Chapter 1

Introduction and Importance of Isocarbostyril alkaloids of Amaryllidaceae family.
1.1. Introduction

The natural products are chemical compounds produced by living organisms found in nature. They can be extracted from tissues of terrestrial plants, marine organisms and microorganism’s fermentation broths. Not only they offer an invaluable source of lead compounds with a wide variety of chemical structures and biological activities, they also play highly significant role in the discovery and development of new drugs for the treatment of human diseases.

During early years of 19th century, it was found that the plant extracts which contained nitrogen bases formed salts with acids. Therefore, they got classified as the vegetable alkalis or alkaloids. Alkaloids may be grouped according to their plant sources e.g. Aconitum, Amaryllidaceae, Cinchona, Curare, Ergot, Opium, Senecio and Vinca. Another classification is based on the structure of the ring system containing the nitrogen atom e.g. piperidine, isoquinoline and indole. Mostly, alkaloids reflect their biosynthetic origin from amino acids such as ornithine, lysine, phenylalanine, tyrosine and tryptophan.

1.2. Amaryllidaceae class of alkaloids.

The Amaryllidaceae class of alkaloids; representing a group of isoquinoline alkaloids, are known to be formed biogenetically by intramolecular oxidative coupling of norbelladines derived from L-phenylalanine and L-tyrosine (Figure-1). The alkaloids under this family mainly consist of 7 structural types, viz. lycorine (6) (1H-pyrrolo [3,2,1-d,e] phenantridine type), crinine (9) (5,10b-ethanophenantridine type), pancratistatin (2) (isocarbostyril type), galanthamine (8) (6H-benzofuro [3a,3,2-e,f]-2-benzazepine type), tazettine (10) (2-benzopyrano [3,4-c] indole type), lycorenine (11) (2-benzopyrano [3,4-g] indole type) and montanine (7) (5,11-methanomorphanthridine type) (Figure-2).

![Figure-1](image-url)
Category

Isocarbostyril type

R= OH, Pancratistatin (2)
R= H, Deoxypancratistatin (3)

R= OH, Narciclasine (4)
R= H, Lycoricidine (5)

1H-pyrrolo [3,2,1-d,e] phenanthridine type

Lycorine (6)

5,11- methanomorphanthridine type

Montanine (7)
6H-benzofuro [3a,3,2-e,f]-2-benzazepine type

Galanthamine (8)

5,10b-ethanophenanthridine type

Crinine (9)

2-benzopyrano [3,4-c] indole type

Tazettine (10)

2-benzopyrano [3,4- g] indole type

Lycorenine (11)

Figure-2: Types of Amaryllidaceae plant alkaloids.
1.3. Importance of Amaryllidaceae alkaloids.

These classes of alkaloids have been known for their medicinal and also toxic values since ancient Greek\(^1\). It dates back to at least the 4\(^{th}\) century BC, when Hippocrates used oil from the Daffodil bulbs Narcissus poeticus L. for the treatment of cancer\(^1\). Lycorine (6), the first member of this class was studied for its antitumor activity.\(^3,4\) The antitumor potential of other amaryllidaceae isocarbostyril derivatives e.g. narcilasine (4), lycoricidine (5), and pancratistatin (2), have long been studied and recently reviewed.\(^5\) Other than anti-tumor activities, these alkaloids were also known to exhibit anti viral and anti feedant activities. Presently, galanthamine (8) which belongs to this class of alkaloid is commercially successful as acetylcholinesterase inhibitor (used for the treatment of Alzheimer’s disease) and is marketed under the trade name Razadyne.\(^\circ\)

1.4. Natural sources of the Isocarbostyril alkaloids of Amaryllidaceae family.

One of the most widely reported source of narcilasine (4)\(^2\) is the bulbs of Narcissus incomparabilis (yield 0.2 g/kg during flowering stage of the plant) and can also be isolated from Zephyranthes flava, a tropical and subtropical plant cultivated in India\(^2\). The deoxy analog of 4, generally known as lycoricidine (5) was first discovered in L. radiata in 1968\(^18\) and is isolated in good amount from wild Hawaiian bulbs of H. littoralis (yield ~ 0.12 g/kg).\(^18b\) The structurally similar analog of 4, pancratistatin (2) was found in low quantities in plant bulbs such as P. maritinum, Z. flava, H. kalbreyeri and P. littorale,\(^7\) however, the rare tropical bulbs of H. littoralis seem to be the good source of 2.

1.5. Biological Evaluation of Isocarbostyril type of Amaryllidaceae alkaloids:

1.5.1. Antitumor activity of natural analogs.

a) Derivatives of Narcilasine (4).
The biosynthetic derivative of narciclasine (4), 2-O-β-D-glucopyranoside derivative kalbreclasine (12)\textsuperscript{2} provoked significant activation of splenic lymphocytes \textit{in vitro}, a feature characteristic of immunostimulants.\textsuperscript{2b} Another natural β-D- glucose derivative 13 showed cytotoxic activities against Artemia \textit{salina} and anti tumor properties against potato discs infected with Agrobacterium \textit{tumifaciens} (53 % inhibition of crown gall) which are comparable to 4. Recently, the \textit{trans}-dihyronarciclasine (14)\textsuperscript{2c} was found to be active against murine P-388 lymphocytic leukemia cells (Fig-3).

\textbf{b) Derivatives of lycoricidine (5).}

The \textit{trans}-dihydrolycoricidine (15), a derivative of 5 in which the B/C rings are \textit{trans} linked, is shown to possess antitumor activity\textsuperscript{2d} against melanoma subpanel cell lines, NSC lung, colon, brain and renal cell lines.

\textbf{c) Derivatives of pancratistatin (2).}

The 2-O- β-D-glucopyranoside derivative\textsuperscript{2e} 16 of pancratistatin (2) was found to promote germination of seeds and the growth of roots. Other natural derivatives such as 17, 18, 19 and 20 exhibited appreciable cytotoxicities (Fig-4).
1.5.2. Antitumor activity of unnatural analogues.

Unnatural analogues – SAR studies.

The search for the minimum pharmacophore and to understand the mode of the action, resulted in the syntheses and biological screening of many unnatural derivatives which provided essential information about some of the functional groups required for the activity. As a result, series of derivatives and analogues were synthesized (fully or semisynthetic) to obtain more active and/or soluble products (Fig-5). Some of these analogues were selected for the preclinical development as anticancer agents and also their structure–activity relationship was explored. Several key positions in the molecule were modified. For example, one such modification involved the reduction of the lactam carbonyl group to obtain amine hydrochloride salt of lycoricidine (21) but this modification was found to have low impact on cytotoxicity. Fully synthetic compounds 22 and 23 in which the lactam carbonyl was replaced by lactone functionality, showed no significant cytotoxic activity against L1210 murine leukemia cells. All these modifications indicate that the lactam carbonyl is essential for cytotoxic activity.

Three deoxy analogs of these alkaloids 24, 25, 26, showing weak antitumor activity, revealed that the C2, C3, and C4 hydroxyl groups were essential for showing the
cytotoxicity and can be considered as a minimum required pharmacophore. Another modification concerning the B/C ring junction stereochemistry (e.g. 27, 28 and 29) were also carried out and all these compounds showed weaker cytotoxicity than the parent natural products. The cytotoxicity of 29 against P388 murine leukemia and several human cancer cell lines was found to be about 1000 times less potent than pancratistatin (2).

Modification of the benzodioxole moiety of 2 was investigated through the synthesis of the analogs i.e., the methoxy analog 30 and β-carboline analog 31 and evaluation of their activity revealed that these are 100 times less potent against P388 lymphocytic leukemia and other human cancer cell lines (BXPC-3, MCF-7 and KM20L2). These results show that an intact methylenedioxyphenyl or benzodioxole functionality is essential for significant cancer cell cytotoxicity in these classes of natural compounds. The only unnatural analogue that has been found to be 10 times more active than 2 is phenpanstatin (32) (Fig-5).

Since, pancratistatin (2) exhibited very low water solubility (53 μl/ml), to increase aqueous solubility phosphate prodrugs were also synthesized, but they all displayed markedly lower cytotoxicity in vitro.
1.5.3. Mechanism of action.

Narciclasine (4) and lycoridicine (5) were first identified as plant growth inhibitors involved in inhibiting the synthesis of proteins and the development of chloroplasts. Narciclasine (4), although, not effective in in vitro protease activity, is known to inhibit protein synthesis at the ribosomal level and is also described as an anti mitotic substance displaying colchicines-like activities. It was also found to be an inhibitor for peptide bond formation in eukaryotic ribosomes considering its ability to bind to the 60-S ribosomal subunit or more precisely to the peptidyltransferase center. It is also known to prevent the transfer of the N-acetylleucyl residue from CACCA-leu-Ac onto puromycin. Therefore, it is postulated that these alkaloids are inhibitors of the transpeptidation reaction. Narciclasine (4) induces apoptosis by triggering the activation of the initiator caspases (caspase-8 and -10) in human MCF-7 breast and PC-3 prostate carcinoma cells. Unlike 4, pancratistatin (2) is known to induce rapid apoptosis in neuroblastoma cells in murine P388, lymphocytic leukemia and M-5076 ovary sarcoma models in vivo by disruption of the mitochondrial membrane potential and DNA fragmentation.
1.5.4. Conclusions from SAR studies of pancreatistatin.

There has been serious research activity over the years concerning SAR with pancreatistatin analogues in order to identify active pharmacophore and to discover more bioavailable derivative or potential prodrugs. From these studies, it has emerged that regions crucial for maintaining the activity of 2 are as follows:

1) The presence of phenolic and / or phenanthridone functionalities as a potential donor-acceptor pair is essential.\(^{17}\)

2) The piperonyl type ring A is must for enhanced activity. Changes in the substitution pattern or functionalities elsewhere in the aromatic core structure leads to decreased activity.\(^{14}\)

3) The correct spacial orientation of the peripheral hydroxyl functions in the aminocyclitol-type ring C and the aminoinositol moiety must remain essentially intact, except for variations of substituents, functionalities and configuration at C\(_1\).

4) The \textit{trans} stereochemistry of the B/C ring junction is essential for activity.\(^{15}\)

Therefore, the potential of variation in structure appears to be the greatest in the region between C\(_1\) and C\(_8\)/C\(_9\)/C\(_{10}\) portion of the aromatic core.

1.5.5. Conclusions from SAR studies in narciclasine.

A comprehensive view of the cytochrome P450 3A4 inhibitory pharmacophore has been developed through SAR studies on 4 which reveal the following points.

1) A small substituent such as hydroxyl and acetoxy contributes to cytochrome interaction at H-bond acceptor region at C\(_1\).

2) The cytochrome interaction is enhanced when a bulky lipophilic substituent such as silyl group is placed at C\(_2\) or C\(_4\) indicating a strong interaction with a large hydrophobic binding pocket in the cytochrome active site.

3) A double bond between the C\(_1\)-C\(_{10b}\) elicits strong interaction with the cytochrome alone.
1.6. Summary

1. Since the discovery of narciclasine (4), the first member of isocarbostyril class of alkaloids in 1967, the search for new natural compounds from the Amaryllidaceae plant family has resulted in the isolation of other isocarbostyrils such as 2, 3 and 5.

2. The search for new anticancer drug analogues that resemble the structural motifs of natural products represents an endeavor with the potential for discovery as well as the eventual deciphering of the mode of action of these compounds.

3. It is clear from the results of the biological screening that none of the unnatural derivatives rival the potency of either 2 or 4.

4. Amaryllidaceae constituents represent ideal targets on which synthetic design may be practiced in esthetic manner. There is no doubt that the activity in this field will continue at the interface of biology (activity screening) and chemistry (synthetic design). The potential for discovery in both disciplines is enormous and valuable results will surely be forthcoming.
1.7. References


