SYNTHESIS AND INHIBITION STUDIES OF STEVIAMINE STEREOISOMERS, NOVEL TRIAZOLE- AND TETRAZOLE- FUSED FIVE-MEMBERED IMINOCYCLITOLS

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by

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submitted

in fulfillment of the requirements of the degree of DOCTOR OF PHILOSOPHY

to the

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This thesis is dedicated to my beloved parents

Mr. S. Santhanam *and* ***Mrs. S. Kalaivani***

For their love, endless support

And encouragement

CERTIFICATE

This is to certify that the thesis entitled "**SYNTHESIS AND INHIBITION STUDIES OF STEVIAMINE STEREOISOMERS, NOVEL TRIAZOLE- AND TETRAZOLE- FUSED FIVE-MEMBERED IMINOCYCLITOLS",** being submitted by **Mr. VENKATESAN S** 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. VENKATESAN S 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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Venkatesan S

ABSTRACT

The thesis titled "**SYNTHESIS AND INHIBITION STUDIES OF STEVIAMINE STEREOISOMERS, NOVEL TRIAZOLE- AND TETRAZOLE- FUSED FIVE-MEMBERED IMINOCYCLITOLS"** presents the research work carried out on the synthesis of novel iminocyclitols from a single starting material viz., tri-*O*-benzyl-D-glucal. Their inhibition studies against commercially available glycosidases are also discussed.

Iminocyclitols, also called iminosugars or azasugars, are sugar derivatives in which the endocyclic oxygen atom has been replaced with a basic nitrogen atom. They are inhibitors of glycosidases due to which they find immense applications as drug molecules against various diseases. This is clearly evident from the fact that quite a few iminosugar based drugs have already hit the market and many more are in their earlier/advanced clinical trials.

The research work embodied in the thesis has been divided into three chapters

Chapter I highlights the synthesis of two new stereoisomers of steviamine, an indolizidine alkaloid isolated in 2010, namely 1,8a-di-*epi*-(+)-steviamine and 2,3-di-*epi*-(–)-steviamine, starting from tri-*O*-benzyl-D-glucal. The key step in the synthesis was a modified Julia olefination of a 2-formyl-polyhydroxy-pyrrolidine with a sulfone derived from ethyl acetoacetate to install the side chain required for the construction of piperidine ring of steviamine stereoisomers. Glycosidase-inhibition studies revealed that 2,3-di-*epi*-(–)-steviamine is a selective inhibitor of α-galactosidase. Since inhibitors of α-galactosidase have been found to be promising chemical chaperones for Fabry's disease, this molecule is likely to find potential applications in this direction.

Chapter II describes design, synthesis and glycosidase inhibiton studies of novel triazole- and tetrazole- fused five-membered iminocyclitols synthesized through intramolecular Huisgen 1,3-dipolar cycloaddition reaction as the key step. We have utilized

the protected polyhydroxylated pyrrolidines as common intermediates for the synthesis of triazole and tetrazole fused iminocyclitols. One of the triazole fused iminocyclitols synthesized by us displayed good inhibitory activity against α-glucosidase (IC_{50} = 55 μM) as compared to corresponding five-membered amino modified iminocyclitols.

Chapter III discusses design, synthesis and glycosidase inhibiton studies of novel triazolo-δ-lactams through intermolecular Huisgen 1,3-dipolar cycloaddition reaction as the key step. We have utilized the protected polyhydroxylated pyrrolidines as common intermediates for the synthesis of triazolo-δ-lactams. They displayed moderate but selective inhibition against α-glucosidase (IC_{50} = 112 μM) and β-galactosidase (IC_{50} = 135 μM).

सार

थीसिस शीर्षक "स्टेवियामीन स्टीरियोइज़र्स, नोवेल ट्राइज़ोइल- और टेट्राज़ोल-फ्यूज्ड फाइव-एम्मेड इमिनोसाइक्लिओल्स" के सिथेसिस और इनबिबिशन स्टडीज का शीर्षक "उपन्यास इमिनोक्लिक्लिओल्स के संश्लेषण पर किए गए शोध कार्य को एक प्रारंभिक सामग्री से प्रस्तुत करता है, जैसे त्रि-ओ-बेंज़िल-डी-शर्करा। व्यावसायिक रूप से उपलब्ध ग्लाइकोसिडेस के खिलाफ उनके अवरोध अध्ययन पर भी चर्चा की गई है।

इमिनोसाइक्लिटोल, जिसे इमिनासुगर या अज़सूगर भी कहा जाता है, चीनी डेरिवेटिव होते हैं जिसमें एंडोक्साइक ऑक्सीजन परमाणु को मूल नाइट्रोजन परमाणु के साथ बदल दिया गया है। वे ग्लाइकोसिडेस के अवरोधक हैं जिसके कारण उन्हें विभिन्न बीमारियों के खिलाफ दवा अणुओं के रूप में अत्यधिक अनुप्रयोग मिलते हैं। यह इस तथ्य से स्पष्ट रूप से स्पष्ट है कि कुछ इमिनासुगर आधारित दवाओं ने पहले से ही बाजार को मारा है और कई और अपने पहले / उन्नत नैदानिक परीक्षणों में हैं।

थीसिस में शामिल शोध कार्य को तीन अध्यायों में विभाजित किया गया है

अध्याय I स्टीवियामीन के दो नए स्टीरियोइज़ोमर्स के संश्लेषण पर प्रकाश डाला गया है, जो कि 2010 में पृथक एक इंडोलिज़िडाइन एल्कालोइड है, अर्थात् 1,8 ए-डी-एपीआई -(+)-स्टेवाइमीन और, 2,3-डी-एपीआई -(-)-स्टेवाइमीन, से शुरू त्रि-ओ-बेंज़िल-डी-ग्लू कैल। संश्लेषण में महत्वपूर्ण कदम एक 2-फॉर्मिल-पॉलीहाइड्रॉक्सी-पाइरोलिडाइडिन का एक संशोधित जूलिया ओलेफ़िनेशन था जिसे एथिल एसीटोएसेटेट से व्युत्पन्न एक सल्फोन के साथ स्टेवाइमीन स्टीरियोइओसमर्स की पाइपरिडाइन रिंग के निर्माण के लिए आवश्यक साइड चेन स्थापित करने के लिए किया गया था। ग्लाइकोसिडेज़-अवरोध अध्ययन से पता चला है कि 2,3-डी-एपीआई-(-)-स्टीवियान α-गैलेक्टोसिडसे का एक चुनिंदा अवरोधक है।अवरोधक फैब्रिक रोग के लिए

रासायनिक चाप रोनों का वादा करने के लिए पाए गए हैं, इस अणु को इस दिशा में संभावित अनुप्रयोगों की संभावना है।

अध्याय II में उपन्यास त्रिज्या के डिजाइन, संश्लेषण और ग्लाइकोसिडेस इनहिबिटन अध्ययनों का वर्णन किया गया है- और टेट्राज़ोल- मुख्य चरण के रूप में इंट्रामोल्यूलर ह्यूज़ेन 1,3-द्विध्रुवीय साइक्लोडिशन प्रतिक्रिया के माध्यम से संश्लेषित पांच-ज्ञात इमिनोसाइटिटलोज़ को जोड़ा गया है। हमने संरक्षित पॉलीहाइड्रोक्साइलेटेड पाइरोलिडाइन्स का उपयोग त्रिकोणीय और टेट्राज़ोल फ्यूज्ड इमिनोक्लिक्लिओल्स के संश्लेषण के लिए सामान्य मध्यवर्ती के रूप में किया है। हमारे द्वारा संश्लेषित ट्रायज़ोल फ़्यूज्ड इमिनोक्लिक्लिओल्स में से एक ने α-ग्लूकोसिडसे (मैंसी$_{50}$ = 55 माइक्रोन) के खिलाफ अच्छी अवरोधक गतिविधि प्रदर्शित की है, जो कि पांच-ज्ञात एमिनो संशोधित इमिनोसाइटिटलों की तुलना में है।

अध्याय III मुख्य चरण के रूप में इंटरमोल्यूलर 1,3-द्विध्रुवीय चक्रवात प्रतिक्रिया के माध्यम से उपन्यास ट्राज़ोलो-δ-लाक्टाम्स के डिजाइन, संश्लेषण और ग्लाइकोसिडेस अवरोध अध्ययनों पर चर्चा करता है। हमने ट्राज़ोलो-δ-लाक्टाम्स के संश्लेषण के लिए संरक्षित पॉलीहाइड्रोक्साइलेटेड पाइरोलिडाइन्स का उपयोग सामान्य मध्यवर्ती के रूप में किया है। उन्होंने α-ग्लूकोसिडसे (मैंसी$_{50}$ = 112 माइक्रोन) और β-गैलेक्टोसिडसे (मैंसी$_{50}$ = 135 माइक्रोन) के खिलाफ मध्यम लेकिन चुनिंदा अवरोध प्रदर्शित किया।

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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 $KMnO_4$ 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} as KBr pellets 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 $CDCl_3$, D_2O or DMSO-d_6 with Me_4Si as the internal standard. The multiplicities are denoted as s-singlet, brs-broad singlet, d-doublet, brm-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.

COMMON ABBREVIATIONS

Ac	:	acetyl
Ac_2O	:	acetic anhydride
AcOH	:	acetic acid
ADMP	:	aminodeoxy-DMDP
AIBN	:	azobisisobutyronitrile
atm	:	atmospheric pressure
aq	:	aqueous
Bn	:	benzyl
Boc	:	tertiary-butoxycarbonyl
^{t}Bu	:	tertiary butyl
c	:	concentration
cat	:	catalyst
Cbz	:	carbobenzyloxy
COSY	:	correlation spectroscopy
CSA	:	camphor-10-sulfonic acid
DCC	:	N, N'-dicyclohexylcarbodiimide
DCM	:	dichloromethane
DEAD	:	diethyl azodicarboxylate
DMAP	:	4-dimethylaminopyridine
DMF	:	*N*, *N*'-dimethylformamide
DMSO	:	dimethylsulfoxide
DPP-4	:	dipeptidyl peptidase IV
dr	:	diastereomeric ratio
EGC II	:	endoglycocermidase II
equiv	:	equivalent
ESI	:	electrospray ionization
Et	:	ethyl
EtOAc	:	ethyl acetate
g	:	gram

GalNAcase	:	*N*-acetyl-α-D-galactosaminidase
h	:	hour
HRMS	:	high resolution mass spectrometry
HSQC	:	heteronuclear single quantum coherence
Hz	:	hertz
IC_{50}	:	half maximal inhibitory concentration
Imi	:	imidazole
^{i}Pr	:	isopropyl
IR	:	infrared
K_i	:	inhibition constant
LAH	:	lithium aluminum hydride
LDA	:	lithium diisopropylamide
LiHMDS	:	lithium hexamethyldisilamide
m-CPBA	:	meta-chloro perbenzoic acid
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
NAc	:	*N*-acetyl
NCS	:	*N*-chlorosuccinimide
NIS	:	*N*-iodosuccinimide
nM	:	nanomolar
NMO	:	*N*-methylmorpholine *N*-oxide
NMR	:	nuclear magnetic resonance

NOESY	:	nuclear overhauser effect spectroscopy
Nu	:	nucleophile
PANF	:	polyacrylonitrile fiber
Pd/C	:	palladium on activated carbon
PPh_3	:	triphenyl phosphine
Ph	:	phenyl
PPTS	:	pyridinium *p*-toluenesulfonate
py	:	pyridine
p-TSA	:	para-toluenesulfonic acid
RCM	:	ring closing metathesis
R_f	:	retention factor
ref	:	reference
rt	:	room temperature
TBAF	:	tetra-*N*-butyl ammonium fluoride
TBS	:	tertiary-butyldimethyl silyl
TCDI	:	1,1′-thiocarbonyldiimidazole
TES	:	trimethyl silyl
TFA	:	trifluoroacetic acid
THF	:	tetrahydrofuran
TLC	:	thin layer chromatography
TMS	:	trimethyl silyl
Tr	:	trityl (triphenyl methyl)
Ts	:	tosyl (*p*-toluenesulfonyl)
6N HCl	:	6 normal solution of hydrochloric acid