A CONCISE STRATEGY TOWARDS (–)-POCHONICINE STEREOISOMERS AND SYNTHESIS OF NOVEL HOMOAZANUCLEOSIDES

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by

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Submitted

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*This thesis is dedicated to my wife **Kirti**, my beloved daughter **Ishita***

*And my parents **Mr. Vilas** and **Mrs. Sangita***

CERTIFICATE

This is to certify that thesis entitled **"A Concise Strategy towards (–)-Pochonicine Stereoisomers and Synthesis of Novel Homoazanucleosides",** being submitted by **RAHUL VILAS SALUNKE** to Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him. Mr Rahul Vilas Salunke has worked under my supervision and guidance and has fulfilled all the requirements for the submission of a Ph.D. thesis, which to my knowledge has reached the requisite standard and is worthy of consideration for the award of Ph.D. degree.

The work embodied in this thesis has not been submitted, in part or full, to other University or Institute for the award of any degree or diploma.

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Rahul Vilas Salunke

ABSTRACT

The thesis titled "**A Concise Strategy towards (–)-Pochonicine Stereoisomers and Synthesis of Novel Homoazanucleosides"** presents the the work carried out on the synthesis of six stereoisomers of (–)-pochonicine, indolizidine alkaloids, novel tricyclic azasugars and homoazanucleosides from a single starting tri-*O*-benzyl-D-glucal. Enzyme inhibition studies against five commercially available glycosidase were also discussed.

Chapter I describes the synthesis of stereoisomers of (–)-pochonicine a novel pyrrolizidine alkaloid, indolizidine alkaloids and tricyclic azasugars. We have reported a concise synthesis of six stereoisomers of (–)-pochonicine through a one-pot *N*-detosylative intramolecular epoxide opening as a key step. Enroute towards (–)-pochonicine stereoisomers we have also synthesized some novel indolizidine alkaloids through a ring expansion reaction. Novel tricyclic alkaloids were also synthesized. Glycosidase inhibition demonstrated (–)-pochonicine stereosiomers are good inhibitors of β-glucosidase and β-*N*-GLcNAcase, however indolizidine alkaloids showed inhibition only against β-glucosidase.

Chapter II describes the synthesis of novel homoazanucelosides and peptidyl homoazanucleosides, hybrid molecules containing three biologically important moieties namely amino acid, azasugar and nucleobase from common starting material tri-*O*-benzyl-D-glucal. In this process, we have also demonstrated the pluri-potency of polyhydroxypyrrolidine synthesized previously in our lab. Glycosidase inhibition studies revealed that all the homoazanucleosides having free amino group did not show any inhibitory activity. However their acetamido derivatives were found to be sub-millimolar inhibitors of β-*N*-acetyl-glucosaminidase.

सार

थीसिस "(-) की ओर एक संक्षिप्त रणनीति (-)-पोकोनिसिन स्टीरियोआयसोमर्स और उपन्यास होमो-अज़ान्यूक्लिओसाइड्स का संश्लेषण" - (-) के छह स्टीरियोआयसोमर्स के संश्लेषण पर किए गए काम को प्रस्तुत करता है। (-) पोकोनिसिन, इंडोलिजिडीन एल्कालॉइड्स, नया ट्राइसाइक्लिक अज़ाशुगर और होमो-अज़ान्यूक्लिओसाइड्स ट्राई-ओ-बेंजिल-डी-ग्लुकलसे शुरू करके पांच व्यावसायिक रूप से उपलब्ध ग्लाइकोसिडेज के खिलाफ एंजाइम निषेध अध्ययन पर भी चर्चा की गई।

अध्याय। (-) के स्टीरियोइसोमर्स के संश्लेषण का वर्णन करता है - एक नया पाइरोलिज़िडिन एल्कलॉइड, इंडोलिज़िडिन एल्कलॉइड्स और ट्राईसाइक्लिक अज़ाशुगर। हमने (-) के छह पोकोनिसिन स्टीरियोआयसोमर्स के एक संक्षिप्त संश्लेषण की सूचना दी है - एक महत्वपूर्ण एक- पात्र एन-डिटोसाइलेटिव इंट्रामोलीक्यूलर एपॉक्साइड के माध्यम से से (-)-पोकोनिसिन की ओर अग्रसर - पोकोनिसिन स्टीरियोइसोमर्स हमने एक अंगूठी विस्तार प्रतिक्रिया के माध्यम से कुछ नए इंडोलिज़िडिन एल्कलॉइड को भी संश्लेषित किया है। नए ट्राइसाइक्लिक अल्कलॉइड को भी संश्लेषित किया गया था। ग्लाइकोसिडेस निषेध का प्रदर्शन (-) - पोकोनिसिन स्टीरियोसोमर्स बीटा-ग्लूकोसिडेस और बीटा-एन-एसिटाइलग्लूकोसिडेस के अच्छे अवरोधक हैं, हालांकि इंडोलिज़िडिन एल्कलॉइड केवल बीटा-ग्लूकोसिडेस के खिलाफ निषेध दर्शाते हैं।

अध्याय ॥ में उपन्यास होमोअज़ान्यूक्लिओसाइड्स और पेप्टिडिल होमोअज़ान्यूक्लिओसाइड्स के संश्लेषण का वर्णन किया गया है, जिसमें तीन जैविक रूप से महत्वपूर्ण एकक अर्थात् अमीनो एसिड, अज़ाशुगर और न्यूक्लियोबेस युक्त आम अणु ट्राई-ओ-बेंजिल-डी-ग्लुकल हैं। इस प्रक्रिया में, हमने अपनी प्रयोगशाला में पहले से संश्लेषित पॉलीहाइड्रॉक्सीरिपोलिडिन की प्लुरी-पोटेंसी का भी प्रदर्शन किया है। ग्लाइकोसिडेज निषेध अध्ययनों से पता चला है कि मुक्त अमीनो समूह वाले सभी होमोअज़ान्यूक्लिओसाइड्स ने कोई निरोधात्मक गतिविधि नहीं दिखाई थी। हालाँकि, उनके एसिटामिडो डेरिवेटिव को बीटा-एन-एसिटाइलग्लूकोसिडेस का उप-मिलिम्मल अवरोधक पाया गया।

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GENERAL EXPERIMENTAL CONSIDERATIONS

All solvents employed were purified by standard procedures. Anhydrous solvents were dried over sodium wire (THF, diethyl ether, benzene) or molecular sieves (CH_2Cl_2, $CHCl_3$, DMF).

Nitrogen or Argon gas used for creating inert atmosphere was freed from oxygen prior to entry into reaction vessel.

Commercially sourced TLC plates were used, and the spots were visualized by exposure to iodine, or by dipping in $KMnO_4$ and ninhydrin solution. Column chromatography was carried out on silica gel (230–400 mesh) using mixtures of hexane and ethyl acetate as eluent unless otherwise mentioned.

Optical rotations were recorded on an Autopol V (Rudolph Research Flanders, NJ) instrument. All the rotations were measured at 589 nm (sodium D' line).

All melting points reported in this thesis are uncorrected and were taken on an electric melting point apparatus (Ambassador, India).

Freeze-drying of samples was done on a Freezone 2.5 (Labconco, USA) lyophilizer.

Inhibition studies were carried out on Biotek Synergy 2 microplate reader.

IR spectra were taken within the range 4000–600 cm^{-1} either as KBr pellets or neat on a Nicolet (Madison, USA) FT-IR spectrophotometer (Model Protégé 460).

^{1}H-NMR spectra were recorded on a 300 MHz or 400 MHz Bruker Spectrospin DPX FT-NMR instruments. The solvents employed were $CDCl_3$, CD_3OD, D_2O or DMSO-d_6 with Me_4Si as the internal standard. The multiplicities are denoted as s-singlet, br s-broad singlet, d-doublet, br m-broad multiplet, t-triplet, q-quartet, dt-doublet triplet and m-multiplet. ^{13}C-

NMR spectra were recorded at 75 MHz or at 100 MHz instrument. The chemical shifts are reported in δ values (parts per million, ppm) relative to the internal standard Me_4Si.

High-resolution mass spectra were recorded with a Q-TOF Bruker instrument, using electrospray ionization (ESI) as the ionization method.

X-ray crystallography

Suitable crystal of compounds, were obtained by slow evaporation of their saturated solutions in chloroform-methanol solvent mixture or water. BRUKER AXS SMART-APEX diffractometer equipped with CCD area detector (Kα=0.71073Å, monochromator: graphite). Frames were collected at T=298K by ω, φ and 2θ-rotation with full quadrant data collection strategy (four domains each with 600 frames) at 10s per frame with SMART. The measured intensities were reduced to F^2 and corrected for absorption with SAINT. Structure solution and refinement were carried out with the SHELXTL package by direct methods. Non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in idealized positions, and a riding model was used for the refinement. Images were created with the program Diamond.

COMMON ABBREVIATIONS

ent	:	Enantiomer
Cbz	:	Carbobenzyloxy
2,2-DMP	:	2,2-Dimethoxy propane
Boc	:	Tertiary-butoxycarbonyl
epi	:	Epimer
aq	:	Aqueous
Bn	:	Benzyl
Conc.	:	Concentrated
LDA	:	Lithium diisopropyl amide
c	:	Concentration
cat	:	Catalyst
COSY	:	Correlation Spectroscopy
HSQC	:	Heteronuclear Single Quantum Coherence
NOESY	:	Nuclear Overhauser Effect Spectroscopy
HMBC	:	Heteronuclear Multiple Bond Correlation
HETCOR	:	Heteronuclear Correlation
DCC	:	*N*, *N*'-dicyclohexylcarbodiimide
DCM	:	Dichloromethane
DEAD	:	Diethyl azodicarboxylate
DMAP	:	4-dimethylaminopyridine
DMF	:	*N*, *N*'-dimethylformamide
DMSO	:	Dimethylsulfoxide
ppm	:	Parts per million
dr	:	Diastereomeric ratio
DEPT	:	Distortionless Enhancement by Polarization Transfer
equiv	:	Equivalent
ESI	:	Electrospray ionization
CSA	:	Camphor sulphonic acid
g	:	Gram
GalNAcase	:	*N*-acetyl-α-D-galactosaminidase
h	:	Hour
HRMS	:	High Resolution Mass Spectrometry

Hz	:	Hertz
IC_{50}	:	Half maximal Inhibitory Concentration
^{i}Pr	:	Isopropyl
IR	:	Infrared
K_i	:	Inhibition Constant
LAH	:	Lithium Aluminum Hydride
Me	:	Methyl
mg	:	Milligrams
min	:	Minute
mL	:	Milliliters
mM	:	Millimolar
mmol	:	Millimoles
µM	:	Micromolar
M.p.	:	Melting Point
Ms	:	Mesyl
MS	:	Molecular sieves
MHz	:	Megahertz
m/z	:	mass-to-charge ratio
NBS	:	*N*-Bromosuccinimide
NIS	:	*N*-Iodosuccinimide
nM	:	Nanomolar
NMO	:	*N*-Methylmorpholine *N*-oxide
NMR	:	Nuclear magnetic resonance
Nu	:	Nucleophile
Pd/C	:	Palladium on activated carbon
Ph	:	Phenyl
p-TSA	:	Para-toluenesulfonic acid
TsCl	:	Para-toluenesulfonyl chloride
Ts	:	Para-toluenesulfonyl
R_f	:	Retention factor
ref	:	Reference
rt	:	Room temperature
TBAF	:	Tertiary-butyldimethyl silyl fluoride

TBS	:	Tertiary-butyldimethyl silyl
TBSCl	:	Tertiary-butyldimethyl silyl chloride
TBSOTf	:	Tertiary-butyldimethyl silyl triflate
TBDPS	:	Tertiary-butyl diphenyl silyl
TMSCN	:	Trimethyl silyl cyanide
THF	:	Tetrahydrofuran
TLC	:	Thin layer chromatography
TMS	:	Tetramethyl silane
liq	:	Liquid
DIPEA	:	Diisopropyl ethyl amine
Py	:	Pyridine
DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid
ATP	:	Adenosine triphosphate
HIV	:	Human immunodeficiency virus
AIDS	:	Acquired immunodeficiency syndrome
US FDA	:	United states Food and Drug Administration
HBV	:	Hepatitis B virus
HCV	:	Hepatitis C virus
mRNA	:	Messenger Ribonucleic acid
AON	:	Antisense oligonucleotide
PNP	:	Purine nucleoside phosphorylase
Ipc	:	Diisopinocamphyl
de	:	Diastereomeric excess
mCPBA	:	Meta chloro perbenzoic acid
TFA	:	Trifluoroacetic acid
Fmoc	:	Fluorenylmethyloxycarbonyl
DMTrCl	:	Dimethoxy trityl
CbzCl	:	Carboxybenzyl chloride
DAST	:	Diethylaminosulfur trifluoride
cAMP	:	Cyclic adenosine mono phosphate
cGMP	:	Cyclic guanosine mono phosphate
NAD^+	:	Nicotinamide adenine dinucleotide

$NADP^+$	:	Nicotinamide adenine dinucleotide phosphate
FAD	:	Flavin adenine dinucleotide
FMN	:	Flavin mono nucleotide
dGTP	:	Deoxy guanosine triphosphate