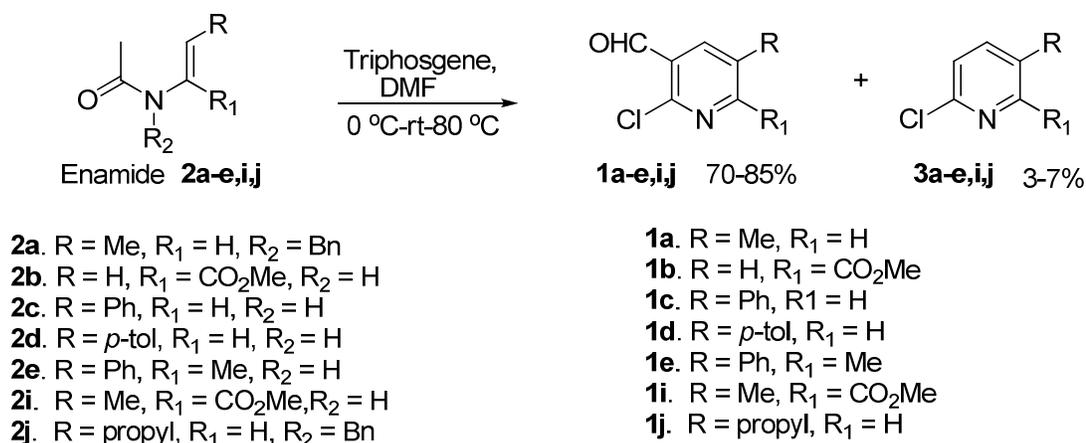
In conclusion, efficient reaction conditions are disclosed for the synthesis of new tetrazole derivatives from Baylis Hillman allyl amines. The compounds were found to exert cytotoxic activity on the five human cancer cell lines. Compounds **5b**, **5f**, **5l**, and **5o** are particularly more active than remaining compounds against liver carcinoma (Hep G2) and lung adenocarcinoma (A 549) cancer cell lines. Compounds **5e**, **5f**, **5g** and **5o** displayed significant activity against prostate (DU 145) cancer cell line. The hit compound **5o** could bind to DNA and form stable complex, may act as a potential genotoxic agent for cancer therapy. Overall, these compounds are promising agents since only a very few tetrazole derivatives are described in the literature as having anticancer activity.

Chapter IV: Facile synthesis of thieno[2,3-*b*]pyridine derivatives from 2-chloro nicotinaldehydes as antimicrobial agents.

This chapter dealt with the introduction to thienopyridines, summarizes some of the earlier methods to thienopyridines synthesis and describes the synthesis of thieno[2,3-*b*]pyridine derivatives and their evaluation of antimicrobial activity.

Considering this recent interest in 2,3-carbon disubstituted thieno[2,3-*b*]pyridines, the development of methods which allow access to the biologically active compounds is an area of continuing research. In this chapter, a protocol for preparation of a wide variety of 2,3-carbon disubstituted thieno[2,3-*b*]pyridines from readily accessible 2-chloro nicotinaldehydes and methyl thioglycolate was described. The synthesized thienopyridines are evaluated for *in vitro* antimicrobial activity against various organisms.

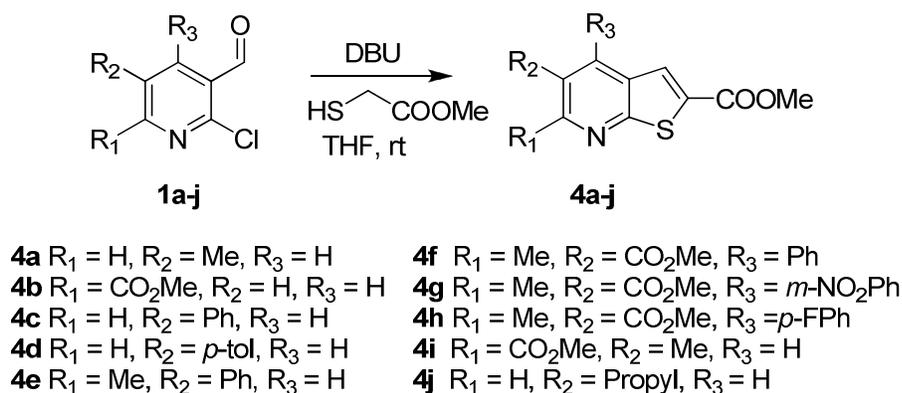
Initially various substituted 2-chloronicotinaldehydes (**1a-j**) which are one of the starting materials for the synthesis of new thieno[2,3-*b*]pyridines were synthesized using modified Vilsmeier cyclisation conditions from various enamides according to our reported procedure in good yields. The synthesized substituted 2-chloronicotinaldehydes are listed in **Figure 1**. Thus various enamides (**2a-e,i,j**) were prepared in different methods involving different starting materials and subjected to modified Vilsmeier reaction leads to corresponding substituted 2-chloronicotinaldehydes (**Scheme 1**).



Scheme 1

Synthesis of thieno[2,3-*b*]pyridines (**4a-j**) are achieved efficiently from 2-chloro nicotinaldehydes by the treatment of methyl thioglycolate using DBU as mild base. During

the optimization studies, the reaction was carried out in different bases such as NaH, K₂CO₃, NaOMe in MeOH, NEt₃, and also with hindered amines DABCO and DBU. It was observed that only with DBU, the desired thieno derivatives are obtained at ambient temperature in very good yields. The reaction failed to occur in other bases giving different side products along with a little amount of required product. In order to evaluate the generality of this method, several analogues of substituted thienopyridines were synthesized with different 2-chloronicotinaldehydes (**Scheme 2**). The reactions proceeded very efficiently with good yields in less reaction time. To demonstrate general utility of the method, we applied these conditions to a variety of 2-chloro quinoline-3-carbaldehydes (**4k-l**) prepared according to earlier reported procedure. In all the cases, the reactions occurred smoothly affording corresponding thieno[2,3-*b*]quinolines in good yields.



Scheme 2

All the synthesized new compounds (**4a-j**) were screened for their antimicrobial activity by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards. The antibacterial activity is tested on six different organisms (Gram-positive and Gram-negative), *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with respect to the references Penicillin and Streptomycin. Interestingly, out of six organisms, almost all the thienopyridines displayed good antibacterial activity especially against the most compromising resistant strain, *P. aeruginosa*. Compound **4j** exhibited considerable antibacterial activity against *S. eperidimis*. Compound **4a** showed moderate activity against several strains. Some of the thienoquinolines exhibited moderate activity against *K. pneumoniae* organism. Minimum inhibitory concentration (MIC) in µg/mL values

for all the compounds are listed in **Table 1**. It is observed that most of the thienopyridine derivatives exhibited good antibacterial activity compared to the thienoquinoline derivatives.

All the compounds were also screened for their antifungal activity against two representative microorganisms *Yeast* and *Filamentous fungi* viz. *Candida albicans*, *Candida rugosa*, *Saccharomyces cerevisiae*, *Aspergillus flavus* with respect to standard Amphotericin B (50) by paper disc diffusion method. Zone of inhibition (mm) were determined for the compounds and the screening results indicate that the compounds **4a**, **4b** and **4j** of all the compounds exhibited moderate antifungal activity. Compounds **4a** and **4b** exhibited antifungal activity especially on *S. cerevisiae* strain, whereas compound **4j** have antifungal effect against *C. albicans* and *C. rugosa* strains.

Table 1. Antibacterial activity of prepared thieno[2,3-*b*]pyridine derivatives:

Compound	MIC ($\mu\text{g/mL}$)					
	<i>B. subtilis</i>	<i>S.aurues</i>	<i>S.epidermidis</i>	<i>E. coli</i>	<i>P.aeruginosa</i>	<i>K. pneumoniae</i>
4a	75	75	150	75	18.75	150
4b	150	150	150	150	9.375	150
4c	150	150	150	150	9.375	150
4d	150	150	150	150	37.5	150
4e	150	150	150	150	18.75	150
4f	150	150	150	150	9.375	150
4g	150	150	150	150	37.5	150
4h	150	150	150	150	9.375	150
4i	150	150	150	150	18.75	150
4j	150	150	37.5	150	18.75	150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

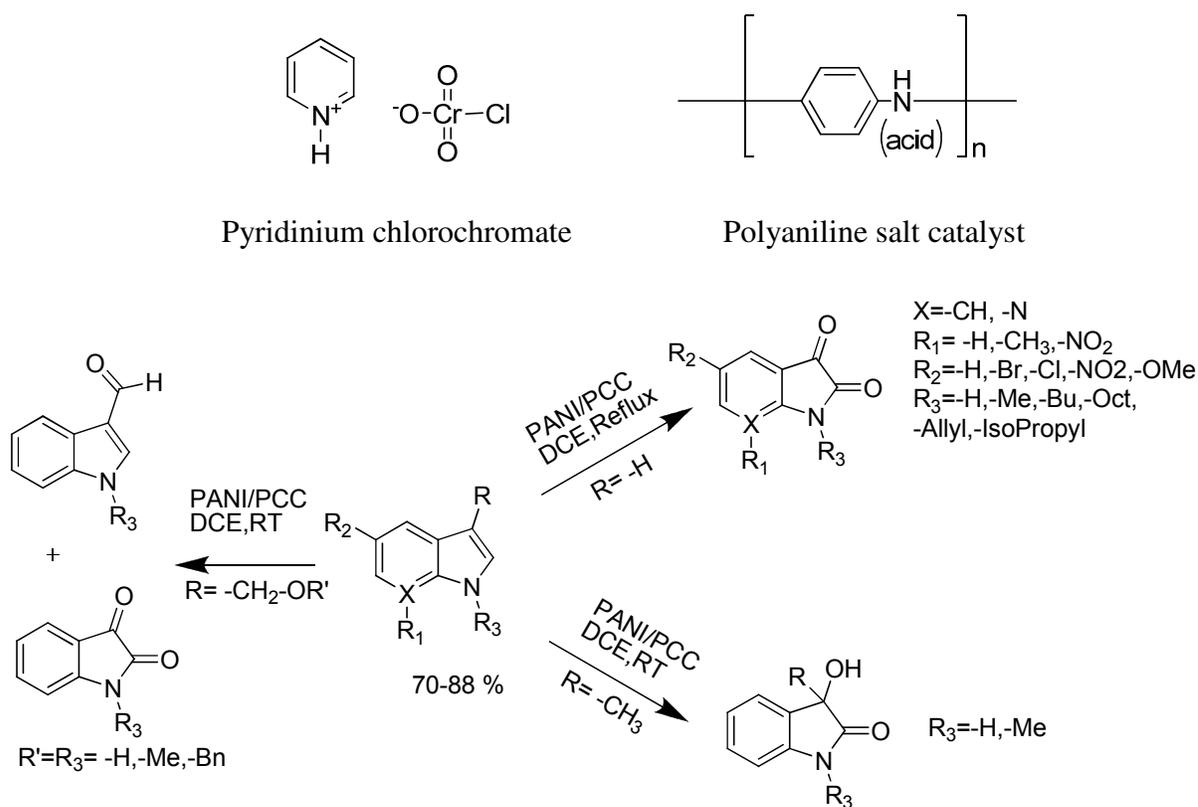
In conclusion, various new thienopyridine derivatives were synthesized in an efficient manner using DBU as mild base. The other bases failed to give desired products and the reaction consists simple workup procedure, good yields and mild conditions. All the

synthesized compounds were screened for *in vitro* antimicrobial activity against several organisms and most of the compounds exhibited very good antibacterial activity against *P.aeruginosa* strain.

Chapter V: Synthesis of biologically active Isatin derivatives from oxidation of Indoles using PCC/PANI combination.

This chapter dealt with the introduction to isatins, summarizes some of the earlier methods to synthesis of isatins from indoles and its derivatives and describes the novel synthesis of biologically active isatin derivatives from indoles using PCC/PANI combination.

As part of our ongoing development of efficient protocols for the preparation of organic compounds, we recently used polyaniline salt (PANI) catalyst as simple, mild, and reusable polymer-based acid catalyst for the synthesis of quinoxalines, benzimidazoles and benzodiazepines. Herein, a novel and efficient method for the conversion of indoles into isatins is disclosed. Pyridinium chlorochromate (PCC) was used for the first time as oxidizing agent for the oxidation of indoles to isatins.



Scheme 1

Interestingly, oxidation of 3-alkyl indoles by this procedure gave 3-hydroxy-3-alkyl oxindoles. On the other hand, indol-3-alkanols gave mixtures of isatins and 3-formyl indoles (**Scheme 1**).

In summary, a new method has been reported for the preparation of isatins and oxindoles by the oxidation of indoles using PCC catalyzed by polyaniline salt for the first time. The advantages of the polyaniline salt catalyst are ease of synthesis and handling, versatility, simple workup procedure, mildness and recyclability. The use of an inexpensive and water tolerant reagent system makes this procedure quite simple, more convenient, and environmentally friendly for the preparation of synthetically and biologically potent isatins in a one-pot operation.