SMALL MOLECULES MODULATORS OF HEDGEHOG -GLI (Hh) PATHWAY AS POTENTIAL THERAPEUTIC AGENTS

RAMANUJ (091756)

ACHYUT (091757)

UNDER THE SUPERIVISION OF DR. NEERAJ MAHINDROO

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

May-2013

Submitted in partial fulfillment of the Degree of Bachelor of Pharmacy

DEPARTMENT OF PHARMACY

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT

CONTENTS

CERTIFICATE

This is to certify that the thesis entitled "SYNTHESIS OF SMALL MOLECULES MODULATORS OF HEDGEHOG -GLI (Hh) PATHWAY AS POTENTIAL THEREPEUTIC AGENTS", submitted by **Achyut Kathuria** (091757) and **Ramanuj Sharma**(091756) in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy to the Jaypee University of Information Technology, Waknaghat is a record of bonafide research work carried out by them under my supervision and guidance. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

27/05/2013

Dr. Neeraj Mahindroo
Associate Professor
Department of Pharmacy
Jaypee University of Information Technology
Waknaghat, Solan
Himachal Pradesh - 173234

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the possibility to complete this project. I want to thank the **Department of Pharmacy, Jaypee University of Information Technology,Wakhnaghat**, for giving me permission to commence this project in the first instance, to do the necessary research work and to use departmental data. We would like to extend our grateful thanks to **Prof. R.S.Chauhan,** Head, Department of Pharmacy, for providing the best facilities during the project. He gave and confirmed this permission and encouraged me to go ahead with my project.

I am deeply indebted to my supervisor **Dr.Neeraj Mahindroo** JUIT whose help, stimulating suggestions and encouragement helped me in all the time of research for and writing of this project report.

My seniors from the Department of Pharmacy supported me in my research work. I want to thank them for all their help, support, interest and valuable hints. Especially, I am obliged to Dr. Kuldeep Singh and Dr. Uday banu for all their guidance on this project. I would like to give my special thanks to God and my Parents whose patience and love enabled me to complete this work.

SUMMARY

The Hedgehog (Hh) signaling pathway plays an important role in the regulation of cell differentiation and organ formation during normal vertebrate embryonic development, ensuring that developing tissues attain their correct size, location, and cellular content.[13, 14]

Hh pathway becomes inactive in most adult tissues; with the exception of role in tissue maintenance and repair.[13]Inappropriate reactivation of the pathway in adult tissues has been linked to the development of several human cancers.[13] The Hh signaling pathway therefore represents a potential therapeutic target for new anticancer treatments. On the other hand, the Hh pathway agonists might have therapeutic role in neurodegenerative diseases and cerebral ischemia by neuroprotection and neuroregenration.

We designed and synthesized small molecules modulator of Hedgehog Gli (Hh) pathway as potential therapeutic agents based on a hit discovered during design of Hh-Gli pathway inhibitors.(Mahindroo*et al.* unpublished data) Different amines and benzoic acids were coupled to prepare eight compounds which were purified by column chromatography and characterized by proton NMR.

LIST OF FIGURES AND TABLES

LIST OF ABBREVIATION

Hh-Hedgehog

Shh-Sonic Hedgehog

Ihh-Indian Hedgehog

Dhh-Desert Hedgehog

PNS-Peripheral Nervous System

CNS-Central Nervous System

Ptch-Patched

GNPS-Granule Neuron Precursors

Smo- Smoothend

CGNP-Cerebellar Granular Neuron Precursors

Ci-CubitusInterrutus

HSC-Hedgehog Signaling Complex

Fu-Fused

Sufu-Suppressor of Fused

NMR-Non Magnetic Resonance

TLC-Thin Layer Chromatography

TEA-Tri Ethyl Amine

DIPEA- Diisoporpyl ethyl amine

DMF-Di Methyl Formamide

HBTU-O-Benzotriazole-N, N, N, N-Tetra-Methyl-Uronium-Hexafluro-Phosphate

1.0 INTRODUCTION

1.0.1 Glossary and Abbreviations:-

- **Hedgehog (Hh):** It belongs to the family of protein ligands that regulate cell proliferation and differentiation during embryonic development [2].
- **Smoothened (SMO):** A seven-transmembrane GPCR (G-protein coupled receptors)-like protein that transmits the Hh signal upon stimulation, leading to activation of the Gli transcription factors.
- **Glioma-associated oncogene (Gli):** **Gli1** is a protein originally isolated in human glioblastoma. **Zinc finger protein Gli2** also known as **Gli family zinc finger 2** is a protein that in humans is encoded by the *GLI2*gene. The protein encoded by this gene is a transcription factor. **Zinc finger protein Gli3** is a protein that in humans is encoded by the *GLI3*gene. This gene encodes a protein that belongs to the type of zinc finger proteins subclass of the Gli family. They are characterized as DNA-binding transcription factors and are mediators of sonic hedgehog (Shh) signaling[2, 4].
- **Patched (PTCH):** The Hh receptor, a 12-transmembrane protein found at the cell surface or in primary cilia that binds to Hh to initiate ligand-dependent signaling [2].
- **Sonic Hedgehog (SHh):**It is one of the three proteins in the mammalian signaling pathway family called hedgehog. SHh is the best studied ligandof the hedgehog signaling pathway. It plays a key role in regulating vertebrate organogenesis, such as in the growth of digits on limbs and organization of the brain.
- **INDIAN HEDGEHOG (IHh):**It is a protein which in humans is encoded by the IHh gene.
- **Desert Hedgehog(DHh):**It is a gene .The protein encoded by this gene is involved in cell signaling.It is named for the Desert Hedgehog.

CHAPTER 2: REVIEW OF LITERATURE

2.0 THE HEDGEHOG(Hh) PATHWAY

In a growing embryo, cells develop differently in the head or tail end of the embryo. The hedgehog pathwayhelps in forming segments which develop into different body parts. This pathway gives cells information needed to develop the embryo properly. Different parts of the embryo have different concentrations of hedgehog signaling proteins. The pathway also has role in repair in the adults. The pathway malfunctioning leads to diseases like basal cell carcinoma.[1]The pathway takes its name from its polypeptide ligand, an intercellular signaling molecule called Hedgehog (*Hh*) found in fruit flies of the genus Drosophila. Hedgehog, a Drosophila's segment polarity gene product, is involved in establishing the basis of the fly body plan. The hedgehog molecule is also important in embryogenesis and metamorphosis.This pathway is a major regulator of embryonic development.Mutations that decrease its activity have been associated with severe defects in nervous system development. Hedgehog continues to function in normal as well as disease dadult tissues, regulating both cell proliferation and the production of growth and angiogenic factors. Thus modulation of hedgehog signaling might provide therapeutics for neural diseases, including neurodegenerative disorders; and brain tumors, particularly medulloblastoma.[2]The following sections describe the discovery and utility of small molecule agonists and antagonists of this pathway and their potential as novel types of therapeutics.

The hedgehog (Hh) pathway is one of a small collection of pathways that control the number and types of cells formed during development in species ranging from *Drosophila* to humans. The most well studied Hh-pathway ligand, sonic hedgehog (Shh), has been shown to participate in central nervous system (CNS) development.Another ligand, desert hedgehog (DHh), is essential for the proper formation of the peripheral nervous system (PNS)[2] .

2.0.1Hedgehog Pathway Regulation[9]

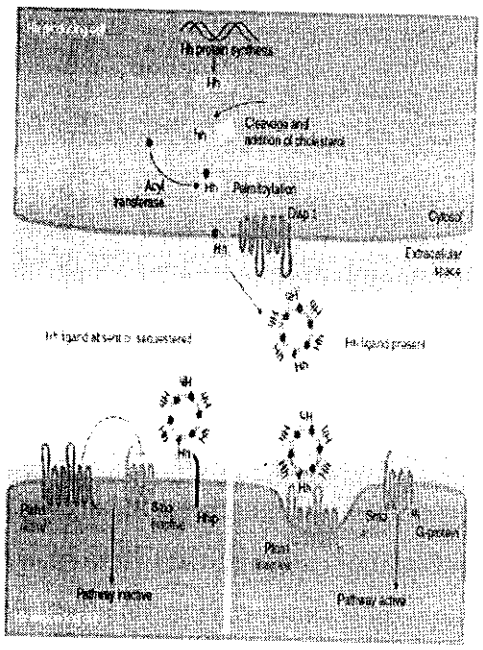


Figure 1: Hh pathway regulation

The regulation of Hh protein is through transduction mechanism. Following translation, the full length Hh protein auto catalytically cleaves and a cholesterol moiety is added in the process. Further, palmitoyl group is added by a dedicated acyl transferase. There secretion into the extracellular space in the form of multimers is mediated by the action of Disp1. In the absence of Hh ligand, or when Hh is sequestered by an inhibitory protein like Hhip, Ptch1 represses Smo and the downstream pathway is inactive. In the presence of Hh that is free to bind to Ptch1, the repression of Ptch1 on Smo is released and Smo activates downstream pathway components through G-proteins [9] which mediates the gene expression by controlling transcription factors.

2.0.2 HEDGEHOG PATHWAY Regulation

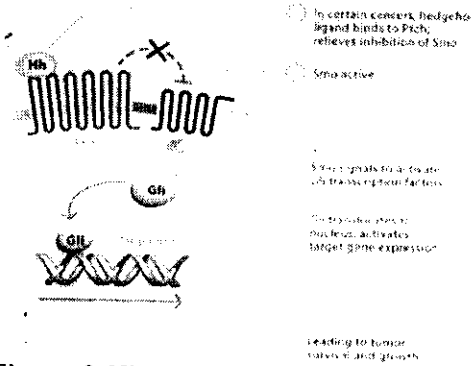


Figure 2: Hh pathway role in cancer

The signaling is initiated by the biding of the Hedgehog ligand to the receptors present on Patched(Ptch) which is 12-transmemberane protein receptor this activates smoothened (SMO) a 7-membrane spanning receptor.Downstream of SMO is multi-protein complex known as HSC(Hedgehog signaling complex) which comprises of transcriptional factors Gli. Hence transcriptional factors are activated.

The Gli translocates to the nucleus and activates gene expression of CyclinD1 and BCL hence due to over expression of this gene can lead to growth of tumor and other dysfunctions.

2.1The role of the Hedgehog pathway in embryonic development[20]

It functions by time- and position-dependent expression patterns to ensure that developing tissues attain their correct size, location and cellular content.

Skin

It is responsible for forming a variety of epidermal structures that differ among vertebrates. During the development of a normal hair follicle, Sonic Hedgehog is expressed in the thickening embryonic epithelial layer, and its receptor Patched (PTCH) is expressed in the underlying dermal layer[20]

Cerebellum

The granule cells regulate the activity of Purkinje cells, which are large neurons responsible for motor coordination output in the cerebellum cortex.The binding of Sonic Hedgehog to PTCH relieves the repression of target gene activation and results in the proliferation of GNPs (Granule neuron precursor) [20]

Pancreas

Down regulation of Hh expression is required to initiate mammalian pancreatic development Beta cell role. [20]

Gut

The gastrointestinal tract develops from the embryonic gut tube, which is composed of two different germ layers: endoderm, which differentiates into the epithelial lining, and mesoderm.[20]

2.2 HEDGEHOG IN DISEASES

Hedgehog pathway and neurodegenerative diseases [18]

The importance of the Hh pathway in embryonic neural development has been thoroughly documented but the role of Hh signaling in the adult brain is less well understood.

Hedgehog pathway and Parkinson disease [19]

It is caused by the loss of dopaminergic neurons. Activation of Hh can protect them against toxic effect during embryonic development.

Hh and peripheral neuropathy

Treatment with Hh protein has been shown to be protective and/or enhance regeneration in PNS injury models. Diabetes mellitus is a chronic metabolic disease accompanied, in a significant number of cases, by peripheral neuropathies (pain and loss of sensation as the main symptoms) and ultimately, by nerve-fiber degeneration.

Hh pathway and cancer

Misregulations of Hh signaling causes cancer in various tissues.Ptch mutations are associated with basal cell carcinoma as well as medulloblastoma and rhabdomyosarcoma[20]. Ptch can be considered as tumor suppressor,similarly loss of function mutation in suppressor of fused (Sufu) have been identified in some medulloblastoma cells.

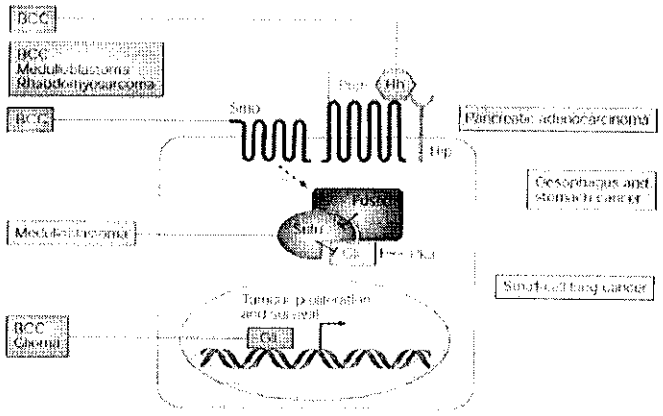


Figure 3[15,16]:Hhsignaling, Stem Cells and Cancer

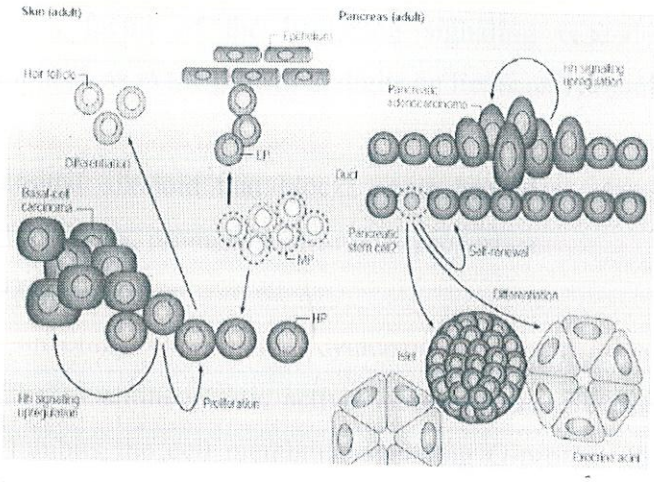


Figure 4[17]:Hh signaling in skin and pancreas.

Ep=epithelium progenitor.Mp=multipotent.Hp=hair follicle progenitor.

2.3Agonist and Antagonist of Hh pathway[27]

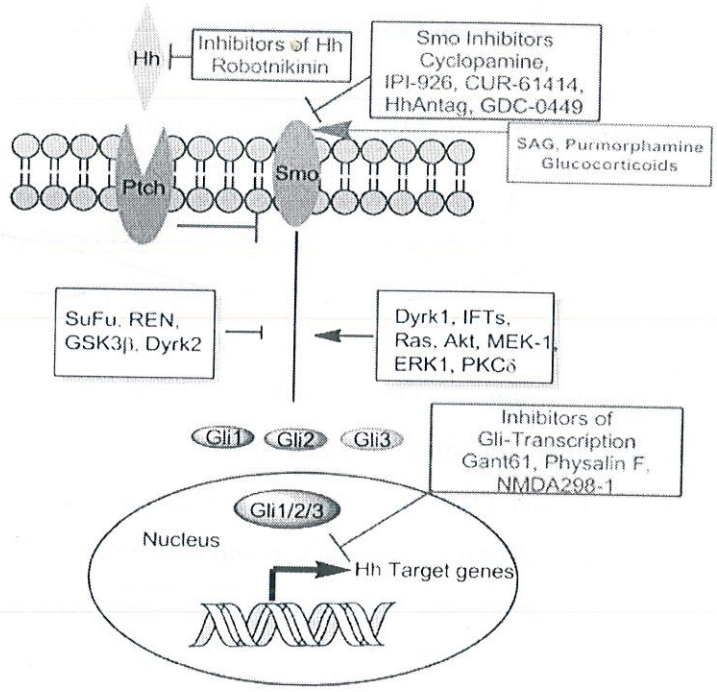


Figure 5: Hh pathway modulators.

2.3.1 Robotonikinin[5] (1)

A small-molecule chemical inhibitor of Sonic hedgehog (SHh) signaling, that blocks hedgehog signaling through specific binding and blockade of SHh signaling factor. It is the first characterized hedgehog inhibitor whose molecular mechanism of action is through blockade of

SHh protein and not a factor of the hedgehog signaling cascades.Robotnikinin is an embryogenesis inhibitor such as in the growth in digits on limbs and organization of the brain.

2.3.2 Cyclopamine[3]

Cyclopamine is a steroidal alkaloid that blocks sonic hedgehog signaling. It demonstrates teratogenic properties, as well as, promising anti-tumor properties.

2.3.3 Saridegib (IPI-926)[5]

Saridegib (IPI-926) is an orally bioavailable, cyclopamine-derived inhibitor of the Hedgehog (Hh) pathway with potential antineoplastic activity. Specifically, Hedgehog pathway inhibitor IPI-926 binds to and inhibits the cell membrane-spanning G-protein coupled receptor SMO, which may result in the suppression of Hh pathway signaling and a decrease in tumor cell proliferation and survival. SMO is activated upon binding of Hh ligand to the cell surface receptor (PTCH); inappropriate activation of Hh signaling and uncontrolled cellular proliferation may be associated with SMO mutations. The Hh signaling pathway plays an important role in proliferation of neuronal precursor cells in the developing cerebellum and other tissue

2.3.4 CUR 61414

CUR 61414 is a proline derivative used as a mediator of hedgehog signaling pathways(G-024856/CUR-61414).

2.3.5 Vismodegib(GDC-0449)[2]

Vismodegib is a drug for the treatment of basal-cell carcinoma (BCC). The approval of vismodegib on January 30, 2012, represents the first Hedgehog signaling pathway targeting agent to gain U.S. Food and Drug Administration (FDA) approval. The drug is also undergoing clinical trials for metastatic colorectal cancer, small-cell lung cancer, pancreatic cancer, medulloblastoma and chondrosarcoma as of June 2011.The drug was developed by the biotechnology / pharmaceutical company Genentech, which is headquartered at South San Francisco, California, USA

2.3.6 GANT61[3]

GLI antagonist that inhibits GLI1 and GLI2-induced transcription .Inhibits the hedgehog (Hh) signaling pathway downstream of SMO and SUFU causing GLI1 nuclear accumulation.Displays antiproliferative and antitumor activity *in vivo*.

2.4 LATESTDEVELOPMENTS

2.4.1 Naturally occurring small molecule inhibitor of Hh-gli pathway.

The aberrant hedgehog (Hh)/GLI signaling pathway causes the formation and progression of a variety of tumors. To search for Hh/GLI inhibitors, compounds were screened for naturally occurring inhibitors of the transcriptional activator GLI1 by using a cell-based assay. It was identified zerumbone, zerumboneepoxies, staurosporinone, 6-hydroxystaurosporinone, arcyriaflavinC and 5, 6-dihydroxyarcyriaflavin A as inhibitors of GLI-mediated transcription. These compounds also inhibited GLI2-mediated transactivation. Semi quantitative RT-PCR and Western blotting analysis further revealed that they decreased Hh-related component expressions.

2.4.2 Zerumbone[21]

Zerumbone is one of the compounds that suppress the expression of the antiapoptotic protein Bcl2. The suppression of Bcl2 expression might be due to the inhibition of GLI-mediated transcription.Zerumbone is a sesquiterpene phytochemical from a type of edible ginger known as "ZingiberZerumbet Smith" grown in Southeast Asia or "Zingiberaromaticum". Zerumbone is currently being explored for its effects on cancer in general, Leukemia in particular, as well as HIV.

2.4.3 Staurosporine[23]

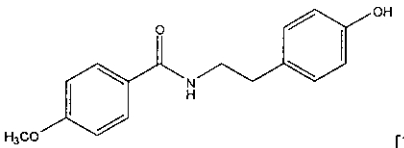
Staurosporine is a natural product originally isolated in 1977 from the bacterium *Streptomycin staurosporeus*. Potential inhibitor of Hhpathway, same as cyclopamine acts on smo and deactivates it hence no further activation of transcriptional factors occurs.

CHAPTER 3

AIM OF THE PROJECT

The main goal of this project is to synthesize such modulators (agonist/antagonist) molecules that can affect Hedgehog-Gli pathway and can be used as therapeutic agents.

Our main aim is to go target Gli-mediated transcription with small molecule modulators of this pathway rather than targeting at level of Smo, upstream in the Hh-gli pathway against which most of the current modulators are designed. The lead compound (NMDA225[26]) was discovered during earlier project by Mahindroo et al. (unpublished data). It showed Hh-Gli agonist activity.We concentrated in synthesizing NMDA225 [26] analogues keeping linker part constant and changing the head part and tail part of the compound.



NMDA225 [26]

CHAPTER 4

MATERIALS

4.1 MATERIALS:

i. RBF 50,100,250 ml
ii. FUNNELS
iii. BEAKERS 20,50,100,250,500 ml
iv. MEASURING CYLINDERS
v. GLASS RODS
vi. FILTER PAPERS
vii. ROTATORY EVAPORATOR
viii. HEATING BATH TUB
ix. COLUMN
x. PARRAFIN FILM
xi. SEPERATING FUNNEL
xii. TLC PLATES
xiii. TEST TUBES
xiv. MICROPIPPETE
xv. EPPENDORF
xvi. CAPILLARY TUBES
xvii. HEATING OVEN

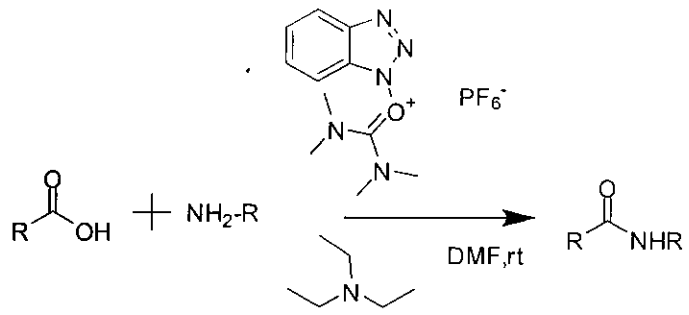
4.2CHEMICALS

i. TYRAMINE
ii. 2-CHLOROBENZOIC ACID
iii. 3-CHLORO BENZOIC ACID
iv. 4-CHLOROBENZOIC ACID
v. 2-FLUROBENZOIC ACID
vi. 3-FLUROBENZOIC ACID
vii. 4-FLUROBENZOIC ACID
viii. CYCLOPENTANONE
ix. TRYPTOPHAN
x. ISATIN
xi. DI ETHYL ETHER
xii. N-BUTANOL
xiii. DIPEA
xiv. SODIUM BICABONATE
xv. SODIUMSULFATE AN HYDROUS
xvi. SILICA GEL GF254
xvii. SILICA GEL FOR COLUMN CHROMATOGRAPHY
xviii. ETHYL ACETATE
xix. HEXANE
xx. METHANOL
xxi. DCM
xxii. ETHANOL
xxiii. HBTU
xxiv. DMF
xxv. ACTEOPHENONE
xxvi. DIETHYLAMINE
xxvii. HCL
xxviii. GLACIAL ACETIC ACID
xxix. HYDRAZINE HYDRATE
xxx. CYCLOHEXALAMINE

CHAPTER 5

REACTION RESULT AND DISCUSSION

1.1 GENERAL REACTION



5.2REACTION MECHANISM

O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate

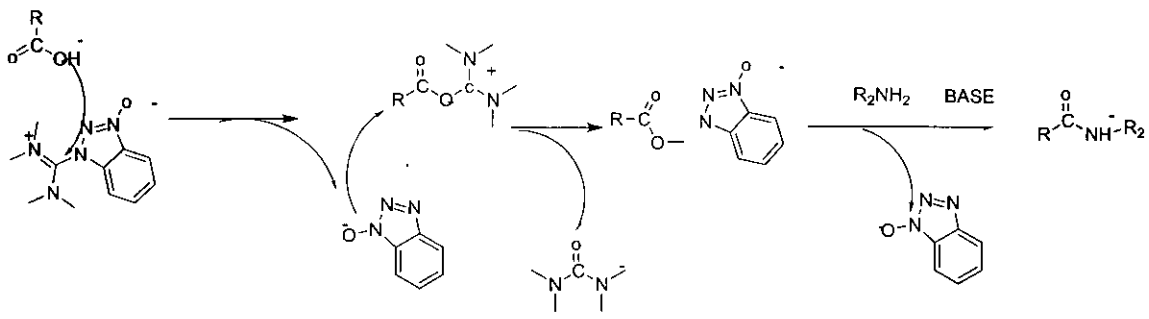
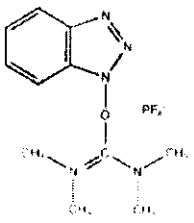
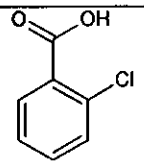
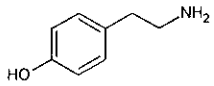
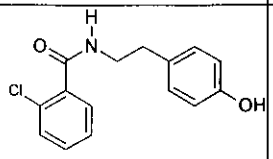
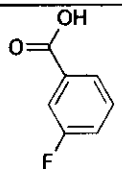
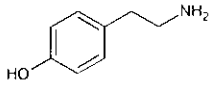
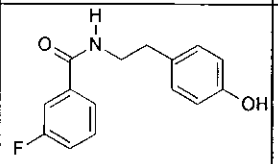
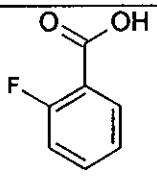
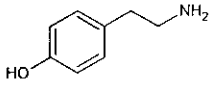
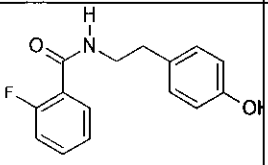
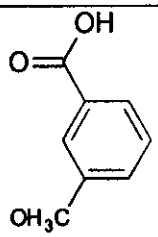
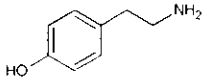
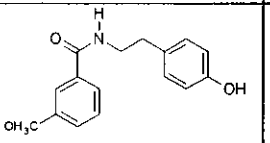
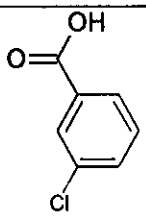
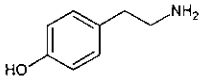
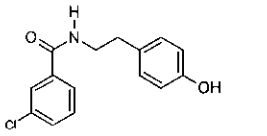


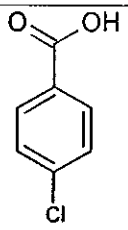
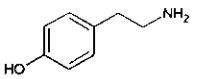
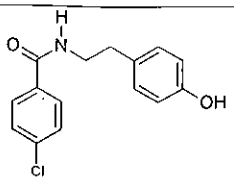
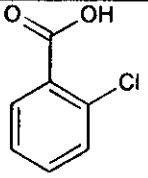
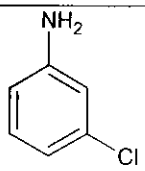
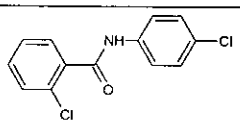
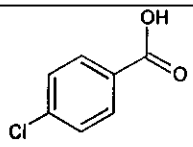
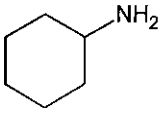
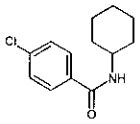
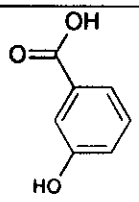
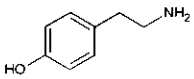
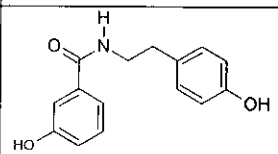
Figure: Mechanism of reaction of HBTU coupling



HBTU ($C_{11}H_{16}F_6N_5OP$)

5.3REACTION CHART:

S.No	REACTANT 1	REACTANT 2	PRODUCT CODE	PRODUCT STRUCTURE	TLC Conditions	Rf
1.			JUIT-PY-RS001		30% ETHYL ACETATE IN HEXANE	0.5
2.			JUIT-PY-AK003		25% ETHYL ACETATE IN HEXANE	0.5
3.			JUIT-PY-AK004		20% ETHYL ACETATE IN HEXANE	0.5
4.			JUIT-PY-AK005		25% ETHYL ACETATE IN HEXANE	0.6
5.			JUIT-PY-AK006		25% ETHYL ACETATE IN HEXANE	0.6

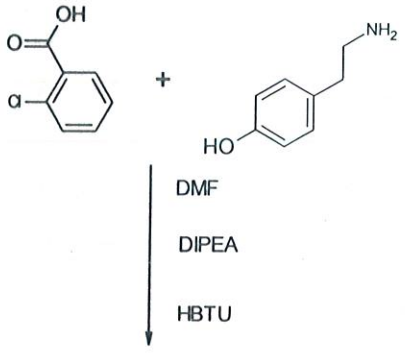
6.			JUIT-PY-RS003		15% ETHYL ACETATE IN HEXANE	0.5
7.			JUIT-PY-AK007		20% ETHYL ACETATE IN HEXANE	0.4
8.			JUIT-PY-RS004		15% ETHYL ACETATE IN HEXANE	0.6
9.			JUIT-PY-AK008		25% ETHYL ACETATE IN HEXANE	

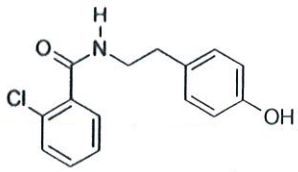
General Procedure

Appropriate benzoic acid (1eq) was dissolved in DMF and HBTU (1.5 eq) and DIPEA (3 eq) were added to it. The mixture was allowed to stand for 30 min and then appropriate amine (2 eq.) was added to it. The reaction was monitored by TLC and on completion was worked up by adding water followed by extraction with ethyl acetate. The organic layer was washed successively with water and brine and dried over anhydrous sodium sulphate. The solvent was removed in vacuo and the residue was chromatographed over silica gel (60-120 mesh size) eluting with hexane ethyl acetate gradient.The column was monitored by TLC. The fractions that

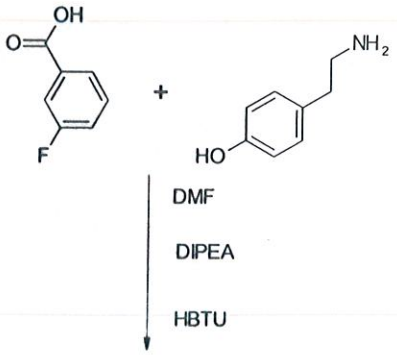
contained the product were combined and solvent was removed in vacuo to get the desired product.

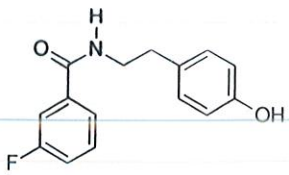
5.4JUIT-PY-RS001





5.8 JUIT-PY-AK003



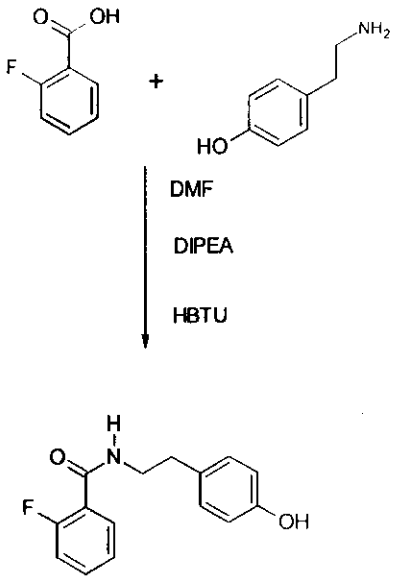


Result:-

The compound was synthesized using the general procedure. TLC condition 25% ethyl acetate in hexane.R_f was 0.5

^{1}H NMR (DMSO.d_6, δ, ppm): 6.3121-7.7894(8H, m, Ar-H), 4.0986(1H, s, NH), 2.7128-2.6776 (2H, t, J=14.08 CH_2). 3.3668 – 3.4182 (2H, dd, J= 6.2, J=14, CH_2)

5.9 JUIT-PY-AK004



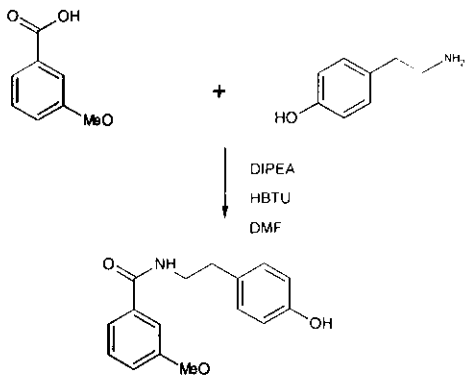
Result :-

The compound was synthesized using the general procedure. TLC condition 20% ethyl acetate in hexane.R_f was 0.5

^{1}H NMR (DMSO.d_6, δ, ppm): 7.3350-7.7194(8H, m, Ar-H), 3.459(1H, s, NH)

3.4734-3.4229 (2H, dd, J=6.72, 13.44, CH_2), 2.7255-2.6880 (2H, t, J=14.68, CH_2).

5.10JUIT-PY-AK005

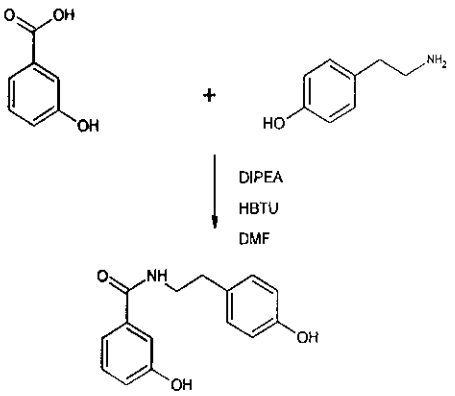


Result:-

The compound was synthesized using the general procedure. TLC condition 25% ethyl acetate in hexane.R_f was 0.6 .

^{1}H NMR (*DMSO*.d_6, δ, ppm): 7.023-7.7194(8H, m, Ar-H) , 3.8353(1H , s, NH), 3.6489-3.67980(2H,dd, J=6.96, 12.88, CH_2), 2.8424-2.88768 (2H, t, J=13.76, CH_2).

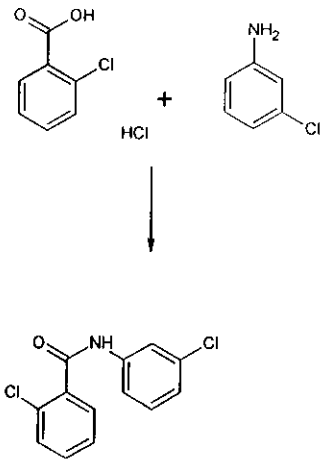
5.10JUIT-PY-AK008



RESULT:-

The compound was synthesized using the general procedure. TLC condition 25% ethyl acetate in hexane.

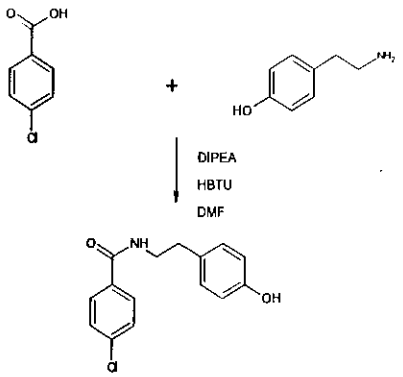
5.11JUIT-PY-AK007



Result:-

The compound was synthesized using the general procedure. TLC condition 20% ethyl acetate in hexane.Rf was 0.4

5.12 JUIT-PY-RS003



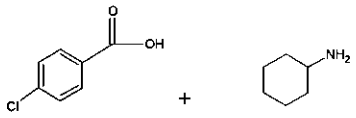
Result:-

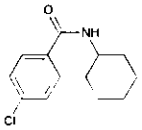
The compound was synthesized using the general procedure. TLC condition 15 % ethyl acetate in hexane.Rf was 0.5

^{1}H NMR was done of this compound.

^{1}H NMR (DMSO.d_6, δ, ppm): 6.6987-6.7452(2H, t, Ar-H),7.0064-7.0267(2H, d, Ar-H), 7.3866-7.040477(2H, t, Ar-H), 7.8227-7.9293(2H, m, Ar-H), 3.4605-3.511(2H, dd, J=12 J=6 , CH2), 2.7986 – 2.8570 (2H, t, J=23, CH_2)

5.13REACTION:JUIT-PY-RS004





Result:-

The compound was synthesized using the general procedure. TLC condition 15 % ethyl acetate in hexane.Rf was 0.5

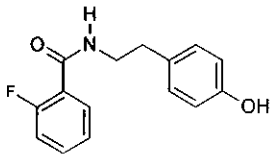
^{1}H NMR was done of this compound.

^{1}H NMR (CDCl$_3$.d$_6$, δ, ppm): 7.6733-7.7004(2H, m, Ar-H), 7.3703-7.4108(2H, m, Ar-H) 6.00(1H , s, NH), 1.1571 – 2.1732 (11H, m, CH & CH$_2$).

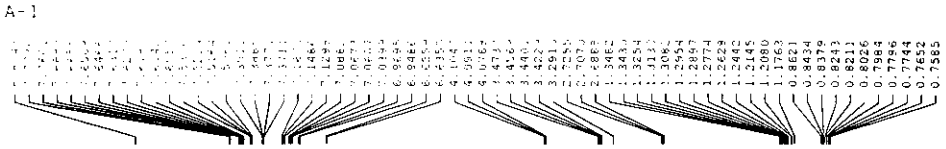
CHAPTER 6

NMR SPECTRA

6.1 SPECTRA JUIT-PY-AK004



A-1



BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters

NAME	Apr11-2013
EXPNO	31
PROCNO	1

F2 - Acquisition Parameters

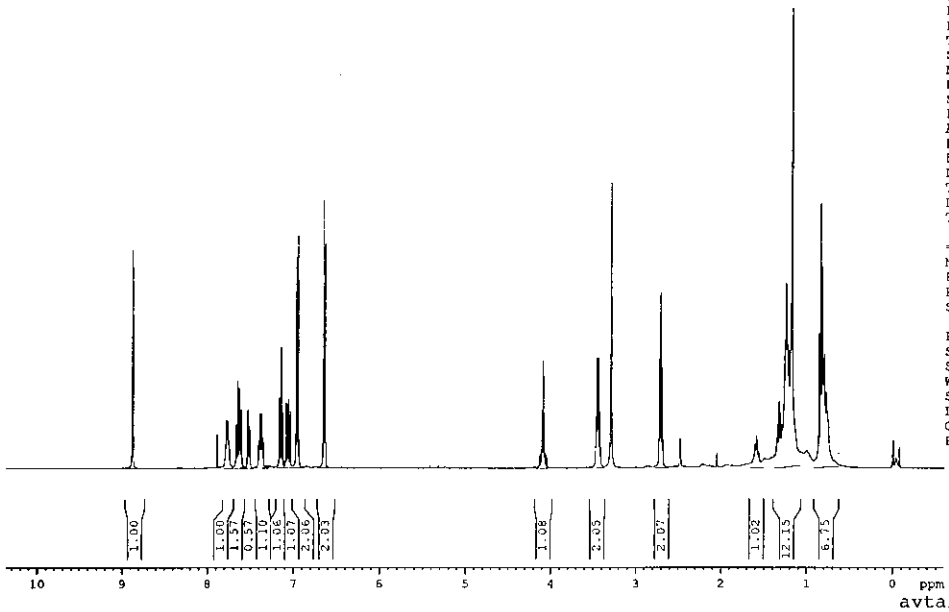
Date_	20130411
Time	16.37
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	8
DS	2
SWH	12019.230 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	228
DW	41.600 usec
DE	6.00 usec
TE	294.7 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

NUC1	1H
P1	10.90 usec
PL1	-3.00 dB
SFO1	400.1324710 MHz

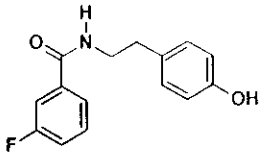
F2 - Processing parameters

SI	32768
SF	400.1300132 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

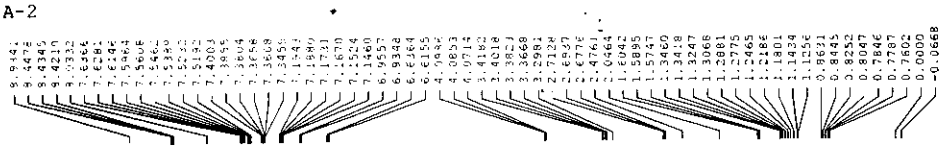


avtar_saifpu@yahoo.co.in

6.2 SPECTRA JUIT-PY-AK003



A-2



BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters

NAME	Apr11-2013
EXPNO	41
PROCNO	1

F2 - Acquisition Parameters

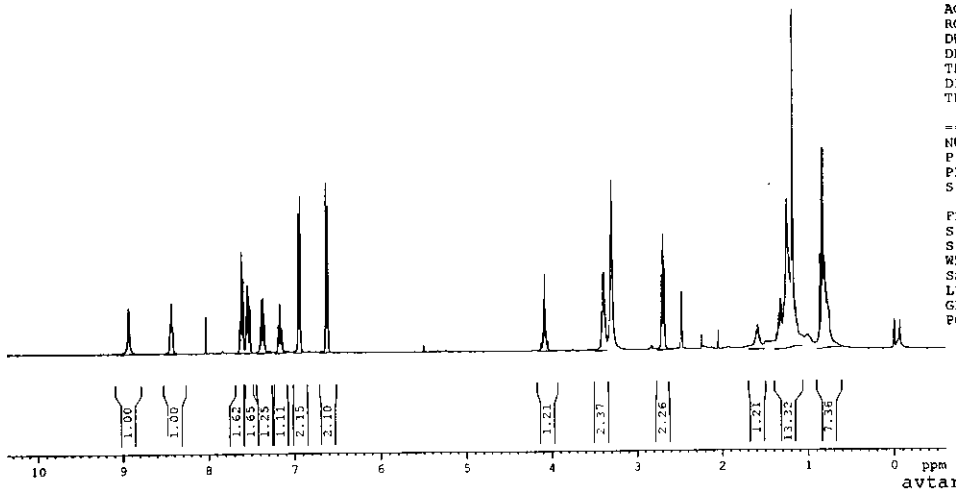
Date_	20130411
Time	16.42
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	8
DS	2
SWH	12019.230 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	181
DW	41.600 usec
DE	6.00 usec
TE	294.7 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

NUC1	1H
P1	10.90 usec
PL1	-3.00 dB
SFO1	400.1324710 MHz

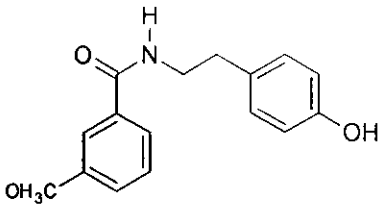
F2 - Processing parameters

SI	32768
SF	400.1300133 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

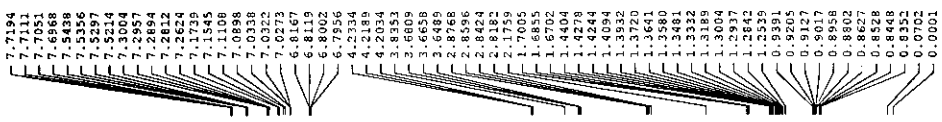


avtar_saifpu@yahoo.co.in

6.3 SPECTRA JUIT-PY-AK005



A-3



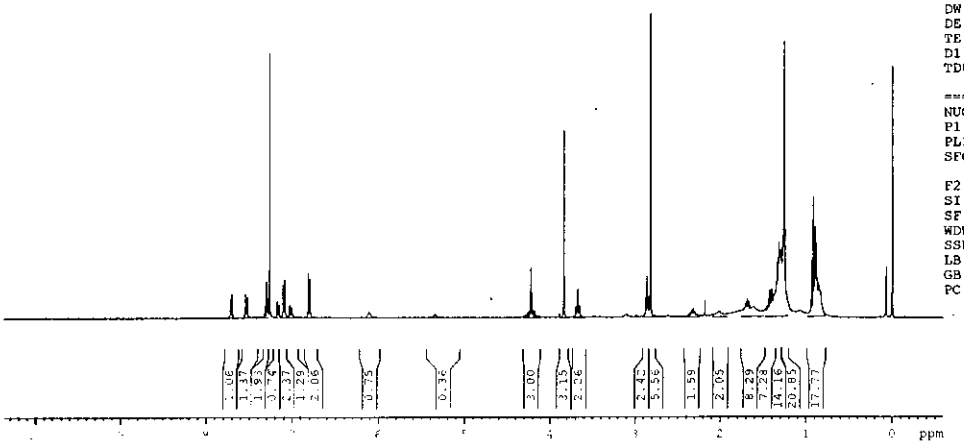
BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters
NAME Apr11-2013
EXPNO 52
PROCNO 1

F2 - Acquisition Parameters
Date_ 20130411
Time 16.48
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 12019.230 Hz
FIDRES 0.183399 Hz
AQ 2.7263477 sec
RG 456
DW 41.600 usec
DE 6.00 usec
TE 294.7 K
D1 1.00000000 sec
TD0 1

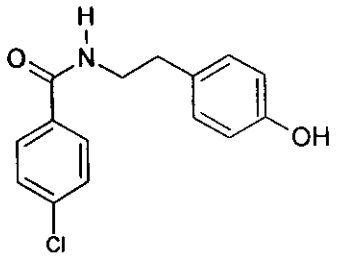
======== CHANNEL f1 ========
NUC1 1H
P1 10.90 usec
PL1 -3.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300086 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

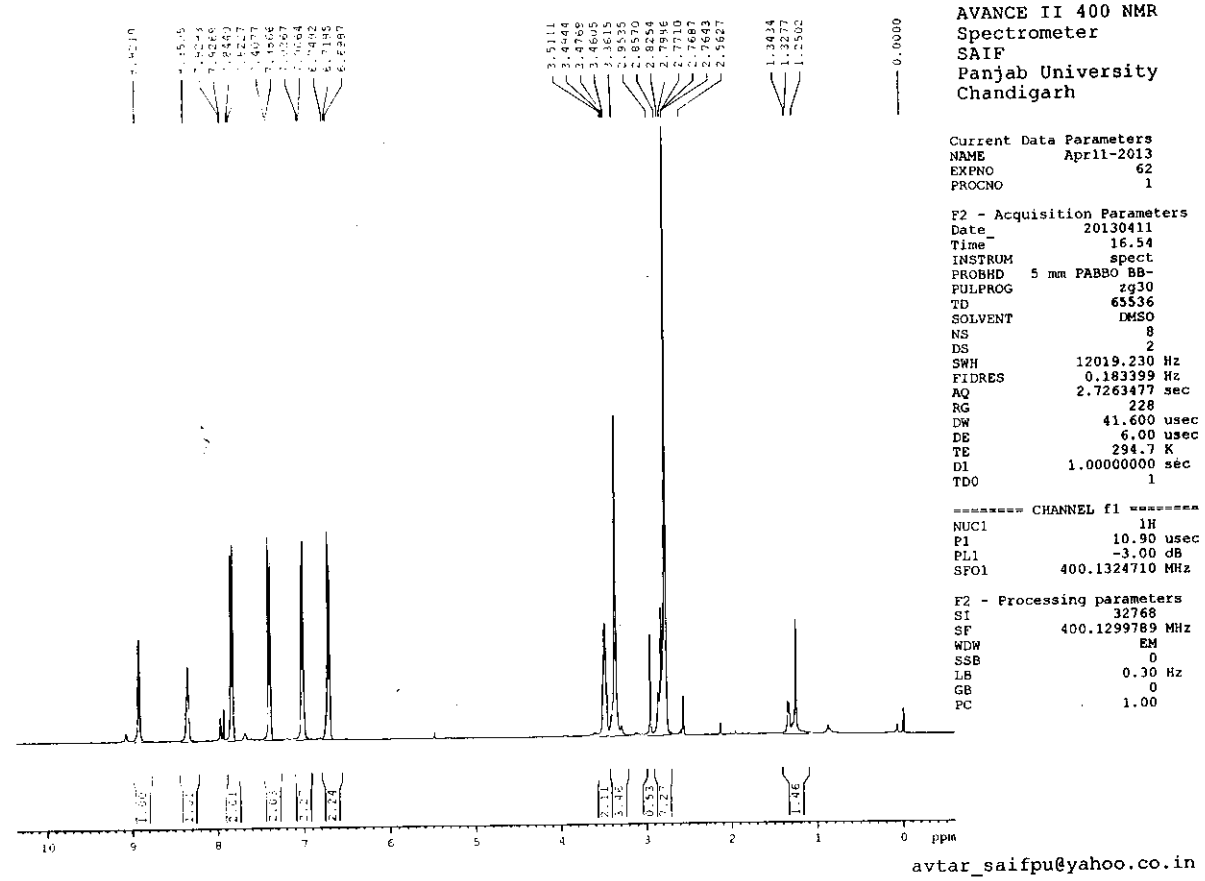


avtar_saifpu@yahoo.co.in

6.4 SPECTRA JUIT-PY-RS003



A-4

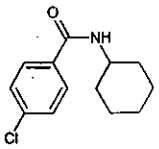


BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

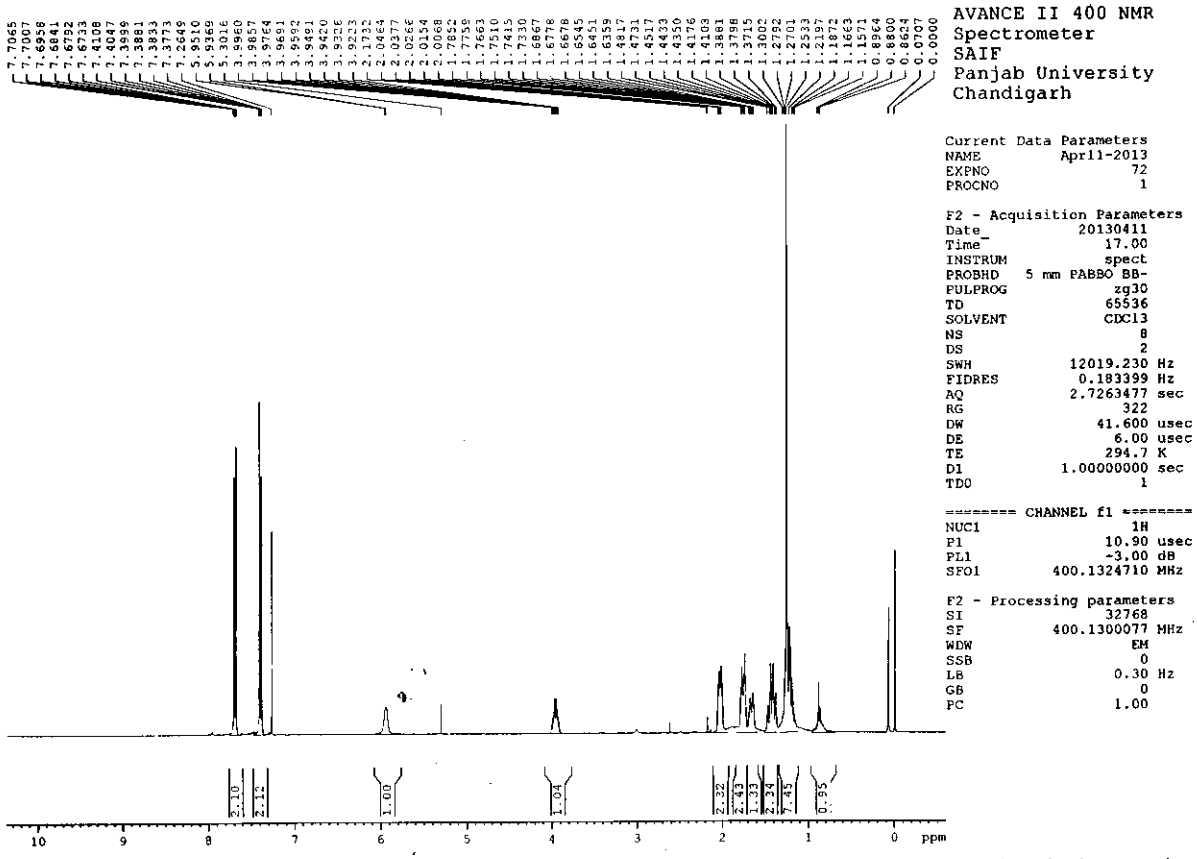
Current Data Parameters	
NAME	Apr11-2013
EXPNO	62
PROCNO	1
F2 - Acquisition Parameters	
Date_	20130411
Time	16.54
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	8
DS	2
SWH	12019.230 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	228
DW	41.600 usec
DE	6.00 usec
TE	294.7 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.90 usec
PL1	-3.00 dB
SFO1	400.1324710 MHz
F2 - Processing parameters	
SI	32768
SF	400.1299789 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

avtar_saifpu@yahoo.co.in

6.5 SPECTRA JUIT-PY-RS004



A-5



BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

Current Data Parameters	
NAME	Apr11-2013
EXPNO	72
PROCNO	1
F2 - Acquisition Parameters	
Date_	20130411
Time	17.00
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	12019.230 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	322
DW	41.600 usec
DE	6.00 usec
TE	294.7 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.90 usec
PL1	-3.00 dB
SFO1	400.1324710 MHz
F2 - Processing parameters	
SI	32768
SF	400.1300077 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

avtar_saifpu@yahoo.co.in

CONCLUSION

In this study, we report the synthesis of novel modulators (agonists/antagonist) of Gli1-mediated transcription that can act as potential therapeutics in Neurodegenerative disorder and for Neurorestoration. The NMR of five compounds out of nine were done in order to characterize the compunds and confirm the structure (JUIT-PY-AK001,JUIT-PY-AK002,JUIT-PY-AK003,JUIT-PY-RS003,JUIT-PY-RS004). The compounds would be evaluated for biological activity.

BIBLIOGRAPHY

1. Kimball's Biology Pages, The Hedgehog Signaling Pathway
   The Hedgehog Pathway and Neurological Disorders Tammy Dellovade, 1Justyna T. Romer, Tom Curran,2 and Lee L. Rubin1 Annu. Rev. Neurosci. 2006. 29:539–63

2. Ingham, Philip W.; Nakano, Yoshiro; Seger, Claudia (2011). "Mechanisms and functions of Hedgehog signalling across the metazoa". Nature Reviews Genetics 12 (6): 393–406.doi:10.1038/nrg2984. PMID 21502959

3. Christiane Nusslein-volhard the identification of genes controlling development in flies. Max-Planck-Institut für Entwicklungsbiologie, Abteilung Genetik, Tübingen,Spemannstr.

4. H.Onishi and M. Katano, "Hedgehog signaling pathway as a therapeutic target in various types of cancer," Cancer Science, vol. 102, no. 10, pp. 1756–1760, 2011.

5. Hedgehog-Gli Signaling Pathway Inhibitors as Anticancer Agents NeerajMahindroo,* ChandanamaliPunchihewa, and NaoakiFujiiDepartment of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis 38105, Tennessee ReceiVedNoVember 10, 2008 page no. 3829-3833

6. Bhardwaj, G.; Murdoch, B.; Wu, D.; Baker, D. P.; Williams, K. P.; Chadwick, K.; Ling, L. E.; Karanu, F. N. et al. (2001). "Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation". Nature Immunology 2 (2): 172–80.doi:10.1038/84282. PMID 11175816.

7. REVIEW Activation of the hedgehog-signaling pathway in human cancer and the clinical implications L Yang, G Xie, Q Fan and J Xie. Page no.470-471

8. Non-cell-autonomous signaling by Shh in tumors: challenges and opportunities for therapeutic targets Maarten F Bijlsma&HenkRoelink† University of California Berkeley, Department of Molecular and Cell Biology, Berkeley, USA page no. 693-695.

9. Alessandra Pierani, Susan Brenner-Morton, Chin Chiang, Thomas M Jessel, A Sonic Hedgehog–Independent, Retinoid-Activated Pathway of Neurogenesis in the Ventral Spinal Cord, *Cell* Volume 97, Issue 7, 25, Pages 903-915, June 1999

10. Tammy Dellovade, Justyna T. Romer, Tom Curran, Lee L. Rubin, "The Hedgehog Pathway and Neurological Disorders", Annual Review of Neuroscience Vol. 29: 539-563, July 2006

11. Nduka M. Amankulor, Dolores Hambardzumyan, Stephanie M. Pyonteck, "Sonic Hedgehog Pathway Activation Is Induced by Acute Brain Injury and Regulated by Injury-Related Inflammation", The Journal of Neuroscience, 29(33):10299 –10308, August 19, 2009

12. Hynes M, Porter JA, Chiang C, Chang D,Tessier-Lavigne M, et al. Induction of midbrain dopaminergic neurons by sonic hedgehog. *Neuron* 15:35–44, 1995.

13. Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancerand implications for therapy. *Trends Pharma Sci.* 2009;30:303-312.

14. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov.* 2006;5:1026-1033.

15. Watkins, D. N. et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422, 313–317 (2003).

16. Evidence that HH signalling is activated in SCLC and that constitutive activation of the pathway is required for cancer maintenance.

17. Thayer, S. P. et al. Hedgehog an early and late mediator of pancreatic cancer tumorigenesis. Nature 425, 851–855 (2003)

18. A. Caricasole et al Wnt Signaling in Neurodegeneration and Neuro-Oncology Bioscience Reports, Vol. 25, Nos. 5/6, October/December 2005 (2005) DOI: 10.1007/s10540-005-2893-6

19. Tammy Dellovade et al The Hedgehog Pathway and Neurological Disorders Annu. Rev. Neurosci.2006. 29:539–63 0147-006X/06/0721

20. Ryan B. Corcoran and Matthew P. Scott A mouse model for medulloblastoma and basal cell nevus syndrome Journal of Neuro-Oncology 53: 307–318, 2001

21. Kerr JF. Shrinkage necrosis: A distint mode of cellular death. J Pathol. 1971;105:13–20. doi: 10.1002/path.1711050103.

22. Bambakidis, N. C., Wang, R., Franic, L., and Miller, R. H. (2003). Sonic hedgehog induced neural precursor proliferation after adult rodent spinal cord injury. J. Neurosurg. Spine 99, 70-75.

23. Journal of Biology and Life Science ISSN 2157-6076 2013, Vol. 4, No. 2 page 155.

24. Kurtova, A. V., Balakrishnan, K., Chen, R., Ding, W., Schnabl, S., Quiroga, M. P. Sivina, M. Wierda, W. G., Estrov, Z., Keating, M. J., Shehata,(2009). Diverse marrow stromal cells protect CLL cells from spontaneous and druginduced apoptosis: Development of a reliable and reproducible system to assess stromal cell adhesion-mediated drug resistance. Blood 114, 4441–4450.

25. Laner-Plamberger, S., Kaser, A., Paulischta, M., Hauser-Kronberger, C., Eichberger, T.,and Frischauf, A. M. (2009). Cooperation between GLI and JUN enhances transcriptionof JUN and selected GLI target genes. Oncogene 28, 1639–1651.

26. Neeraj Mahindroo Hedgehog-Gli Signaling Pathway Inhibitors as Anticancer Agents. J Med Chem. Author manuscript; available in PMC 2010 July 9. Published in final edited form as doi: 10.1021/jm801420y

27. Neeraj Mahindroo,Michele C. Connelly, Chandanamali Punchihewa, "Structure-Activity Relationships and Cancer-Cell Selective Toxicity of Novel Inhibitors of Glioma-Associated Oncogene Homolog 1 (Gli1)-Mediated Transcription, J Med Chem; 52(14): 4277–4287., 2009 July 23