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Doctoral Dissertation (A Commentary on Published Articles)

Amaryllidaceae Alkaloids of Montanine type and Their Derivatives as Potential Drugs

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Field of Study: Pharmacognosy and Nutraceuticals

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The Declaration

I declare that all the results included in this dissertation are original. It contains no material submitted, in whole or in part, to accomplish a degree or diploma by either a university or an institute. To the best of my knowledge, this thesis contains no previously published or written material by another individual, except for parts where an acknowledgment or a reference is stated.

Hradec Králové, January 2023 Negar Maafi MSc.

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Negar Maafi

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ABBREVIATIONS

3,4-DHBA	3,4-dihydroxybenzaldehyde
AA	Amaryllidaceae alkaloid
Aβ	Amyloid-beta
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
BBB	Blood-brain barrier
BuChE	Butyrylcholinesterase
Ca3H	Coumarate-3-hydroxylase
Ca4H	Cinnamate-4-hydroxylase
CC_{50}	Half-maximal cytotoxicity concentration
CDK5	Cyclin dependent kinase-5
CNS	Central Nervous System
cv.	Cultivar
DAST	Diethylaminosulfur trifluoride
EC_{50}	Half-maximal effective concentration
*Ee*AChE	Electric eel Acetylcholinesterase
eEF1A	Eukaryotic translation elongation factor 1A
*Eq*BuChE	Equine serum Butyrylcholinesterase
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
GC-MS	Gas chromatography-mass spectroscopy
GI_{50}	Half-maximal growth inhibitory concentration
GP	Growth percentage
GSK-3β	Glycogen synthase kinase-3 beta
*h*AChE	Human Acetylcholinesterase
*h*BuChE	Human Butyrylcholinesterase
HDAC	Histone deacetylase
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
HRMS	High-resolution mass spectrometry
IC_{50}	Half-maximal inhibitory concentration
i.p.	Intraperitoneal injection
LD_{50}	Median lethal dose
MAO	Monoamine oxidase
MDR	Multidrug-resistant
MIC	Minimal inhibitory concentration
MRSA	*Staphylococcus aureus* subsp. *aureus* methicillin-resistant strain
MS	Mass spectroscopy
MsCl	Methanesulfonyl chloride
Mtb	*Mycobacterium tuberculosis*
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
N4OMT	Norballadine 4'-*O*-methyltransferase
NBS	Norbelladine synthase
NFTs	Neurofibrillary tangles
NMDAR	*N*-methyl-d-aspartate receptor
NMR	Nuclear magnetic resonance
NR	Noroxomaritidine reductase
NSCLC	Non-small cell lung cancer
PAL	Phenylalanine ammonia-lyase
P-gp	P-glycoprotein
POP	Prolyloligopeptidase

SAR	Structure-activity relationship
SERT	Serotonin transporter
SI	Selectivity index
S_N2'	Second-order nucleophilic substitution
$SOCl_2$	Thionyl chloride
SRB	Sulforhodamine B
subsp.	Subspecies
TB	Tuberculosis
TC_{50}	Half-maximal toxic concentration
TLC	Thin-layer chromatography
TYDC	Tyrosine decarboxylase
WST-1	Water-soluble tetrazolium salts

1 INTRODUCTION

The universal role of plants in treating disease is undeniable as various thriving civilizations have adopted significant nature-based systems of medicine throughout history, regardless of the underlying philosophical premise. For example, western medicine with origins in Mesopotamia and Egypt, the Unani (Islamic) and Ayurvedic (Hindu) systems centered in the west of Asia and the Indian subcontinent, and those of the Orient (China, Japan, Tibet, and others). [1] Though the true origins of plants' exploitation by humans are lost in prehistory, the transition from the oral transmission of medical information to the development of writing communication methods led to the permanence of medical knowledge; As in Papyri (for example, the Egyptian Papyrus Ebers ca. 1600 B.C.), baked clay tablets (about 660 cuneiform tablets ca. 650 B.C. from Ashurbanipal's library at Nineveh), parchments and manuscript herbals, printed herbals (invention of printing ca. 1440 A.D.), pharmacopeias and other works of reference (first London Pharmacopoeia in 1618; first British Pharmacopoeia in 1864), and most recently electronic storage of data. Similar records exist for Chinese medicinal plants (texts from the 4th century B.C.), Ayurvedic medicine (2500–600 B.C.), and Unani medicine (Kitab-Al-Shifa, the Magnum Opus of Avicenna, 980–1037 A.D.). [1,2]

Natural products are, up to this date, still plentiful sources for discovering new potential therapeutic agents to treat diseases. More than 75% of small molecules as anticancer drugs are either natural products or directly derived from them. [3] In the early 19th century, the isolation of some pure opium alkaloids from natural sources paved the way for drug discovery and became the leading event in the development of modern pharmacy. Undoubtedly, the plant kingdom still holds many undiscovered molecules with medicinal value as many plants are constantly being screened for their possible pharmacological values, particularly for anti-inflammatory, antiparasitic, cytotoxic, antibiotic, and neuroprotective properties. [1,4] Not only can the active molecules isolated from plants provide potential medicines in therapy, but they also might be valuable as lead molecules to be modified chemically or even serve as a template for the design of synthetic molecules by incorporating the pharmacophore. The development of atracurium from tubocurarine, dextromethorphan from morphine, and verapamil from papaverine are some examples of medications achieved by the chemical development of their natural structures. [1]

The observed biological activity of plants is due to the presence of metabolites in both primary and secondary forms. Primary metabolites directly contribute to plant growth and development, while secondary metabolites such as phenols, terpenes, saponins, and alkaloids are plant-environment mediated and ensure the plant's survival in its surroundings. [5] One of plants' most prominent group of

secondary metabolites is the alkaloids, bitter nitrogen-containing byproducts derived from amino acids. Over 10,000 different alkaloids have been discovered in species from over 300 plant families. [2]

Amaryllidaceae alkaloids (AAs) are one of the most critical groups of alkaloids, generated exclusively by plants of Amaryllidaceae. [6,7] Extensive traditional usage of this family is apparently due to the presence of AAs. [8] These compounds have demonstrated properties such as cytotoxic, antiproliferative, anti-inflammatory, and antibacterial activity, as well as a wide range of physiological effects, such as acetylcholinesterase (AChE) inhibitory and butyrylcholinesterase (BuChE) inhibitory in numerous studies. [7-9] Galanthamine is an AA that has been approved by FDA in 2001 for the treatment of mild to severe stages of Alzheimer's disease (AD) as a natural selective acetylcholinesterase inhibitor (AChEI). [3,8] Among these secondary metabolites from Amaryllidaceae, montanine-type alkaloids have been investigated infrequently due to their limited availability in these plants, while they demonstrated to possess antiproliferative, antimalarial, antirheumatic, and recently antimicrobial activity. [10-14] In the current dissertation, inspired by earlier discovery of the possibility of crinane-type ring transformation to montanine-type nucleus and availability of montanine in our research group (previously isolated from *Hippeastrum* x *hybridum* cv. Ferrari) in grams, we had the chance to shed light on biological values of these rarely studied alkaloidal skeleton type. [10-12] Furthermore, by preparing semisynthetic derivatives and chemical modifications in the framework of montanine-type core, we planned to enhance the biological potency and establish the structure-activity relationship (SAR).

2 AIMS OF DISSERTATION

This dissertation aims to evaluate the potency of montanine-type AAs and their derivatives on various primary biological tests to introduce novel molecules with therapeutic potential in treating diseases, study their SAR, and suggest chemical modifications to enhance their activity.

Individual goals in detail:

- Comprehensive literature peruse, concentrating on montanine-type AAs and their potential biological activities, and the existing knowledge of the impact of their structural modifications on activity.
- Intramolecular rearrangement of widely available haemanthamine ring to montanine-type scaffold and its subsequent derivatization.
- Preparation of the analogs of the selected derivatives from priorly isolated montanine (from *Hippeastrum* x *hybridum* cv. Ferrari) for a better comprehension of SAR.
- Determination of the legitimate structure of prepared compounds by utilizing spectroscopic methods (such as MS, NMR, HRMS, and optical rotation).
- Evaluation of the prepared derivatives against various biological tests (inhibition activity against cholinesterases, cytotoxicity, antimycobacterial, antifungal, and antibacterial activity).

3 THEORETICAL PART

3.1 Plants of the Amaryllidaceae: Distribution and Ethnobotany

The Amaryllidaceae are a large family of perennial, herbaceous, and bulbous flowering plants, consisting of ca. 85 genera and ca. 1100 species with extensive distribution throughout the tropics, South Africa, Mediterranean zone, Andean region, and temperate regions of Asia. [3,15,16] The most prominent genera of Amaryllidaceae are *Galanthus*, *Nerine*, *Hymenocallis*, *Narcissus*, *Pancratium,* and *Hippeastrum*. [1] Over one-third of all the known species are found in South Africa, with *Crinum* and *Haemanthus* as the most notable genera. [17] In the Andes and Mediterranean regions, significant proliferation of Amaryllidaceae species can be found in Peru (*Eustephia coccinea*), Argentina (*Hippeastrum parodii* and *Zephyranthes carinata*), Turkey (*Narcissus tazetta*), and Bulgaria (*Galanthus nivalis* and *Leucojum aestivum*). [17-19] Popular ornamental varieties of Amaryllidaceae, such as daffodils (genus *Narcissus*), snowdrops (genus *Galanthus*), and snowflakes (genus *Leucojum*), can also be found in parts of Europe with a mild climate. [16,19-21] For centuries, Amaryllidaceae plants have not only been cultivated as ornamental plants but also as folk herbal medicines against various diseases in many countries and nations. [8]

Evidence shows that ethnic usage of *Narcissus* in the Mediterranean region can be dated to the times of Hippocrates and Pliny, as a pessary prepared from *Narcissus* oil was used for treating uterine tumors and topical balm of the plant extract for anticancer purposes. [7,16] Undoubtedly, the Amaryllidaceae has a long history in the traditional medicinal practices of the aboriginal people of South Africa. All three major tribes, namely the Sotho, Xhosa, and Zulu, are known to use extracts and decoctions of Amaryllidaceae bulbs for various diseases, from coughs and colds, renal and hepatic conditions to mental illness as well as terminal illnesses such as cancer. San rock paintings of *Brunsvigia* species in the Lesotho highlands attest to the usage of the Amaryllidaceae by first inhabitants of the region. [16] Moreover, the consumption of *Crinum* and *Haemanthus* for anti-infection purposes is extensively recorded in ethnopharmacological literature over the southern African region. Ethnic consumption of these plants is not only restricted to southern Africa, as *Crinum asiaticum* L. is reported to be the most widely used Amaryllidaceae plant with listings from six nations, in Bangladesh, India, Indonesia, Myanmar, South Pacific, and Thailand, to alleviate fever and disinfect wounds. [17]

3.2 Biosynthesis of Amaryllidaceae Alkaloids: Norbelladine Pathway

The most specific characteristic of Amaryllidaceae plants is the consistent presence of an exclusive group of isolated alkaloids from this family's genera, known as AAs. The majority of this large and still expanding group of alkaloids is not known to occur in any other family of plants. [15] Biosynthesis of AAs with their diverse and complex skeleton involves a series of biochemical reactions like oxidation,

reduction, hydroxylation, methylation, phenol-phenol coupling, and oxide bridge formation. [22,23] Although novel AAs are still being discovered; radiolabeling observations show that they all share a common biochemical pathway with a critical intermediate, norbelladine, which is subsequently *O*-methylated and then undergoes cyclization to generate various basic skeletons of these alkaloids (**Figure 1**). [22]

Aromatic amino acids, L-phenylalanine and L-tyrosine, are used to produce norbelladine in Amaryllidaceae plants. [15,22,24,25] In the initial stages of AAs biosynthesis, the enzyme phenylalanine ammonia-lyase (PAL) catalyzes the elimination of ammonia group on L-phenylalanine to generate *trans*-cinnamic acid. Two consequent hydroxylation reactions on the aromatic ring catalyzed by cytochrome P450s, cinnamate-4-hydroxylase (Ca4H) and coumarate-3-hydroxylase (Ca3H), followed by the removal of two carbons, lead to the generation of the C_6C_1 precursor, 3,4-dihydroxybenzaldehyde (also known as protocatechuic acid; 3,4-DHBA). [26] The pathway leading to the production of 3,4-DHBA from L-phenylalanine is known as the phenylpropanoid pathway, which is phylogenetically available in most plant species. In Amaryllidaceae, it is reported that *trans*-cinnamic acid, *p*-coumaric acid, and caffeic acid were intermediate molecules that eventually led to the production of 3,4-DHBA. [15,22,26] On the other hand, the enzyme responsible for tyramine biosynthesis is tyrosine decarboxylase (TYDC) which is the key regulatory enzyme in many alkaloid-containing plants. TYDC controls the sufficient supply of tyramine for synthesizing a wide range of isoquinoline alkaloids, including the well-known narcotic analgesics morphine and codeine. [26] Norbelladine, as the key intermediate, is formed through the condensation of 3,4-DHBA and tyramine, catalyzed by the norbelladine synthase (NBS) and/or the noroxomaritidine reductase (NR) or a combination of both enzymes (**Figure 1**). [15,22,26]

Generated norbelladine from the condensation of 3,4-DHBA and tyramine can form cherylline-type AAs after a sequence of various biochemical reactions like methylation, hydroxylation, dehydration, cyclization, and tautomerization, or can be methylated by norbelladine 4'-*O*-methyltransferase (N4OMT) to 4'-*O*-methylnorbelladine, as a central intermediate for the biosynthesis of most AAs (**Figure 1**). [22,24-26] 4'-*O*-methylnorbelladine can take one of three pathways of intramolecular oxidative couplings: *ortho-para'*, *para-para'*, and *para-ortho'*. The *para-ortho'* C-C coupling leads to galanthamine-type AAs, whereas the *ortho-para'* phenol coupling elaborates lycorine- and homolycorine-types of AAs. The *para-para'* phenol-phenol' coupling reaction generates the crinane- (α- and β-), narciclasine-, pretazettine-, and montanine-types (**Figure 1**). [15,24,26] Nevertheless, genes involved in the biosynthesis of AAs are poorly investigated, and transcriptomic research and genome sequencing for Amaryllidaceae plants have only started recently. [27-29]

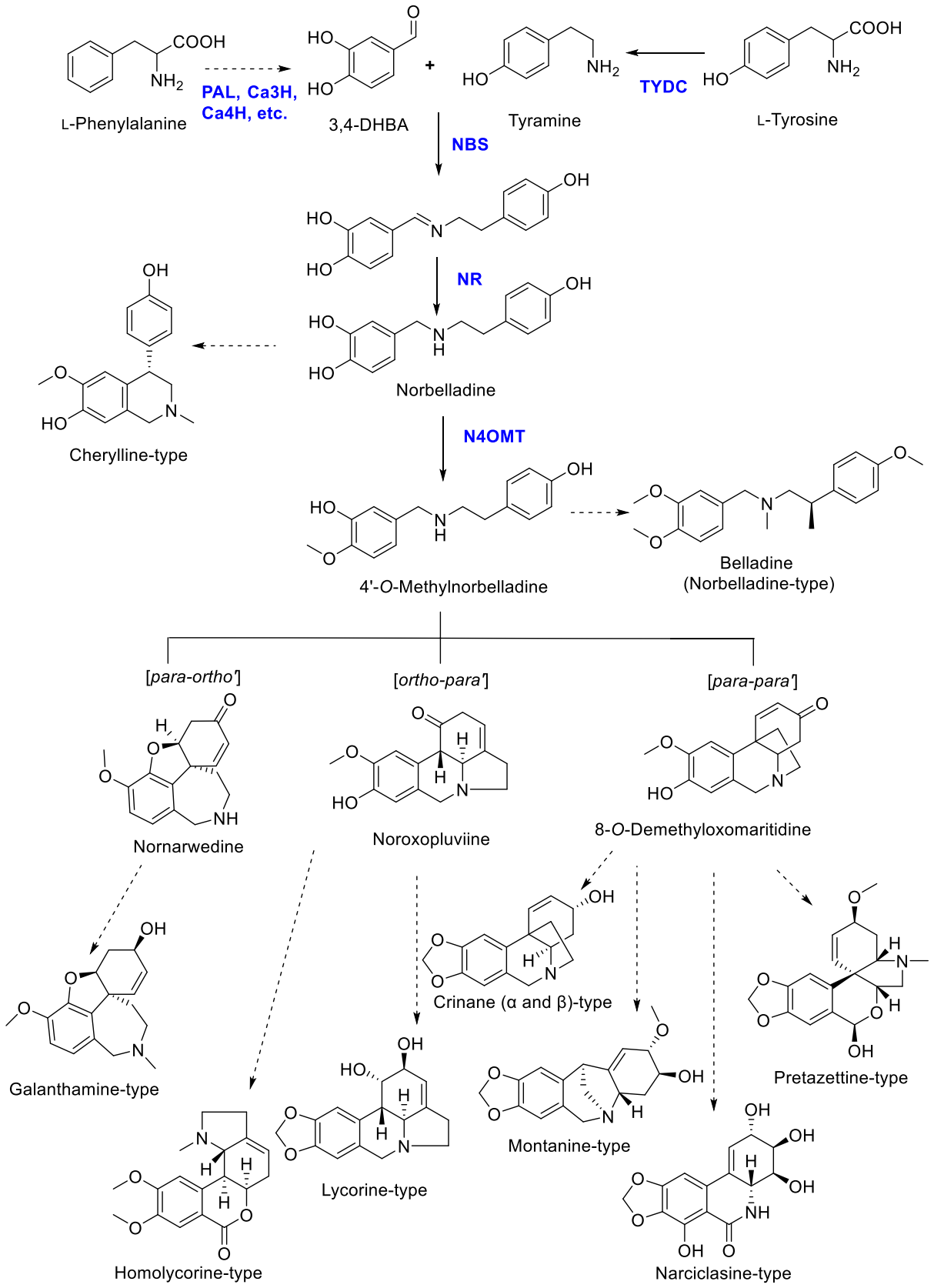


Figure 1. Proposed biosynthetic pathways of Amaryllidaceae alkaloids. A solid arrow represents one enzymatic reaction, while a dashed arrow represents multiple enzymatic steps.

3.2.1 Detailed Biosynthesis of Montanine-type Alkaloids

There are quite a few studies investigating the detailed biosynthetic pathway of montanine-type alkaloids. In 1973, it was suggested that the successful chemical transformation of an 11-hydroxy-substituted crinane-type nucleus into montanine-type core could support a theory; the 5,11-methanomorphanthridine skeleton is derived biosynthetically from *O*-methylnorbelladine by *para-para'* coupling followed by subsequent rearrangement of a haemanthamine-like intermediate. Moreover, it was proposed that if a haemanthamine-like intermediate is involved in this nucleus rearrangement, then hydroxylation on C11 of the crinane skeleton has a different stereochemical state from what is present in haemanthamine. [30,31] This theory was introduced through a feeding experiment on *Haemanthus coccineus* with asymmetrically labeled precursors. The results established that in the biological conversion of *O*-methylnorbelladine to a 5,11-methanomorphanthridine alkaloid, montanine, *pro*-S hydrogen from C2 of the precursor is lost, and *pro*-R hydrogen at C2 is retained at C11 of the montanine skeleton, which agrees with *para-para'* coupling of the precursor in the generation of the 5,11-methanomorphanthridine nucleus. [30] Later, in 1976, 11-hydroxyvittatine was proposed as the primary precursor in montanine and haemanthamine biosynthesis in *Rhodophiala bifida*, mentioning the higher conversion rate of 11-hydroxyvittatine to haemanthamine in comparison with montanine is caused by skipping the necessary ring transformation step in the production of haemanthamine. Therefore, rearrangement of 11-hydroxyvittatine to pancracine and the following 2-*O*-methylation of pancracine was proposed as a possible montanine biosynthetic pathway in *R. bifida*. [32] Feinstein and Wildman have demonstrated the proposed biosynthetic pathway of montanine and its relation to other crinane-type alkaloids as in **Figure 2**. A similar biosynthetic pathway affirming 11-hydroxyvittatine as an intermediate molecule has been asserted in other studies with slight modifications. [15,29]

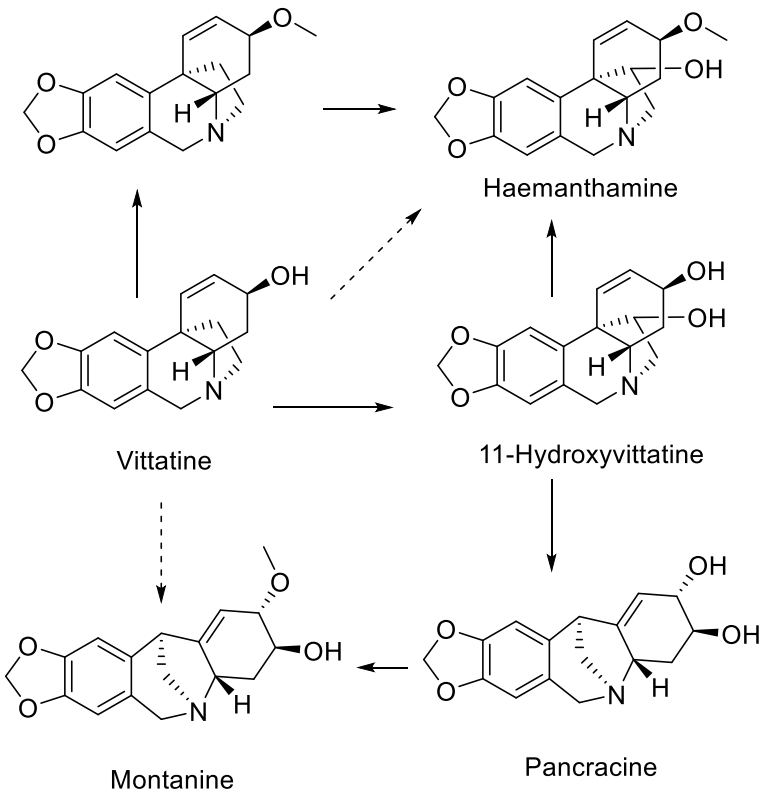


Figure 2. Proposed biosynthetic pathway for montanine and pancracine, according to Feinstein and Wildman. [15,32]

Alternatively, another possible biosynthetic pathway for montanine-type AAs was described by Jin et al. in 2007; after aromatization of a belladine-type intermediate, intramolecular addition of the *p'*-position of the aromatic ring to the benzylic position of the oxidized quinonoid generates cherylline-type alkaloids. Further addition of the secondary amine to the dienone intermediate leads to the production of montanine-type alkaloids (**Figure 3**). [23] Based on this proposed pathway, the involvement of a chemically stable cherylline-type precursor in the multistep transformation of norbelladine-type intermediates into montanine-type nucleus has also been utilized as a new bioinspired strategy for the synthetic approach to 5,11-methanomorphanthridine alkaloids. [33]

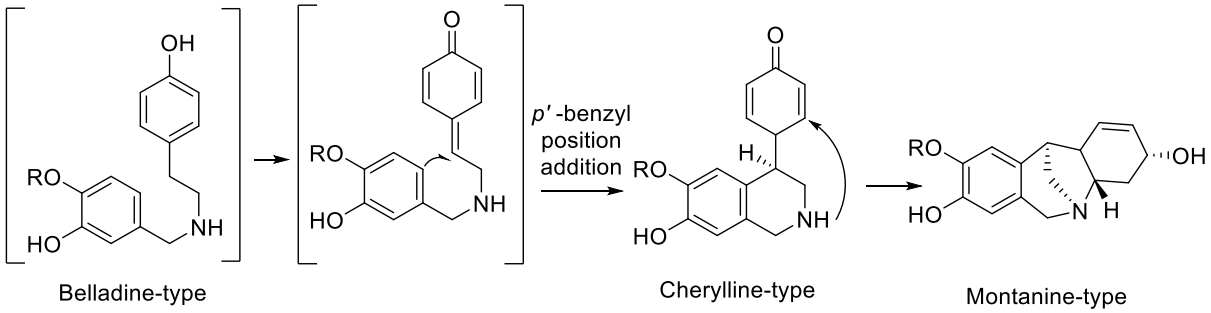


Figure 3. Proposed biosynthetic pathway for montanine-type, according to Jin et al. [23]

3.3 Structure Types of Amaryllidaceae Alkaloids

Alkaloids are one of the plants' largest groups of secondary metabolites, usually derived from amino acids. [2] The nitrogen atom can be part of an aromatic heterocycle or stand out from the carbon ring, such as mescaline. Initially, alkaloids were discovered only from higher plants, but later the definition was expanded to a broad range of natural products such as fungi and animals. [34] These secondary metabolites are stored in plants mainly as water-soluble salts formed with common carboxylic acids, namely citric, lactic, oxalic, acetic, maleic, and tartaric. [2]

Several approaches exist to classify alkaloids, such as chemical, taxonomic, biological, and biosynthetic. The most general classification is based on their chemical skeleton as they are organized based on a typical heterocyclic core, as in isoquinoline, indole, quinolone, quinazoline, pyrrolizidine, and tropane alkaloids. Another common method to classify the alkaloids is according to their natural origin and plant families, such as Amaryllidaceae, Solanaceae, and Rutaceae alkaloids, or based on a genus, such as the *Catharanthus* (or Vinca) alkaloids. [34]

Although alkaloids are known as poisonous compounds (for example, strychnine or coniine), some of them are used in medicine as therapeutic agents. [2] Evidently, the exploitation of alkaloids by humans has a long history of thousands of years. Discovered papyri of ancient times in Egypt (from about 4000 years ago) imply the utilization of alkaloid-containing crude drugs such as opium, *Hyoscyamus niger* containing hyoscyamine, and *Colchicum* extract containing colchicine. Furthermore, arrows poisoned with a mixture of toxic constituents from *d*-tubocurarine, C-curarine, and a bisindole alkaloid from the *Strychnos* were often used by native peoples of South America for hunting. [34] However, it was not until the early 19th century that alkaloids could be reproducibly isolated and structurally elucidated on account of new advances in separation techniques, such as chromatography and mass spectroscopy (MS). [2]

As previously stated, the specific characteristic of Amaryllidaceae is the abundant presence of a unique group of alkaloids mainly containing isoquinoline core. [3] Since lycorine was isolated as the first AA from *Narcissus pseudonarcissus* in 1877, until now, more than 600 structurally diverse AAs have been isolated from this family, and yet the number is still expanding. [3,8,22,35] These alkaloids are considered to be produced by intramolecular oxidative coupling of norbelladine. [8] Due to the unique chemical structure and promising biological potency of these compounds, their investigations have been progressively accentuated in recent years. [3] These compounds have been displayed to be biologically active as anticancer, antiproliferative, anti-inflammatory, antiparasitic, and antibacterial molecules. In addition, they can modulate the central nervous system (CNS) functions, such as acting as AChE/BuChE and prolyloligopeptidase (POP) inhibitors. [7-9,22,36] As earlier stated, galanthamine, as a highly available

AA in most plants of Amaryllidaceae, was launched in 2001 in the U.S. and Europe for symptomatic treatment of AD under the commercial brands Reminyl® or Razadyne®.[3,8,37]

AAs can be classified into different categories according to their molecular skeletons and ring systems.[8] So far, different approaches have been adopted to classify AAs into 5, 12, or even 17 categories.[3,7,8,22,35] Though in addition to all minor miscellaneous alkaloids isolated from plants of this family, a total number of 42 different chemical scaffolds have been discovered.[35] Here, a standard classification based on the biosynthetic origin and skeleton ring type is presented, which divides AAs into nine main types, including norbelladine, cherylline, galanthamine, lycorine, homolycorine, crinane (α- and β-), narciclasine, pretazettine, and montanine (**Table 1**).[22,24,35] Though Norbelladine-type AAs do not contain an isoquinoline backbone, they are recognized as a primary structural type of AAs due to the importance of norbelladine in the biosynthesis of AAs.[8]

Table 1. Basic structure types of the Amaryllidaceae alkaloids and their ring types.

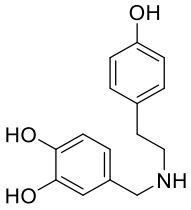
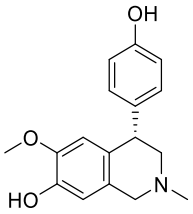
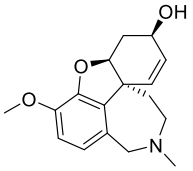
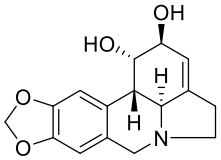
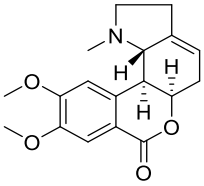
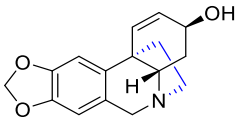
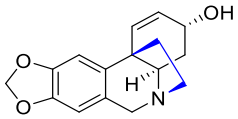
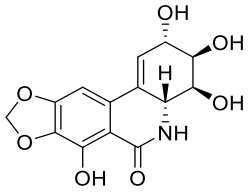
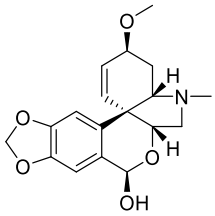
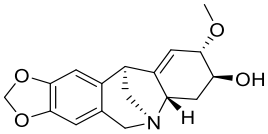
Structure type	Structure	Ring-type	Representative alkaloids
Norbelladine		*N*-(3,4-Dioxybenzyl)-4-oxyphenethylamine	Norbelladine
Cherylline		Tetrahydroisoquinoline	Cherylline, gigantelline
Galanthamine		6H-Benzofuro[3a,3,2-e,f]-2-benzazepine	Narwedine, galanthamine
Lycorine		Pyrrolo[d,e]phenanthridine	Lycorine, galanthine
Homolycorine		2-Benzopyrano-[3,4-g]indole	Homolycorine, hippeastrine

Table 1. Basic structure types of the Amaryllidaceae alkaloids and their ring types (continuation).

Structure type		Structure	Ring-type	Representative alkaloids
Crinane	**Haemanthamine (α-Crinane)**		5,10b-Ethanophenanthridine	Haemanthamine, vittatine
	Crinine (β-Crinane)		5,10b-Ethanophenanthridine	Crinine, ambelline
Narciclasine			Lycoricidine	Narciclasine, pancratistatin
Pretazettine			2-Benzopyrano[3,4-c]indole	Pretazettine, tazettine
Montanine			5,11-Methanomorphanthridine	Pancracine, montanine

From the minor structural type of AAs, galanthaindole, cripowelline, ismine, plicamine, garciline, and galasine can be named. [35] Alkaloids with new chemical skeleton types and atypical compounds are constantly being described from Amaryllidaceae plants. In recent years, two new chemical skeletons of narcikachnine- and carltonine-type AAs have been isolated and presented as promising biologically active scaffolds. [9,18,38-40] Moreover, different skeleton types might be available in these plants as dimers, such as isolated cliniatines A and B (consisting of 2,6-dimetylpyridine linker between a lycorine-type part and a galanthamine-type part of the alkaloid), and clivimine B from *Clivia miniata*. [41,42]

3.4 Montanine-type Alkaloids: Structural Characteristics and Phytochemistry

These alkaloids contain a distinctive 5,11-methanomorphanthridine nucleus. [31,43] The first montanine-type alkaloids, montanine, manthine, manthidine, and coccinine, were isolated by Wildman et al. in 1955 from *Haemanthus* species. [44] Though later, in 1960, Inubushi et al. described the 5,11-methanomorphanthridine nucleus for montanine, manthine, and coccinine, in addition to reporting the discovery of brunsvigine. [31] Furthermore, during their exploration of the structure and absolute

configurations of haemanthamine and crinamine, as they were hoping to find some method for replacing the C11 hydroxyl with hydrogen, they obtained an unexpected rearrangement in the crinane nucleus. Later in the Chapter 3.10.1. Derivatives of Montanine-type Alkaloids of this commentary, this phenomenon will be explained in details.

Characteristically, montanine-type alkaloids have a 15-carbon membered skeleton and are distinguished by variation of substituents such as hydroxyl, methoxy, and acetyl groups on C2, C3, C4, and C11a of 5,11-methanomorphanthridine nucleus. [6] Thus far, 13 alkaloids with a montanine-type framework have been isolated from Amaryllidaceae plants, indicating the scaffold's infrequent presence in these plants. [6,8] Additionally, two newfound alkaloids of this type, montabuphine and lycolongirine C, are the only compounds with a defined β-configuration for the 5,11-methanobridge. Otherwise, the proton at C4a is β-oriented in all previously isolated compounds, and the methanobridge between N5 and C11 has an α-oriented configuration. [6] Therefore, the accuracy of the elucidated structure for montabuphine and lycolongirine C is questionable and requires more investigation. [6,45] All naturally discovered molecules containing 5,11-methanomorphanthridine scaffold are illustrated in **Figure 4**.

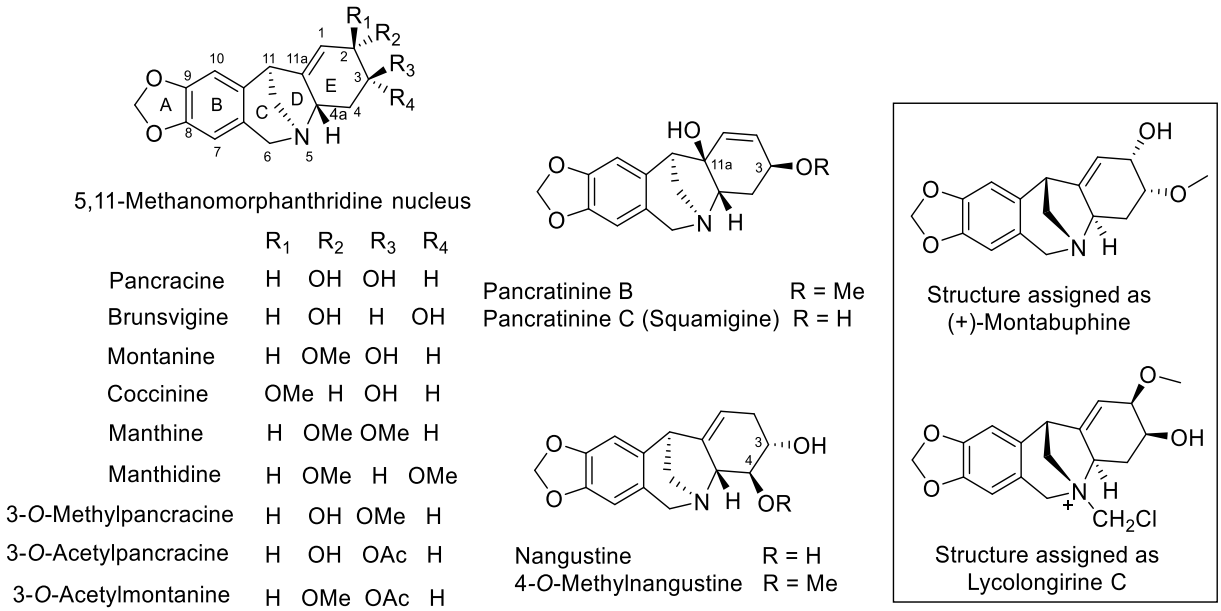


Figure 4. Structures of montanine-type Amaryllidaceae alkaloids

Montanine is the leading representative alkaloid of the class, with methoxy and hydroxyl substitution on C2 and C3, respectively. Other montanine-type alkaloids with typical C2 and C3 substitutions are pancracine (and its natural derivatives), coccinine, manthine, brunsvigine, and manthidine. Montanine is the primary alkaloid produced in *R. bifda* and *Scadoxus multiflorus*. [29,46,47] It also can be found in many plants of the *Haemanthus* and *Rhodophiala* genera, as well as *Hippeastrum*. [6,48-53] Chemometrics

evaluations for identifying the constituents of *Hippeastrum elegans* demonstrated that the maximum montanine quantity occurs in the 9th month of cultivation compared to 5, 7, 11, 13, and 15 months of horticulture. At the same time, Pancratinine C reaches its maximum intensity in the 11th month of the plant's growth. [54] Coccinine is an isomer of montanine with a β-oriented methyl group on C2. To the best of our knowledge, *Haemanthus humilis* is the most prominent natural source of coccinine as the dominant alkaloidal metabolite in the plant. [55] Reportedly, the alkaloid extracts of *H. coccineus* and *Haemanthus sanguineus* are composed predominantly of coccinine, while *Haemanthus montanus* contains primarily the α-oriented *O*-2-methyl-isomer, montanine. [50]

Pancracine was discovered in 1968 by Wildman et al. as another AA with a 5,11-methanomorphanthridine nucleus. [56] The presence of this alkaloid culminates in the flowering stage (in April) rather than the preflowering stage (in November) in *Amaryllis belladonna* L. bulbs, as it was surveyed using high-performance thin layer chromatography (HPTLC) for comparing the relative amounts of the alkaloids present in growth stages. [57]

Manthine is another montanine-type AA, which could be isolated naturally from a few plants of *Haemanthus* genera only in a trivial amount. [44,50] However, it has been derived semisynthetically from the chemical rearrangement of haemanthamine to the montanine-type skeleton by adding methoxide solution to mesylated haemanthamine. [12] Brunsvigine and manthidine have also been isolated from plants of Amaryllidaceae only in trivial amounts. Hydroxyls of brunsvigine and methoxy groups of manthidine stand on C2 and C3 in an α-configuration. [31,44,48,50]

Nangustine is one of the recently characterized members of this AA class, isolated from the bulbs of *Narcissus angustifolius* subsp. *transcarpathicus* collected in Ukraine. Significantly, nangustine varies from its congeners regarding the substituents on C3 and C4 rather than the usual C2 and C3 substitution pattern on the E ring. Pancratinine B and pancratinine C (also named squamigine), both isolated primarily from *Pancratium canariense* in minor amounts, are characterized by the presence of a double bond between C1 and C2 and with a hydroxy group located at C11a. [58]

In 1995, Viladomat et al. reported the isolation of montabuphine from bulbs of *Boophone flava* (recently known as *Crossyne flava)*, describing the β-orientation of methano-bridge for the first time.[59] This report attracted attention by hypothesizing the possibility of the natural occurrence of both enantiomeric forms of the bridge in the 5,11-methanomorphanthridine skeleton. Though perceived spectroscopic and spectrometric data from the total synthesis of (+)-montabuphine found the accuracy of illustrated structure for montabuphine controversial. The legitimate structure of this AA is yet to be described. [45,60] Lycolongirine C is another newly discovered molecule with a β-configuration of the 5,11-methanobridge, isolated from *Lycoris longituba*. [61] However, lycolongirine C is assumed to be an

artifact generated during the extraction and isolation process. [6] **Table 2** summarizes all the Amaryllidaceae plants reported to contain alkaloids with the montanine framework.

Table 2. Sources of montanine-type Amaryllidaceae alkaloids; * Asterisk indicates species in which the presence of alkaloid is only detected by methods such as gas chromatography-mass spectroscopy (GC-MS), and the compound was not isolated solely.

Montanine	*Haemanthus amarylloides*	44
	Haemanthus coccineus (Haemanthus tigrinus)	31,44,50
	Haemanthus montanus	44,50
	Haemanthus multiflorus (Scadoxus multiflorus)	46,62
	Haemanthus pauculifolius	48
	Haemanthus deformis	48
	*Haemanthus sanguineus**	50
	Haemanthus humilis	55
	Hippeastrum argentinum	63
	Hippeastrum x *hybridum* cv. Ferrari	11,53
	Hippeastrum x *hybridum* cv. Double King*	53
	Hippeastrum x *hybridum* cv. Pretty Nymph*	53
	Hippeastrum x *hybridum* cv. Spartacus*	53
	*Hippeastrum elegans**	54
	Hippeastrum aulicum	64
	Hippeastrum vittatum	51,65
	Rhodophiala bifida	29,47,66
	*Rhodophiala mendocina**	49
	*Rhodophiala montana**	52
	*Rhodophiala pratensis**	52,67
	*Lycoris longituba**	68
	*Lycoris chinensis**	68
	*Lycoris squamigera**	68
	*Lycoris albiflora**	68
	*Pyrolirion albicans**	69
	*Habranthus jamesonii**	49
Pancracine	*Pancratium maritimum*	56,70-72
	Pancratium canariense	58,73
	Pancratium sickenbergeri	74
	Hippeastrum vittatum	75
	Hippeastrum x *hybridum* cv. *Ferrari*	11,53
	Hippeastrum argentinum	63
	Hippeastrum x *hybridum* cv. Double King*	53
	Hippeastrum x *hybridum* cv. Pretty Nymph*	53
	*Hippeastrum elegans**	54
	Rhodophiala bifida	56
	*Rhodophiala Montana**	52
	*Rhodophiala pratensis**	52

Table 2. Sources of montanine-type Amaryllidaceae alkaloids; * Asterisk indicates species in which the presence of alkaloid is only detected by methods such as gas chromatography-mass spectroscopy (GC-MS), and the compound was not isolated solely (continuation).

Pancracine	*Rhodophiala splendens**	[52]
	*Rhodophiala mendocina**	[49]
	Narcissus cv. Professor Einstein	[76]
	*Narcissus poeticus**	[77]
	Narcissus angustifolius subsp. *transcarpathicus*	[78]
	Lycoris radiata	[79]
	*Pyrolirion albicans**	[69]
	*Galanthus ikariae**	[80]
	Amaryllis belladonna	[57]
Coccinine	*Haemanthus amarylloides*	[44]
	Haemanthus coccineus (Haemanthus tigrinus)	[31,44,50]
	Haemanthus deformis	[48]
	Haemanthus montanus	[50]
	*Haemanthus sanguineus**	[50]
	Haemanthus humilis	[55]
	Haemanthus albiflos (Haementhus albomaculatus)	[81]
Manthine	*Haemanthus amarylloides*	[44]
	*Haemanthus montanus**	[50]
	Haemanthus coccineus (Haemanthus tigrinus)	[31]
Manthidine	*Haemanthus coccineus (Haemanthus tigrinus)*	[44]
	Haemanthus pauculifolius	[48]
	Haemanthus deformis	[48]
	Haemanthus amarylloides	[31]
Brunsvigine	*Brunsvigia radulosa (Brunsvigia cooperi)*	[31,82]
	Hippeastrum reticulatum	[83]
Pancratinine B	*Pancratium canariense*	[58]
Pancratinine C (Squamigine)	*Pancratium maritimum**	[84]
	Pancratium canariense	[58]
	Lycoris radiata	[79]
	Lycoris squamigera	[85]
	*Lycoris longituba**	[68]
	*Rhodophiala pratensis**	[52]
	*Hippeastrum elegans**	[54]
Nangustine	*Narcissus angustifolius* subsp. *transcarpathicus*	[78]
	Rhodophiala bifida	[47]
3-*O*-Methylpancracine	*Lycoris radiata*	[79]
3-O-Acetylpancracine	*Rhodophiala bifida*	[29,66]
4-*O*-Methylnangustine	*Hippeastrum argentinum*	[63]
Montabuphine	*Crossyne flava (Boophone flava)*	[59]
Lycolongirine C	*Lycoris longituba*	[61]
3-*O*-Acetylmontanine	*Rhodophiala bifida*	[66]

3.5 Biological Activity of Montanine

The first evidence for montanine as a potential compound with *in vitro* cytotoxicity and growth inhibition activity on cancerous cell lines was reported in 2008 as the main compound responsible for the cytotoxic value of *H. vittatum's* bulbs extract. [65] So far, montanine has been subjected to numerous cytotoxic studies compared to other alkaloids from this skeleton type, suggesting montanine as a potent cytotoxic compound against multiple cell lines. However, its mechanism of action with pharmacological details has not been explained yet. **Table 3** summarizes obtained results from antiproliferation investigations of montanine and its naturally occurring congers which will be discussed in the following chapters.

Montanine also was subjected to toxicity evaluation studies *in vivo*. When administered to male mice at 60, 70, or 100 mg/kg i.p., it modified motor activity, attenuated the respiratory rate, and induced violent body tremors and clonic convulsions leading to death, respectively, in 20, 10, and 100% of the cases. Lesser doses (10 and 30 mg/kg) did not lead to any noticeable motor or behavioral disturbance and did not cause death within 24 h of administration. Female mice showed more resistance to the adverse effects of montanine injection since only death occurred in doses 85 mg/kg or higher. The calculated LD_{50} for montanine was 64.7 mg/kg for male and 67.6 mg/kg for female mice. [51]

Montanine is described to inhibit AChE in a dose-dependent pattern, suppressing more than 50% of the enzyme at 1 mM concentration. With lower concentrations of 500 µM and 100 µM, 30–45% of AChE inhibition activity was detected. Although montanine has suppressed the AChE activity at higher concentrations than galanthamine, further studies concerning its SAR and interaction with AChE, along with investigations to comprehend the relation of AChE activity role with cognition through *in vivo* memory studies, are needed. [86] In another study, montanine showed nonsignificant IC_{50} values of more than 100 µM against both AChE and BuChE enzymes. Moreover, it did not show any impressive activity against POP. [11]

Table 3. Impact of montanine-type Amaryllidaceae alkaloids on the proliferation of cancerous and non-cancerous cell lines using *in vitro* assays.

Montanine-type Alkaloid	Cell Line	Method of Assay/Time of Treatment	Value and Type of Half-Maximal Inhibitory Concentration		References
Montanine	Jurkat	WST-1/48 h	1.04 ± 0.14	IC_{50} - µM	[53]
	MOLT-4	WST-1/48 h	1.26 ± 0.11	IC_{50} - µM	[53]
	A549	WST-1/48 h	1.09 ± 0.31	IC_{50} - µM	[53]
	HT-29	WST-1/48 h	1.35 ± 0.47	IC_{50} - µM	[53]
	PANC-1	WST-1/48 h	2.30 ± 0.45	IC_{50} - µM	[53]
	A2780	WST-1/48 h	1.67 ± 0.29	IC_{50} - µM	[53]
	HeLa	WST-1/48 h	1.99 ± 0.22	IC_{50} - µM	[53]
	MCF-7	WST-1/48 h	1.39 ± 0.21	IC_{50} - µM	[53]
	SAOS-2	WST-1/48 h	1.36 ± 0.49	IC_{50} - µM	[53]
	MRC-5	WST-1/48 h	1.79 ± 0.50	IC_{50} - µM	[53]
	A549	MTT/48 h	1.9 ± 0.4	IC_{50} - µM	[55]
	HCT-15	MTT/48 h	6.8 ± 0.5	IC_{50} - µM	[55]
	SK-MEL-28	MTT/48 h	23.2 ± 1.9	IC_{50} - µM	[55]
	MCF-7	MTT/48 h	4.4 ± 0.4	IC_{50} - µM	[55]
	MDA-MB-231	MTT/48 h	3.4 ± 0.9	IC_{50} - µM	[55]
	Hs578T	MTT/48 h	3.6 ± 1.7	IC_{50} - µM	[55]
	HT-29	SRB/not specified	0.71 ± 0.1	IC_{50} - µg/mL	[65]
	H460	SRB/not specified	0.57 ± 0.57	IC_{50} - µg/mL	[65]
	RXF393	SRB/not specified	0.65 ± 0.01	IC_{50} - µg/mL	[65]
	MCF-7	SRB/not specified	0.74 ± 0.02	IC_{50} - µg/mL	[65]
	OVCAR3	SRB/not specified	0.84 ± 0.11	IC_{50} - µg/mL	[65]
Pancracine	Jurkat	WST-1/48 h	5.07 ± 0.31	IC_{50} - µM	[76]
	MOLT-4	WST-1/48 h	2.71 ± 0.25	IC_{50} - µM	[76]
	A549	WST-1/48 h	2.29 ± 0.43	IC_{50} - µM	[76]
	HT-29	WST-1/48 h	2.60 ± 0.51	IC_{50} - µM	[76]
	A2780	WST-1/48 h	5.08 ± 0.43	IC_{50} - µM	[76]
	HeLa	WST-1/48 h	5.03 ± 0.36	IC_{50} - µM	[76]
	MCF-7	WST-1/48 h	2.68 ± 0.37	IC_{50} - µM	[76]
	SAOS-2	WST-1/48 h	2.20 ± 0.25	IC_{50} - µM	[76]
	MRC-5	WST-1/48 h	5.15 ± 0.34	IC_{50} - µM	[76]
	A2780	SRB/48 h	8.3 ± 0.5	GI_{50} - µM	[73]
	SW1573	SRB/48 h	4.3 ± 0.7	GI_{50} - µM	[73]
	T47-D	SRB/48 h	6.5 ± 2.5	GI_{50} - µM	[73]
	WiDr	SRB/48 h	9.1 ± 1.0	GI_{50} - µM	[73]
Coccinine	A549	MTT/48 h	5.9 ± 0.8	IC_{50} - µM	[55]
	HCT-15	MTT/48 h	16.8 ± 1.8	IC_{50} - µM	[55]
	SK-MEL-28	MTT/48 h	>50	IC_{50} - µM	[55]
	MCF-7	MTT/48 h	7.9 ± 0.9	IC_{50} - µM	[55]
	MDA-MB-231	MTT/48 h	13.8 ± 0.8	IC_{50} - µM	[55]
	Hs578T	MTT/48 h	5.3 ± 0.4	IC_{50} - µM	[55]
Manthine	A549	MTT/72 h	3	GI_{50} - µM	[12]
	SK-MEL-28	MTT/72 h	4	GI_{50} - µM	[12]
	U373	MTT/72 h	5	GI_{50} - µM	[12]
	MCF-7	MTT/72 h	4	GI_{50} - µM	[12]
	Hs683	MTT/72 h	3	GI_{50} - µM	[12]
	B16F10	MTT/72 h	3	GI_{50} - µM	[12]

One study highlights the psychopharmacological properties of montanine, including anxiolytic, antidepressant, and anticonvulsive effects. It manifests that i.p. injection of 10 µl/g of montanine lessens locomotor activity and has tranquilizing, anxiolytic, antiepileptic, and antidepressant effects in mice. Its general depressant activity is declared based on reduced sleep latency in mice and the tendency to increase pentobarbital-induced sleeping time, though the mechanism of action is not yet clarified. This effect can be accredited to the suppression of pentobarbital metabolism or an action in sleep regulation. In addition, the decline in the number of rearings and crossings of mice in the open field test proves the central activity of montanine since rearing is assumed as a sign of the excitability level of the CNS. Montanine also demonstrated a dose-dependent anxiolytic-like activity when assessed in the elevated plus maze test as it increased the percentage of open-arms entries and the time spent in those arms. The elevated plus maze is a frequently used model to evaluate the anxiolytic properties of drugs. The recurrence and the time spent in the open arms are the primary factors of anxiety measurement, as lingering in an open area is undesirable in rodents. [51]

Additionally, obtained outcomes from the pentylenetetrazole-induced seizure test model suggest that montanine may interact with the GABA receptor on the benzodiazepines site in the mouse's brain. Hence, the anxiolytic and hypnotic effects of montanine are likely the result of its combined action on several neurotransmitter receptor systems, including $GABA_A$ receptors. [51]

Antidepressant activity is also attributed to this alkaloid; Administration of montanine before the forced swimming test reduced total immobility time and enhanced struggling behavior in rodents. Though, montanine did not affect long-term memory retention when given 3–10 mg/kg i.p. in post-training sessions. [51] Scrutinizing the antidepressant activity of montanine, distinct research exhibited that it has an affinity for serotonin transporter protein (SERT), with an IC_{50} value of 36.6 µg/mL (121.3 µM) *in vitro* while not interacting with blood-brain barrier (BBB) efflux transporter P-glycoprotein (P-gp). [50]

Montanine showed activity against pathogenic *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538), and *Staphylococcus epidermides* (ATCC 12228) with the minimum quantities required for activity of 5, 20, 5 and 15 µg respectively. This alkaloid also exhibited inhibitory activity against *Saccharomyces cerevisae* (ATCC 2601) with the minimum required quantity of 10 µg for the activity. [13]

The antiparasitic activity of montanine and eight other AAs were evaluated against *Trypanosoma cruzi* compared to benznidazole as a standard drug. The study aimed to present prospective drug development starting points as new therapeutic solutions for Chagas disease in an adapted *in vitro* anti-*T. cruzi* phenotypic assay. Montanine was among the active compounds with an average IC_{50} value

similar to the standard, 1.99 µM versus 1.56 µM of benznidazole. Nonetheless, montanine with a Selectivity Index (SI) value of 2.53 concerning Vero cells was not specific to *T. cruzi*, even though it showed low toxicity for HepG2 cells (TC_{50} = 46.1 µM). [87]

The anti-inflammatory activity of montanine was preliminarily evaluated by determining the sample antichemotaxis property, which did not exhibit outstanding value in the examined dose. [13] Later, montanine was evaluated for its antiarthritic activity on antigen-induced and collagen-induced arthritis animal models and its impact on lymphocyte proliferation and the invasiveness of fibroblast-like synoviocytes *in vitro*. The alkaloid significantly lessened the development of empirical arthritis in both acute and chronic models, in a dose-dependent manner, with the lower dose being more effective in arthritis severity. Accordingly, it is hypothesized that increased availability of montanine leads to an acute activation that culminates in mechanisms of desensitization of the receptor, reducing the activation of receptor cell signaling and consequently decreasing the biological effect seen at lower doses. The obtained results designate montanine as a potential candidate for dealing with autoimmune diseases, such as arthritis. [14] Two related studies regarding the comprehension of the montanine's biosynthesis regulation in *R. bifida* and its antirheumatic effect are followed by a patent granted to the scientific group of Professor Zuanazzi in 2020. [14,29,88] The registration of US20200000798A1 patent invention describes the montanine isolation method thoroughly with substantial yield and much faster method than described previously from *R. bifida*. [88]

3.6 Biological Activity of Pancracine and 3-*O*-Methylpancracine

Two studies have contributed to the cytotoxic effect of pancracine against diverse human cancer cell lines *in vitro* (**Table 3**). Pancracine exhibited solid antiproliferative activity against A549 cells and antiproliferative and cytotoxic effects on MOLT-4 cells. [76] Further investigation to establish the mechanism of the apoptosis induced by pancracine in MOLT-4 cells denotes pronounced high activity of caspases. This was transmitted through the upregulation of p53 phosphorylated on Ser392, p38 mitogen-activated protein kinase phosphorylated on Thr180 and Tyr182, and upregulation of p27. Treatment with pancracine downregulated the proliferation of A549 cells due to cell cycle arrest in the G1 phase, associated with the downregulation of phosphorylated retinoblastoma tumor suppressor protein, with the collateral upregulation of p27 and downregulation of Akt phosphorylated on Thr308.[89]

This AA was demonstrated to have a weak AChEI effect (31.84 ± 0.29 % of enzyme inhibition rate) when tested with a 10 µg/mL concentration. [80] Though IC_{50} of pancracine for inhibition of both AChE and BuChE is reported to be more than 200 µM. [63,80]

Pancracine also has been subjected to anti-infective studies. [57,72,78] The antibacterial and antifungal activity of pancracine was discussed using an agar diffusion technique against Gram-positive bacterium *S. aureus*, two Gram-negative bacteria *E. coli* and *P. aeruginosa,* and *Candida albicans*. It displayed a minimal inhibitory concentration (MIC) value of 188 µg/mL against both *S. aureus* and *C. albicans*, in addition to 16 mm of inhibition zone for *P. aeruginosa*. [57]

Pancracine exhibited poor performance against *Trypanosoma brucei rhodesiense* and *T. cruzi* with IC_{50} of 0.7 and 7.1 µg/mL, respectively, but no activity against *Leishmania donovani*. In order, the IC_{50} values of 0.75 and 0.70 mg/mL for two strains of *Plasmodium falciparum* K1 and NF54 also represent insignificant activity. Nevertheless, since any cytotoxicity effect on L-6 cells (rat skeletal myoblasts) was not observed, selective inhibition of pancracine against *T. brucei rhodesiense* and *P. falciparum* can be conceded. [78] Recently, the antiviral ability of pancracine also came to attention by reporting the inhibition of pseudotyped human immunodeficiency virus (HIV)−1 with EC_{50} of 18.5 µM. Moreover, it interrupted dengue virus replication (EC_{50} of 0.36 µM), maintaining a low cytotoxicity profile on monocytic cell line THP-1 (CC_{50} of 25.93 µM). [72]

Naturally occurring 3-*O*-methylpancracine was evaluated for its antiproliferative property against SH-SY5Y cells, demonstrating cytotoxicity even at the minor concentration of 6.25 µM. In the same study, the neuroprotective ability of pancracine was screened against $CoCl_2$, H_2O_2- or $A\beta_{25–35}$-induced cell injuries in SH-SY5Y cells by which pancracine expressed neuroprotective effects against $CoCl_2$- and H_2O_2-induced SH-SY5Y cell death. [79]

3.7 Biological Activity of Other Montanine-type Alkaloids

The only evaluation of coccinine as anticancer compound evidences propitious potency at low micromolar concentrations against a panel of six cancerous cell lines (**Table 3**). [55] Moreover, this isomer of montanine expressed affinity for SERT, with an IC_{50} value of 59.2 µg/mL (196.3 µM); However, it did not show any interaction with P-gp as a transporter. [50]

Further antiproliferative investigation of montanine-type alkaloids showed that semisynthetically obtained manthine has promising antiproliferative activity with single-digit GI_{50}s against a panel of cancerous cell lines (**Table 3**). [12]

Pancratinine C was screened for its neuroprotective potential against $CoCl_2$, H_2O_2- or $A\beta_{25–35}$-induced cell injuries in SH-SY5Y cells. The result showed its apparent neuroprotective effects against $CoCl_2$- and H_2O_2-induced SH-SY5Y cell death with 52.99 ± 3.38 and 70.37 ± 0.42 percent of the control group, respectively, when used in a concentration of 6.25 µM. [79]

Though brunsvigine and manthidine have been known for a long time as montanine-type alkaloids, they have been isolated and investigated infrequently. Recently brunsvigine was tested against AChE and showed to be inactive. [83]

Investigation of nangustine as an antimalaria compound has demonstrated its poor but selective activity against *T. brucei rhodesiense*, *T. cruzi,* and *P. falciparum*. [78,90] It is reported that 4-*O*-methylnangustine isolated from *H. argentinum*, did not express any activity against both AChE and BuChE enzymes. [63]

3.8 Biological Activity of Selected Amaryllidaceae Alkaloids

To make this present review comprehensive, in the following text, selected classes of AAs and their extensively emphasized biological activity will be briefly discussed:

3.8.1 Galanthamine-type Alkaloids

These dibenzofuran nuclei possessing AAs are formed through *para-ortho'* phenol oxidative coupling of 4'-*O*-methylnorbelladine in genera such as *Narcissus*, *Leucojum*, *Pancratium*, *Crinum*, *Galanthus*, *Zephyranthes*, and *Lycoris*. [3,8,25,91,92] Galanthamine, isolated originally from *G. nivalis* L. in the 1940s, is a long-acting, selective, reversible, and competitive AChEI. This enzyme is responsible for acetylcholine (ACh) degradation in the neuromuscular junction, in peripheral and central cholinergic synapses, and in parasympathetic target organs. Additionally, galanthamine stimulates pre- and postsynaptic nicotinic receptors, which can increase the release of neurotransmitters, thus directly stimulating neuronal function. [15,92] Galanthamine is approximately 53 times more selective for human erythrocyte AChE than plasma BuChE (AChE IC_{50} = 0.35 µM; BuChE IC_{50} = 18.6 µM). [92] At present, AD is unpreventable and incurable, so the only approved therapeutic option is the relief of the symptoms offered by AChEI therapy. Galanthamine has the approval for use in the US, many European countries, and many Asian countries for treating AD, and it is effective and well-tolerated, improves cognition in short-term, and improves function and daily life quality in patients with mild to moderate symptoms.[15,93]

In addition to galanthamine, other representatives of this group are norgalanthamine, chlidantine, sanguinine, and lycoramine (selected structures are demonstrated in **Figure 5**). [8] Sanguinine has up to 10-fold more inhibitory potency than galanthamine since it has an extra available hydroxyl group for potential interaction with AChE. [94]

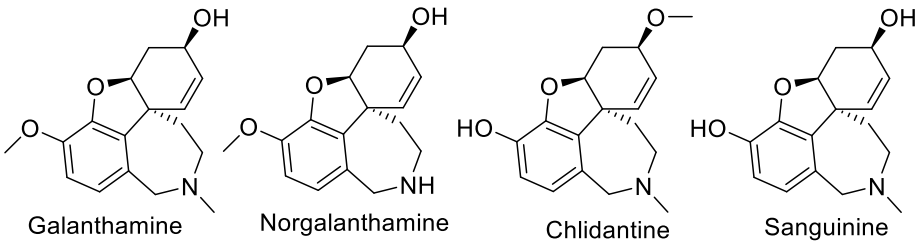


Figure 5. Selected structures of galanthamine-type Amaryllidaceae alkaloids.

3.8.2 Lycorine-type Alkaloids

Formed by *ortho-para'* cyclization of norbelladine, lycorine-type AAs possess a unique pyrrolo[d,e]phenanthridine (15-carbon) nucleus and are the most naturally occurring and the most diverse AAs. [6,8,95,96] Lycorine itself is the first isolated AA from the plant *N. pseudonarcissus* in 1877. [97-99] The significant diversity in lycorine-type alkaloids is mainly because of various conformations of six-membered ring C, and up to this day, 119 alkaloids within the lycorine-type framework are known. [6,8] Other representatives of the lycorine-type skeleton are 1-*O*-acetyllycorine, pseudolycorine, galanthine, sternbergine, norpluviine, amarbellisine, and lycorene (selected structures displayed in **Figure 6**). [8,96] Amaryllidaceae species with an abundant amount of lycorine are reported as *L. radiata*, *L. aestivum*, *Hymenocallis littoralis*, *Hippeastrum equestre*, flowers of *Cilivia nobilis*, *Ammocharis coranica*, *B. radulosa*, and *Crinum macowanii*. [100]

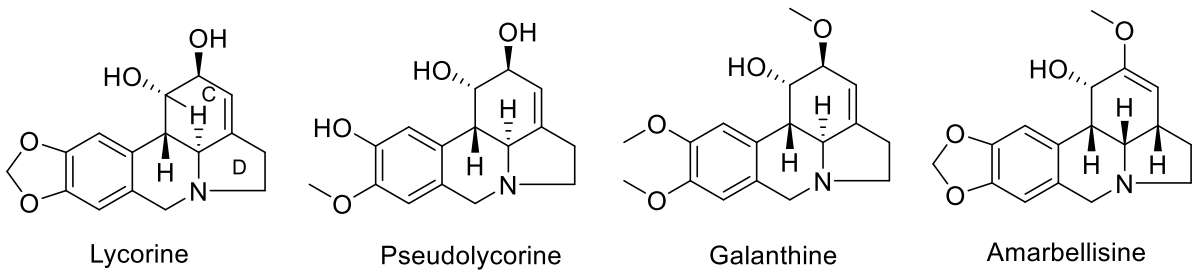


Figure 6. Selected structures of lycorine-type Amaryllidaceae alkaloids.

Among the various pharmacological values manifested by lycorine and its congeners are effects such as antiviral, antibacterial, antifungal, antiparasitic, antioxidant, anti-inflammatory, insecticide, AChE inhibition, suppression of ascorbic acid biosynthesis, RNA inhibitory activity, and control of circadian period length. [95,98,101,102]

Lycorine is recognized as an antiproliferative molecule, active in low micromolar amounts against multidrug-resistant and apoptosis-resistant cancerous cells with selective cytotoxicity dependent on the cell type in tumor cells. [99] Different interaction mechanisms have been explained for the anticancer activity of lycorine and its congeners. Initially, it was postulated that lycorine could inhibit protein biosynthesis. However, some experiments demonstrated that it does not inhibit protein synthesis

initiation. Meanwhile, others showed that it also could not inhibit the peptide bond formation, though it did link to ribosome under certain conditions. [96] Lycorine demonstrated potency in growth suppression and apoptosis induction on human leukemia cells (MCL-1). Also, it can induce apoptosis through downregulation of the cell cycle in leukemia HL-60 and multiplemeyloma KM3 cell lines. [103] The studies have further revealed that lycorine reduces histone deacetylase (HDAC) enzymatic activity, leading to cell cycle arrest and growth inhibition in K562 cells. [104]

Lycorine has considerable inhibitory activity against AChE, which appears to be associated with the presence of two free hydroxyl groups in some of the alkaloids of this skeleton type. Assoanine (IC_{50} = 3.87 µM) and oxoassoanine (IC_{50} = 47.21 µM) are reported as potential AChEI molecules, and their activity attributes to the aromatic ring C, which gives certain planarity to these alkaloids. [15,94] The most active lycorine-type alkaloid against AChE with authentic capacity for clinical development is 1-*O*-acetyllycorine (IC_{50} = 0.96 µM) which has two times more AChE inhibitory potency than galanthamine.[105]

Moreover, lycorine exhibited antimalarial activity in a dose-dependent manner when screened against *P. falciparum* T9.96 and *P. falciparum* K1. [106] It also displayed cytotoxic activity against parasites such as *Trichomonas vaginalis, Entamoeba hystolitica, Tribolium castaneum,* and *Aphis gossypii.* [95,107,108]

3.8.3 Narciclasine-type Alkaloids

The narciclasine-type alkaloids are generated through *para-para´* phenol oxidative coupling of 4'-*O*-methylnorbelladine and possess a phenanthridine or phenanthridone/isocarbostiryl backbone (also called lycoricidine ring), with different degrees of oxidation and/or aromatization on the ring C. [6,20,24] Narciclasine was isolated for the first time in 1967 from bulbs of several *Narcissus* species. [109,110] Up to this date, about 33 alkaloids of narciclasine-type have been isolated. [35] Pancratistatin and narciclasine are the main representatives of narciclasine-type AAs (**Figure 7**). [24]

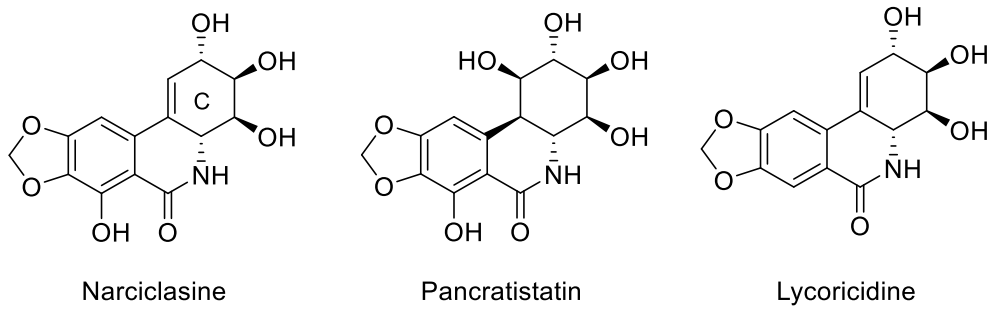


Figure 7. Selected structures of narciclasine-type Amaryllidaceae alkaloids.

Preliminary biological evaluation of narciclasine indicated its impressing antimitotic properties in 1967.[110] Later, inhibition of cell growth by blocking protein biosynthesis and binding to the 60S subunit

of ribosomes was shown to be a possible mechanism of action for narciclasine. [109] Afterward, it was reported that narciclasine possesses a broad cytotoxic activity against a variety of tumor cell-lines as the compound was tested on a panel of 60 human cancerous cell lines with a mean IC_{50} of 15.5 nM. Intriguingly, narciclasine showed a selective action on the melanoma cell lines. [109,111] A new direct target of narciclasine, the eukaryotic translation elongation factor 1A (eEF1A), was introduced through an investigation while treating immunodeficient mice implanted with human brain metastatic and apoptosis-resistant VM-48 melanoma cells in their brains. Treated models with narciclasine (1 mg/kg, orally) displayed a significant therapeutic benefit to those treated with the chemotherapeutic substance temozolomide. [112] Due to the high potency and selective cytotoxicity action of narciclasine, this scaffold is being extensively investigated as a lead structure with antitumor properties, both *in vitro* and *in vivo*. [109]

Another essential narciclasine-type AA, pancratistatin, displays a significant anticancer activity. Various mechanism of action for its cytotoxic activity has been suggested, such as: up-regulating the Fas, increase in caspase-3, flip phosphatidyl serine, and destabilization of mitochondrial membrane potential. [99] Pancratistatin can selectively induce cell death in human colon tumor xenografts' study independent of Bax and caspase activation by targeting HT-29 cancer cells *in vivo*. [99,113] Remarkably, pancratistatin and narciclasine are currently at various stages of clinical candidate development, designated for commercialization. [8]

3.8.4 Crinane-type Alkaloids (Haemanthamine- (or α-Crinane-) and Crinine- (or β-Crinane-) types)

The crinane-type (α- and β-) skeleton is generated through *para-para´* phenol oxidative coupling of 4'-*O*-methylnorbelladine. [6] These AAs possess a unique 5,10b-ethanophenanthridine with a bridge link in their skeletons straddling between N5 and C10b, presenting their significant taxonomic feature. They are often categorized into two separate skeleton types, crinine-type (β-crinane), and haemanthamine-type (α-crinane). The distinguishing feature of these two frames is the orientation of the ethano-bridge, which may be either α- or β-oriented, thereby leading to the absolute stereochemical configuration at C4a. [8,114,115]

Hitherto, more than 54 molecules associated with crinane-type nuclei containing a β-oriented 5,10b-ethano bridge moiety are isolated from Amaryllidaceae plants. [8,114,115] In addition to crinine, some of the other representatives of this skeleton are buphanisine, buphanidrine, ambelline, undulatine, distichamine, and bowdensine. [8] The most pronounced distribution and structural diversity of crinine-type (β-crinane) AAs can be found in the African tribe Amaryllidaceae. They are primarily isolated from

Nerine and *Leucojum* genera, and only a few have been reported from the genera *Galanthus*, *Hippeastrum*, *Pancratium,* and *Lycoris*, and none have ever been isolated from *Narcissus*. [6,8,116]

Alkaloids with α-oriented 5,10b-ethano bridge in the mainframe of the crinane nucleus can be classified as haemanthamine-type (α-crinane) alkaloids subgroups. [8,114,117] The alkaloids of the *Narcissus* genus are exclusively of the haemanthamine-type. In contrast, in genera such as *Brunsvigia* and *Boophane*, the crinane-type alkaloids with β-oriented bridge linkage are predominant. [15] Haemanthamine-type (α-crinane) alkaloids also have been isolated from genera *Crinum*, *Lycoris,* and *Pancratium*. [8,116,117] Haemanthamine, per se, is one of the most abundant AA and has been isolated from a wide variety of Amaryllidaceae plants. [117] **Figure 8** illustrates some representatives of crinane-type AAs retaining α- or β-oriented bridge linkers.

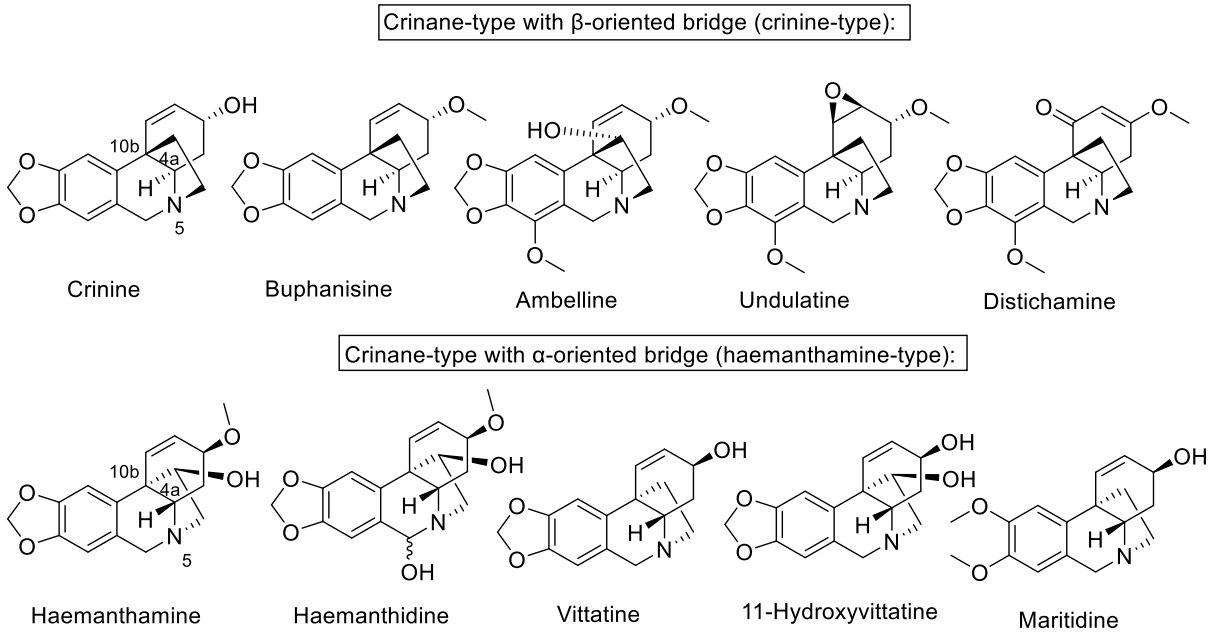


Figure 8. Selected representative alkaloids of α- and β-crinane-type.

Both *in vitro* and *in vivo* studies support the ameliorative effects of crinane-type (α- and β-) compounds as anticancer agents. [115] Haemanthamine has been extensively investigated for its antiproliferative and cytotoxic properties. It has shown substantial growth inhibitory and cytotoxic activity against a wide range of cancer cell lines of different histotypes, such as cervical carcinoma (HeLa), acute lymphoblastic leukemia (MOLT-4), hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7), chronic myeloid leukemia (K562), colon carcinoma (Caco-2), colorectal carcinoma (HT-29), ovarian carcinoma (A2780), and others. [117] The dominant mechanisms of action are reported as prominent inhibition of growth by forming a complex with RNA, inhibition of protein synthesis by binding to the peptidyl transferase center of the 60S ribosomal subunit, activating a p53-dependent antitumor response, the direct binding of haemanthamine to ribosomes and specific inhibition of ribosome biogenesis. [28,29,117]

Haemanthidine is structurally associated with haemanthamine, with the only difference being in the presence of an additional hydroxyl group attached to C6 of the nucleus. Despite this slight difference, haemanthidine still possesses the similar antiproliferative property with a mechanism of action comparable with haemanthamine, through induction of cell cycle arrest and apoptosis on various cancerous cell lines. [117] Both alkaloids also demonstrated potent antimalarial characteristics against a chloroquine-sensitive strain of *P. falciparum* F32 with IC_{50} value of 1.3 µM for haemanthamine, and IC_{50} value of 1.2 µM for haemanthidine. [118]

3.8.5 Biological Activity of Minor Amaryllidaceae Alkaloids

In recent years, few new classes of AAs with fascinating biological activity have been isolated from these plants. Most noteworthy among them are narcikachnine-type and carltonine-type AAs.

Narcikachnine-type alkaloids are built from both the galanthamine- and galanthindole-type. So far, five alkaloids of this class have been isolated from *Zephyranthes* and *Narcissus* genera. [18,39,40] Isolated narcibaduliine from bulbs of *N. pseudonarcissus* cv. Carlton displayed inhibitory activity on both AChE and BuChE, with IC_{50} value of 3.29 ± 0.73 µM for *h*AChE, and 3.44 ± 0.02 µM for *h*BuChE. [40] The best *h*BuChE inhibition activity of narcikachnine-type AAs belongs to narcieliine with IC_{50} value of 1.3 ± 0.3 µM. It also showed IC_{50} values of 18.7 ± 2.3 µM against *h*AChE. [18]

Carltonine-type AAs are also among the recently discovered class with significant bioactivity against acetylcholinesterase. [9] Initially, they were categorized inappropriately as belladine-type AAs, though later they were classified as carltonine-type since their biosynthesis interfered with galanthindole-type structure. [119] Carltonine A–C were isolated for the first time from bulbs of *N. pseudonarcissus* cv. Carlton. Carltonine A and carltonine B showed IC_{50} values in the nanomolar range (*h*BuChE IC_{50} = 910 nM, and 31 nM, respectively). Selected structures of narcikachnine-type and carltonine-type AAs are illustrated in **Figure 9**.

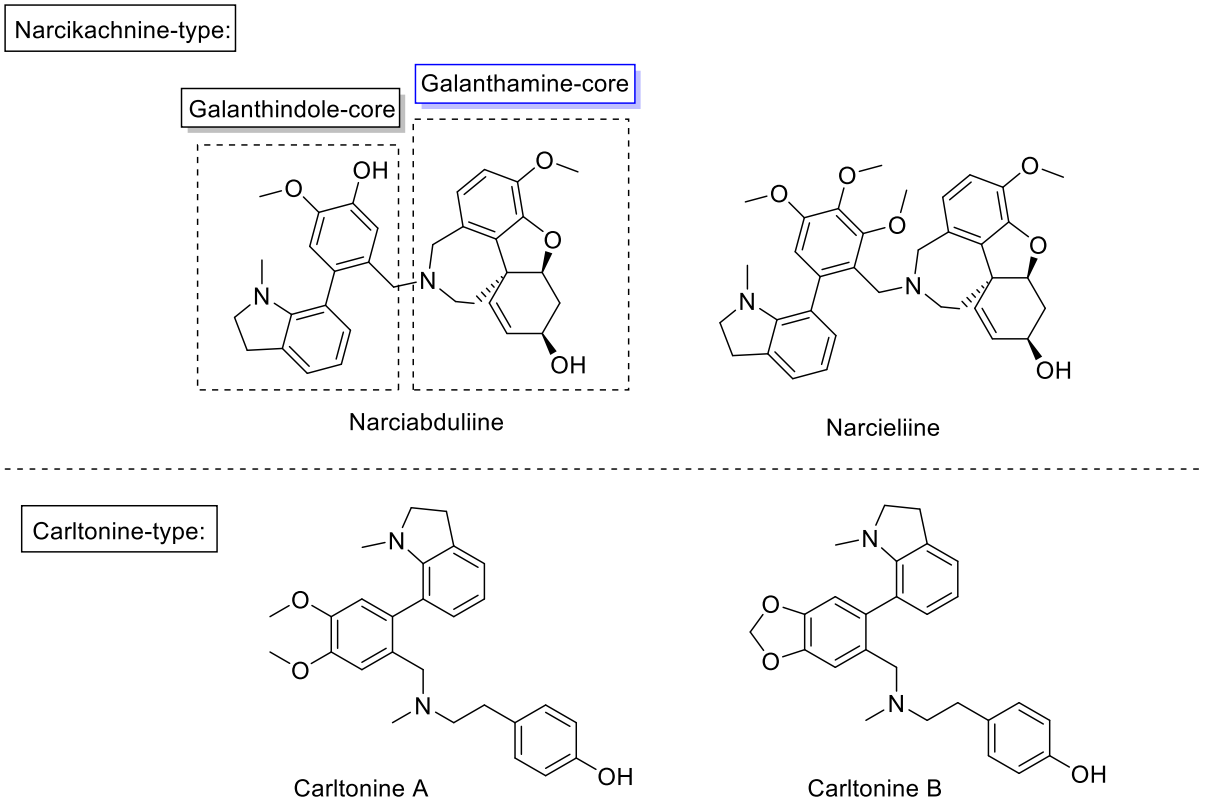


Figure 9. Selected Amaryllidaceae alkaloids of narcikachnine-type and carltonine-type.

3.9 History of Total Synthesis of Montanine-type Alkaloids

The isolation and bioactivity screening of AAs from natural sources confronts limitations due to several difficulties. Not only do many of these compounds naturally occur in trivial amounts, but the insufficiency and unsustainability of the current isolation and purifying methods are restricting issues. Moreover, inadequate knowledge of the AA biosynthetic pathway has limited the opportunity for the biotechnology engineering of plants or microorganisms to produce these alkaloids. Chemical synthesis can be beneficial in covering the demand for AAs, though producing these complex compounds can be challenging. [24]

Influenced by the pioneer synthesis studies by Overman et al. and Hoshino et al. in 1991, several attempts for the chemical production of montanine-type alkaloids have been made. [120,121] During the following two decades, in various approaches to the assembly of the 5,11-methanomorphanthridine skeleton, five general types of synthons were included, and the closure of the ring C, D, C/D, or E was involved in the last step in the construction of the nucleus (**Figure 10**). [33,45,60,90,121-137] Later, the synthetic evolution of synthons I–V conducted the development of many novel approaches such as aza-cope rearrangement/Mannich cyclization, allenylsilane imino ene reaction, [3+2] cycloaddition, and chemoenzymatic methods in chemical synthesis on montanine-type alkaloids. [33,60,90,121,122,127,128,135-137]

Lately, total synthesis of the 5,11-methanomorphanthridine core by imitating their natural biosynthetic pathway in plants to obtain the complex structure has been practiced synthetically. [33] The prior knowledge of the biogenetic origins of the montanine-type AAs (vittatine-intermediated or chyrelline-intermediated, **Figure 2** and **Figure 3**, respectively) has drawn attention to the involvement of the chemically stable cherylline-type intermediate in the multistep modification of norbelladine-type intermediate into the montanine-type scaffold. A retrospective synthesis plan considering the chyrelline intermediate elaborated the development of a new strategy, exploiting oxidative dearomatization/intramolecular aza-Michael addition for the asymmetric synthesis of five montanine-type AAs. [33]

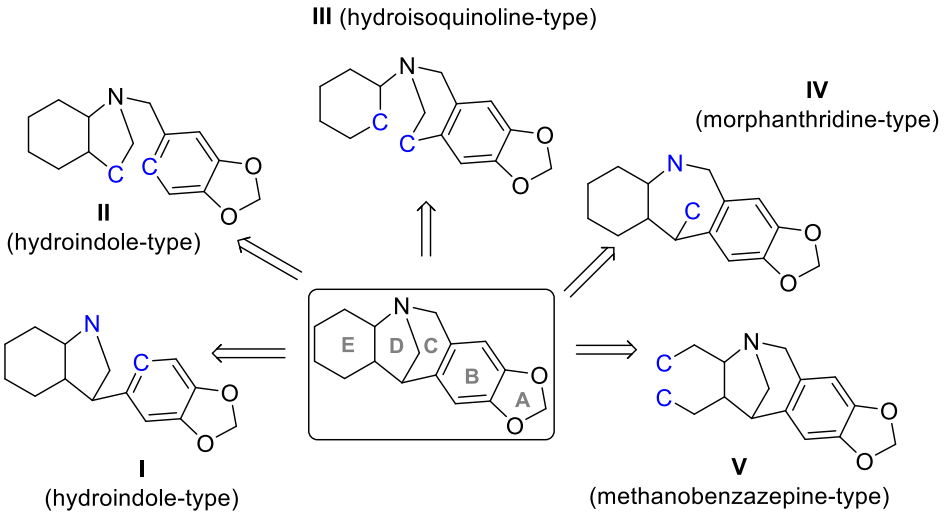


Figure 10. Five general used synthons in non-bioinspired strategies for assembling the 5,11-methanomorphanthridine skeleton. [33]

3.10 Semisynthetic Derivatives of Amaryllidaceae Alkaloids: Synthesis and Biological Activity

A fair number of studies have promoted semisynthetic derivatization of AAs, not only to enhance the potency and metabolic profile of the existing active compounds but also to describe SAR for these alkaloids and their derivatives. [97]

For the discovery of novel lead anticancer agents, many derivatives from the five most common classes of AAs, including lycorine-, homolycorine-, pretazettine-, crinane- (mainly α-), and narciclasine-types, were prepared and evaluated for antiproliferative activities. [73,138,139]

As stated before, narciclasine showed to be a promising antiproliferative compound, exhibiting antitumor properties in apoptosis-resistant as well as in apoptosis-sensitive cancer cells by disturbing the composition of actin cytoskeleton at nontoxic concentrations (IC_{50} values of 30–90 nM). [140] Further chemical manipulation over the narciclasine backbone generated several derivatives with variable

stability, weaker activity, or even utterly inactive *in vitro*. However, one semisynthetic derivative of narciclasine, narciclasine-4-*O*-β-*D*-glucopyranoside, demonstrated higher *in vivo* antitumor activity against human orthotopic glioma models in mice by both intravenous and oral administration routes compared to narciclasine at nontoxic doses. This effort regarding semisynthetic derivatives of AAs introduced the prodrug from narciclasine as a potential compound to combat brain tumors. [140]

Considering lycorine's frequently manifested antiproliferative characteristic, its bountiful availability in Amaryllidaceae plants has enabled researchers to develop new anticancer agents derived from lycorine. [95,101,141] In one study, thirty-five C1 and C2-ether analogs of this natural alkaloid were synthesized and evaluated for their antiproliferative property *in vitro* on a panel of cancerous cells with varying levels of apoptosis resistance (A549, MCF-7, T98G, Hs683, SK-MEL-28, and B16F10). Results indicate a strong correlation between lipophilicity factor and antiproliferative activity, conceding cell permeability properties as an essential determinant in the design of lycorine-based anticancer agents with silyl ether substituent (mean GI_{50} = 4.5 μM, clog*P* = 5.9) being equipotent with lycorine (mean GI_{50} = 4.6 μM, clog*P* = -0.8). [141]

In another study, a set of seven natural AAs (tazettine, pancracine, hippeastrine, vittatine, 11-hydroxyvittatine, haemanthamine, and haemanthidine) and their 32 derivatives were evaluated against four cancerous cell lines (A2780, SW1573, T47-D, and WiDr) to investigate SAR and specify the best candidate for further investigation. The best cytotoxicity outcomes were achieved with compounds derived from pancracine, haemanthamine, and haemanthidine. Regarding hippeastrine, modification of lactone moiety and introduction of nitrogen atom induced selectivity and higher activity in the derived molecule for T47-D and WiDr cell lines. [73]

Given that haemanthamine and haemanthidine displayed considerable bioactive potencies such as cytotoxicity and antiparasitic, their ubiquitous presence in plant material provided the opportunity for developing several semisynthetic derivatives for screening on various biological tests. [73,142-145]

Cedrón et al. prepared thirty-one derivatives of haemanthamine, haemanthidine, and 11-hydroxyvittatine, which were subsequently evaluated for their *in vitro* antimalarial activity against chloroquine-sensitive strains of *P. falciparum* F32. One of the derivatives of 11-hydroxyvittatine expressed the best activity among all tested compounds with IC_{50} of 0.8 ± 0.06 μM, 16 times better than its fundamental compound, 11-hydroxyvittatine with IC_{50} of 13.2 ± 1.4 μM. Moreover, the derivatization elaborated the discussion of SAR, clarifying the necessity of the methoxy group presence at C3 and the free hydroxyl group at C11 for the activity of haemanthamine derivatives. [118]

Evaluation of lycorine derivatives on the same *Plasmodium* strain did not indicate any improvement in IC_{50} compared with lycorine (IC_{50} = 0.13 μM). However, the obtained results led to a better

comprehension of SAR; the best antiplasmodial activity was obtained with lycorine derivatives possessing free hydroxyl groups at C1 and C2 or esterified as acetates or isobutyrates. The double bond between C3 and C4 also influenced the activity. [97] Remarkably, in a set of twenty-one hippeastrine derivatives, with modifications performed on hippeastrine such as functional group transformations, structural simplification, and preparation of dimers, hippeastrine dimers showed to be the most active compounds against *P.falciparum* F32 with IC_{50} less than 4.0 μM. [146]

Though α-crinane-type AAs like haemanthamine and haemanthidine did not express significant activity against AChE or BuChE, their availability in Amaryllidaceae plants provided an accessible source to a complex nucleus for further chemical modification. [117,142]

Among twelve derivatives of haemanthamine, 11-*O*-(2-methylbenzoyl)haemanthamine and 11-*O*-(4-nitrobenzoyl)haemanthamine revealed the most intriguing profile, both being AChEIs on a micromolar scale with glycogen synthase kinase-3 beta (GSK-3β) inhibition properties. [142] In the successive study, significant AChE inhibition was displayed by 11-*O*-(3-nitrobenzoyl)haemanthamine with an IC_{50} value of 4.0 ± 0.3 μM, and 1-*O*-(2-methoxybenzoyl)haemanthamine demonstrated the most substantial human BuChE (*h*BuChE) inhibition with IC_{50} value of 3.3 ± 0.4 μM. [147] In the case of ambelline, ester derivatives have shown noticeable potency as BuChE inhibitor agents as three compounds, 11-*O*-(1-naphthoyl)ambelline, 11-*O*-(2-methylbenzoyl)ambelline, and 11-*O*-(2-methoxybenzoyl)ambelline, showed IC_{50} values of 0.10 ± 0.01, 0.28 ± 0.02, and 0.43 ± 0.04 μM, respectively. [148] Though vittatine per se is not active against AChE or BuChE, three out of twelve ester derivatives showed to be active with single-digit IC_{50}s in μM against BuChE with 3-*O*-(2-Nitrobenzoyl)vittatine as the most active compound with IC_{50} value of 1.4 ± 0.1 μM. [11]

Lycorine-derived compounds also were subjects of AChE inhibition studies. [149,150] Semisynthetic compound, 2-*O*-tert-butyldimethylsilyl-1-*O*-(methylthio)methyllycorine, showed to be a dual inhibitor of *h*AChE and *h*BuChE with IC_{50} values of 11.40 ± 0.66 μM and 4.17 ± 0.29 μM, respectively. The SAR study indicated that the acylated or etherified derivatives of lycorine and lycorin-2-one were more selective for *h*BuChE than *h*AChE. [149]

3.10.1 Derivatives of Montanine-type Alkaloids

Though compelling as potential bioactive molecules, yet scarcity of unique scaffolds of montanine-type alkaloids has restricted the chance of their thorough investigation. [12] Many synthetic approaches for the assembly of 5,11-methanomorphanthridine nucleus have been adopted, involving many complicated steps with unsatisfactory yields. [10] Montanine-type nucleus has been proposed to be achievable through a semisynthetic rearrangement of widely obtainable haemanthamine. [10,12,31] The transformation possibility of the haemanthamine ring to the montanine-type skeleton through an

intramolecular rearrangement was reported for the first time by Inubushi et al. in 1960 while they were trying to define the absolute configuration of haemanthamine and crinamine. During an experiment designed to find a method for replacing the C11 hydroxyl in these alkaloids with hydrogen, haemanthamine was reacted with methanesulfonyl chloride (MsCl) in pyridine and hydrolyzed by alkalized water. The result was the generation of a compound with a montanine-type scaffold, isomeric but not identical to montanine and coccinine, named isohaemanthamine. The rearrangement failed with dihydrohaemanthamine and epihaemanthamine, indicating that the presence of a double bond and a specific configuration of the hydroxyl group is necessary for the rearrangement. [31] The mechanism of this transformation is hypothesized regarding the intramolecular nucleophilic substitution of the electron-rich aromatic moiety on the activated C11-hydroxyl (**A** intermediate in **Figure 11**) to form the new bond between C10a and C11, eventually refiguring to the arenium ion **B**. Afterwards, the link between C10a and C10b breaks due to S_N2' attack of a nucleophile at C2, followed by translocation of the C1–C2 alkene to the C1–C10b position resulting the montanine-type skeleton **C**. [12,31]

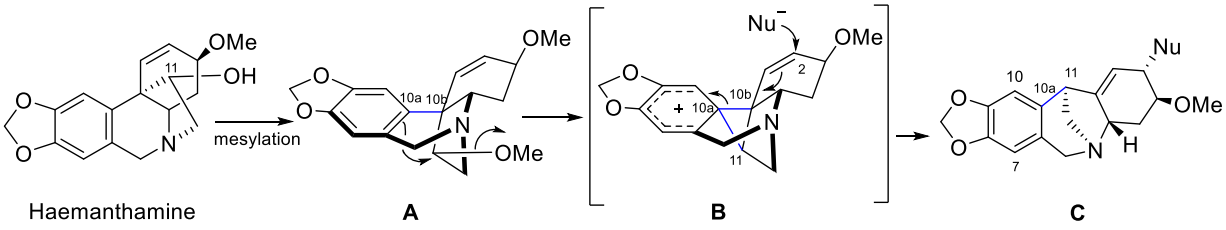


Figure 11. The suggested mechanism for haemanthamine core rearrangement to montanine-type nucleus. [12]

The reaction was performed using a solution of 600 mg of haemanthamine in 10 mL of dry pyridine, treated with 0.5 mL of MsCl, and allowed to stand stirring for 8 hours at 0 °C. Later, the mixture was poured into 50 mL of water containing 6 g of sodium bicarbonate, allowing the solution to stand overnight and extracting it with chloroform afterward. After drying the extract over magnesium sulfate and evaporation of the solvent, crystallization of the residue from ethyl acetate furnished 540 mg (90%) of 3-*O*-methylpancracine (isohaemanthamine). Without the use of pyridine, the reaction yield drops dramatically; a solution of 600 mg of haemanthamine was added to a finely dispersed suspension of 200 mg of potassium in benzene and stirred for 15 min. After adding 0.20 mL of MsCl at 0 °C, the mixture was left to stand stirring for an hour. Afterward, the excessive potassium was removed with wet ether, and the organic layer was washed with water and dried. The residue obtained after evaporation was passed twice over alumina to yield 274 mg (46%) of unreacted haemanthamine and 65 mg (11%) of isohaemanthamine. It Is also reported that neither ethanesulfonyl chloride nor *p*-toluenesulfonyl chloride was effective in this conversion. [31]

Equally, the transformation of the haemanthamine skeleton to a montanine-type can be achieved in the presence of different halogenating agents like thionyl chloride ($SOCl_2$), thionyl bromide, and diethylaminosulfur trifluoride (DAST), resulting in C2 halogen-substituted molecule with 5,11-methanomorphantridine framework (compound **1** in **Figure 12**). Several trials were approached to optimize the yield by assessing different ratios of $SOCl_2$ and the presence of the base in the mixture. Using excessive amounts of $SOCl_2$ (20 equivalent) in DCM optimized the yield for the halogen-substituted molecule **1** up to 71% as the only product of the reaction. [10] Moreover, as mentioned previously, manthine can also be produced semisynthetically by this rearrangement through mesylation and the addition of sodium methoxide as the nucleophile. [12] Therefore, the preparation of montanine-type alkaloids from the haemanthamine ring supplies an immense reservoir of these biologically attractive complex molecules via a reasonable semisynthetic route with acceptable yield, compared with total synthesis and natural isolation.

Up to this date, this approach enabled several studies to synthesize montanine-type derivatives compounds and evaluate their potencies as possible therapeutic candidates in disease. Govindaraju et al. have generated a library of C2-substituted derivatives of montanine-type scaffold by utilizing different nucleophiles and reaction conditions for the acknowledged ring transformation, not only on haemanthamine but also on another α-crinane-type alkaloid, haemanthidine (**Figure 12**). The synthesized compounds went under screening for their *in vitro* antiproliferative properties against a panel of six cancerous cell lines, including cells resistant to several pro-apoptotic stimuli, such as human A549 non-small cell lung cancer (NSCLC), human glioblastoma U373, and human SK-MEL-28 melanoma, as well as tumor models, which are prone primarily to apoptosis-inducing stimuli, such as human Hs683 anaplastic oligodendroglioma, human MCF-7 breast adenocarcinoma, and mouse B16F10 melanoma. The results of this evaluation are expressed in **Table 4**. [12] Accordingly, anticancer screening of manthine and compounds **1**–**14** (**Figure 12**) indicated that manthine possesses the most significant antiproliferative activity among all C2-substituted montanine-type derivatives. Although replacing the C2 methoxy group with an ethoxy, as in compound **3**, reduced the antiproliferative potency, ether substitutions of a three-membered carbon chain with unsaturated bonds retrieved the activity, as in **4** and **5**. Regardless of the significant antiproliferative activity of compound **7** against tested cell lines while carrying a large indole group at position C2, overall data analysis indicates better activity for compounds with a small C2-substituent, as observed in derivatives with chloride **1**, alcohol **2** (3-*O*-methylpancracine)**,** and bromide **13**. The activity of compound **13**, in which the halogen atom has the inverted configuration, indicates that the α-C2 configuration is not a critical factor. Quaternization of the nitrogen atom and modification of the aromatic ring led to activity loss.

Moreover, the variations in cell line sensitivities to both **1** and **13** suggest that they are not indiscriminate alkylating agents but have distinct intracellular targets. [12]

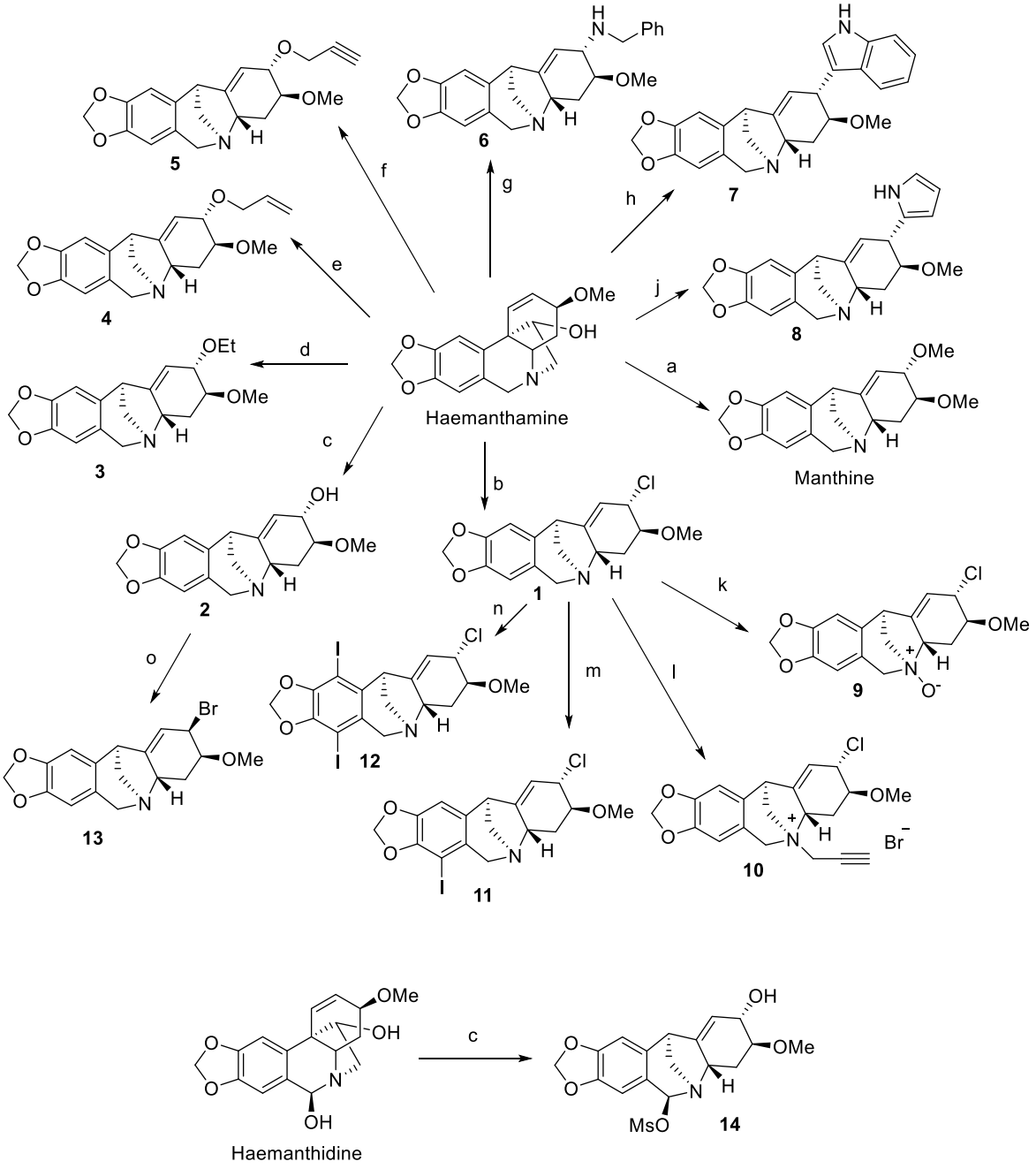


Figure 12. Preparation of C2-substituted montanine-type derivatives by Govindaraju et al. Reagents and conditions for synthesis: (a) MsCl, py, 0 °C, 8 h, then MeOH, NaH (yield 65%); (b) MsCl, Et_3N, DCM, rt, 48 h (yield 55%); (c) MsCl, py, 0 °C, 8 h, then aqueous $NaHCO_3$ (yield 74% for **2**; 46% for **14**); (d) MsCl, py, 0 °C, 8 h, then EtOH, NaH (yield 65%); (e) MsCl, py, 0 °C, 8 h, then allyl alcohol, NaH (yield 68%); (f) MsCl, py, 0 °C, 8 h, then propargyl alcohol, NaH (yield 50%); (g) MsCl, py, 0 °C, 8 h, then $BnNH_2$, NaH (yield 67%); (h) MsCl, py, 0 °C, 8 h, then indole, NaH (yield 62%); (j) MsCl, py, 0 °C, 8 h, then pyrrole, NaH (yield 55%); (k) *m*-CPBA, DCM, rt, 30 min (yield 70%); (l) propargyl bromide, MeCN, rt, 24 h (yield 78%); (m) NIS (1.2 eq), $In(OTf)_3$, MeCN, 45 °C, 12 h (yield 72%); (n) NIS (2.4 eq), $In(OTf)_3$, MeCN, 45 °C, 12 h (yield 55%); (o) CBr_4, Ph_3P, DCM, rt, 4 h (yield 64%).[12]

Table 4. Results of *in vitro* antiproliferative evaluation of C2-substituted manthine and montanine-type derivatives reported by Govindaraju et al. [12]

Compound	GI_{50} *in vitro* values (μM)					
	Resistant to apoptosis			Sensitive to apoptosis		
	A549	SK-MEL-28	U373	MCF-7	Hs683	B16F10
Manthine	**3**	**4**	**5**	**4**	**3**	**3**
1	**6**	26	51	**17**	**6**	**7**
2	**5**	**8**	31	**13**	**4**	**8**
3	59	>100	>100	82	>100	40
4	**10**	**14**	**20**	**20**	**7**	**7**
5	23	28	42	28	24	**10**
6	59	65	72	44	67	**10**
7	**18**	**9**	**9**	**23**	**24**	**4**
8	86	67	>100	68	95	**11**
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	78	>100	>100	78	71	39
13	**9**	**18**	**25**	**19**	**5**	**7**
14	>100	>100	>100	>100	>100	72

In another study, Cedron et al. synthesized a few halogenated derivatives of montanine-type skeleton while they were determined to elucidate the absolute configuration of 5,11-methanomorphantridine compounds generated from haemanthamine ring transformation. Aside from optimizing the reaction yield for **1** by using a large excess of $SOCl_2$ (20 equivalent) in DCM, they successfully synthesized other halogenated compounds possessing montanine-type core through the rearrangement of haemanthamine and haemanthidine (**Figure 13**). Subsequently, compounds **1**, **15,** and **16**, together with pancracine, as a molecule with a basic montanine-type framework, were studied for their *In vitro* activity against *P. falciparum* F-32 using chloroquine as standard (**Table 5**). Since these three derivatives showed higher activity than the natural alkaloid pancracine, it can be concluded that introducing one or more halogens to 5,11-methanomorphantridine scaffold leads to the enhancement of antimalarial activity. [10]

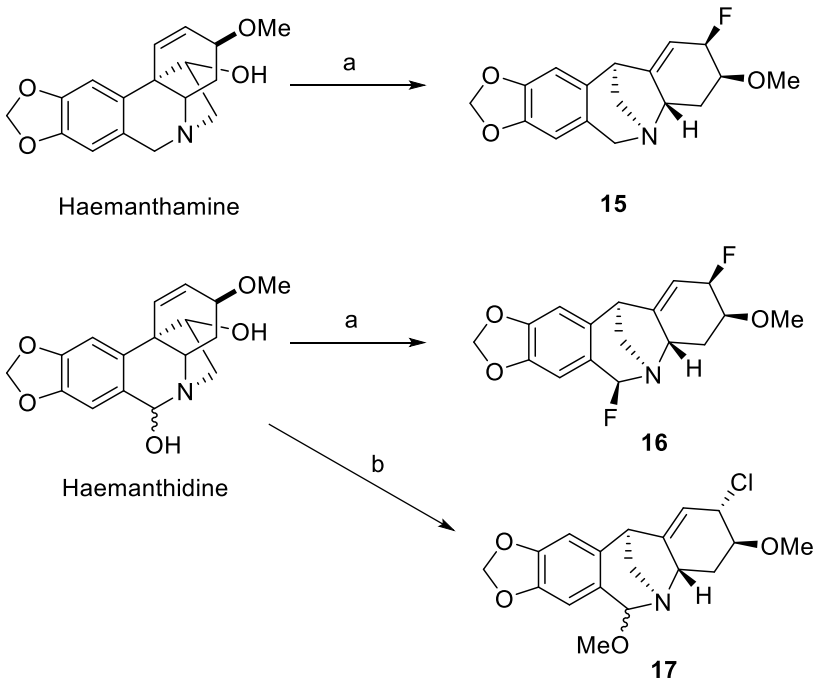


Figure 13. Prepared derivatives of montanine-type Amaryllidaceae alkaloids via rearrangement of haemanthamine and haemanthidine rings by Cedron et al. Reagents and conditions for synthesis: (a) DAST, DCM, -78 °C, 24 h (60% yield for **15**; 49% yield for **16**); (b) 20 eq. $SOCl_2$, DCM, reflux, 5 h (yield 74%).[10]

Table 5. *In vitro* study results of derivatives **1**, **15**, **16,** and pancracine against *P. falciparum* F-32 by Cedron et al.[10]

Compound	IC_{50} (µg/mL)
Pancracine	0.9 ± 0.04
1	0.4 ± 0.02
15	0.6 ± 0.04
16	0.7 ± 0.04
Chloroquine	0.013

Later in a separate study, Cedron et al*.* tested compounds **1** and **17**, along with pancracine, for their antiproliferative property evaluation against the human solid tumor cell lines A2780, SW1573, T-47D, and WiDr (**Table 6**).[73] The result outlined that substituting the hydroxyl group on C2 of pancracine with chlorine and having a methoxy group on the C3 position expresses the same activity as in pancracine while introducing a methoxy group at C6 (**17**) diminishes the activity. Considering similar Log*P* values for **1** and **17** (2.39 and 2.55, respectively), the steric hindrance on C6 is assumed as a critical element in the activity of these chemical structures.[73] **Table 6** shows the achieved results of *in vitro* antiproliferative evaluation in this study.

Table 6. *In vitro* antiproliferative evaluation of compounds **1**, **17**, and pancracine against cell lines A2780, SW1573, T-47D, and WiDr by Cedron et al. [73]

Compound	A2780	SW1573	T47-D	WiDr
Pancracine	8.3 ± 0.5	4.3 ± 0.7	6.5 ± 2.0	9.1 ± 1.0
1	3.4 ± 1.0	3.9 ± 0.7	8.8 ± 1.0	7.5 ± 2.0
17	75.2 ± 25.0	≥100	≥100	≥100

4 OVERVIEW OF THE PUBLICATIONS (COMMENTARY OF THE PUBLISHED WORK)

The outlined results of this submitted dissertation are outcomes of doctoral study in the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, from 2018 to 2022. I am the author and co-author of eight articles published in indexed journals by leading databases, concluding six original works and two review articles. This research focuses on shedding light on various biological activities of montanine-type AAs derivatives and evaluating them as potential drugs. I used the rearrangement of widely available haemanthamine, previously isolated in our research group by Hulcova et al. and Kohelova et al., to achieve 3-*O*-methylpancracine as a fundamental compound with a montanine-type structure. [18,144] A generated library of semisynthetic ester and ether C2-substituted derivatives using 3-*O*-methylpancracine was screened for AChE and BuChE inhibitory, antiproliferative, and antimicrobial activity. Analogs of active compounds were prepared on position C3 of the mother scaffold using montanine, previously isolated from bulbs of *H.* cv. Ferrari by Al Shammari et al., to discuss the SAR. [11,53]

4.1 Derivatives of Montanine-type Alkaloids and Their Implication for the Treatment of Alzheimer's Disease: Synthesis, Biological Activity and *In silico* Study [151]

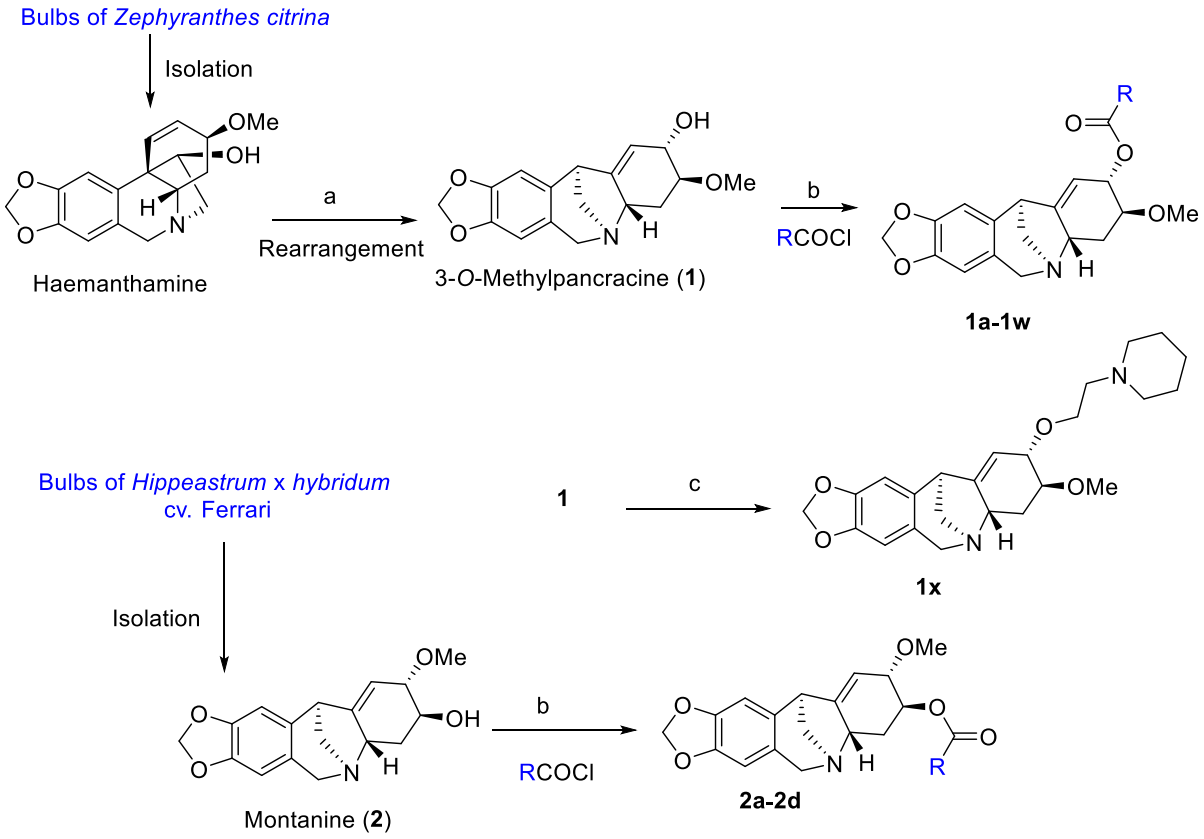
AD's most prominent pathological manifestations are extracellular accumulation of amyloid-beta (Aβ) plaques, formation of intracellular neurofibrillary tangles (NFTs), neuroinflammation, and cholinergic impairments with pronounced deterioration in levels of ACh. This neurotransmitter can be decomposed by two types of cholinesterases, AChE and BuChE, with different catalytic activities. AChE dominantly hydrolyzes the ACh responsible for neurotransmission in the synaptic cleft in a healthy brain. Conversely, BuChE is relatively supportive in ACh hydrolysis and is mainly involved in toxic species clearance.

Since little research has been conducted on the potency of the 5,11-methanomorpanthridine scaffold as a cholinesterase inhibitor, we generated a library of 28 novel derivatives of montanine-type AAs and evaluated their inhibition potency against *h*AChE and *h*BuChE. All prepared compounds were characterized by optical rotation, HRMS, and NMR spectroscopic techniques. The purity of compounds

was assessed via HPLC coupled with photodiode array detection, demonstrating more than 96% purity for all compounds. On account of described haemanthamine ring transformation to a montanine-type scaffold, we used previously isolated and purified haemanthamine from bulbs of *Z. citrina* to prepare 3-*O*-methylpancracine (**1**) (**Figure 14**). From compound **1**, through esterification with corresponding acyl chlorides, 22 compounds **1a**–**1w** were synthesized (**Figure 14**). Subsequent screening of these compounds against *h*AChE and *h*BuChE with a concentration of 100 µM according to a slightly modified Ellman's method demonstrated that disubstituted aromatic derivatives, 2-*O*-(4-chloro-3-nitrobenzoyl)- (**1n**), 2-*O*-(2-chloro-5-nitrobenzoyl)- (**1p**), and 2-*O*-(4-methyl-3-nitrobenzoyl)-3-*O*-methylpancracine (**1o**) have inhibition potency against *h*AChE with more than 75%. IC_{50} values for this compound were measured as 1.6 ± 0.1 µM, 3.2 ± 0.2 µM, and 4.3 ± 0.5 µM, respectively (**Table 7**).

It can be deduced that the subgroup of ester derivatives of **1** with a nitro group in combination with either a halogen (Cl, Br) or a methyl on the aromatic attachment (derivatives **1n**–**1r**) activate the scaffold against *h*AChE, with compound **1n** displaying the lowest IC_{50} bearing ortho-NO_2 and para-Cl substituents. Furthermore, derivatives **1n**–**1p** displayed a selective *h*AChE inhibition profile with SI (*h*BuChE IC_{50}/*h*AChE IC_{50}) higher than 20. By employing formerly isolated montanine (**2**) from bulbs of *H.* cv. Ferrari, the positional isomers of disubstituted aromatic esters were prepared to compare the role of positions C2 and C3 on the E ring of **1** and **2** for *h*AChE inhibition (analogs **2a**–**2d**). Interestingly, the corresponding analogs of montanine **2a** (analog **1o**), **2b** (analog **1n**), and **2d** (analog **1p**) displayed elimination of *h*AChE inhibitory property (IC_{50} for all derivatives > 100 µM).

Concerning *h*BuChE inhibition, **1v** and **2e** expressed selective inhibition potency with IC_{50} values of 1.73 ± 0.05 µM and 6.54 ± 0.74 µM in turn, and a SI for *h*BuChE higher than 15 (*h*AChE IC_{50}/*h*BuChE IC_{50}; **Table 7**), while both containing *N*-piperidine carbamates. Replacement of piperidine (**1v**) with a cyclohexyl moiety (**1w**) resulted in complete activity loss. Due to questioned *in vivo* stability of esters, the corresponding ether **1x** was synthesized. However, shifting to ether linker was associated with the drastic reduction of *h*BuChE inhibition activity (*h*BuChE IC_{50} > 100 µM for **1x**). Summing up the results, it can be extrapolated that position C2 on the E ring of the montanine-type nucleus is more favorable for enhancing AChE and BuChE inhibitory activity.



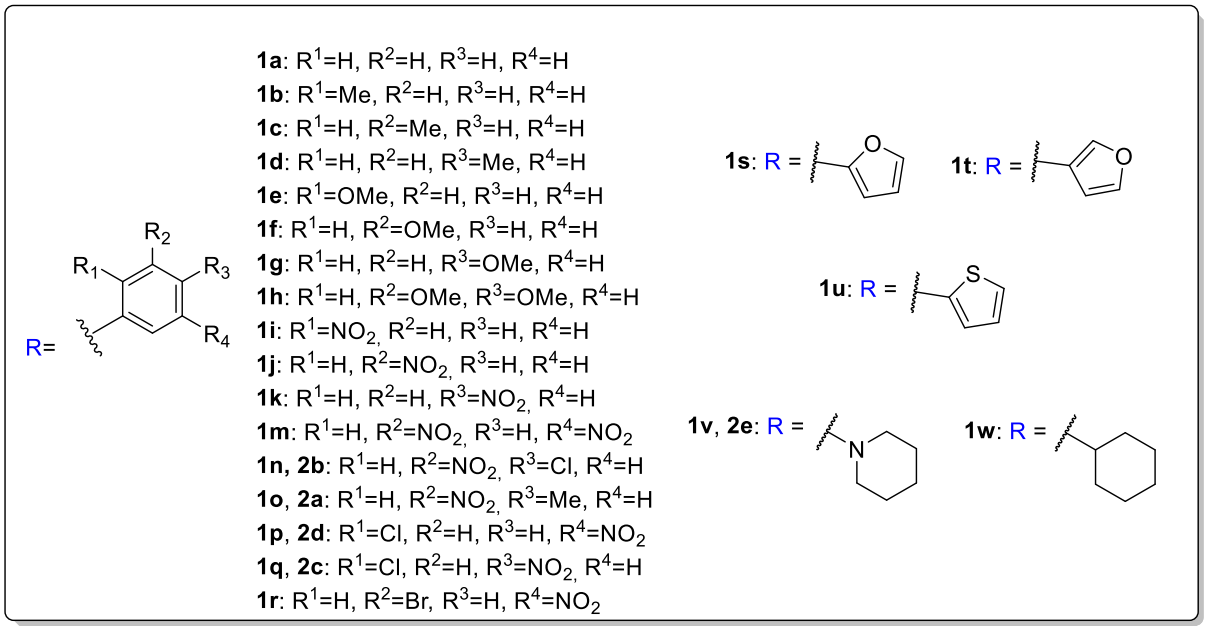


Figure 14. Reaction scheme and synthesized derivatives of montanine-type alkaloids for evaluation against *h*AChE and *h*BuChE (**1a**–**1x**, **2a**–**2e**). Reagents and conditions for synthesis: (a) MsCl, py, 0 °C, 8 h, then aqueous $NaHCO_3$, 25 °C, overnight (yield 70%); (b) RCOCl (1.5–3.0 eq.), DMAP (catalytic amount), py, 80 °C, 5–20 h (yield 8–99%); (c) NaH in Dry THF, under argon gas, 0 °C, 0.5 h, then 1-(2-chloroethyl)piperidine (1 eq.), 25 °C, overnight (yield 16%).

Table 7. *In vitro h*AChE/*h*BuChE inhibition of prepared montanine-type derivatives (**1a**–**1x**, **2a**–**2e**) and calculated BBB score.

Compound	% Inhibition *h*AChE ± SEM[a]	IC_{50}, *h*AChE ± SEM (μM)[b]	SI for *h*AChE[c]	% Inhibition *h*BuChE ± SEM[a]	IC_{50}, *h*BuChE ± SEM (μM)[b]	SI for *h*BuChE[d]	BBB score[e]
3-*O*-Methyl-pancracine (1)	9.2 ± 0.2	> 100	n.c.	9.2 ± 0.1	> 100	n.c.	n.c.
1a	8.5 ± 2.0	>100	n.c.	20.1 ± 0.6	>100	n.c.	n.c.
1b	29.7 ± 4.6	>100	n.c.	30.2 ± 2.6	>100	n.c.	n.c.
1c	18.2 ± 0.4	>100	n.c.	12.9 ± 0.6	>100	n.c.	n.c.
1d	12.4 ± 0.1	>100	n.c.	12.9 ± 0.6	>100	n.c.	n.c.
1e	15.6 ± 0.3	>100	n.c.	30.1 ± 0.8	>100	n.c.	n.c.
1f	19.3 ± 3.3	>100	n.c.	20.3 ± 3.7	>100	n.c.	n.c.
1g	12.5 ± 0.2	>100	n.c.	15.5 ± 0.5	>100	n.c.	n.c.
1h	17.2 ± 2.9	>100	n.c.	18.1 ± 4.2	>100	n.c.	n.c.
1i	34.1 ± 4.1	>100	n.c.	45.4 ± 2.5	>100	n.c.	n.c.
1j	55.3 ± 0.9	>100	n.c.	18.1 ± 1.5	>100	n.c.	n.c.
1k	48.1 ± 0.8	>100	n.c.	26.2 ± 0.8	>100	n.c.	n.c.
1m	70.8 ± 5.2	13.06 ± 0.83	> 7.66	19.2 ± 4.6	>100	< 0.13	2.66
1n	83.7 ± 1.9	**1.61 ± 0.13**	> 62.11	26.5 ± 2.6	>100	< 0.02	3.84
1o	81.5 ± 4.1	**4.32 ± 0.50**	> 23.15	6.8 ± 2.8	>100	< 0.04	3.80
1p	78.7 ± 4.5	**3.18 ± 0.23**	> 31.45	30.0 ± 6.7	>100	< 0.03	3.84
1q	48.6 ± 1.5	>100	n.c.	27.1 ± 1.5	>100	n.c.	n.c.
1r	28.0 ± 0.5	>100	n.c.	31.7 ± 0.7	>100	n.c.	n.c.
1s	15.1 ± 0.2	>100	n.c.	26.9 ± 0.3	>100	n.c.	n.c.
1t	6.8 ± 0.1	>100	n.c.	30.2 ± 0.7	>100	n.c.	n.c.
1u	29.4 ± 3.0	>100	n.c.	49.7 ± 2.9	>100	n.c.	n.c.
1v	8.8 ± 1.5	>100	< 0.02	97.0 ± 0.2	**1.73 ± 0.05**	> 57.80	4.66
1w	1.2 ± 0.2	>100	n.c.	20.8 ± 0.8	>100	n.c.	n.c.
1x	6.5 ± 0.5	>100	n.c.	45.2 ± 2.8	>100	n.c.	n.c.
Montanine (2)	13.7 ± 2.4	>100	n.c.	8.4 ± 4.2	>100	n.c.	n.c.
2a	30.3 ± 1.0	>100	n.c.	20.4 ± 1.2	>100	n.c.	n.c.
2b	4.8 ± 1.1	>100	n.c.	13.8 ± 0.6	>100	n.c.	n.c.
2c	2.3± 0.8	>100	< 0.17	69.8 ± 0.8	16.96 ± 0.28	> 5.90	3.83
2d	1.8 ± 0.6	>100	n.c.	40.1 ± 1.6	>100	n.c.	n.c.
2e	10.9 ± 1.1	>100	< 0.07	88.2 ± 1.3	6.54 ± 0.74	> 15.29	4.64
Galanthamine[f]	98.8 ± 1.1	2.01 ± 0.14	14.6	68.2 ± 1.2	29.31 ± 3.49	0.07	5.01
Eserine[f]	99.8 ± 0.6	0.20 ± 0.0.01	1.50	99.9 ± 0.5	0.30 ± 0.01	0.67	5.02

[a] Compounds were tested at the concentration of 100 μM; [b] Concentration required to decrease enzyme activity by 50%, the values are reported as mean ± SEM of three independent measurements, each performed in triplicate; [c] Calculated SI for *h*AChE as *h*BuChE IC_{50}/*h*AChE IC_{50}; [d] Calculated SI for *h*BuChE as *h*AChE IC_{50}/hBuChE IC_{50}; [e] Calculated *in silico* using BBB score; [f] Reference compound; n.c. means not calculated.

BBB scores for active compounds were calculated *in silico* to predict the ability of compounds to penetrate to CNS area. Compounds **1v** and **2e** pronounced BBB score values more than 4.0, which is assumed as the required threshold to enter the CNS. Compounds **1n**–**1p** showed BBB scores of 3.80–3.84, which is proximate to the 4.0 value, and thus can be assumed they are, at least partly, penetrable

through BBB. Moreover, cytotoxicity tests for compounds **1n** and **1v** were conducted using WAST-1 assay on a panel of cancer cell lines Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7, SAOS-2 and normal lung fibroblasts MRC-5 (**Table 8**). Compound **1n** displayed cytotoxic activity against eight of the selected cell lines with less than 25% cell viability, while derivative **1v** was non-toxic (viability > 90%).

Table 8. Cytotoxicity of derivatives **1n** and **1v** (c = 10 μM) on ten human cancer and normal cell lines. Values represent cell proliferation after treatment with analogs and are expressed as a percentage of the proliferation of untreated control cells. Each value is a mean of three independent experiments. Doxorubicin is the reference drug (c = 1 μM)

Cell line	1n	1v	DOX
Jurkat	3 ± 2	98 ± 8	0 ± 3
MOLT-4	8 ± 8	93 ± 12	0 ± 1
A549	2 ± 1	101 ± 12	66 ± 16
HT-29	19 ± 3	98 ± 9	77 ± 12
PANC-1	95 ± 7	92 ± 6	59 ± 9
A2780	45 ± 30	99 ± 14	5 ± 1
HeLa	4 ± 1	95 ±9	7 ± 10
MCF-7	13 ± 7	91 ± 6	41 ± 7
SAOS-2	26 ± 7	99 ± 10	73 ± 8
MRC-5	5 ± 5	98 ± 6	40 ± 4

In vitro results were justified by conducting docking studies for the foremost active compounds against *h*AChE and *h*BuChE, **1n** and **1v,** respectively. The *h*AChE protein complex with PDB entry 4EY6 and *h*BuChE with PDB entry 4BDS were selected for *in silico* study, and for comparative purposes, we also investigated the binding modes of **1n** and **1v** in their corresponding inactive enzymes, *h*BuChE and *h*AChE, in turn. We comprehend the existing potency of the formation of the carbamylated adduct due to the presence of a carbamate group in the ligand structure **1v**. The most energetically favored poses of the two main active compounds are represented in **Figure 15** and **Figure 16** for *h*AChE and *h*BuChE enzymes, respectively. Docking of **1n** in the *h*AChE complex (**Figure 15**; A and B) shows that the ligand stretches the cavity gorge while the 5,11-methanomorpanthridine core is aligned towards the cavity entrance. Interaction of ligand with the protein includes hydrogen bond with amides between Phe295 and Arg296, π-π interactions with Phe297, and hydrogen bond of the nitro group of the 4-chloro-3-nitrobenzene moiety with Gly120 and Tyr133. Though ligand **1v** (**Figure 16;** C and D) shows a similar binding position to **1n** in *h*AChE site, the interaction with the backbone amide is missing.

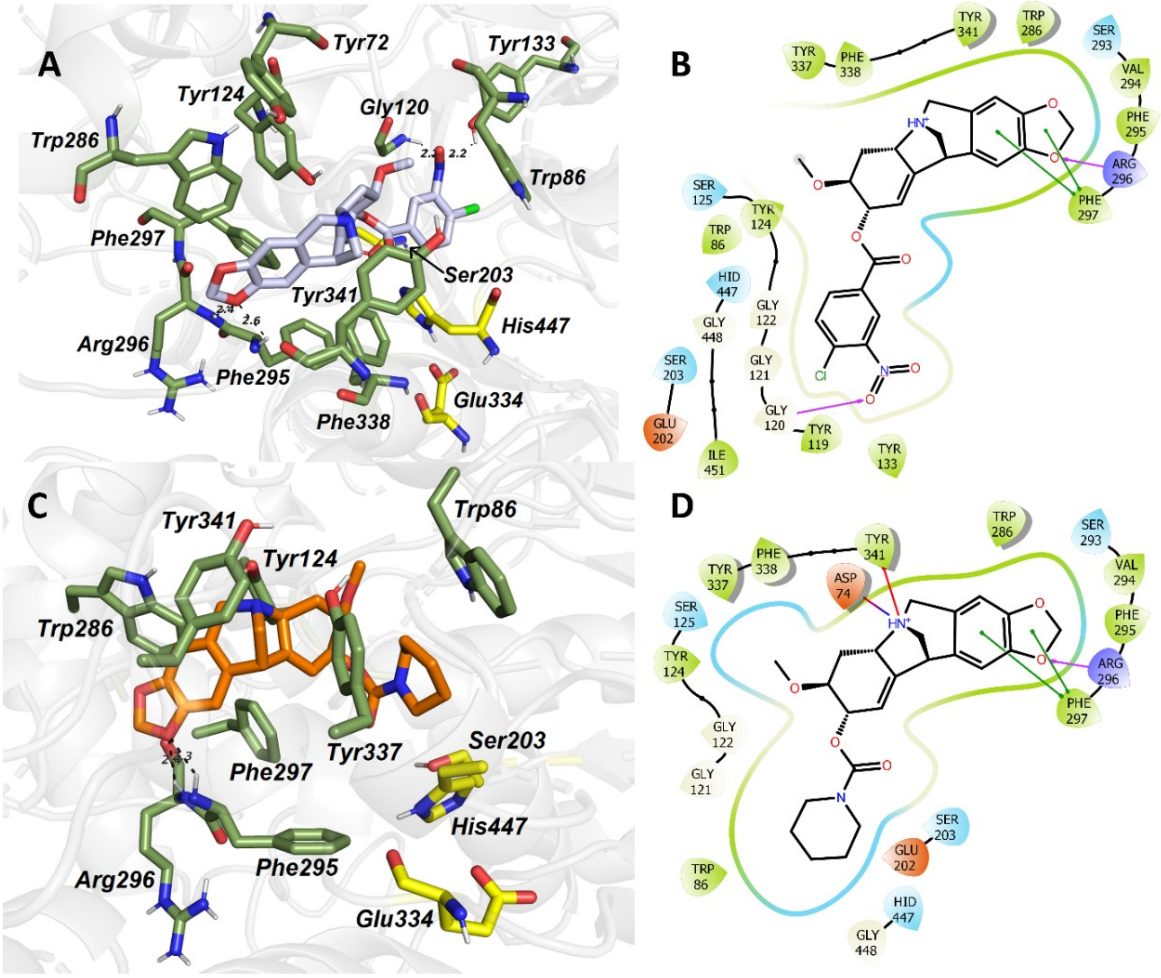


Figure 15. 3D and 2D illustrations of top-scored docking pose for compounds **1n** (A and B) and **1v** (C and D) in the *h*AChE active site (PDB entry: 4EY6).

The top-scored docking position for **1v** in the *h*BuChE active site (**Figure 16**; C and D) revealed a similar dimensional orientation as **1n** (**Figure 15**; A and B). Since the **1v** contains a carbamate group, we justify the mechanism of action involving the carbamylation of Ser198 from the catalytic triad. Though considering the protrusion of the carbamate group from the cavity gorge, we can also assume that the compound binds reversibly to *h*BuChE. Though, in the case of *in silico* study on *h*BuChE, we cannot indeed point to any determinant that determines the active agent (**1v**) from the inactive one (**1n**).

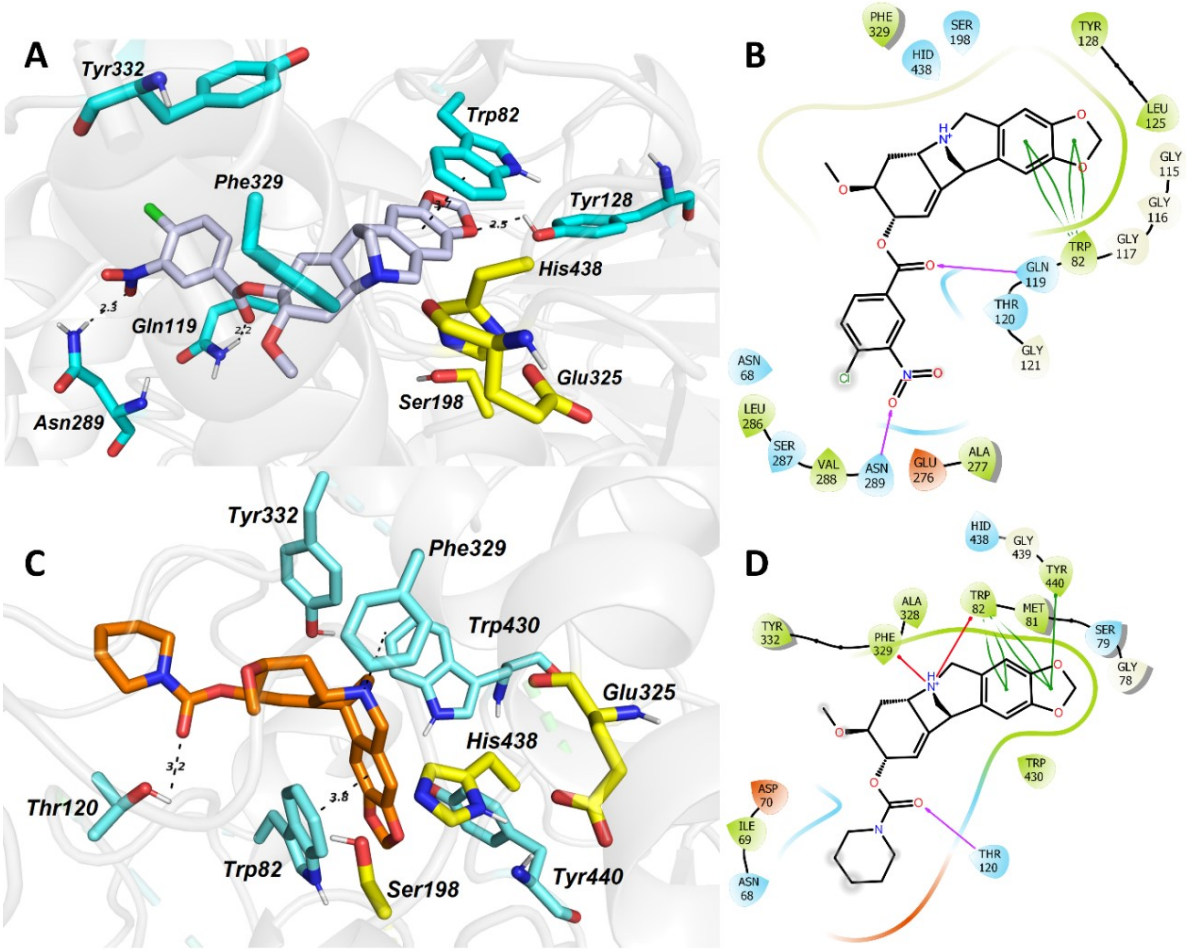


Figure 16. 3D and 2D illustrations of top-scored docking pose for compounds **1n** (A and B) and **1v** (C and D) in the *h*BuChE active site (PDB entry: 4BDS).

4.2 Semisynthetic Derivatives of Selected Amaryllidaceae Alkaloids as a New Class of Antimycobacterial Agents [152]

Tuberculosis (TB) is a devastating infectious disease caused by species of pathogenic bacteria in the family Mycobacteriaceae. *Mycobacterium tuberculosis* (Mtb) is the most common causative agent of TB. In 2018, one-quarter of the world's population was assumed to have a latent TB infection, and an estimated 10 million individuals manifested active TB, resulting in 1.5 million deaths. Current therapeutic options for non-resistant TB include isoniazid, rifampicin, ethambutol, and pyrazinamide as first-line medications, administrated for a minimum duration of six months. The significant rise in cases of Mtb multidrug-resistant (MDR) strains is a distressing issue in combat with TB. This rising complication has propelled to the forefront of investigations for novel antitubercular agents with activity against multiple strains of Mtb possessing new pharmacophores and modes of action.

The current study was influenced by the successful chemical modification of other classes of AAs, like haemanthamine, ambelline, and vittatine, aiming to enhance their inhibition potency against cholinesterases. [11,142,147,148] Nine AAs of various structural types and 19 esters derivatives of

galanthamine, 3-*O*-methylpancracine, vittatine, and maritidine were selected for *in vitro* antitubercular activity evaluation against three strains of *Mycobacterium*, Mtb H37Ra, *M. aurum*, and *M. smegmatis*, using a modified Microplate Alamar Blue Assay. Derivatives of galanthamine, vittatine, and maritidine were prepared by other colleagues in the department, and this commentary only focuses on the results obtained from 3-*O*-methylpancracine derivatives.

Compounds **3a**–**3g** were novel molecules derived by aromatic ester substitution on the free hydroxyl group on the C2 position of 3-*O*-methylpancracine (**Figure 17**). Previously isolated haemanthamine in grams from bulbs of *Z. citrina* was used to prepare excessive amounts of 3-*O*-methylpancracine through the ring rearrangement reaction. Their structures were elucidated by MS, HRMS, and NMR spectroscopic techniques. The results from the antimycobacterial screening of montanine, 3-*O*-methylpancracine, and seven aromatic ester derivatives of 3-*O*-methylpancracine are demonstrated in **Table 9**.

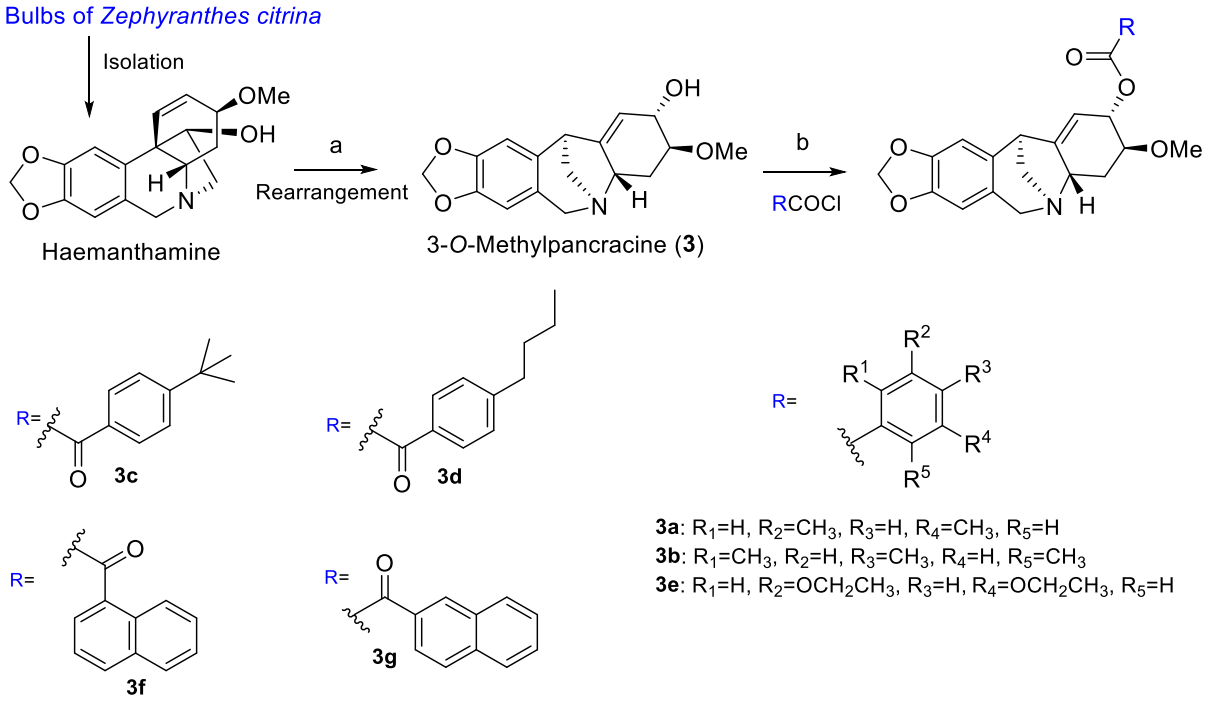


Figure 17. Scheme of C2-substituted ester derivatives of 3-*O*-methylpancracine screened for antimycobacterial activity. Reagents and conditions for synthesis: (a) MsCl, py, 0 °C, 8 h, then aqueous $NaHCO_3$, 25 °C, overnight (yield 70%); (b) RCOCl (1.5–3.0 eq.), DMAP (catalytic amount), py, 80 °C, 5–20 h (yield 42–100%).

Table 9. *In vitro* measured MIC against Mtb H37Ra, *M. aurum,* and *M. smegmatis*, cytotoxicity against HepG2 cells (IC_{50}), SI, and calculated lipophilicity (log*P*, clog*P*) of montanine, 3-*O*-methylpancracine, and seven derivatives of 3-*O*-methylpancracine.

Alkaloid/derivative	Mtb H37Ra (µg/mL)	Mtb H37Ra (µM)[a]	*M. smegmatis* (µg/mL)	*M. aurum* (µg/mL)	HepG2 IC_{50} (µM)	SI[b]	log*P*[c]	clog*P*[c]
3-*O*-Methylpancracine (**3**)	≥ 500	≥ 500	≥ 500	≥ 500	n.s.	n.c.	0.62	0.61
Montanine	≥ 500	≥ 500	≥ 500	≥ 500	n.s.	n.c.	0.62	0.61
3a	7.81	16.6	15.625	7.81	40.6 ± 7.6	2.45	3.72	4.20
3b	7.81	16.1	15.625	7.81	31.2 ± 5.4	1.94	4.21	4.70
3c	**3.91**	7.9	7.81	7.81	24.3 ± 2.4	3.08	4.45	5.03
3d	**3.91**	7.9	7.81	7.81	20.5 ± 0.7	2.59	4.49	5.29
3e	**3.91**	7.8	15.625	7.81	24.9 ± 1.7	3.15	3.17	4.49
3f	**3.91**	7.9	**3.91**	**3.91**	27.7 ± 4.7	3.51	3.75	4.38
3g	15.625	31.8	15.625	15.625	n.s.	n.c.	3.75	4.38
Isoniazid[d]	0.25	1.82	31.25	3.91	n.s.	n.c.	-0.64	-0.67
Rifampicin[d]	0.0062	0.0075	6.25	0.39	n.s.	n.c.	2.70	3.71
Ciprofloxacin[d]	0.25	0.75	0.125	0.0156	n.s.	n.c.	1.32	-0.62

[a] Calculated from MIC (µM/mL), [b] SI values are calculated as IC_{50} HepG2/MIC against Mtb H37Ra (in µM), [c] Log*P* and cLog*P* parameters are calculated in ChemDraw software v18.1.; [d] Standard; n.s. stands for not studied; n.c. stands for not calculated.

All of the evaluated semisynthetic compounds derived from the 5,11-methanomorpanthridine nucleus showed activity against selected strains of *Mycobacterium* with MIC values of more than 15.63 µg/mL, while montanine and 3-*O*-methylpancracine were utterly inactive. Compound **3f** with 1-naphthoyl substitution on the C2 position displayed the highest potency against all three strains of *Mycobacterium* with a MIC value of 3.91 µg/mL equal to 7.9 µM. Compounds **3c**–**3f** (with 4-tert-butylbenzoyl for **3c**, 4-butylbenzoyl for **3d**, and 3,5-diethoxybenzoyl for **3e**) were active against Mtb H37Ra with MIC values of 7.9 µM (at the concentration of 3.91 µg/mL). Compared with other derivatives of 3-*O*-methylpancracine, which were omitted in this article due to inactivity, it can be concluded that bulky substituents and surrogates with long aliphatic chain in the para position of the aromatic moiety are associated with the improvement of the antimycobacterial activity. Surprisingly this rule also could be deduced from evaluating other AAs structural modifications.

Since antituberculosis agents are known for their risk of hepatotoxicity, *in vitro* cytotoxicity of compounds on hepatocellular carcinoma cells HepG2, using MTT assay, was surveyed as a hepatotoxicity model. SI values were calculated as the ratio of IC_{50} HepG2 to MIC against Mtb H37Ra in µM, knowing that a SI value greater than 10 indicates an acceptable hepatotoxicity profile for the compound. Active compounds were tested at an initial concentration of 50 µM, and the IC_{50} values were subsequently determined. Measured IC_{50} values for compounds **3a**–**3g** were in the range of 20.5 ± 0.7 µM to 40.6 ± 7.6 µM, with compound **3a** being the least toxic compound in the series with a SI

value of 2.45. SI values were between 1.9 to 3.51, which denotes the necessity of structural optimization to eliminate the risk of hepatotoxicity.

Lipophilicity of the novel derivatives was evaluated *in silico* regarding the importance of the physicochemical properties of an antimycobacterial agent to transport through the cell transmembrane. It is postulated that higher lipophilicity is associated with better activity against Mtb. All the screened derivatives possess log*P* values 3.17–4.49 and clog*P* 4.20–5.29, indicating their acceptable lipophilicity profile as antimycobacterial agents.

4.3 Chemical and Biological Aspects of Montanine-Type Alkaloids Isolated from Plants of the Amaryllidaceae Family [43]

This article reviews the existing literature focusing on chemical and biological aspects of montanine-type AAs, aiming to point out their biological potencies despite being infrequently explored.

Amaryllidaceae is one of the 20 most fruitful alkaloid-containing plant families, and thus far, more than 600 structurally diverse alkaloids with a wide range of bioactivity have been discovered from their phytochemistry analysis. Lycorine was the first AA isolated from *N. pseudonarcissus* in 1877. Its isolation initiated many phytochemical investigations on Amaryllidaceae plants to find new potential molecules with promising bioactivity. The Discovery of galanthamine is the most prominent achievement of this research, providing an FDA-approved medication in the treatment of mild to severe stages of AD.

Biosynthesis of AAs is conducted through the norbelladine pathway with aromatic amino acids phenylalanine and tyrosine as precursors in the production of the fundamental intermediate, 4'-*O*-methylnorbelladine, which is generated from the condensation of 3,4-DHBA (produced from L-phenylalanine) and tyramine (formed from decarboxylation of L-tyrosine). As a result of their condensation, a Schiff base is generated, which is reduced to norbelladine and eventually methylated to 4'-*O*-methylnorbelladine. This key intermediate can undertake three modes of intramolecular oxidative couplings: *ortho-para*', *para-para*', and *para-ortho'*, which leads to forming the primary structural types of AAs (**Figure 1**). It is postulated that by *para-para'* coupling of 4'-O-methylnorbelladine and subsequent rearrangement of a haemanthamine-like intermediate, the 5,11-methanomorphanthridine core is produced, which is the main framework of montanine-type alkaloids as its illustrated in **Figure 2**. Another suggested biosynthetic pathway proposes the involvement of a stable cherylline-type intermediate generated from intramolecular addition in belladine-type skeleton, followed by addition of the secondary amine to the dienone. However, the first theory with *para-para'* coupling of 4'-*O*-methylnorbelladine has more supporting evidence than the involvement of a cherylline-type intermediate (**Figure 3**).

To date, 14 AAs containing the pentacyclic 5,11-methanomorphanthridine core in their skeleton are recognized (**Figure 4**). The placement of the carbon-carbon double bond on the E ring of the montanine-type can divide them into two subgroups; Alkaloids montanine, manthine, manthidine, pancracine, coccinine, brunsvigine, 3-*O*-methylpancracine, 3-*O*-acetylpancracine, and 3-*O*-acetylmontanine belong to the subgroup with located double bond between C1 and C11a. Nangustine and 4-*O*-methylnangustine also possess a double bond between C1 and C11a while having substituents on C3 and C4 rather than standard C2 and C3. Pancratinine B and pancratinine C (also called squamigine) belong to the second subgroup characterized by a double bond between C1 and C2 and a hydroxy group on C11a. Montabuphine is another reported AA with a 5,11-methanomorphanthridine skeleton with a double bond between C1 and C11a, although with described β-orientated methano-bridge rather than the standard α configuration for the first time. The structure analysis of (+)-montabuphine, prepared via total synthesis, found the previously illustrated structure for naturally isolated montabuphine to be controversial, and its legitimate structure is yet to be revised.

Montanine-type alkaloids are mainly found in genera *Haemanthus*, *Hippeastrum,* and *Pancratium*. They were also isolated in trivial amounts from species such as *L. radiata* and *N. angustifolius* subsp*. transcarpathicus.* Montanine is the primary alkaloid found in *R. bifda* and *S. multiflorus* (**Table 2**).

Numerous studies have presented AAs as antiproliferative, cytotoxic, antifungal, antibacterial, antimalarial, and anticholinesterase agents. Montanine-type AAs are one of the most intensely investigated classes of AAs as potent anticancer compounds against diverse cancer cell lines. In addition, they have displayed anxiolytic, anti-depressive, and anticonvulsive activities, as well as anti-inflammatory properties. Furthermore, montanine, per se, has been investigated for its AChE inhibition, antirheumatic, and antimicrobial impacts.

Cytotoxicity of montanine, pancracine, coccinine, and manthine have been outlined against various cancerous cell lines, utilizing standard colorimetric assays based on either the reduction of the tetrazolium salt in WST-1 and MTT to formazan or an alternative assay based on the measurement of cellular protein content, using the protein-binding dye sulforhodamine B (SRB). Among these, the most pronounced anticancer activity belongs to montanine, which was presented as a significantly potent antiproliferative alkaloid in three studies, despite the variation in utilized assays and types of cancer cell lines. Its evaluated IC_{50} value against ten cancer cell lines reported as 1.04–2.30 µM using WST-1 assay after 48 hours of treatment. Another study confirmed the solid antiproliferative property of montanine on a panel of six cancer cell lines, including MCF-7, Hs578T, MDA-MB-231, HCT-15, A549, and SK-MEL-28 (IC_{50} values range 1.9–23.2 µM using MTT assay after 48 hours of treatment). In another study, sub-micromolar IC_{50} values were reported for montanine against five cancer cell lines (HT-29,

H460, RXF393, MCF-7, and OVCAR3) using SRB assay. Regarding the different types of solid and leukemic cancer cell lines used in cytotoxicity investigations, it can be presumed that montanine-type AAs are promising agents in treating human cancer diseases.

Montanine-type alkaloids are scarce in plants; therefore, little research has been conducted on other biological activities of these compounds. Montanine and pancracine were evaluated for their antimicrobial activity; montanine showed more significant activity than pancracine against pathogenic *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermis*. While in another study, pancracine was reported to display inhibition activity against *S. aureus* and *P. aeruginosa* and moderate activity against *C. albicans*.

Nangustine and pancracine were inactive to weakly active against the protozoans *T. brucei rhodesiense*, *T. cruzi*, *L. donovani,* and *P. falciparum, in vitro*. In addition, none of the montanine-type AAs showed significant inhibitory activity against cholinesterases. However, da Silva et al. have advanced the role of montanine in neurological disorders by demonstrating its ability to decrease locomotor activity, tranquilize, relieve stress, and decrease epilepsy in mice. The mechanism of action might involve interaction with the GABA receptor on the benzodiazepines site in the mouse's brain. Hence, the anxiolytic and hypnotic effects of montanine are likely the result of its combined action on several neurotransmitter receptor systems, including $GABA_A$ receptors.

Montanine has successfully regulated the development of experimental arthritis in both acute and chronic cases in animals, indicating the potency of montanine for regulating autoimmune disorders like arthritis. The potential role of this alkaloid is also described through *in vivo* and *in vitro* models for suppressing inflammatory diseases such as rheumatoid arthritis, ulcerative colitis, acute pulmonary disease, and inflammatory infections.

The scarcity of 5,11-methanomorphanthridine scaffolds in Amaryllidaceae is a limiting issue in their thorough biological investigations. Therefore, several attempts for their total synthesis have been established, though they all include complicated multistep reactions with unsatisfactory yields. Old papers explained an accidentally discovered route to generate the 5,11-methanomorphanthridine nucleus by a semisynthetic intramolecular transformation of a haemanthamine-type ring. This easily achievable structure rearrangement has paved the way for the design and synthesis of pilot derivatives of montanine-type AAs, and their subsequent evaluation on various biological tests to not only improve the activity by the chemical modification of the skeleton but also to describe SAR for these compounds.

4.4 Recent Progress on Biological Activity of Amaryllidaceae and Further Isoquinoline Alkaloids in Connection with Alzheimer's Disease [38]

This review summarizes available pharmacological targets in treatment of multifactorial neurodegenerative disorder AD. It also points out recently isolated or newly investigated isoquinoline alkaloids, between 2010 and 2021, with the potency to target AD. Several enzymes and receptors are assumed to be potential targets in drug discovery against AD, such as AChE and BuChE with their pivotal role in the regulation of ACh, *N*-methyl-d-aspartate receptor (NMDAR), GSK-3β, cyclin-dependent kinase 5 (CDK5), POP, and monoamine oxidase (MAO). It is noteworthy to mention that the source of enzymes used for *in vitro* evaluation of cholinesterase inhibition can have a critical role in obtained results. For example, using electric eel acetylcholinesterase (*Ee*AChE), *h*AChE, mouse brain AChE, equine serum BuChE (*Eq*BuChE), or *h*BuChE may lead to different IC_{50}s for one compound.

Natural products, and specifically alkaloids, have represented an immense source of clinical drugs due to their structural diversity. Alkaloids could be extracted from plant material with solvents such as methanol and ethanol and isolated by column chromatography, flash chromatography, HPLC, and preparative TLC.

In the last five years, two new structural types of AAs, named narcikachnine- and carltonine-type, have been isolated from Amaryllidaceae plants. Both new classes demonstrated promising biological activity against enzymes connected with AD. Up to this date, five AAs of narcikachnine-type have been isolated from *Z. citrina*, *N. pseudonarcissus* cv. Carlton, *N. pseudonarcissus* cv. Dutch Master, and *N. poeticus* cv. Pink Parasol. Newly isolated narcikachnine-type AAs (narciabduliine, narcieliine, narcimatuline, and narcipavline) and their *in vitro* activities against enzymes connected with AD, are displayed in **Figure 18**. Carltonine-type AAs are named carltonine A, B, and C, which have been isolated from *N. pseudonarcissus* cv. Carlton. Carltonine A and B demonstrated fascinating selective inhibitory activity against BuChE with IC_{50}s in the nanomolar range. Carltonine A also showed POP inhibition ability with an IC_{50} value of 143 ± 21 μM, which is comparable to berberine (IC_{50} = 142 ± 21 μM), a recognized natural POP inhibitor. The structures and biological activities of these three carltonine-type alkaloids are illustrated in **Figure 18**. Furthermore, other newly isolated alkaloids belonging to previously known classes of AAs, such as lycorine-, galanthamine-, and homolycorine-type, have been discovered in the past five years, and they have been evaluated for their potency against AD. Among these, the best inhibitory activity against AChE was demonstrated by *N*-norgalanthamine (IC_{50}, *Ee*AChE = 2.76 ± 0.56 μM) and 11β-hydroxygalanthamine (IC_{50}, *Ee*AChE = 3.04 ± 0.61 μM), which respectively, they were isolated from *P. maritimum* and *L. longituba*. *N*-methylcrinasiadine, belonging to narcislasine-type AAs and isolated from *L. longituba*, displayed intriguing inhibition activity against *Ee*AChE with an IC_{50} value of 4.23 ± 1.13 μM (**Figure 18**).

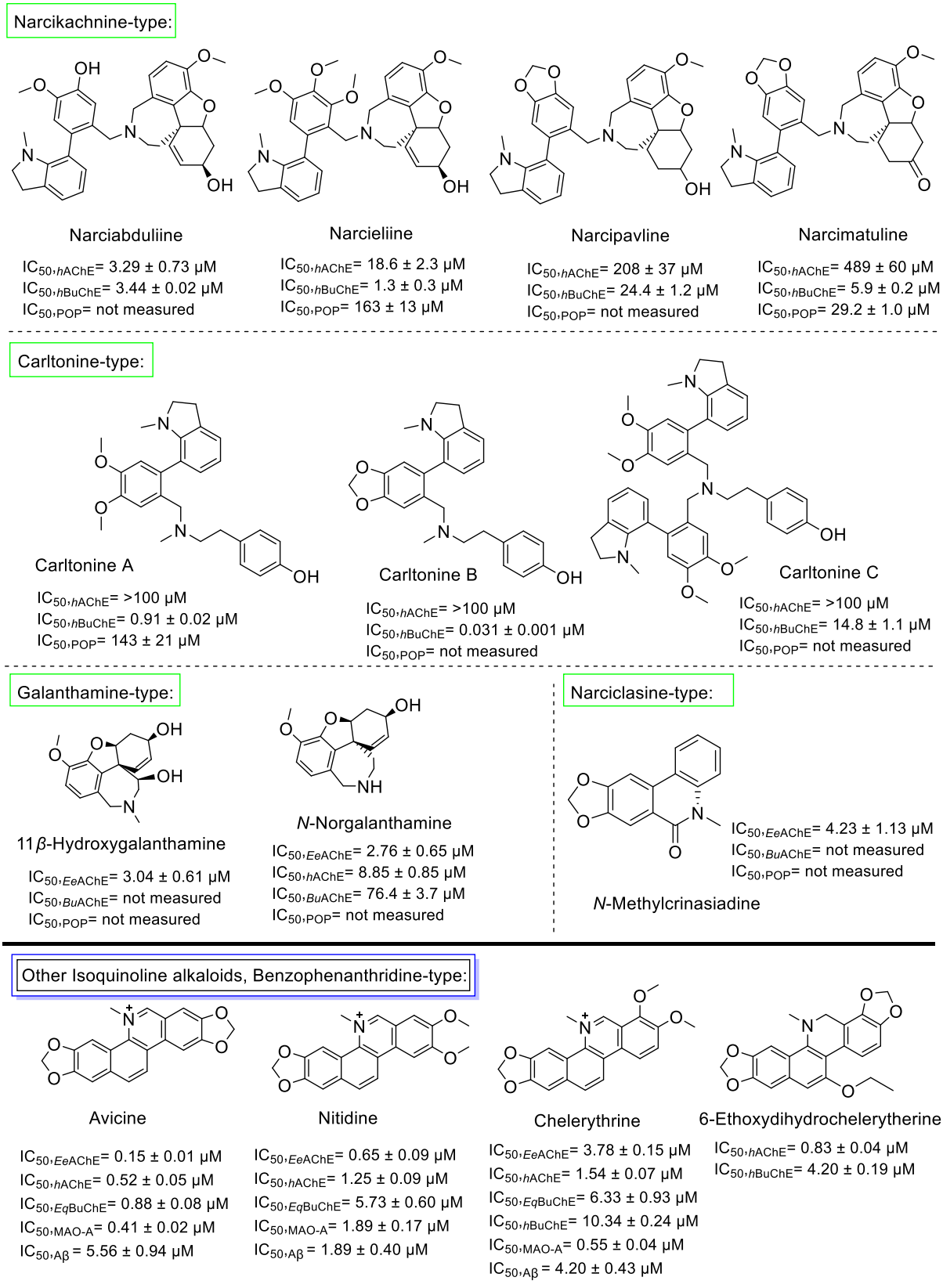


Figure 18. Selected examples of narcikachnine-, carltonine-, galanthamine-, and narciclasine-type Amaryllidaceae alkaloids, and selected examples of isoquinoline alkaloids with their potential bioactivity profile concerning Alzheimer's disease.

Several other isoquinoline alkaloids (not from Amaryllidaceae) were isolated in the past decades and subsequently studied for their potency against AD. Most potent examples of these alkaloids, mainly possessing benzophenanthridine core, are demonstrated in **Figure 18** along with their evaluated potential activity against AD. Avicine and nitidine, both isolated from *Zanthoxylum rigidum,* and chelerythrine isolated from *Chelidonium majus*, are reported as a multitarget substrates with promising inhibitory activity against *h*AChE (IC_{50}s, 0.52 ± 0.05 μM, 1.25 ± 0.09 μM, and 1.54 ± 0.07 μM, respectively), *Ee*BuChE (IC_{50}s, 0.88 ± 0.08 μM, 5.73 ± 0.60 μM, and 6.33 ± 0.93 μM, respectively), and MAO-A (IC_{50}s, 0.41 ± 0.02 μM, 1.89 ± 0.17 μM, and 0.55 ± 0.04 μM, respectively). In addition, these compounds exhibited significant $A\beta_{1-42}$ anti-aggregation feature with an IC_{50} values of 5.56 ± 0.94 μM, 1.89 ± 0.40 μM, and 4.20 ± 0.43 μM, in turn. Furthermore, 6-ethoxydihydrochelerythrine inhibited both the *h*AChE and *h*BuChE enzymes with IC_{50} values of 0.83 ± 0.04 μM and 4.20 ± 0.19 μM, respectively.

Influenced by the fascinating bioactivity of AAs and other isoquinoline alkaloids in targeting AD-related factors, their chemical structure has inspired researchers to develop new active substances in drug discovery against AD. A study inspired by recently presented carltonine-type alkaloids reported the total synthesis of compounds with the same fundamental backbone. Seven synthesized compounds out of twenty demonstrated solid and selective *h*BuChE inhibitory character, with IC_{50} values less than 1 μM. The most potent compound is illustrated in **Figure 19**. Esther derivatives of crinane-type (α- and β-) AAs, using haemanthamine and ambelline as the lead structure, also led to the development of new semisynthetic derivatives with improved potency against AChE and/or BuChE. The attractive multipotent characteristic of berberine and its abandonment in natural sources allowed the development of a series of 22 ether analogs, all displaying *h*AChEI ability in the micromolar to sub-micromolar range. The most potent compounds are illustrated in **Figure 19** with their biological activity profile.

Overall, the presented review showed that AAs and isoquinoline alkaloids present a source of chemical structures with promising neuropharmacological features, essential for further exploration and optimization of the chemical skeleton as multitarget candidates for the treatment of AD.

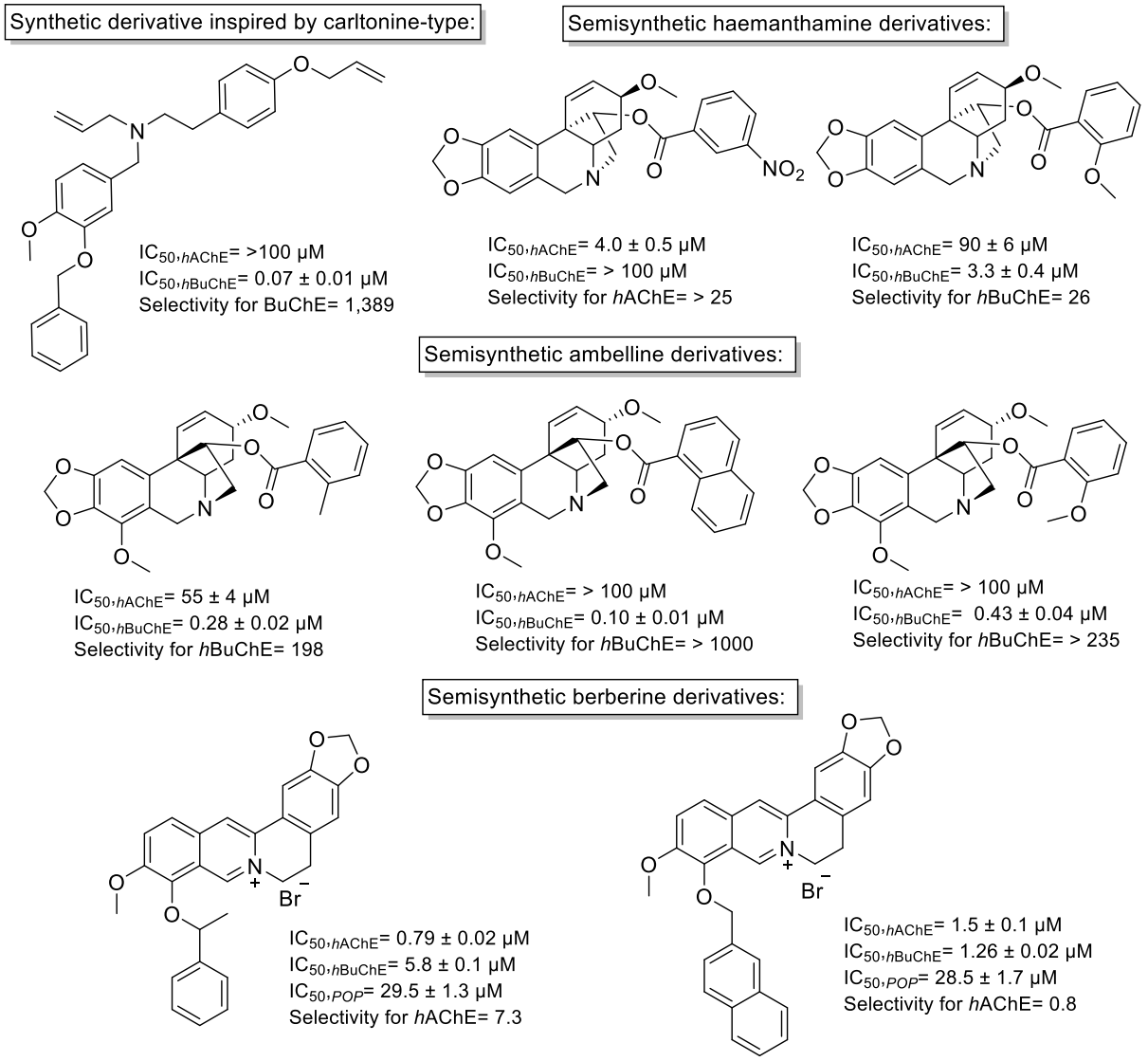


Figure 19. Selected examples of synthetic or semisynthetic derivatives inspired by carltonine- and α-crinane-type Amaryllidaceae alkaloids and derivatives of berberine with their potential bioactivity profile concerning Alzheimer's disease.

5 CONCLUSION

Chemical modifications on the framework of AAs have gained much attention in the assignment of novel potent molecule discovery. Derivatization of AAs such as lycorine, haemanthamine, tazettine, and narciclasine to improve their AChE/BuChE inhibitory, cytotoxicity, antibacterial, antiparasitic activities are examples of these chemical modifications. [73,100,117,138,149] Montanine-type AAs are demonstrated to have antiproliferative, antimalarial, antirheumatic, and antimicrobial activity. [43] Although the scarcity of their 5,11-methanomorphanthridine nucleus in plants of Amaryllidaceae has made the opportunity of their thorough investigation infrequent. [10,12] Fortunately, earlier investigations described the possibility of the crinane-type ring transformation to the montanine-type nucleus and utilized it for derivatization of 5,11-methanomorphanthridine core. [10,12,31] Considering the

unexplored biological potency of this class of AAs and inspired by using the haemanthamine ring rearrangement to montanine-type and the possibility of its further derivatization, we aimed to fill the knowledge gap about the biological potencies of montanine-type AAs.

In this commentary, we investigated and published the results obtained from the potency evaluation of 3-*O*-methylpancracine and montanine derivatives in treating AD and TB. Screening of 28 new derivatives of montanine-type AAs (23 derived from 3-*O*-methylpancracine, and 5 derived from montanine) against *h*AChE and *h*BuChE identified three compounds (2-*O*-(4-chloro-3-nitrobenzoyl)-, 2-*O*-(2-chloro-5-nitrobenzoyl)-, and 2-*O*-(4-methyl-3-nitrobenzoyl)-3-*O*-methylpancracine; **1n**, **1p**, **1o** respectively in **Figure 14**) with significant selective inhibitory potency for *h*AChE (IC_{50} < 5 μM). From the SAR study, it can be concluded that aromatic substitution containing a nitro group and a halogen (Cl, Br) on C2 can activate the scaffold against *h*AChE. However, mounting the same substituents on montanine (free hydroxyl group on C3) led to activity elimination. One analog with *N*-piperidine carbamate, showed selective *h*BuChE inhibition activity (IC_{50} = 1.73 ± 0.05 μM). Repeating the same substitution on C3 of montanine reduced the activity to an IC_{50} value of 6.54 ± 0.74 μM. It can be proposed that the mechanism of action involves the carbamylation of residues in *h*BuChE. We also conducted the *in silico* studies to justify the activity of compounds against *h*AChE and *h*BuChE and to comprehend the SAR. We have also calculated the BBB score for active compounds to evaluate their ability to penetrate CNS. According to the low cytotoxicity of *N*-piperidine carbamate derivative of 3-*O*-methylpancracine (2-*O*-((piperidine-1-yl-carbonyl)oxy)-3-*O*-methylpancracine, **1v** in **Figure 14**) and its acceptable BBB score, we can recommend it for further structure optimization as a selective *h*BuChE inhibitor.

Moreover, screening of the synthesized derivatives of the 5,11-methanomorphanthridine nucleus indicated the activation of the inert scaffold against *Mycobacterium* after chemical modification. Montanine, 3-*O*-methylpancracine, and seven aromatic ester derivatives of 3-*O*-methylpancracine were evaluated against Mtb H37Ra, *M. aurum*, and *M. smegmatis.* The most active derivatives were subjected to IC_{50} determination against HepG2 cells as hepatotoxicity model, and thus the SI was also calculated. For the prediction of lipophilicity and cell permeability, log*P* and clog*P* were calculated *in silico*. Interestingly, all derivatives demonstrated significant activity against all selected *Mycobacterium* strains, with MIC values ranging between 3.91–15.63 μg/mL and Mtb H37Ra as the most sensitive strain. We asserted that aromatic ester substitutions on position C2 with 4-tert-butylbenzoyl, 4-butylbenzoyl, 3,5-diethoxybenzoyl, and 1-naphthoyl give the maximum potency, and not only on montanine-type skeleton but also on derivatives of other classes of AAs such as galanthamine, vittatine, and maritidine. Among derivatives of 3-*O*-methylpancracine, 2-*O*-(1-naphthoyl)-3-*O*-methylpancracine with a MIC value of 7.9 μM against all strains has the highest activity. Though the

lipophilicity profile of synthesized compounds is favorable as antimycobacterial agents, they might have a potential risk of hepatotoxicity. Nevertheless, further structure development and stabilization, and optimizing the toxicity profile are required.

Considering the stability issues associated with the ester group in our derivatives, we decided to prepare the analog of active compounds with ether as the linker to aromatic substitution on the C2 position of 3-*O*-methylpancracine. In addition, ester analogs of active compounds were prepared using montanine as starting material to compare the impact of the substitute position on the scaffold. Figure **20** illustrates this complementary batch, and the results of their screening against Mtb H37Ra, *M. aurum*, and *M. smegmatis* are demonstrated in **Table 10**.

By comparison of the results from **Tables 9** and **10**, it can be justified that replacing the bulky aromatic substitutions (4-tert-butylbenzoyl, 4-butylbenzoyl, 1-naphthoyl and 2-naphthoyl, 3,5-dimethylbenzoyl, 3,5-diethoxybenzoyl, and 2,4,6-trimethylbenzoyl) on the C3 position of montanine-type skeleton decrease the antimycobacterial activity but does not lead to its complete elimination (**2f**–**2l**). Bulky aromatic substitution with ether linker to C2 position also leads to a slight drop in activity which might be explained due to a slight decrease in lipophilicity parameter of derivatives containing ether linker (clog*P* values of 4.20–5.29 for **3a**–**3g** with ester linker, and 4.11–4.93 for **3h**–**3l** with ether linker). Therefore, it can be concluded that the antimycobacterial activity of montanine-type AAs derivatives is not dependent on the presence of the ester linker or the substitution position on C2 or C3 but mainly depends on the chemical modification using a bulky substitute. These results are yet unpublished, and in the near future, they will be published along with results from antimycobacterial investigations on other AAs classes within our research group.

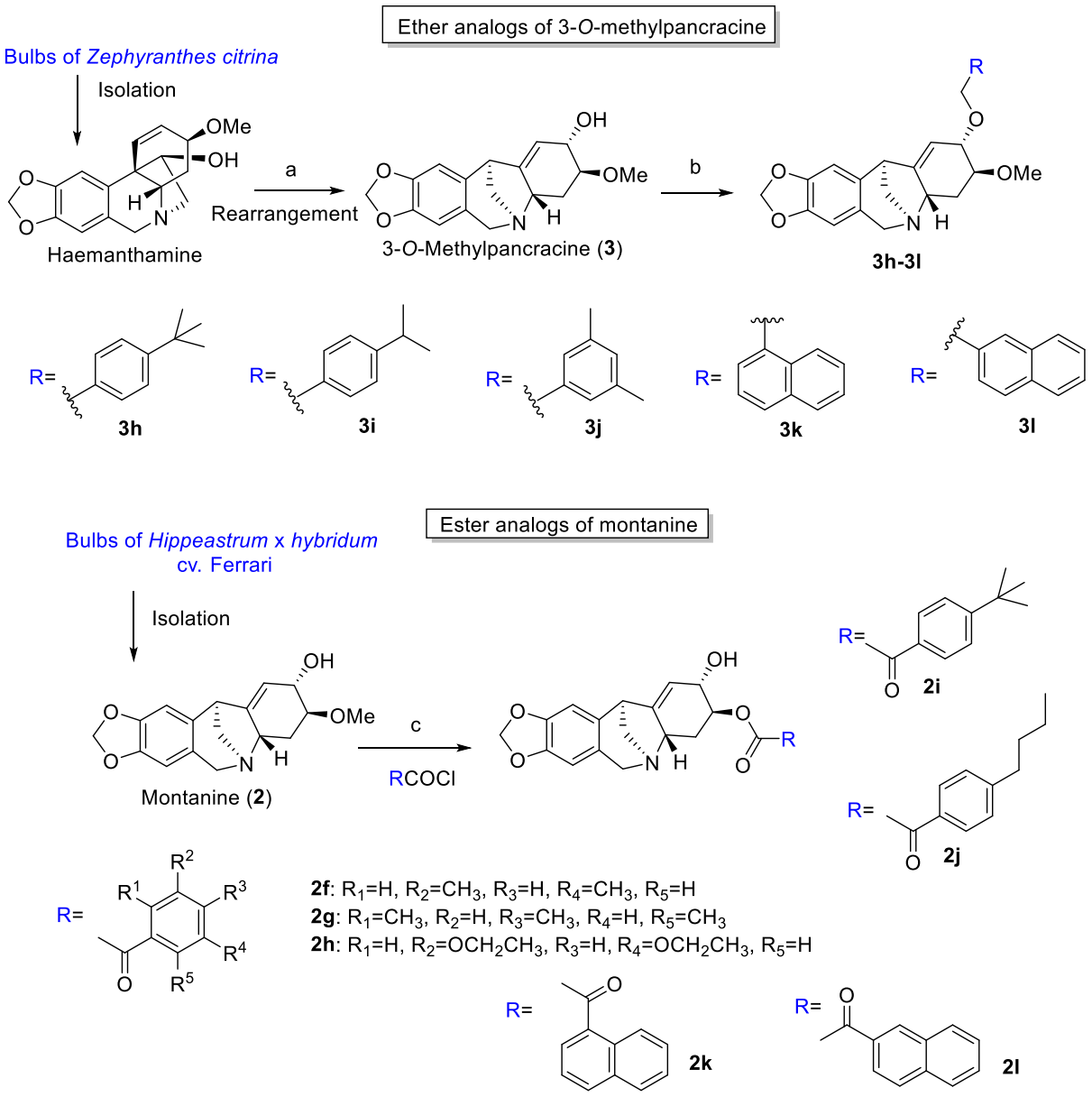


Figure 20. Reaction scheme and synthesized derivatives of 3-*O*-methylpancracine (**3**) and montanine (**2**) for evaluation against Mtb H37Ra, *M. aurum*, and *M. smegmatis* (**3h**–**3l**, **2f**–**2l**). Reagents and conditions for synthesis: (a) MsCl, py, 0 °C, 8 h, then aqueous $NaHCO_3$, 25 °C, overnight (yield 70%); (b) NaH in Dry THF, under argon gas, 0 °C, 0.5 h, then corresponding aryl alkyl halide (1 eq.), 25 °C, overnight (yield 13.7–40.9%); (c) RCOCl (1.5–3.0 eq.), DMAP (catalytic amount), py, 80 °C, 5–20 h (yield 28.3–100%).

Table 10. *In vitro* measured MIC of five ether derivatives of 3-*O*-methylpancracine (**3h**–**3l**) and seven ester derivatives of montanine (**2f–2l**) (analogs of previously reported active compounds) against Mtb H37Ra, *M. aurum,* and *M. smegmatis* and their calculated lipophilicity (log*P*, clog*P*).

Alkaloid/derivative	Mtb H37Ra (μg/mL)	Mtb H37Ra (μM)[a]	*M. smegmatis* (μg/mL)	*M. aurum* (μg/mL)	log*P*[b]	clog*P*[b]
3h	12.5	27.93	12.5	6.25	4.42	4.93
3i	12.5	26.60	12.5	12.5	3.95	4.54
3j	25	54.83	25	12.5	3.69	4.11
3k	12.5	26.15	6.25	12.5	3.71	4.29
3l	12.5	26.15	12.5	12.5	3.71	4.29
2f	15.625	33.24	15.625	7.81	3.72	4.20
2g	15.625	32.28	7.81	7.81	4.21	4.70
2h	25	47.17	12.5	25	3.17	4.49
2i	7.81	15.68	**3.91**	**3.91**	4.45	5.03
2j	15.625	31.37	**3.91**	**3.91**	4.49	5.29
2k	16.625	33.79	7.81	7.81	3.75	4.38
2l	16.625	33.79	7.81	7.81	3.75	4.38
Isoniazid[c]	0.25	1.82	31.25	3.91	-0.64	-0.67
Rifampicin[c]	0.00625	0.0075	6.25	0.39	2.70	3.71
Ciprofloxacin[c]	0.25	0.75	0.125	0.0156	1.32	-0.62

[a] Calculated from MIC (μM/mL), [b] Log*P* and cLog*P* parameters are calculated in ChemDraw software v18.1.; [c] Standard.

Since montanine-type alkaloids have been mainly explored as potential anticancer molecules and inspired by previous reports of anticancer activity of AAs derivatives, we included the cytotoxicity test in our generated library evaluation. [12,76,89,141,153]

We screened selected montanine-type derivatives on a 10 μM single dose against a panel of human cell lines of various histotypes; cancer cell lines such as Jurkat (acute T-cell leukemia), MOLT-4 (acute lymphoblastic leukemia), A549 (lung carcinoma), HT-29 (colorectal adenocarcinoma), PANC-1 (pancreas epithelioid carcinoma), A2780 (ovarian carcinoma), HeLa (cervix adenocarcinoma), MCF-7 (breast adenocarcinoma), SAOS-2 (osteosarcoma), and normal human cell MRC-5 (normal lung fibroblasts) to determine the compounds overall toxicity. Doxorubicin was used as a positive control with a concentration of 1 μM, and WST-1 proliferation assay was used to measure the cytotoxicity of compounds after 48 hours of treatment. The growth percent (GP) value was calculated for each screened compound as the mean of the viable cells in the percent of all the treated cell lines. For the most active compounds, IC_{50} values were calculated in the concentration range of 0.1–100 μM.

Aliphatic and aromatic esters or ethers of 3-*O*-methylpancracine did not express any significant cytotoxic activity against the selected cell line panel. Interestingly, manthine and a few derivatives of montanine demonstrated cytotoxicity against the selected cell line panel. The structure of 15 screened

derivatives of montanine and the procedure for preparation of manthine are illustrated in **Figure 21** (Compounds **2a**–**2c** were already published as inactive molecules against *h*AChE, and compounds **2f**–**2k** were synthesized in order to complete the SAR study for antimycobacterial activity). Obtained results of the preliminary screening are expressed in **Figure 22**.

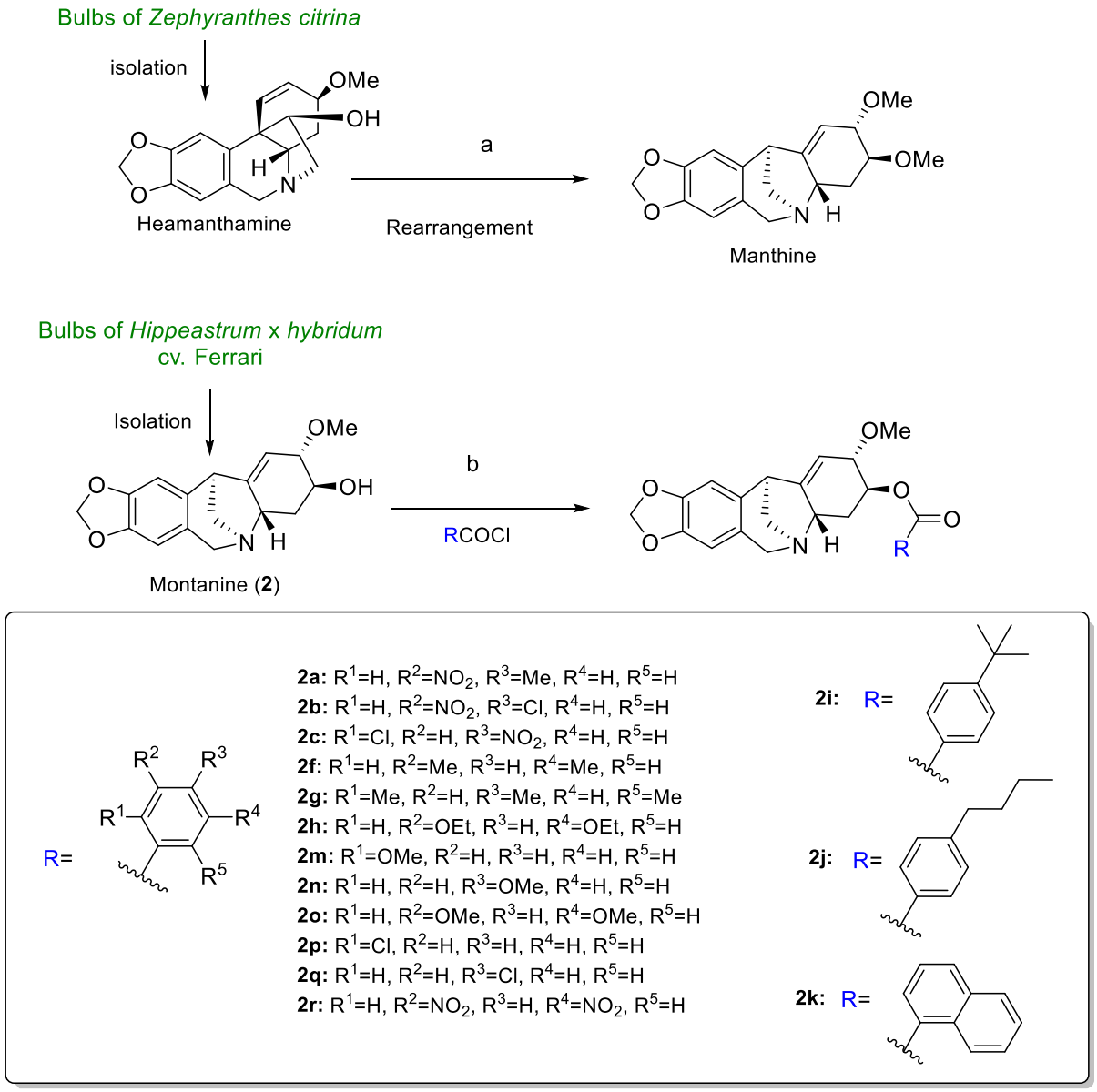


Figure 21. The preparation procedure for manthine and 15 aromatic ester derivatives of montanine. Synthesized compounds were screened for cytotoxicity activity on a panel of cancer cell lines. Reagents and conditions for synthesis: (a) MsCl, py, 0 °C, 8 h, then cold suspension of MeOH in dry THF treated with NaH, 25 °C, overnight (yield 61.6%); (b) RCOCl (1.5–3.0 eq.), DMAP (catalytic amount), py, 80 °C, 5–20 h (yield 21.3–100%).

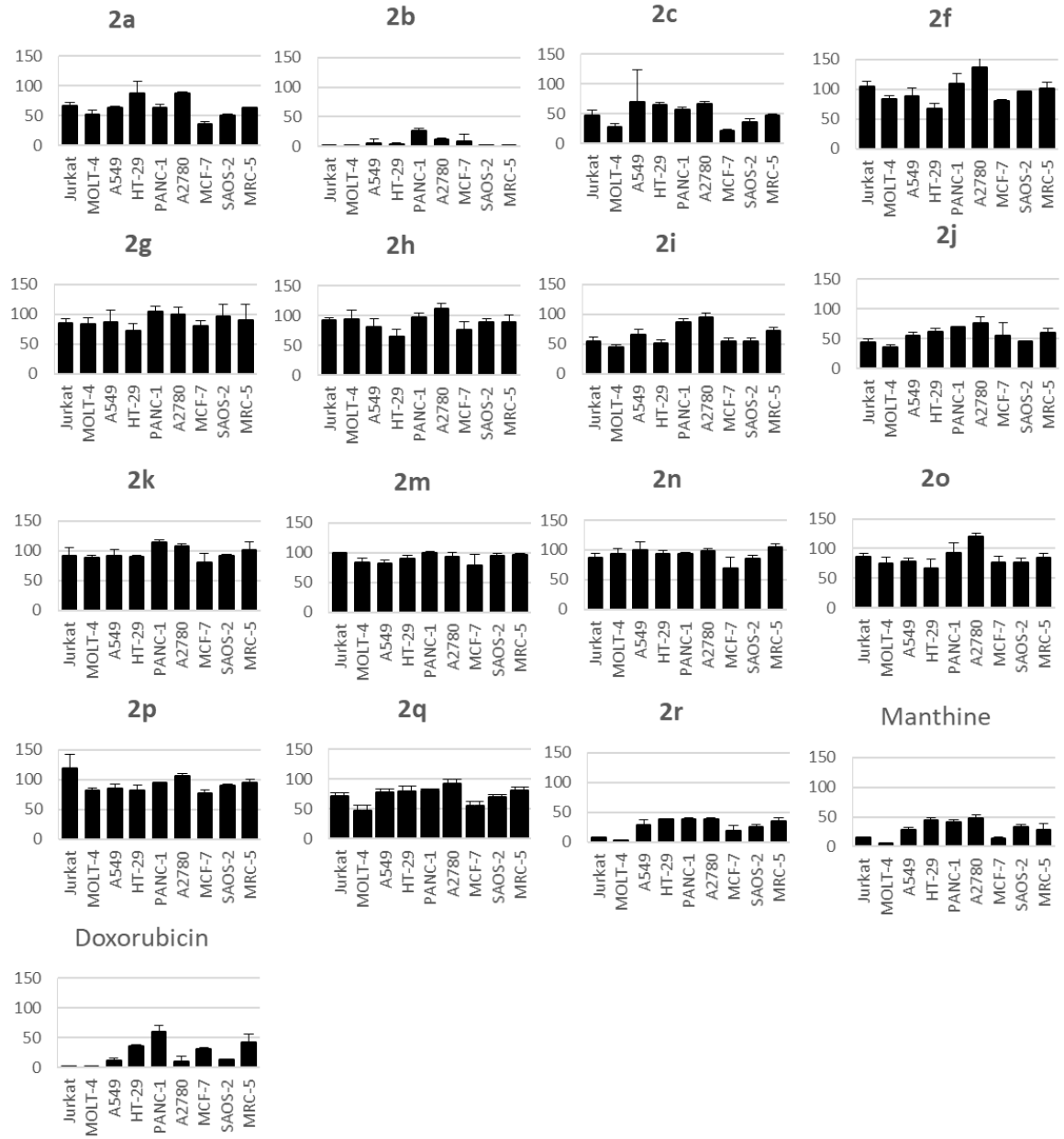


Figure 22. Antiproliferative activity of the screened compounds (manthine, **2a–2c**, **2f–2k**, and **2m–2r**; and doxorubicin as the positive control). Each graph exhibits the antiproliferation activity for each compound on nine cell lines. Cells were treated with a concentration of 10 µM, and their proliferation was measured by WST-1 assay after 48h of treatment. The antiproliferation potency is expressed as a percentage of control cells (0.1% DMSO treated, proliferation 100%). Each value is the mean of three independent measurements ± standard deviation. The horizontal line borders the 50% value. Doxorubicin was tested at 1 µM concentration.

Manthine was prepared according to the previously described procedure and by using isolated haemanthamine from bulbs of *Z. citrina*, available in our research group from phytochemical investigations.[12,18] Recently, our research group reported cytotoxicity of montanine on nine cancer cell lines with a mean GP of 20 µM (GP concentration range of 2–36 µM) by introducing MOLT-4, Jurkat, and MCF-7 as the most sensitive cell lines. The measured IC_{50} value for montanine against cancer cell lines was reported in the range of 1.04–2.30 µM. [53] In the current study, our preliminary cytotoxicity

evaluation of the primary alkaloid manthine, together with a series of 15 semisynthetic derivatives of montanine (**2a–2c**, **2f–2k,** and **2m–2r**) indicated that manthine and two prepared derivatives, 3-*O*-(4-chloro-3-nitrobenzoyl)montanine as **2b**, and 3-*O*-(3,5-dinitrobenzoyl)montanine as **2r**, posses potential antiproliferative effect while keeping the same pattern of highest sensitivity for MOLT-4, Jurkat, and MCF-7 cell lines. Both derivatives have a 3-nitro group of the benzoyl moiety but differ in additional substituents. IC_{50} values of manthine, **2r**, and **2b**, together with previously reported IC_{50} for montanine, are demonstrated in **Table 11**. Though manthine showed interesting potency, it was not as cytotoxic as montanine. Compounds **2b** and **2r** showed strong IC_{50} values against cancer cell lines ranging between 1.41–8.06 μM and 1.18–2.95 μM, respectively. However, they did not surpass the potency of montanine to prohibit cell proliferation. Though montanine, **2b,** and **2r** displayed a high antiproliferative potency, none of the molecules exhibited a selective antiproliferative activity against cancer cells by exempting non-cancer MRC-5 cells from cytotoxic effect.

Table 11. IC_{50} values of montanine, manthine, and **2b** and **2r** on human cancer and non-cancer cells [a,b].

Cell type	2b	2r	Manthine	Montanine[53]
Jurkat	3.4 ± 1.04	2.58 ± 1.07	3.02 ± 1.05	1.04 ± 0.14
MOLT-4	1.56 ± 1.09	2.95 ± 1.08	3.51 ± 1.2	1.26 ± 0.11
A549	1.41 ± 1.09	1.55 ± 1.19	6.18 ± 1.15	1.09 ± 0.31
HT-29	2.11 ± 1.05	2.16 ± 1.17	19.69 ± 1.19	1.35 ± 0.47
PANC-1	8.02 ± 1.06	1.88 ± 1.11	7.69 ± 1.18	2.30 ± 0.45
A2780	8.06 ± 1.06	2.7 ± 1.14	12.94 ± 1.16	1.67 ± 0.29
MCF-7	1.89 ± 1.07	1.65 ± 1.12	2.68 ± 1.08	1.39 ± 0.21
SAOS-2	2.13 ± 1.06	1.18 ± 1.17	4.06 ± 1.13	1.36 ± 0.49
MRC-5	1.54 ± 1.06	2.18 ± 1.17	3.6 ± 1.14	1.79 ± 0.50

[a] Results are expressed in μM; [b] Results are expressed as the mean values ± standard deviations of at least three independent replications.

Surprisingly, two derivatives of haemanthamine with identical aromatic ester substitutes, 11-*O*-(4-chloro-3-nitrobenzoyl)haemanthamine and 11-*O*-(3,5-dinitrobenzoyl)haemanthamine, were recently reported to possess antiproliferative effect. Calculated IC_{50} for 11-*O*-(4-chloro-3-nitrobenzoyl)haemanthamine against cancer cell lines ranges between 0.2–10.1 μM while screening the ligand 4-chloro-3-nitrobenzoylchloride did not show any cytotoxic effect on cells. [153] Overall, we report chemical modifications on the C3 position of 5,11-methanomorphanthridine skeleton to be more promising for antiproliferative activity than C2. We conclude that 4-chloro-3-nitrobenzoyl and 3,5-dinitrobenzoyl substitution on C3 of the montanine-type scaffold can lead to a non-selective antiproliferative effect comparable to montanine potency. In addition, it can be deduced that 4-chloro-3-nitrobenzoyl attachment might also enhance the cytotoxic activity of other AAs, although the ligand per se is not cytotoxic. Results of antiproliferative activity of montanine-type alkaloids are at the

moment being prepared for publication together with molecular details of mechanisms of actions for **2b** and montanine.

Lastly, along with antimycobacterial screening, we evaluated the antifungal and antibacterial potency of the generated library of montanine-type derivatives. The preliminary antibacterial and antifungal screening was conducted using the microdilution broth method according to EUCAST instructions (The European Committee on Antimicrobial Susceptibility Testing) with slight modifications. [154,155] A panel of eight bacterial strains of clinical importance was selected for antibacterial screening (four Gram-positive strains: *S. aureus* subsp. *aureus* ATCC 29213, CCM 4223, *S. aureus* subsp. *aureus* methicillin-resistant strain (MRSA) ATCC 43300, CCM 4750, *S. epidermidis* ATCC 12228, CCM 4418, *Enterococcus faecalis* ATCC 29212, CCM 4224; and four Gram-negative strains: *E. coli* ATCC 25922, CCM 3954, *Klebsiella pneumoniae* ATCC 10031, CCM 4415, *Acinetobacter baumannii* ATCC 19606, DSM 30007, *P. aeruginosa* ATCC 27853, CCM 3955). Eight clinically critical fungal strains (four yeast: *C. albicans* ATCC 24433, CCM 8320, *Candida krusei* ATCC 6258, CCM 8271, *Candida parapsilosis* ATCC 22019, CCM 8260, *Candida tropicalis* *ATCC 750, CCM 8264;* and four mold strains: *Aspergillus fumigatus* ATCC 204305, *Aspergillus flavus* CCM 8363, *Absidia corymbifera* CCM 8077, *Trichophyton interdigitale* ATCC 9533, CCM 8377) were selected to screen the antifungal activity. Both positive (microbe solely) and negative (cultivation medium and DMSO) controls and internal quality standards (ciprofloxacin) were involved in the assays. Antibacterial and antifungal activity was expressed as minimum inhibitory concentration (MIC) in µM. Visual inspection was done for MIC endpoints evaluation.

Derivatives of montanine-type AAs did not show significant antifungal activity on the eight selected fungal strains. Surprisingly, four aromatic ester derivatives of 3-*O*-methylpancracine were active mainly against Gram-positive pathogens and *K. pneumoniae* (**Table 12**). We have previously reported compounds **1c** and **1f** as inactive compounds against AChE/BuChE. [151] Compound **1y** belongs to the same batch with 3,5-dimethoxybenzoyl substitution on C2 of 3-*O*-methylpancracine. Among these aromatic esters, compound **1f**, 2-*O*-(3-methoxybenzoyl)-3-*O*-methylpancracine, showed the most interesting antimicrobial activity and therefore is going under further microbiological screening, such as screening against clinically isolated *Klebsiella* and *Staphylococcus* stains, checkerboard study, toxicity evaluation on human normal kidney cell lines HK-2, and human cancerous liver cell line HepG2, and *in vivo* toxicity evaluation on *galleria mellonella* model. The manuscript from the achived results of this study is under preparation right now and will be published soon. Analogs **3m** and **2s**, in turn, were synthesized to assess the impact of change in the linker group from ester to ether and to determine the impact of 3-methoxybenzoyl group on the C3 position of montanine. Compounds **1c**, **1f**, **1y**, **3m**, and **2s** are illustrated in **Figure 23**. From the result of antibacterial screening (**Table 12**), it can be concluded that changing the linker in **1f** to ether or placing it on C3 of the scaffold eliminates the activity.

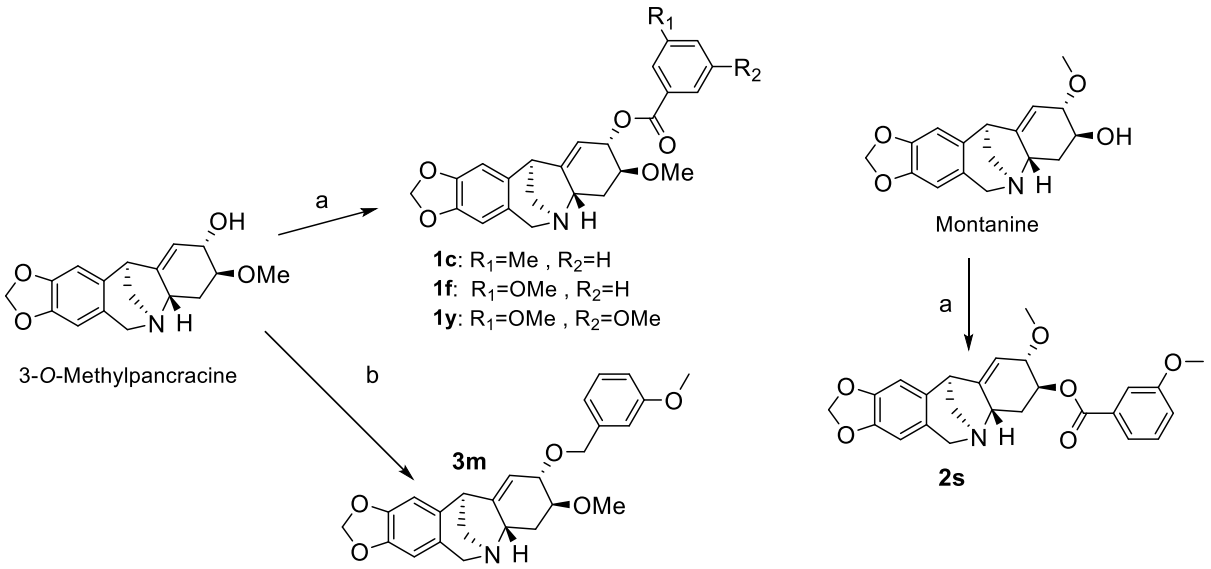


Figure 23. Scheme of selected synthesized derivatives of 3-*O*-methylpancracine and montanine for antibacterial evaluation. Reagents and conditions for synthesis: (a) RCOCl (1.5–3.0 eq.), DMAP (catalytic amount), py, 80 °C, 5–20 h (yield 96–100%); (b) NaH in Dry THF, under argon gas, 0 °C, 0.5 h, then corresponding aryl alkyl halide (1 eq.), 25 °C, overnight (yield 35%).

Table 12. Antibacterial activity of selected montanine-type Amaryllidaceae alkaloids derivatives.

Strains		MIC [μM]							
		Montanine	**3-*O*-Methylpancracine**	**1y**	**1c**	**1f**	**3m**	**2s**	**CIP**
SA	24h	>500	>500	**7.81**	**31.2**	**3.9**	250	125	3.1
	48h	>500	>500	**15.6**	**31.2**	**7.81**	>500	500	-
MRSA	24h	>500	>500	**31.25**	62.5	**31.25**	500	125	1.5
	48h	>500	>500	62.5	62.5	**31.25**	>500	500	-
SE	24h	>500	>500	**31.25**	**31.25**	**31.25**	250	125	0.4
	48h	>500	>500	125	62.5	**31.25**	>500	500	-
EF	24h	>500	>500	125	>125	**31.25**	>500	>500	3.1
	48h	>500	>500	>125	>125	**31.25**	>500	>500	-
EC	24h	>500	>500	>125	>125	>125	>500	>500	0.1
	48h	>500	>500	>125	>125	>125	>500	>500	-
KP	24h	>500	>500	**31.25**	62.5	**15.62**	>500	>500	0.02
	48h	>500	>500	**31.25**	62.5	**15.62**	>500	>500	-
ACI	24h	>500	>500	>125	>125	>125	>500	>500	1.6
	48h	>500	>500	>125	>125	>125	>500	>500	-
PA	24h	>500	>500	>125	>125	>125	>500	>500	1.6
	48h	>500	>500	>125	>125	>125	>500	>500	-

CIP = Ciprofloxacin; SA: *Staphylococcus aureus* ATCC 29213; MRSA: methicillin-resistant *Staphylococcus aureus* ATCC 43300; SE: *Staphylococcus epidermidis* ATCC 12228; EF: *Enterococcus faecalis* ATCC 29212; EC: *Escherichia coli* ATCC 25922; KP: *Klebsiella pneumoniae* ATCC 10031; ACI: *Acinetobacter baumannii* ATCC 19606; PA: *Pseudomonas aeruginosa* ATCC 27853. The cultivation was performed in Cation-adjusted Mueller-Hinton broth at 35 ± 2 °C. The tested compounds were dissolved in DMSO to produce stock solutions. The final concentration of DMSO in the testing medium did not exceed 1% (v/v) of the total solution composition. Antibacterial activity, expressed as MIC (in μM), was evaluated after 24 and 48 h of static incubation in a dark and humidified atmosphere at 35 ± 2 °C.

In conclusion, we represent derivatives of montanine-type AAs with their unique 5,11-methanomorphanthridine core among potential candidates to treat diseases such as AD, TB, bacterial

infections, and human cancers. This study deduced that minor substitution on C2 of montanine-type AAs is essential for cytotoxic activity. On the contrary, 3-*O*-methylpancracine as starting material for chemical modification could be ideal for developing a non-toxic antimicrobial or AChE/BuChE inhibitor drug. Investigation of the impact of hydrogenation of double bond between C1 and C11a, quaternization of the nitrogen atom, and modification of the aromatic ring could help the better comprehension of SAR for antimicrobial activity in the future.

6 ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmacognosy and Pharmaceutical Botany

Candidate: **Negar Maafi**

Supervisor: **Prof. Ing. Lucie Cahlíková, Ph.D.**

Title of Doctoral Thesis: **Amaryllidaceae alkaloids of montanine type and their derivatives as potential drugs.**

Based on the knowledge of the biological activity of montanine-type alkaloids of Amaryllidaceae, these alkaloids were selected for the preparation of their semisynthetic derivatives to study the relationship between structure and the activity. Derivatives of montanine-type alkaloids were prepared using haemanthamine and montanine as starting substances. The montanine-type alkaloid 3-*O*-methylpancracine was prepared using intramolecular rearrangement of haemanthamine. Previously isolated montanine from bulbs of *Hippeastrum* x *hybridum* cv. Ferrari was used in synthesize of derivatives. In total, more than 80 aliphatic and aromatic derivatives of montanine and 3-*O*-methylpancracine have been prepared. All compounds were identified using 1D- and 2D-NMR, MS, HRMS, and so forth. The prepared derivatives were screened for a wide range of biological activities (inhibitory potential against *h*AChE/*h*BuChE, antimycobacterial, antibacterial and antifungal activity, cytotoxicity, and others).

In the *h*AChE/*h*BuChE study, the derivative 2-*O*-(4-chloro-3-nitrobenzoyl)-3-*O*-methylpancracin with an IC_{50} value of 1.6 ± 0.1 µM had an interesting selective *h*AChE inhibitory activity. The derivative 2-*O*-((piperidin-1-yl-carbonyl)oxy)-3-*O*-methylpancracine (IC_{50} = 1.73 ± 0.05 µM) showed promising *h*BuChE inhibitory activity. As part of the antimycobacterial screening against *Mycobacterium tuberculosis* H37Ra, *M. aurum,* and *M. smegmatis*, promising activity was obtained for 2-*O*-(1-naphthoyl)-3-*O*-methylpancracine (MIC= 7.9 µM against all tested strains). 2-*O*-(3-Methoxybenzoyl)-3-*O*-methylpancracine was the most effective compound against *Staphylococcus aureus* and *Klebsiella pneumoniae* with MIC values of 7.9 and 15.6 µM, respectively. Montanine derivatives 3-*O*-(4-chloro-3-nitrobenzoyl)montanine and 3-*O*-(3,5-dinitrobenzoyl)montanine showed interesting cytotoxic activity against the tested tumor lines with IC_{50} values in the range of 1.41–8.06 µM and 1.18–2.95 µM, in turn.

Keywords: Montanine, 3-*O*-Methylpancracin, Alkaloids, Biological activity, Acetylcholinesterase, Butyrylcholinesterase, Antimycobacterial, Cytotoxicity.

7 ABSTRAKT

Karlova Univerzita v Praze, Farmaceutická fakulta v Hradci Králové

Katedra farmakognózie a farmaceutické botaniky

Kandidát: **Negar Maafi**

Školitel: **Prof. Ing. Lucie Cahlíková, PhD.**

Název disertační práce: **Amaryllidaceae alkaloidy montaninového typu a jejich deriváty jako potenciální léčiva.**

Na základě znalostí o biologické aktivitě alkaloidů montaninového typu Amaryllidaceae byly tyto alkaloidy vybrány pro přípravu jejich polosyntetických derivátů za účelem studia vztahu struktura vs. aktivita. Deriváty alkaloidů montaninového typu byly připraveny za využití haemanthaminu a montaninu jako výchozích látek. Montaninový alkaloid 3-*O*-methylpankracin byl připraven za využití intramolekulárního přesmyku z haemanthaminu. Montanin byl pro přípravu polosyntetických derivátů izolován z cibulí *Hippeastrum x hybridum* cv. Ferrari. Celkem bylo připraveno více než 80 alifatických a aromatických derivátů montaninu a 3-*O*-methylpankracinu. Všechny sloučeniny byly identifikovány za využití 1D- a 2D-NMR, MS, HRMS apod. Připravené deriváty byly screeningově testovány na celou řadu biologických aktivit (např. inhibiční potenciál vůči *h*AChE/*h*BuChE, antimykobakteriální, antibakteriální a antifugální aktivita, cytotoxicita a další).

V rámci *h*AChE/*h*BuChE studie zajímavou selektivní *h*AChE inhibiční aktivitou disponoval derivát 2-*O*-(4-chlor-3-nitrobenzoyl)-3-*O*-methylpankracin s hodnotou IC_{50} 1,6 ± 0,1 µM. Slibnou *h*BuChE inhibiční aktivitu vykazoval derivát 2-*O*-((piperidin-1-yl-carbonyl)oxy)-3-*O*-methylpankracin (IC_{50} = 1,73 ± 0,05 µM). V rámci antimykobakteriálního screeningu vůči *Mycobacterium tuberculosis* H37Ra, *M. aurum* a *M. smegmatis* slibná aktivita byla získána pro 2-*O*-(1-naftoyl)-3-*O*-methylpankracin (MIC= 7,9 µM vůči všem testovaným kmenům). 2-*O*-(3-Methoxybenzoyl)-3-*O*-methylpankracin byl nejúčinnější sloučeninou vůči *Staphylococcus aureus* a *Klebsiella pneumoniae* s hodnotami MIC 7,9 a 15,6 µM. Deriváty montaninu: 3-*O*-(4-chlor-3-nitrobenzoyl)montanin a 3-*O*-(3,5-dinitrobenzoyl)montanin vykázaly zajímavou cytotoxickou aktivitu vůči testovaným nádorovám liniím s hodnotami IC_{50} 1,41–8,06 µM a 1,18–2,95 µM.

Klíčová slova: Montanin, 3-*O*-Methylpancracin, Alkaloidy, Biologická aktivita, Acetylcholinesteráza, Butyrylcholinesteráza, Antimykobakteriální, Cytotoxicita.

8 LIST OF PUBLICATIONS

8.1 Publications Included in the Dissertation

P1 **Maafi, N.**; Mamun, A.A.; Janďourek, O.; Maříková, J.; Breiterová, K.; Diepoltová, A.; Konečná, K.; Hošťálková, A.; Hulcová, D.; Kuneš, J.; Kohelová, E.; Koutová, D.; Šafratová, M.; Nováková, L.; Cahlíková, L. Semisynthetic Derivatives of Selected Amaryllidaceae Alkaloids as a New Class of Antimycobacterial Agents. *Molecules* **2021**, *26*, 6023. (IF_{2021}= 4.927)

Full text: **https://doi.org/10.3390/molecules26196023**

Author's contribution: Preparation of the semisynthetic derivatives from montanine-type alkaloids, preparation of derivatives for biological assay, collecting spectrometric data of included derivatives for supplementary material, interpretation of the NMR spectra, preparation of the supplementary material, and proofreading the final manuscript.

P2 **Maafi, N.**; Pidaný, F.; Maříková, J.; Korábečný, J.; Hulcová, D.; Kučera, T.; Schmidt, M.; Al Shammari, L.; Špulák, M.; Catapano, M.C.; Mecava, M.; Prchal, L.; Kuneš, J.; Janoušek, J.; Kohelová, E.; Jenčo, J.; Nováková, L.; Cahlíková, L. Derivatives of Montanine-type Alkaloids and Their Implication for the Treatment of Alzheimer's Disease: Synthesis, Biological Activity and *In silico* Study. *Bioorg. Med. Chem. Lett.* **2021**, *51*, 128374. (IF_{2021}= 2.940)

Full text: **https://doi.org/10.1016/j.bmcl.2021.128374**

Author's contribution: Preparation of the semisynthetic derivatives from montanine-type alkaloids, preparation of derivatives for biological assay, collecting spectrometric data of derivatives for supplementary material, interpretation of the NMR spectra, participating in writing the manuscript's draft, preparation of the supplementary material, and proofreading the final manuscript.

P3 Koutová, D.; **Maafi, N.**; Havelek, R.; Opletal, L.; Blunden, G.; Řezáčová, M.; Cahlíková, L. Chemical and Biological Aspects of Montanine-Type Alkaloids Isolated from Plants of the Amaryllidaceae Family. *Molecules* **2020**, *25*, 2337. (IF_{2021}= 4.927)

Full text: **https://doi.org/10.3390/molecules25102337**

Author's contribution: Comprehensive literature review about montanine-type alkaloids, participation in writing the manuscript's draft, proofreading the final draft.

P4 Cahlíková, L.; Vrabec, R.; Pidaný, F.; Peřinová, R.; **Maafi, N.**; Mamun, A.A.; Ritomská, A.; Wijaya, V.; Blunden, G. Recent Progress on Biological Activity of Amaryllidaceae and Further Isoquinoline Alkaloids in Connection with Alzheimer's Disease. *Molecules* **2021**, *26*, 5240. (IF_{2021}= 4.927)

Full-text: **https://doi.org/10.3390/molecules26175240**

Author's contribution: Data collection and evaluation for the draft manuscript and reading the final manuscript.

8.2 Publications not Included in the Dissertation

P5 Kohelová, E.; Peřinová, R.; **Maafi, N.**; Korábečný, J.; Hulcová, D.; Maříková, J.; Kučera, T.; Martínez González, L.; Hrabinova, M.; Vorčáková, K.; Nováková, L.; De Simone, A.; Havelek, R.; Cahlíková, L. Derivatives of the β-crinane Amaryllidaceae Alkaloid Haemanthamine as Multi-target Directed Ligands for Alzheimer's Disease. *Molecules* **2019**, *24*, 1307. (IF_{2021}= 4.927)

Full-text: **https://doi.org/10.3390/molecules24071307**

Author's contribution: Preparation of the semisynthetic derivatives from crinine-type alkaloids, preparation of derivatives for biological assay.

P6 Peřinová, R.; **Maafi, N.**; Korábečný, J.; Kohelová, E.; De Simone, A.; Mamun, A.A.; Hulcová, D.; Marková, J.; Kučera, T.; Jun, D.; Šafratová, M.; Maříková. J.; Andrisano, V.; Jenčo, J.; Kuneš, J.; Martinez, A.; Nováková, L.; Cahlíková. L. Functionalized Aromatic Esters of the Amaryllidaceae Alkaloid Haemanthamine and Their *In vitro* and *In silico* Biological Activity Connected to Alzheimer's Disease. *Bioorg. Chem.* **2020**, *100*, 103928. (IF_{2021}= 5.307)

Full-text: **https://doi.org/10.1016/j.bioorg.2020.103928**

Author's contribution: Preparation of the semisynthetic derivatives from crinine-type alkaloids, preparation of derivatives for biological assay.

P7 Mamun, A.A.; Pidaný, F.; Hulcová, D.; Maříková, J.; Kučera, T.; Schmidt, M.; Catapano, M.C.; Hrabinová, M.; Jun, D.; Múčková, L.; Kuneš, J.; Janoušek, J.; Andrýs, R.; Nováková, L.; Peřinová, R.; **Maafi, N.**; Soukup, O.; Korábečný, J.; Cahlíková, L. Amaryllidaceae Alkaloids of Norbelladine-type as Inspiration for Development of Highly Selective Butyrylcholinesterase Inhibitors: Synthesis, Biological Activity Evaluation, and Docking Studies. *Int. J. Mol. Sci.* **2021**, *22*, 8308. (IF_{2021}= 6.208)

Full-text: **https://doi.org/10.3390/ijms22158308**

Author's contribution: Participation in the preparation of the synthetic compounds.

P8 Šafratová, M.; Křoustková, J.; **Maafi, N.**; Suchánková, D.; Vrabec, R.; Chlebek, J.; Kuneš, J.; Opletal, L.; Bucar, F.; Cahlíková, L. Amaryllidaceae Alkaloids from *Clivia miniata* (Lindl.) Bosse (Amaryllidaceae): Isolation, Structural Elucidation, and Biological Activity. *Plants* **2022**, *11*, 3034. (IF_{2021}= 4.658)

Full-text: **https://doi.org/10.3390/plants11223034**

Author's contribution: Writing and original draft preparation

8.3 Conferences

8.3.1 Short Presentation

L1 **Maafi, N.**; Konečná, K.; Janďourek, O.; Diepoltová, A.; Křoustková, J.; Cahlíková, l. Antimicrobial Activity of Semisynthetic Derivatives of Montanine-type Alkaloids. 12th Postgraduate and Postdoc Conference, Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 1st and 2nd February 2022.

L2 **Maafi, N.**; Konečná, K.; Janďourek, O.; Diepoltová, A.; Cahlíková, l. Antimicrobial Activity of Semisynthetic Derivatives of Montanine-type Alkaloids. 49th conference- Synthesis and Analysis of Drugs (Online), Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 16th and 17th September 2021.

L3 **Maafi, N.**; Cahlíková, L.; Mařiková, J.; Hulcová, D. Derivatives of Montanine-type Alkaloids and Their Implication to Alzheimer's Disease: Synthesis, Biological Activity, Docking Study. 11th Postgraduate and 9th Postdoc Conference (Online), Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 27th and 28th January 2021.

L4 **Maafi, N.**; Kuneš, J.; Cahlíková, l. Aliphatic and Aromatic Derivatives of Montanine-type Alkaloids and Their Cytotoxic Activity. 10th Postgraduate and Postdoc Conference Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 22nd and 23rd January 2020.

L5 **Maafi, N.**; Kuneš, J.; Cahlíková, l. Derivatives of Amaryllidaceae Alkaloids of Montanine-type and Their Biological Activities. 9th postgraduate and 7th postdoc conference, Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 23rd and 24th January 2019.

8.3.2 Poster

P1 **Maafi, N.**; Hulcová, D.; Hradiská Breiterová, K.; Konečná, K.; Janďourek, O.; Diepoltová, A.; Křoustková, J.; Cahlíková, L. Investigation of Selected Biological Activities of Montanine-type Alkaloids and Their Derivatives. Recent Progress in Pharmacognosy and Phytochemistry Workshop, Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic, 24th and 25th June 2022.

P2 **Maafi, N.**; Kuneš, J.; Cahlíková, l. Derivatives of Amaryllidaceae Alkaloids of Montanine-type and Their Biological Activities. 10th Congress of the Slovak Pharmaceutical Society, Comenius University in Bratislava, Faculty of Pharmacy, Slovakia, 5th and 6th September 2019.

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