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The Dissertation

Amaryllidaceae alkaloids of genus *Narcissus* and their biological activity

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Field of study: Pharmacognosy and Nutraceuticals

The declaration

I declare that all the results stated in this dissertation are completely original. No material of this thesis has been used to obtain another degree or diploma by either the University or other institutions, except by way of background information and duly acknowledged in the Thesis. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due acknowledgment is made in the text of the Thesis.

Hradec Králové, February 2022 Abdullah Al Mamun

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Abdullah Al Mamun

TABLE OF CONTENT

ABBREVIATIONS

AA	Amaryllidaceae alkaloid
AD	Alzheimer's Diseases
Aβ	Amyloid-β
ACh	Acetylcholine
AChE	Acetylcholinesterase
AAP	Amyloid precursor protein
APX	Ascorbate peroxidase
BBB	Blood-brain barrier
BuChE	Butyrylcholinesterase
BACE1	Beta-site APP Cleaving Enzyme-1, β secretase
CC	Column chromatography
CNS	Central Nervous System
C3H	Coumarate 3-hydroxylase
C4H	Cinnamate 4-hydroxylase
CYP96T1	Cytochrome P450 monooxygenase 96T1
CD	Circular dichroism
GSK3β	Glycogen synthase kinase 3 beta
GCMS	Gas chromatography mass spectroscopy
HRMS	High-resolution mass spectroscopy
HBS	4-hydroxybenzaldehyde synthase
IC_{50}	Half maximal inhibitory concentration
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin 8
LD_{50}	Median lethal dose
MIP-1α	Macrophage inflammatory protein-1 α
NFT	Neurofibrillary tangle
N4OMT	Norbelladine 4'-*O*-methyltransferase

NMR	Nuclear magnetic resonance
TYDC	Tyrosine decarboxylase
NR	Noroxomaritidine reductase
NBS	Norbelladine synthase
TNFα	Tumor necrosis factor α
PAL	Phenylalanine ammonia-lyase
POP	Prolyloligopeptidase
PS1	Presenilin
PHF	Paired Helical Filament
PAMPA	Parallel Artificial Permeation Assay
SAR	Structure-activity relationship
Sp	Species
TLC	Thin layer chromatography
UHPLC	Ultra-high-performance liquid chromatography

1. INTRODUCTION

Natural products play an essential role in our daily life by providing food and medicines. A wide range of various types of plant extracts, as well as isolated constituents, are widely used in traditional and modern medicine for the treatment of significant diseases such as cancer, malaria, neurological disorders, cardiovascular diseases, liver diseases, fungal and bacterial infections, sleep disorders, diabetes, etc. In the fourth century, B.C.E. Greek physician Hippocrates of Cos first used the oil of *Narcissus poeticus* L. for the treatment of uterine tumors[1,2]. In the 18th century, the German pharmacist Friedrich Serturner isolated morphine from *Papaver somniferum* and some other alkaloidal compounds, including thebaine, codeine, papaverine, and noscapine. After that, research on the isolation and identification of active substances from plants increased significantly[3]. Atropine from *Atropa belladonna*, Caffeine from *Coffea Arabica*, Digoxin from *Digitalis purpurea*, Vinblastine and Vincristine from *Catharanthus roseus*, flavonolignans from *Silybum marianum* etc. are other significant bioactive natural compounds. In cancer research, from the 1940s to the end of 2014, 175 small molecules were approved and 85 are derived direct or indirect from natural products[4].

Plant metabolites occur in both primary and secondary forms. Primary metabolites contribute to the plant growth and development, whereas the function of secondary metabolites is to ensure plant survival in its environment. Phenols, terpenes, saponins, and alkaloids are the main classes of secondary metabolites. The total number of the described structures isolated from plants has exceeded 100,000, and many of them play a significant role in the pharmaceutical sector due to their diverse and versatile pharmacological effects[5, 6]. Thus, natural products represent an important source of clinical drugs, especially due to their structural diversity. Alkaloids are one of the most intriguing templates of natural origin. They are derived from various amino acids and can be classified into several structural groups according to their biosynthetic origin[7]. These natural compounds are produced by a wide range of plant families including Amaryllidaceae, Apocynaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae Rutaceae, and others. To date, more than 3,000 different alkaloids have been isolated and identified from 4,000 plant species[8]. One of the most important groups of alkaloids are Amaryllidaceae alkaloids (AAs), which are exclusively produced by plants of the Amaryllidaceae family[9, 10]. The biological activity of Amaryllidaceae plants is related to the presence of AAs. They have demonstrated a wide range of biological activities, including antitumor, antibacterial, antioxidant, antiparasitic, antifungal,

anti-inflammatory, and insect antifeedant effects, as well as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities[11-14]. The most known AA is galanthamine, originally isolated from *Galanthus woronowii* Losinsk[15]. This compound was approved by the FDA for the treatment of mild and severe stages of Alzheimer´s disease in 2001[16]. Other AAs such as lycorine, haemanthamine, montanine, and pancratistatin are studied in terms of their cytotoxic effect, and further AAs showed antimicrobial potential[16]. Therefore, this dissertation thesis is dedicated to this interesting group of secondary metabolites and deals with the isolation of AAs of various structural types from *Narcissus pseudonarcissus* cv. Carlton and synthesis of compounds structurally inspired by these alkaloids. The next aim of this thesis was to evaluate the biological activities of isolated and synthesized compounds in connection with the potential treatment of neurodegenerative, oncological and microbial diseases[12, 17].

2. AIMS OF THE DISSERTATION

The main purpose of the dissertation thesis was the isolation and structural identification of AAs from the concentrated extract of fresh bulbs of *Narcissus pseudonarcissus* cv. Carlton, screening of biological activities, and preparation of compounds structurally inspired by minor AAs.

Individual goals in detail:

- A detailed study of the literature with a focus on alkaloids from the plants of the genus *Narcissus* and their biological activity.
- Isolation of the alkaloids from the bulb extract of *Narcissus pseudonarcissus* cv. Carlton.
- Determination of their structure using spectroscopic methods (NMR, MS, CD spectroscopy, optical rotation, etc.).
- Screening of the biological activities of AAs isolated in sufficient amounts (inhibition activity against cholinesterases, prolyloligopeptidase, screening of cytotoxicity on a panel of cancerous and noncancerous cell lines, antimycobacterial activity, and others).
- Preparation of compounds structurally inspired by isolated minor AAs.
- Investigation of the biological activity of synthetic derivatives for the study of the structure-activity relationship.
- Preparation of the semisynthetic derivatives of the AAs isolated in a higher amount and screening of their biological activities.

3. THEORETICAL PART

3.1. History and traditional use of plants of Amaryllidaceae family

The plants of family Amaryllidaceae are one of the most studied plant taxa comprising three subfamilies, including Amaryllidoideae, Agapanthoideae, and Allioideae. It is an intensively studied alkaloid plant taxa with a distinctive chemotaxonomic characteristic, comprising 85 genera and more than 1600 species. The species of this family are widely distributed throughout the tropical and subtropical regions of the world[10, 18]. They are also cultivated as ornamental plants for their beautiful flowers. They have been widely used in traditional medicine by the indigenous peoples for many centuries[19]. Since the ages, people in Africa, Asia, and the Polynesia region have used various species of Amaryllidaceae for traditional health remedies (Table 1). For example, the southern Sotho and Zulu tribes in South Africa use various species of the genus *Nerine* to prepare herbal decoctions, which help relieve coughs and colds, also in renal and hepatic conditions, relief of back pain, and as a remedy for infertility[20]. One of the most popular plant species among the people of Sotho, Xhosa, Zulu, and San in South Africa is *Boophone disticha*. Herbal preparations made from this plant are used for ailments related to the CNS, wounds, infections, and inflammatory conditions[21]. Various types of extracts from bulbs of *Narcissus tazetta* are used in traditional medicine to treat abscesses, wounds, joint pains, sores, sedatives, hypertension, and ulcers. Moreover, roots are used for the treatment of skin problems, flowers are used for aromatherapy and against cancer[22]. The biological activity of plants is often associated with the content of some specific type of secondary metabolites. Amaryllidaceae plants are well known to contain high concentration of AAs. Until these day, more than 600 AAs have been reported. On the other hand, they also contain other types of secondary metabolites, including flavonoids, flavones, chalcones, chromones, and others[23].

The study of the Amaryllidaceae plant family and AAs itself began in the year 1877, when the first alkaloid lycorine, belonging to the lycorine-type of AAs, was isolated from bulbs of *Narcissus pseudonarcissus.* Interest in these compounds has grown over time due to their diverse and promising pharmacological properties[11]. The most studied genera of this family are *Crinum, Nerine, Narcissus, Galanthus, Hippeastrum, Lycoris, Pancratium,* and *Zephyranthes*[10].

Table 1. Traditional use of selected plants from the family Amaryllidaceae

Plant	Plant part	Traditional use	Country	Ref.
Amaryllis belladona	whole plant	cancer treatment	Egypt	[24]
Boophone disticha	bulb extracts	Hysteria, psychotic treatment, stress-related ailments, anxiety and depression, age-related dementia	South Africa	[21]
Ammocharis coranica	whole plant	Hysteria, mental illness, affliction related to witchcraft	South Africa	[25]
Brunsvigia grandiflora	leaves and roots decoction	cough and cold, renal and liver problems, digestive disturbances	South Africa (Zulu people)	[26]
Crinum asiaticum	bulb	tumors (abdomen)	Zaire, Indochina	[27]
Crinum latifolium	bulb	prostate carcinoma	Vietnam	[28]
Crinum bulbispermum	whole plant	kidney and bladder infection, rheumatism, sores, aching joints	South Africa	[25]
Clivia miniata	dried aerial part, root infusion, bulb decoction	Induce labor, snake bite, urinary infection	South Africa (Zulu people)	[26]
Galanthus woronowii	whole plant	nervous and cardiovascular systems disorders treatment	Russian	[29]
Gethyllis clinearis	infusion of fruits	flatulence, colic, digestive disturbance	South Africa	[26]
Haemanthus coccineus	dried leaves	ulcer, anthrax pustules,	South Africa	[26]
Lycoris radiata	standardized extract	infectious diseases	Korea	[30]
Narcissus pseudonarcissus	bulb, leaves, and flowers	gastric cancer postoperative complications	China	[31]
Narcissus poeticus	essential oil	uterine cancer, perfume industry	Europe	[1, 11, 32]

Table 1. Traditional use of selected plants from the family Amaryllidaceae (continuation)

Plant	Part of the plant	Traditional uses	Country	Citation
Narcissus tazetta	bulb, flowers, root	anti-inflammatory, memorigenic, sedative, abscesses and wounds, dysentery. Bulbs have been used in abscesses, wounds, joint pains, sores, hypertension, as sedatives	Jordan, Turkey,	*22, 33, 34*
Nerine filifolia	bulb decoctions	coughs and colds, renal and hepatic conditions, backpain, and infertility	Southern Africa (Sotho and Zulu tribes)	*35-37*
Nerine bowdenii	bulb	cough and cold, renal, and liver disease	South Africa,	*25, 37*
Zephyranthes carinata.	bulb	skin diseases and parasitosis	Argentina	*38*
Zephyranthes candida	decoction of leaves	diabetes mellitus, infantile convulsions, epilepsy, and tetanus	South America	*36, 39*

3.2. Secondary metabolites of the plant family Amaryllidaceae

The most important group of secondary metabolites of the family Amaryllidaceae are alkaloids. They are one of the most diverse class of secondary metabolites. Alkaloids are usually characterized by the existence of one or more nitrogen atoms in their heterocyclic structure. The biological precursors of most alkaloids are amino acids, such as ornithine, lysine, phenylalanine, tyrosine, tryptophan, and others. Most alkaloids are white crystalline solid substances, and may exist as free bases, as salt, or as *N*-oxides in the plants. Although there are some exceptions, for example, berberine is yellow, coniine, and nicotine are liquids [9]. The function of alkaloids in plants is not yet clear but some studies suggested that they have functioned as a nitrogen reserve, a defense against predators and growth regulators[40]. Alkaloids are commonly found in the following plant families: Amaryllidaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Convolvulaceae, Elaeagnaceae, Erythroxylaceae, Fabaceae, Leguminosae, Liliaceae, Loganiaceae, Menispermaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae, Solanaceae, and Zygophyllaceae[9, 41].

As mentioned above, the most important and studied secondary metabolites of this plant family are AAs, which are divided into several structural groups according to their biosynthesis. However,

other types of alkaloids have also been isolated from this plant family. For example, mesembrane-type alkaloids, which are generally isolated from the Aizoaceae family. Some studies report the presence of this type of alkaloids within some species of Amaryllidaceae such as *Hymenocallis arenicola*, *Crinum oliganthum*, *Narcissus pallidulus,* and *Narcissus triandrus*[42]. The isolation of (-)-capnoidine and (+)-bulbocapnine from *Galanthus nivalis* subsp. *cilicicus* is the first report of the occurrence of classical isoquinoline alkaloids in the Amaryllidaceae family[43]. AAs have also been reported from species that do not belong to Amaryllidaceae. An early report of the isolation of lycorine and acetylcaranine from *Urginea altissima* (Hyacinthaceae), crinamine from tubers of *Dioscorea dregeana* (Dioscoreaceae)[44]. Several AAs of lycorine, haemanthamine, and hostasine have been isolated from *Hosta plantaginea* (Asparagaceae)[45, 46]. In this case, it is necessary to ask whether it is really a product of these plants or it is a contamination. The structural elucidation of AAs, their biological profiles and their synthesis process have been summarized and published in various review articles[16, 47-51].

As mentioned above, various non-alkaloidal compounds have also been isolated from Amaryllidaceae species (Fig. 1). They received much less attention than alkaloids. For example, three flavans aglycones, namely 7-hydroxy-3′,4′-methylenedioxyflavan, 7,4′-dihydroxy-3′-methoxyflavan, and 7-methoxy-2′-hydroxy-4′,5′-methylenedioxyflavan, a glycosyloxyflavan of 7-glucosyloxy-3′,4′-methylenedioxyflavan, have been isolated from the species of *Zephyranthes flava*[52]. *Zephyranthes candida* is reported to contain a flavonoid glycoside: kaempferol-3-*O*-rhamnoglucoside, six ceramides: zephyranamide A-D, Candidamide A, Candidamide B, and four flavans: (*2S*)-3′,7-dihydroxy-4′-methoxyflavan, (*2S*)-4′-hydroxy-7-methoxyflavan, (*2S*)-4′,7-dihydroxyflavan, 7-hydroxy-3′, 4′-methylenedioxyflavan, and two sterols: β-sitosterol and β-daucosterin[53-55]. Seven isoflavonoids, namely biochanin A, daidzein, formononetin, genistein, prunetin, glycitein and glycitine, have been detected in the bulbs of *Zephyranthes robusta* by HPLC-MS method[23].

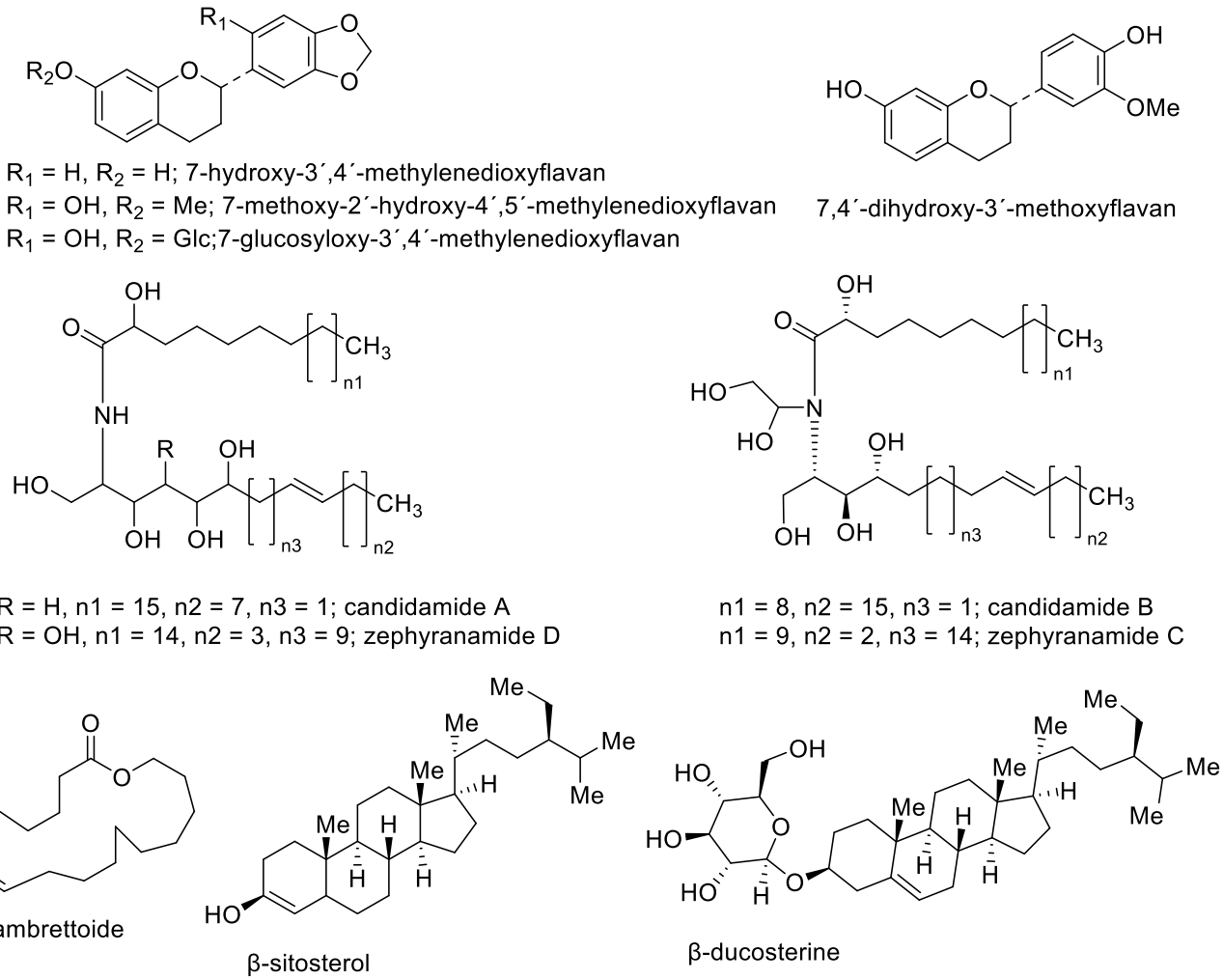


Fig. 1. Examples of nonalkaloidal compounds described in plants of the Amaryllidaceae family

3.3. Biosynthesis of Amaryllidaceae alkaloids: norbelladine pathway

Most of the biosynthetic research done on AAs was carried out in the 1960s and early 1970s. Their structural diversity and unique feature have inspired to investigate their role and biosynthesis in plants. Complete biosynthetic pathways to all structural types of AAs have not yet been determined, but several steps and participating enzymes can be predicted based on reaction types involved in biochemical reactions such as oxidation, reduction, hydroxylation, methylation, tautomerization, phenol-phenol coupling, and oxide bridge formation[56, 57]. The first step in biosynthesis is the condensation of the aromatic amino acids L-phenylalanine and L-tyrosine[18]. The entire biosynthetic pathway can be divided into five steps. A) The amino acid subunits come from the shikimic pathway as primary metabolites. B) Formation of the aldehyde moiety provided by the phenylpropanoid pathway. C) Biosynthesis of tyramine from the amino acid L-tyrosine and

condensation with aldehyde moiety to form the central precursor norbelladine and subsequent *O*-methylation. D) The phenol coupling of the key intermediate norbelladine. E) Subsequent modification and biosynthesis of different types of AAs. Biosynthesis of the crucial precursor 3,4-dihydroxybenzaldehyde (3,4-DHBA) from L-phenylalanine involves a series of reactions. The amino acid L-phenylalanine leads to the production of some intermediate compounds, including trans-cinnamic acid, p-coumaric acid, and caffeic acid, which further leads to the construction of 3,4-DHBA. Reactions are catalyzed by the enzyme of phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), coumarate 3-hydroxylase (C3H), ascorbate peroxidase (APX), and 4-hydroxybenzaldehyde synthase (HBS)[18]. Tyramine is biosynthesized from the amino acid L-tyrosine. In this case, the reaction is catalyzed by the enzyme tyrosine decarboxylase (TYDC). Subsequently, the condensation of tyramine and 3,4-DHBA leads to the formation of norbelladine. The reaction is catalyzed by norbelladine synthase (NBS) and noroxomaritidine reductase (NR); the intermediates are then methylated, in the presence of norbelladine 4′-*O*-methyltransferase (N4OMT), to produce *O*-methylnorbelladine[56, 57](Fig. 2).

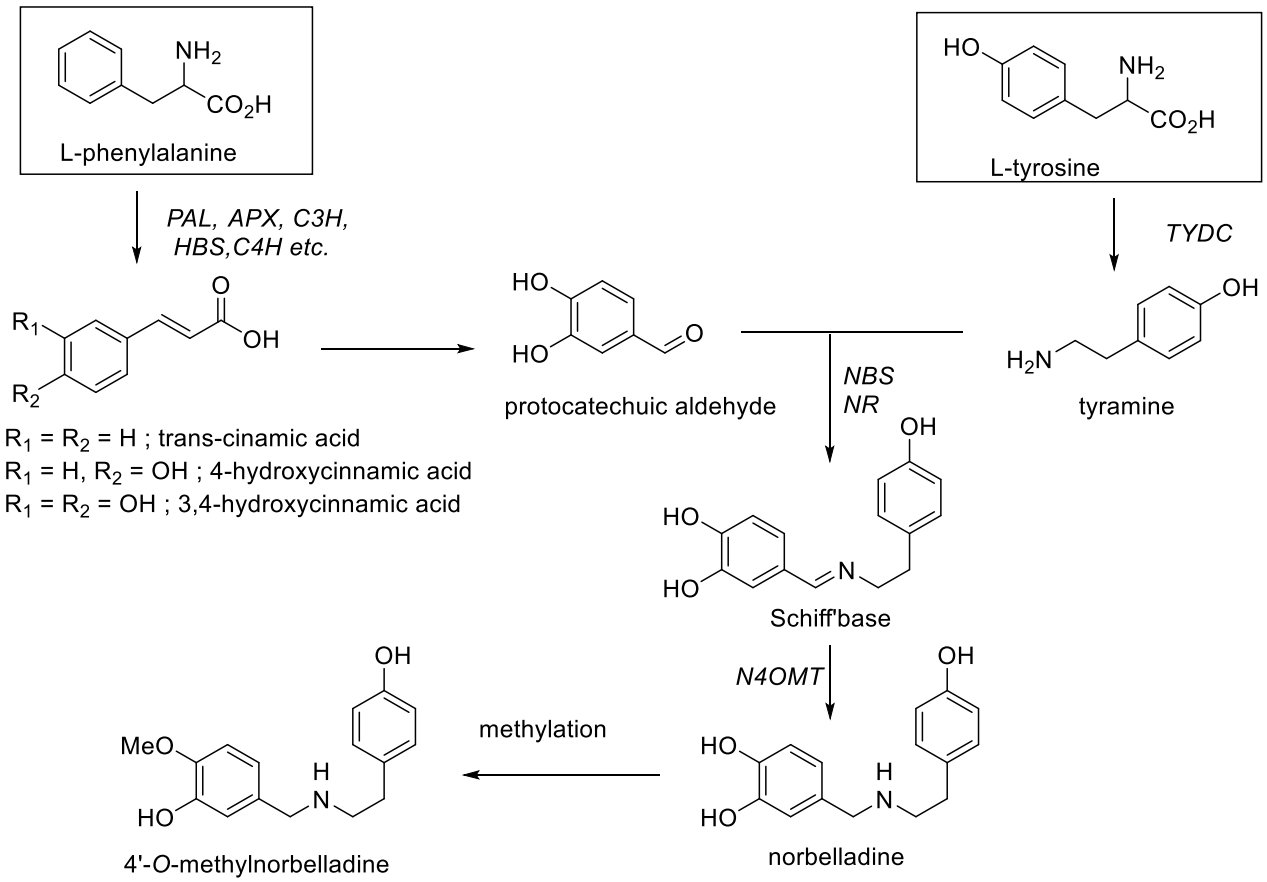


Fig. 2. First steps of the norbelladine pathway

Norbelladine is the first key intermediate in the biosynthesis of AAs. Methylation of norbelladine leads to the production of 4'-*O*-methylnorbelladine, which can undergo further modification to produce alkaloids of norbelladine-type (Fig. 3). The reaction is catalyzed by N4OMT enzyme[58].

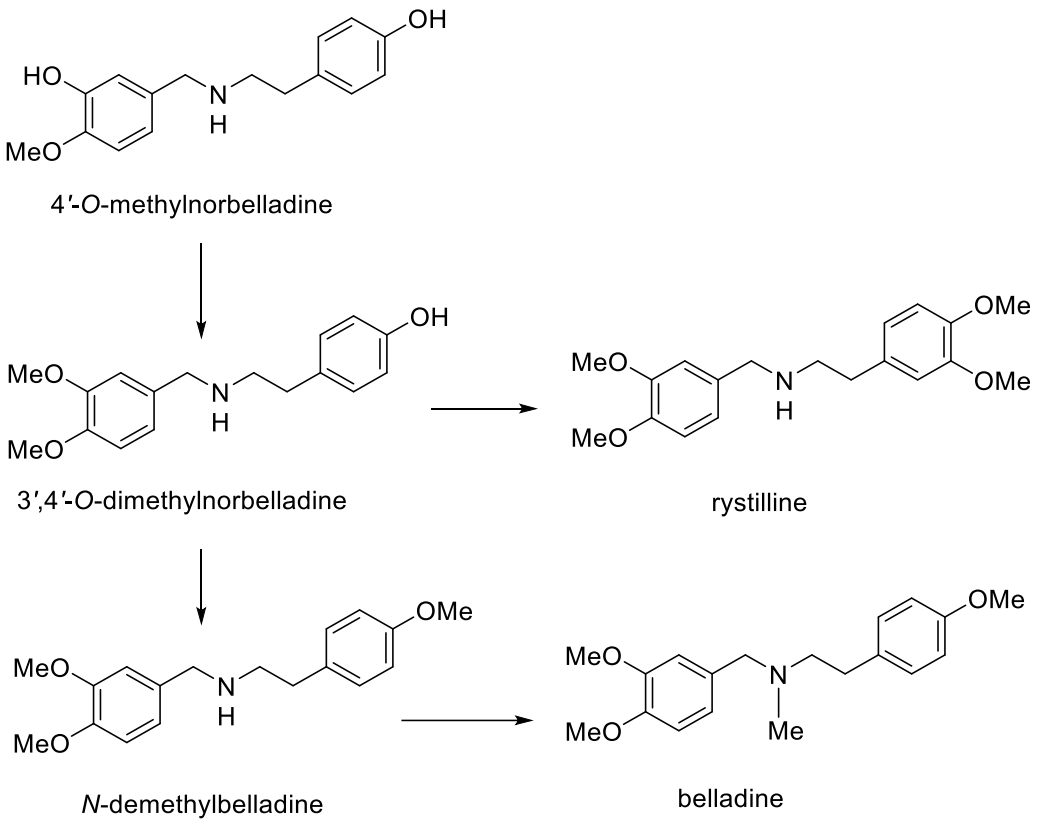


Fig. 3. Biosynthetic pathway of norbelladine- type alkaloid

An important step of biosynthesis is the intramolecular coupling of 4′-*O*-methylnorbelladine to the basic skeletons of AAs. At first, hydrogen is abstracted from the phenol followed by delocalization of the unpaired electron via resonance formation, and thus the free electron is dispersed to the *ortho* and *para* positions. These radicals are then reduced by coupling with other radicals and the coupling of two of these resonance structures, in various combinations, gives a range of cyclic structures. Therefore, the cyclization of the 4′-*O*-methylnorbelladine and the formation of the three C–C phenol coupled scaffolds of nornarwedine (*para-ortho′*), noroxolpluviine (*ortho-para′*) and noroxomaritidine (*para-para′*), which are further reduced into metabolite intermediates, is a key step in the synthesis of AAs. The coupling reaction is catalyzed by several enzymes, including cytochrome P450, laccases, and peroxidases[18]. Most reductase enzymes in alkaloid metabolism come from short-chain alcohol dehydrogenase (SDRs) and the aldo–keto reductases (AKRs). Thus,

different types of reactions such as C-C and C-O bond formations, *O*- and *N*- methylations, demethylations, hydroxylation, oxidations, and reductions occur within the biosynthesis of different types of AAs with the help of various catalytic enzymes[48].

The *para-para'* coupling reaction, catalyzed by cytochrome P450 monooxygenase 96T1 (CYP96T1) and noroxomaritidine reductase (NR), leads to the formation of various structural types of AAs such as crinine-, narciclasine-, tazettine-, and montanine-type [7]. The reduction of noroxomaritidine initiates the formation of crinine-type alkaloids. Further modifications of the structure lead to the formation of narciclasine-, tazettine-, and montanine-types of AAs. The narciclasine-type is obtained by hydroxylation and reduction reactions of the vittatine skeleton. Montanine type is produced by *O*-methylation of pancracine, which comes from 11-hydroxyvittatine by skeletal rearrangement. 11-Hydroxyvittatine is a precursor in the biosynthesis of haemanthamine by methylation, which is further rearranged into tazettine-type AAs (Fig. 4).

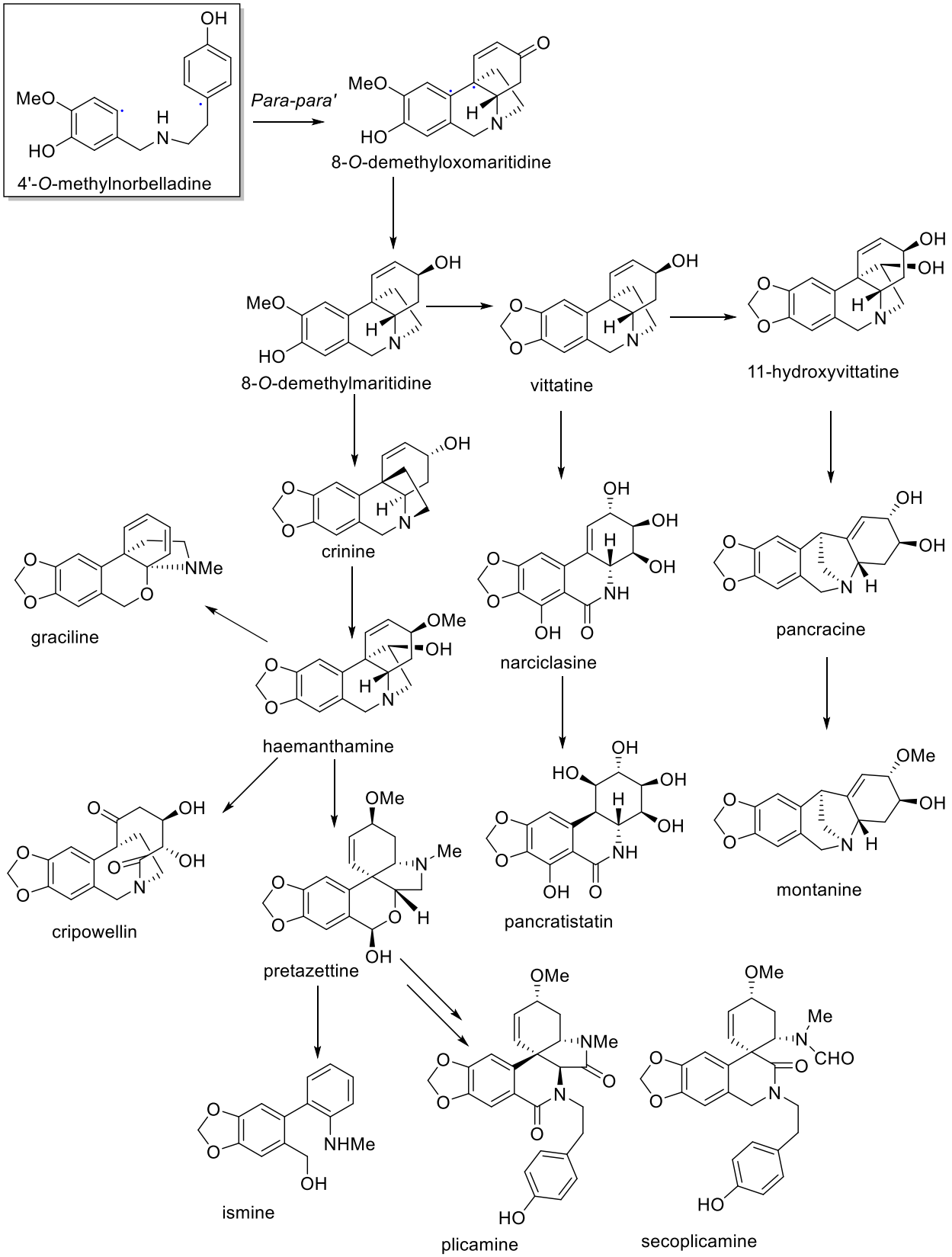


Fig. 4. *Para-para'* oxidative coupling of 4′-*O*-methylnorbelladine

Lycorine-type alkaloids are produced through the *ortho-para'* phenol coupling (Fig. 5). The coupling starts with the production of noroxopluviine, which is immediately converted to norpluviine via reduction. Then, its pathway separates towards lycorine- or lycorenine-type AAs. The formation of the methylenedioxy bridge is the crucial step in forming a lycorine scaffold. The synthetic route to lycorenine-type AAs starts with the hydroxylation of norpluviine followed by a series of modifications, including methylation, oxidation, reduction, etc[18, 59].

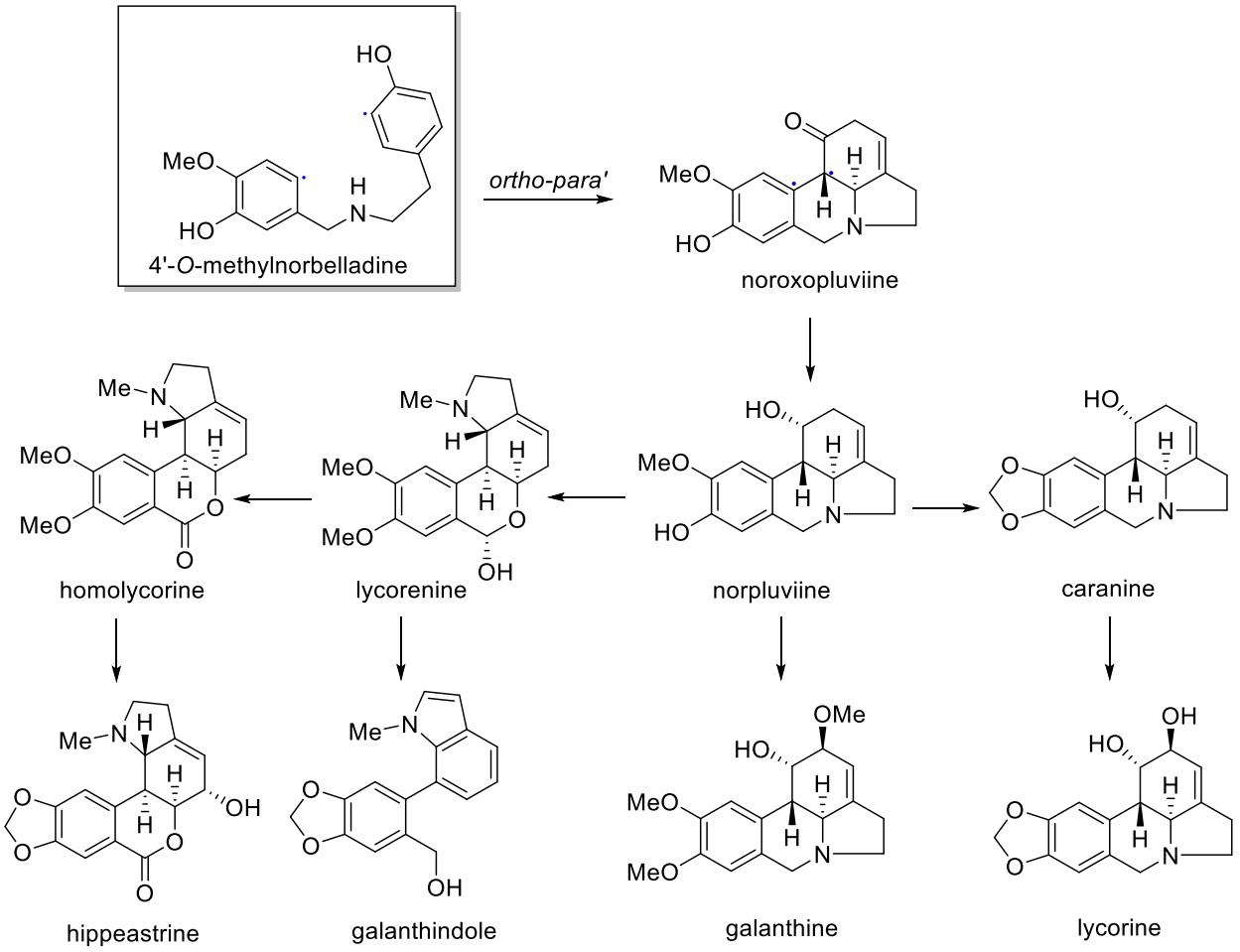


Fig. 5. *Ortho-para'* oxidative coupling of 4′-*O*-methylnorbelladine

Galanthamine type alkaloids are an important and large structural group among AAs. Their structural core is a product of *para-ortho'* phenol coupling (Fig. 6). *N*-Demethylnarwedine is the first galanthamine-type alkaloid produced by this coupling. Spontaneous closure of an ether bridge of 4-*O*-methylnorbelladine structure leads to the production of *N*-demethylnarwedine, which is subsequently converted to norgalanthamine by a stereospecific reduction. The subsequent *N*-methylation step leads to the production of galanthamine. The structure of norgalanthamine can be

converted to norlycoramine via reduction, and *N*-methylation leads to the production of lycoramine. The *N*-methyltransferases catalyze all the *N*-methylation reactions and further modifications of galanthamine and lycoramine which lead to the formation of various AAs of galanthamine-type[18].

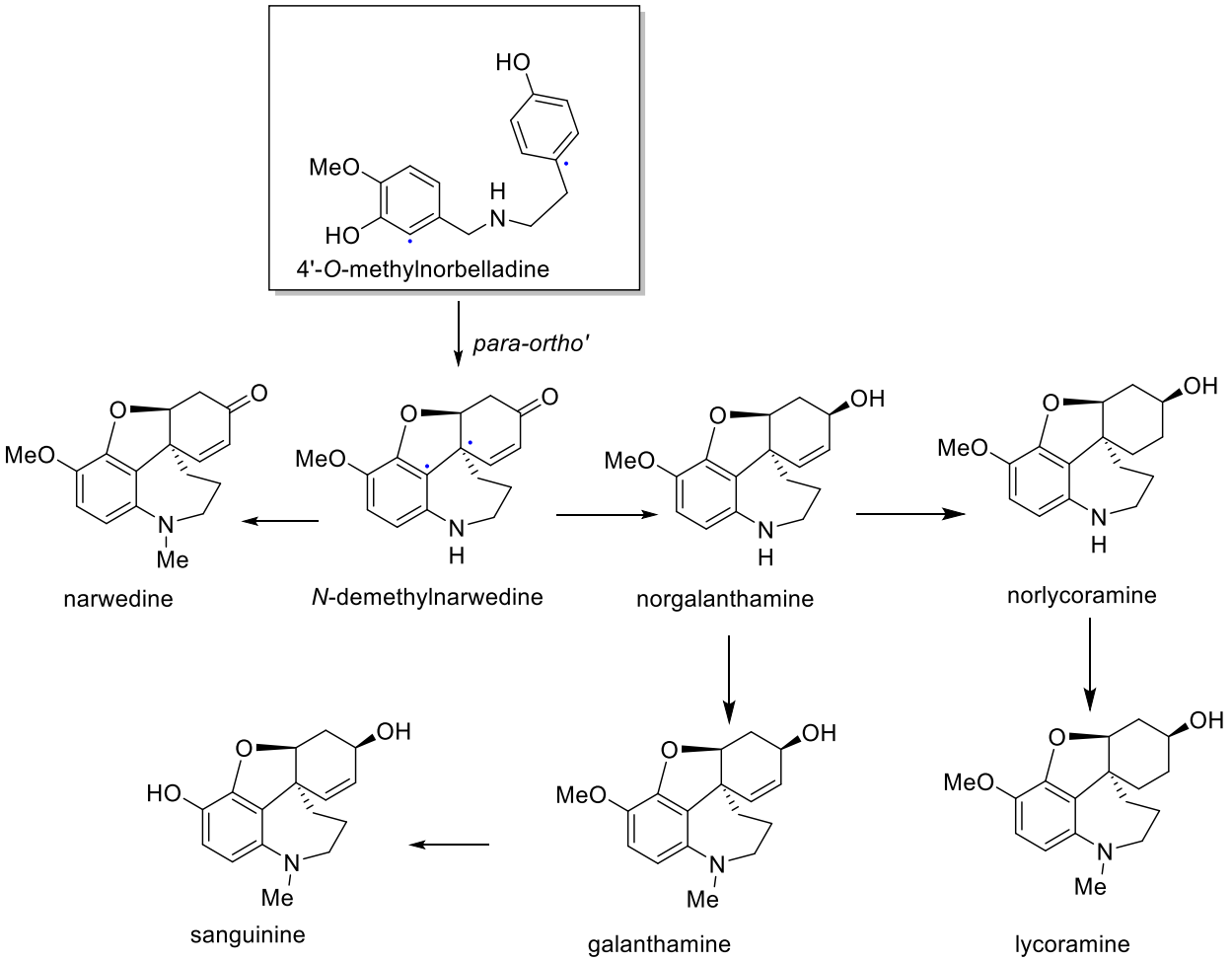


Fig. 6. *Para-ortho'* oxidative coupling of 4′-*O*-methylnorbelladine.

The biosynthesis of cherylline-type alkaloids is started by hydroxylation of the belladine scaffold, and the next step is the cyclization of the phenol moiety. The complete pathway has not yet been fully explained. The belladine skeleton might be converted into 3-*O,N*-dimethylnorbelladine, followed by hydroxylation at the C-2 position, cyclization, and tautomerization (Fig. 7). The enzymes involved in the synthesis of these AAs have not been identified completely identified. Some researchers presume that recombinant N4OMT plays a role in forming cherylline-type alkaloids[18, 60, 61].

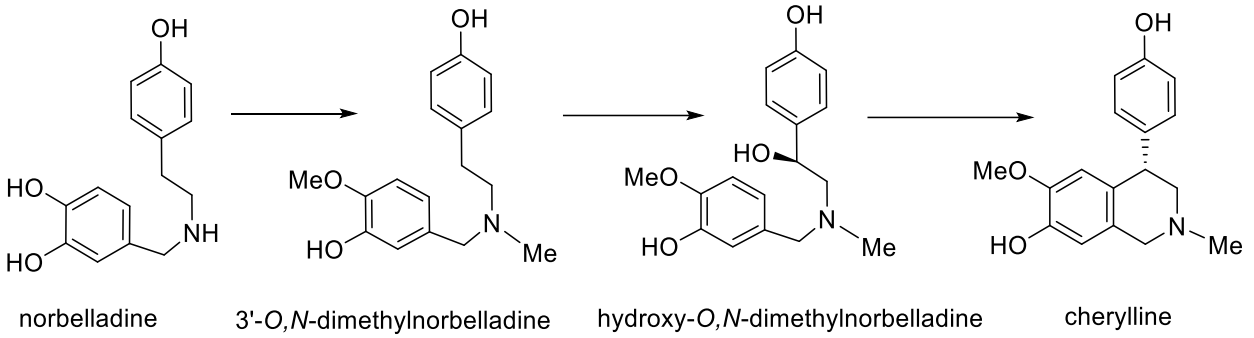


Fig. 7. Biosynthetic pathway of cherylline-type Amaryllidaceae alkaloids

Recently, alkaloids of two new structural types, namely narcikachnine-, and carltonine-type, have been isolated and described from Amaryllidaceae plants. Narcikachnine-type may be the product of condensation of galanthamine-, and galanthindole-type of AAs. The representatives of this structural type (narciabduliine, narcipavline, narcikachnine, narcimatuline, and narcieliine) have been isolated from *Zephyranthes citrina*, *Narcissus poeticus* cv. Pink Parasol, *Narcissus pseudonarcissus* L. cv. Dutch Master, and *Narcissus pseudonarcissus* cv. Carlton [19, 36, 62, 63] (Fig.8.). Unfortunately, all these compounds have been isolated in trace amounts. Therefore, it cannot be assumed that the biosynthesis of these substances will be studied soon. Further, new AAs have been isolated from *Narcissus pseudonarcissus* cv. Carlton and named carltonine A, B, and C. These compounds are dimeric (carltonine A, B) or trimeric (carltonine C) derivatives of norbelladine-type with galanthindole-core in their structure. This new structural type has been named as carltonine-type[13]. All these alkaloids demonstrated promising biological activities connected with potential treatment of AD.

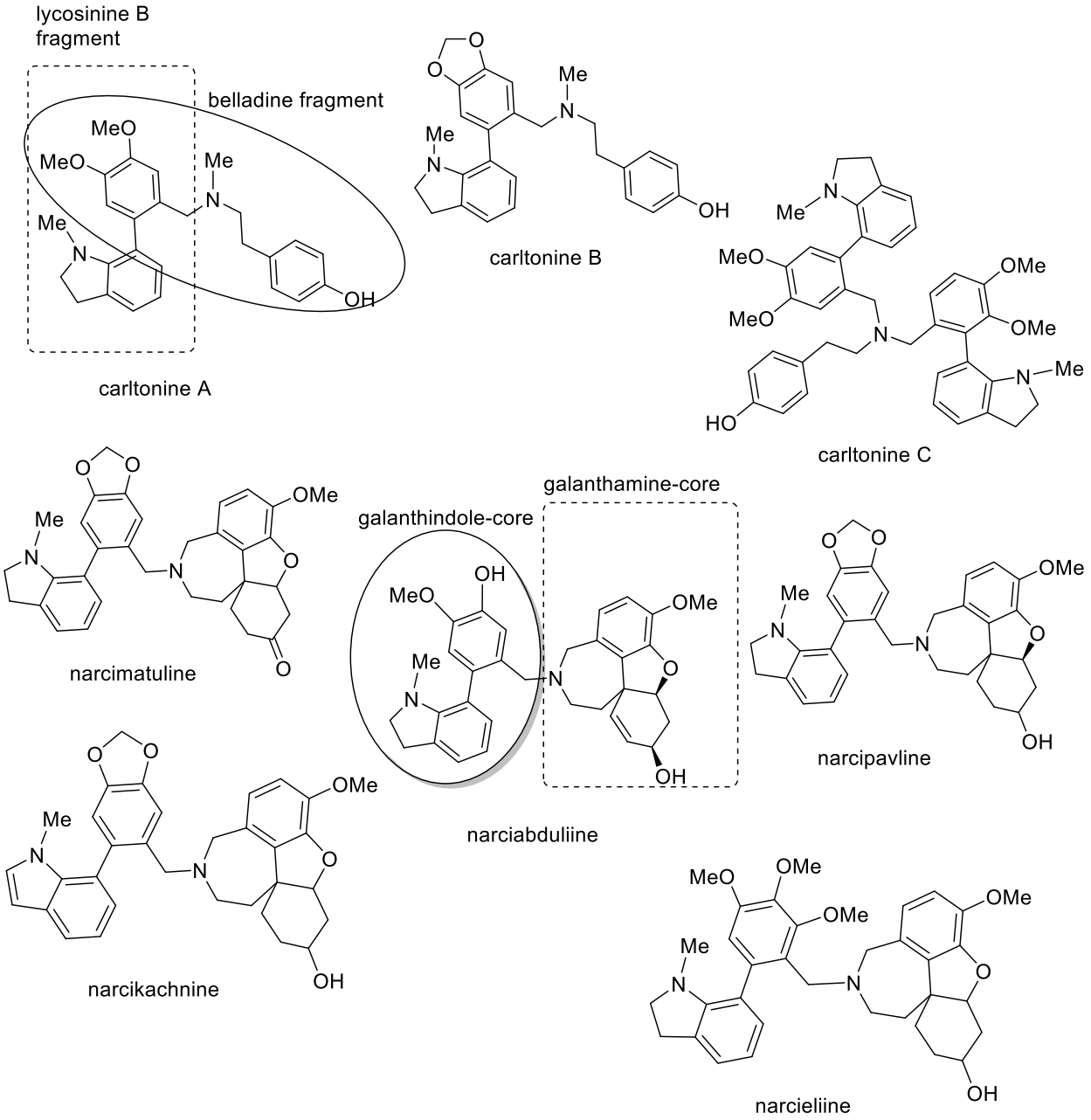


Fig. 8. Recently isolated and described Amaryllidaceae alkaloids of narcikachnine- and carltonine-type

3.4. Structure types of Amaryllidaceae alkaloids and their biological activity

AAs are a large group of secondary metabolites with interesting diversity in their structures. They are classified according to their skeleton structure and named after a representative alkaloid from the class. Nine main structural types of AAs are accepted, namely: norbelladine, homolycorine, lycorine, crinine, haemanthamine, montanine, galanthamine, tazettine, and narciclasine[10, 47, 56] (Table 2). Around 80 % of identified AAs belong to these nine structural types.

Table 2. Basic structure types of the Amaryllidaceae alkaloids and their ring types[18, 56]

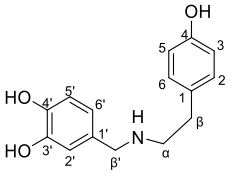
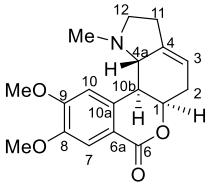
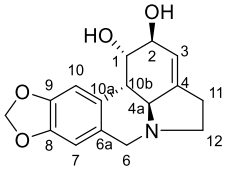
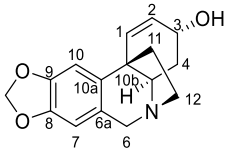
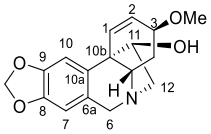
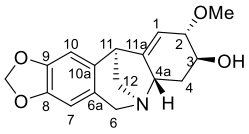
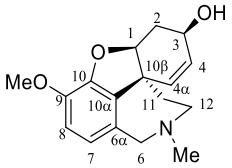
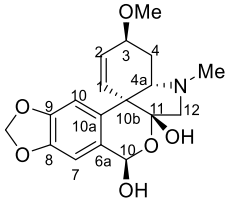
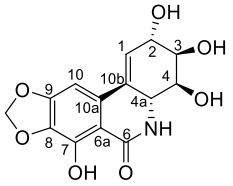
Structure type	Structure	Ring-type	Main representative alkaloid
norbelladine		*N*-(3,4-dioxybenzyl)-4-oxyphenethylamine	norbelladine
homolycorine		2-benzopyrano-[3,4-g]indole	homolycorine
lycorine		pyrrolo[d,e]phenanthridine	lycorine
crinine		5,10b-ethanophenanthridine	crinine
haemanthamine		5,10b-ethanophenanthridine	haemanthamine
montanine		5,11-methanomorphanthridine	montanine
galanthamine		6H-benzof,f]-2-benzazepine	galanthamine
tazettine		2-benzopyrano[3,4-c]indole	pretazettine

Table 2. Basic structure types of the Amaryllidaceae alkaloids and their ring types[18, 56] (continuation)

Structure type	Structure	Ring-type	Main representative alkaloid
narciclasine		lycoricidine	narciclasine

There are also AAs of minor structure types (e.g. galanathindol, cripowelline, plicamine, secoplicamine, ismine, graciline, tyramine, buflavine, augustamine, and zephycandidine), which are usually found in trace amounts or are represented by one alkaloid within structural-type[10]. As mentioned previously, several dimeric AAs have also been isolated from the Amaryllidaceae plants (carltonine-, and narcikachnine-type alkaloids). Further examples of these compounds are: the homodimer alkaloid with a C30 skeleton named digracine isolated from *Galanthus gracilis*[64], and the heterodimer alkaloid pallidiflorine isolated from *Narcissus pallidiflorus*[65] (Fig. 9). All these compounds are presented only in trace amounts in Amaryllidaceae plants; therefore, the biological activity has not been studied.

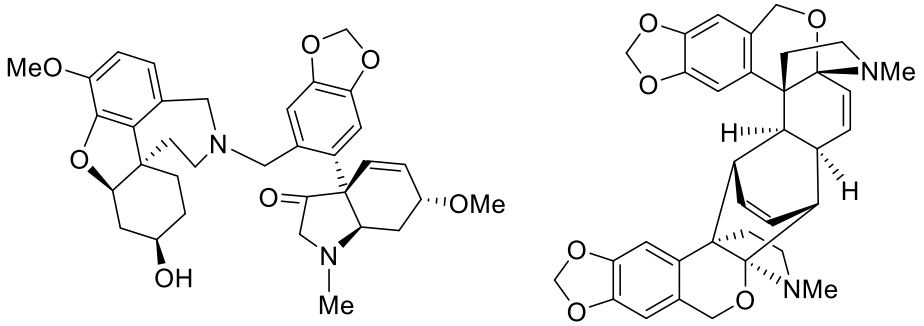
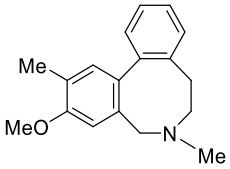
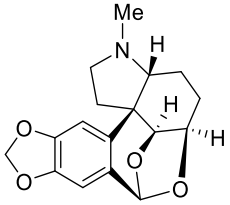
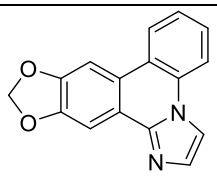


Fig. 9. Examples of dimer Amaryllidaceae alkaloids

Table 3. Examples of minor structural types of Amaryllidaceae alkaloids

Structure type	Structure	Main representative alkaloid	Plant species	Ref.
buflavine		buflavine	*Boophane flava*	*66*
augustamine		augustamine	*Crinum augustum*	*67*
zephycandidine		zephycandidine A	*Zephyranthes candida*	*68*

AAs have shown a wide range of biological activities including antitumor, antiviral, antibacterial, antifungal, antimalarial, analgesic, and cholinesterase inhibitory activity[2, 10, 13, 16]. The well-known AA is galanthamine, which was approved in 2001 under the commercial name Reminyl® for the treatment of mild to severe stages of AD as a selective, reversible, and competitive acetylcholinesterase inhibitor[69]. Narciclasine, pancratistatin, and their derivatives are in the preclinical development as promising antitumor agents. Recently, the anticancer potential of AAs and their synthetic derivatives has been reviewed[70]. In addition, lycorine and its derivatives have been summarized as a new structural scaffold for the development of new drugs[71]. The biological activities of the selected AAs are summarized in Table 4.

Table 4. Biological activity of the selected Amaryllidaceae alkaloids

Alkaloid	Source	Biological activity	Ref.
norbelladine-type and carltonine-type			
belladine	*Narcissus* sp., *Nerine filifolia*	weak acetylcholinesterase (AChE) inhibitory activity	*20, 72*
carltonine A	*Narcissus pseudonarcissus* cv. Carlton	anticholinesterase activity	*13*
carltonine B	*Narcissus pseudonarcissus* cv. Carlton	anticholinesterase activity	*13*
carltonine C	*Narcissus pseudonarcissus* cv. Carlton	anticholinesterase activity	*13*
lycorine type			
lycorine	*Leucojum vernum, Sternbergia lutea, Crinum asiaticum, Narcissus tazetta, Narcissus pseudonarcissus*	apoptosis-inducing effect, antitumor activity, ascorbic acid biosynthesis antiviral, anti-inflammatory, antifungal, antimicrobial, antimalarial, antiretroviral, and AChE inhibitory activity	*73-79*
1-*O*-acetyllycorine	*Nerine bowdenii*	AChE inhibitory	*80, 81*
assoanine	*Lycoris albiflora*	antiproliferative	*70, 82*
amarbellisine	*Amaryllis belladonna*	antiproliferative, antibacterial, and antifungal activity	*82, 83*
galanthamine type			
galanthamine	*Galanthus woronowii,* *Narcissus pseudonarcissus cv.* Carlton, *Leucojum aestivum*	AChE inhibitory	*84-86*
sanguinine	*Narcissus* sp	AChE inhibitory	*87*
norgalanthamine	*Crinum asiaticum*	promoting proliferation of dermal papilla	*88*
haemanthamine type			
haemanthamine	*Leucojum vernum, Galanthus elwesii,*	antiretroviral, antimalarial, and antiprotozoal activity	*79, 89, 90*
haemanthidine	*Zephyranthes robusta*, *Zephyranthes citrina*, *Cyrtanthus elatus*	anticancer activity, anti-inflammatory and antiprotozoal activity	*90-92*

Table 4. Biological activity of the selected Amaryllidaceae alkaloids (continuation)

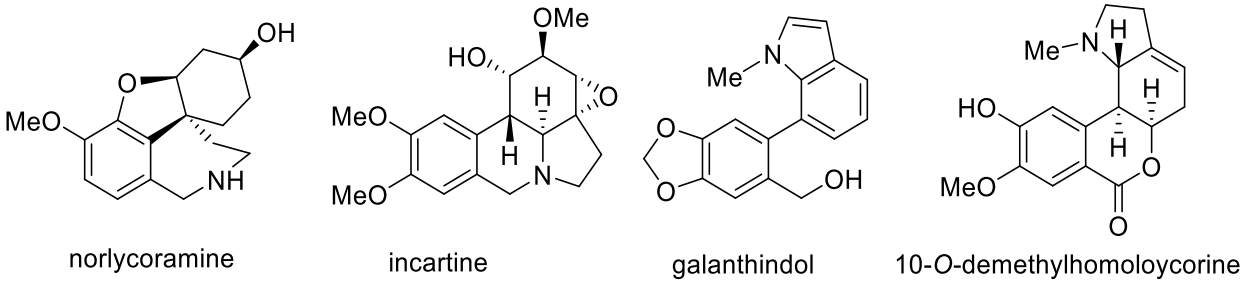
Alkaloid	Source	Biological activity	Ref.
crinine type			
buphanisine	*Nerine bowdenii*	anticancer	*72*
bulbispermine	*Crinum bulbispermum*	antiproliferative and cytotoxic	*93, 94*
distichamine	*Boophone disticha*	anticancer and antibacterial activity, affinity to the serotonin transporter	*95, 96*
homolycorine type			
homolycorine	*Leucojum vernum, Lycoris radiata,*	antitumor and antiretroviral	*49, 79, 97*
lycorenine	*Leucojum vernum*	antitumor, DNA-binding activity	*97, 98*
montanine type			
montanine	*Hippeastrum vittatum*	antiproliferative, anticholinesterase, antimicrobial, and antirheumatic activity	*99-103*
pancracine	*Lycoris radiata, Narcissus cv.* Professor Einstein	antiproliferative, antiparasitic, and antibacterial activity	*14, 103, 104*
narciclasine-type			
narciclasine	*Narcissus* sp.	pleiotropic cytostatic effect, antitumor, apoptosis-inducing cytotoxicity, and anti-inflammatory	*83, 105-107*
pancratistatine	*Haemanthus kalbreyeii*	antiparasitic and anticancer	*108*
tazettine type			
pretazettine	*Narcissus* sp.	antiviral and apoptosis-inducing activity,	*109*

3.5. Biological activity of selected Amaryllidaceae alkaloids

3.5.1. Lycorine and its natural derivatives

Lycorine and its derivatives contain a pyrrolo[d,e]phenanthridine nucleus in their structure. So far, more than 50 lycorine-type alkaloids have been isolated from Amaryllidaceae plants genera such as *Ammocharis, Boophane, Brunsvigia, Crinum, Galanthus, Haemanthus, Hippeastrum, Hymenocallis, Leucojum, Lycoris, Narcisuss, Sternbergia,* and *Zephyranthes*[110, 111]. Some species of the Amaryllidaceae family such as *Ammocharis coranica, Brunsvigia radulosa, Crinum*

macowanii, Hymenocallis littoralis, Hippeastrum equestre, Lycoris radiata, Leucojum aestivum, and *Leucojum aestivum* contain lycorine itself in high concentration (≥ 90 % of all bases)[112].



norlycoramine incartine galanthindol 10-*O*-demethylhomoloycorine

Fig. 10. Examples of lycorine type Amaryllidaceae alkaloids

Lycorine is a promising compound with various biological activities, including antiviral, antibacterial, antiparasitic, anti-inflammatory, anticancer, and other properties (Table 4).

The most important and studied biological activity of lycorine is its cytotoxicity. The first report on cytotoxic activity was published in 1920 by the National Cancer Institute [113]. Lycorine is an excellent anticancer candidate because it has advantageous characteristics such as high specificity to cancer cells, high potency in low concentration, less toxicity to healthy cells, and sensitivity against resistance cells. In some cases, lycorine shows more potential activity than paclitaxel in the xenograft model, while treatment with paclitaxel led to a significant loss of body weight, lycorine showed negligible changes[71, 114]. Lycorine shows potential anticancer activity against many tumor cells, such as BL6 mouse melanoma, Lewis lung carcinoma, and HeLa cells. The molecular mechanism involves inhibition of protein synthesis at the ribosomal level, inhibition of murine macrophage production of tumor necrosis factor-alpha, reduction of nitric oxide production, and induction of inducible nitric oxide synthase (NOS) in lipopolysaccharide-activated macrophages. Lycorine can suppress cell growth by arresting the cell cycle in the G2/M phase and inducing apoptosis in human leukemia cell lines. Additionally, lycorine-type alkaloids such as ungeremine, pseudolycorine, and amarbellisine showed also interesting anticancer activity against various human cancer cell lines (Table 5).

Table 5. Cytotoxic activity of the selected lycorine-type Amaryllidaceae alkaloids against different cancer cell lines

Cell lines	Cytotoxic activity of lycorine-type alkaloids, IC_{50} (μM)				Ref.
	Amarbellisine	Pseudolycorine	Lycorine	Ungeremine	
A549	7.2	7.5	6.5	>10	*74, 115*
Hey1B	nm	nm	1.2	nm	*112*
HCT116	nm	nm	3.0	nm	*115*
HepG2	nm	nm	34.1	nm	*73*
HL60	nm	nm	1.0	9.4 ± 1.3	*116, 117*
Hs683	8.3	7.9	0.9	>10	*74, 116*
K562	nm	nm	1.7	0.8	*74, 116*
MCF7	7.2	nm	4.0	nm	*74*
OE21	6.7	7.7	5.1	>10	*74, 116*
SKMEL-28	8.3	>10	8.5	>10	*74, 116*
U937	nm	nm	1.9	2.5	*74, 118*
U373	7.2 ± 0.4	7.8 ± 0.1	7.6 ± 0.4	83 ± 1	*116, 118*

Cell line abbreviations are as follows: A549 (lung carcinoma), Hey1B (ovarian cancer), HCT116 (colon carcinoma), HepG2 (hepatoma), HL60 (promyelocytic leukemia), Hs683 (neuronal glioma), K562 (myelogenous leukemia), MCF7 (breast cancer), OE21 (oesophageal squamous carcinoma), SKMEL-28 (skin melanoma), U937 (histiocytic leukemia), U373 (glioblastoma astrocytoma); nm stands for not measured.

Lycorine has also been reported to demonstrate broad-spectrum inhibitory activities against several viruses, such as poliovirus, retrovirus HIV-1, coronavirus associated with the severe acute respiratory syndrome (SARS-CoV), herpes simplex, west nile virus, dengue, yellow fever, human enterovirus71 (EV71), influenza virus, hepatitis C, and zika virus vector *Aedes aegypti*[71, 119]. The antiviral effect of lycorine is due to the blocking of viral DNA polymerase activity or elongation of the viral polyprotein during protein synthesis. The SAR study of lycorine reveals that the free hydroxyl groups in C-1 and C-2, benzodioxole group in the A-ring, nitrogen, and C3-C4 double bond are significant for the antiviral activity.

The antibacterial activity of lycorine has been evaluated against several bacterial strains. Lycorine showed activity against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa,* and *Enterobacter cloacae* with MIC (Minimum Inhibitory Concentration) value of 0.24 mg/mL. Lycorine also showed activity against *Micrococcus flavus* and *Salmonella typhimurium* with MIC values of 0.35 mg/mL and 0.48 mg/mL, respectively[120]. Furthermore, ungeremine was effective

in inhibiting the relaxation activity of bacterial topoisomerase IV (EcTopoIV) with an IC_{50} value of 7.3 μM[121]. Topoisomerase enzymes are involved in several crucial cellular processes, including replication, transcription, and recombination.

Furthermore, lycorine-type alkaloids have demonstrated potential antiparasitic activity in many studies. They have shown activity against *Plasmodium falciparum, Tribolium castaneumand,* and *Aphis gossypii*[71]. The antiparasitic mechanism involves inhibition of DNA topoisomerase-I activity, which is crucial for the growth of parasitic cells[122, 123].

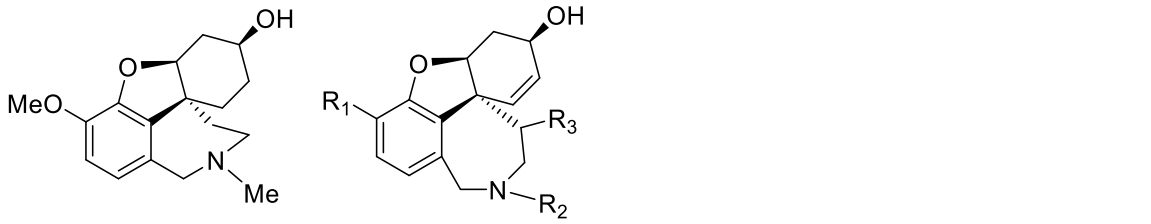
3.5.2. Galanthamine and its natural derivatives

The structural skeleton of galanthamine and its derivatives contain dibenzofuran nucleus (Fig. 11). Galanthamine was isolated for the first time in 1952 from *Galanthus woronowii*[124], later was found in other genera such as *Amaryllis, Galanthus, Hippeastrum, Haemanthus Hymenocallis, Lycoris, Leucojum, Narcissus, Ungernia,* and *Zephyranthes*[10]. Galanthamine is the most important representative of AAs. It was approved by the FDA in 2001 under the commercial name Reminyl® as selective reversible inhibitor of AChE[125]. Galanthamine improves cognitive, behavioral, and functional symptoms while delaying the development of behavioral disturbances and psychiatric symptoms. It shows high selectivity to the AChE over BuChE with no cytotoxicity[126]. In addition, galanthamine binds to the allosteric sites of nicotinic receptors, which causes a conformational change. This allosteric modulation increases the nicotinic receptor's response to acetylcholine. The activation of presynaptic nicotinic receptors increases the release of acetylcholine and its availability[16]. There are four crucial sites in the galanthamine structure: hydroxyl group in C3-position, cyclohexene ring, tertiary amine site, and methoxy group, which are vital for its binding ability to the target receptor. Small changes can significantly increase or decrease AChE inhibition activity. For example, sanguinine isolated from *Lycoris sanguinea* has hydroxyl group instead of methoxy group and AChE inhibition ability of sanguinine (IC_{50} = 0.10 μM) is improved by ten-fold than galanthamine (IC_{50} = 1.07 μM)[127]. Similarly, modification in the tertiary amine site enhances the inhibition activity of AChE. For example, *N*-allylnorgalanthamine and *N*-14-methylallylnorgalanthamine isolated from *Leucojum aestivum* showed AChE inhibition potency with IC_{50} values of 0.18 μM and 0.16 μM, respectively, which is more potent than galanthamine (IC_{50} = 1.07 ± 0.18 μM). Lycoramine isolated from *Zephyranthes robusta* does not contain a double bond in the cyclohexane ring, and this small change is responsible for the dramatic decline in

inhibition activity of AChE (IC_{50} = 55.3 ± 2.5 μM)[87]. The anticholinesterase activity of selected galanthamine-type alkaloids is summarized in Table 6.

Table 6. Cholinesterase inhibition activity of selected galanthamine-type Amaryllidaceae alkaloids

Alkaloid	Cholinesterase inhibition activity of galanthamine-type alkaloids (IC_{50})		Ref.
	AChE (μM)	BuChE (μM)	
galanthamine	1.07 ± 0.18	43.3 ± 1.3	[87]
lycoramine	55.3 ± 2.5	not active	[91]
sanguinine	0.10 ± 0.01	not measured	[87]
N-allylnorgalanthamine	0.18	not measured	[87]
11-hydroxygalanthamine	1.61 ± 0.21	not measured	[87]
N-(14-methylallyl)norgalanthamine	0.16	not measured	[85]



lycoramine

R_1 = OMe, R_2 = Me, R_3 = H; galanthamine
R_1 = OH, R_2 = Me, R_3 = H; sanguinine
R_1 = OMe, R_2 = Me, R_3 = βOH; 11-hydroxygalanthamine
R_1 = OMe, R_2 = allyl, R_4 = H; *N*-allylnorgalanthamine
R_1 = OMe, R_2 = methylallyl, R_3 = H; *N*-(14-methylallyl)norgalanthamine

Fig. 11. Examples of galanthamine-type Amaryllidaceae alkaloids with AChE inhibition activity

3.5.3. Haemanthamine and haemanthidine

The alkaloids haemanthamine and haemanthidine belong to haemantamine-type AAs, which are characterized by 5,10b-ethanophenanthridine bridged ring linkage in their structure (Fig. 12). Representatives of haemanthamine type have α-oriented 5,10*b*-ethano bridge. The orientation of this bridge is important for biological activity, compounds with α-oriented 5,10*b*-ethano bridge often demonstrate significant cytotoxic activity, and compounds with β-orientation are commonly inactive[128]. Haemanthamine is one of the most abundant alkaloids within AAs. In some cases, it is found as a major alkaloid in the plant extracts and can be isolated in gram amounts[13, 19]. It is commonly found in genera *Crinum, Hippeastrum, Hymenocallis, Haemanthus, Narcissus,* and

Zephyranthes. In the latest studies, haemanthamine has been isolated from the species of *Galanthus elwesii*, *Sprekelia formosissima, Urceolina miniata* and *Leucojum vernum*[49, 94, 129]. Haemanthidine is C6-hydroxy-derivative of h aemanthamine, which is present in plants in much lower concentrations and commonly isolated in the form of isomers. Haemanthidine has been isolated from *Zephyranthes robusta*, *Zephyranthes citrina* and *Cyrtanthus elatus*[92] (Table 4).

Both alkaloids demonstrate interesting cytotoxic activity against various cancer cell lines[90, 118, 130]. *In vitro* antiproliferative activity against different cancer lines is summarized in Table 7. The mechanism of antiproliferative activity of haemanthamine involves the inhibition of protein synthesis and blocking the peptide bond formation step of the peptidyl transferase center on the 60S ribosomal subunit. In addition, it can increase nucleolar stress by inhibiting the early stages of ribosome biogenesis, leading to activation of a p53-dependent antitumor pathway[131]. The additional hydroxyl group of haemanthidine is sterically accommodated by the eukaryotic ribosome and further stabilized by additional H-bond formation[131]. Furthermore, haemanthamine and haemanthidine can activate caspases 3-, 7-, 8-, and 9- in p53-null acute T-cell leukemia Jurkat cells, reduce cell viability, and arrested the cell cycle in G1 and G2/M. The cell cycle arrest is accompanied by the activation of $p16^{INK4a}$ protein at the G1/S checkpoint[132].

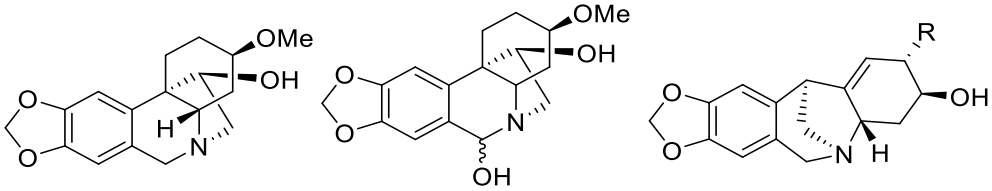


Fig. 12. Examples of haemanthamine-type and montanine-type Amaryllidaceae alkaloids

Table 7. Cytotoxic activity of haemanthamine and haemanthidine

Cell lines	*In vitro* cytotoxic activity of haemanthamine and haemanthidine, IC_{50} (µM)		Ref.
	Haemanthamine	Haemanthidine	
A549	1.1 ± 0.2	4.0 ± 0.4	*118, 130*
A2780	0.7 ± 0.4	2.3 ± 0.4	*130*
AGS	1.0 ± 0.2	1.5 ± 0.3	*130*
BT-549	1.0 ± 0.2	9.6 ± 0.1	*130*
COLO-201	1.0 ± 0.1	9.3 ± 0.3	*130*
HL-60	1.6 ± 0.06	2.0 ± 0.09	*133*
HT-29	0.3 ± 0.1	1.3 ± 0.1	*130*
H1299	1.2 ± 0.2	9.4 ± 0.3	*130*
HeLa	0.6 ± 0.1	1.6 ± 0.2	*130*
Jurkat	1.4 ± 0.3	9.3 ± 0.2	*130*
MOLT-4	1.2 ± 0.1	1.7 ± 0.1	*130*
MCF-7	0.8 ± 0.1	1.8 ± 0.2	*130*
NHDF	0.5 ± 0.1	1.4 ± 0.4	*130*
SW-480	0.7 ± 0.1	1.4 ± 0.1	*130*
SAOS-2	1.1 ± 0.4	9.7 ± 0.4	*130*

Cell line abbreviations are as follows: A549 (lung carcinoma) A2780 (ovarian carcinoma), AGS (gastric carcinoma), BT-549 (breast cancer), COLO-201 (colon carcinoma), HL60 (promyelocytic leukemia), HT-29 (colon carcinoma), H1299 (lung adenocarcinoma), HeLa (cervical adenocarcinoma), Jurkat (lymphoblast), MOLT-4 (T-lymphoblastic leukemia), MCF-7 (breast cancer), NHDF (dermal fibroblast), SW-480 (colon carcinoma), SAOS-2 (osteosarcoma).

Both alkaloids also showed antiprotozoal activity in various studies. Haemanthamine showed activity against *Entamoeba hystolytica, Leshmania donovanii, Trypanosoma brucei rhodensiense* and *Trypanosoma cruzi*[90]. The best activities were observed against *Trypanosoma brucei rhodesiense* (IC_{50} = 0.49 µg/mL), and *Entamoeba hystolytica* (IC_{50} = 0.75 µg/mL). Haemanthidine showed activity against both *Trypanosoma* species with IC_{50} values of 1.1 and 1.4 $\mu g ml^{-1}$ respectively [89]. Both alkaloids demonstrated antimalarial potential against chloroquine-sensitive *Plasmodium falciparum* F32 with the IC_{50} of 1.3 µM and 1.2 µM, respectively[134].

3.5.4. Montanine and pancracine

Montanine and pancracine are montanine type AAs (Fig. 12), characterized by the pentacyclic 5,11-*b*-methanomorphanthridine ring system in their skeleton. The structure of pancracine differs from montanine by the presence of a hydroxyl group instead of methoxy group in the C2 position. Montanine was isolated for the first time in 1955 from *Haemanthus* species [135]. Later, it was found in the genus *Hippeastrum*[103]. Pancracine was isolated from *Pancratium maritimum*, *Narcissus poeticus*, and *Rhodophiala bifida*[136]. Both alkaloids have gained significant attention due to their potent growth-inhibitory effects against various cancer cell lines. *In vitro* antiproliferative activities against various cancer lines of both alkaloids are summarized in Table 8. The cytotoxic mechanism involves the activation of MAPK systems by phosphorylation of p38 MAPK or signaling through Akt kinase[137].

Table 8. Cytotoxic activity of montanine-type Amaryllidaceae alkaloids

Cell lines	Cytotoxic activity of montanine-type Amaryllidaceae alkaloids, IC_{50} (μM)		Ref.
	Montanine	Pancracine	
A549	1.09 ± 0.31	2.29 ± 0.43	*14, 138*
A2780	1.67 ± 0.29	5.08 ± 0.43	*14, 138*
HCT-15	6.8 ± 0.5	nm	*139*
HT-29	1.35 ± 0.47	2.60 ± 0.51	*14, 138*
HeLa	1.99 ± 0.21	5.03 ± 0.36	*14, 138*
Jurkat	1.04 ± 0.14	5.07 ± 0.31	*14, 138*
MOLT-4	1.26 ± 0.11	2.71 ± 0.25	*14, 138*
MCF-7	1.39 ± 0.21	2.68 ± 0.37	*14, 138*
PANIC-1	2.30 ± 0.45	nm	*138*
SAOS-2	nm	2.20 ± 0.25	*14*

Cell line abbreviations are as follows: A549 (lung carcinoma), A2780 (ovarian carcinoma), HCT-15 (colon carcinoma), HT-29 (colon carcinoma), HeLa (cervical adenocarcinoma), Jurkat (lymphoblast), MOLT-4 (T-lymphoblastic leukemia), MCF-7 (breast cancer), PANIC-1 (pancreatic carcinoma), SAOS-2 (osteosarcoma); nm stands for not measured.

Montanine also demonstrates interesting antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Staphylococcus epidermis* with MIC values of 5, 20, 5 and 15 μg, respectively[101]. Furthermore, potential activity has been observed in antigen-induced and collagen-induced arthritis models[103]. Montanine can reduce locomotor activity,

sedative and anxiolytic effects, anticonvulsant and antidepressant effects in mice. The mechanism involves acting on the benzodiazepine site of the GABA receptor in the mouse brain. Thus, the anxiolytic and hypnotic effects of montanine have been observed by combined action on several neurotransmitter receptor systems, including GABA receptors[102].

In vitro antiparasitic activity of pancracine has been studied, and interesting activity was observed against *Trypanosoma brucei rhodesiense* (IC_{50} = 0.7 µg/mL), *Trypanosoma cruzi* (IC_{50} = 7.1 µg/mL), and *Plasmodium falciparum* IC_{50} = 0.75 µg/mL). Pancracine also demonstrated antimicrobial activity against *Staphylococcus aureus*, *Plasmodium aeroginosea,* and *Candida albicans* with MIC values of 22, 16 and 15 µg/mL, respectively [82].

3.5.5. Narciclasine and pancratistatin

Narciclasine and pancratistatin (Fig. 13), both narciclasine type AAs, are characterized by the lycoricidine ring, also called isocarbostyril system, in their skeleton. They are not basic metabolites, since nitrogen has an amidic character[11]. Narciclasine was isolated for the first time in 1967 from *Narcissus tazetta*, *Narcissus pseudonarcissus*, *Narcissus incomparabilis,* and *Narcissus triandrus*[83, 140]. Further, it has been isolated from other genera of *Lycoris, Hymenocallis, Habranthus, Pancratium Sternbergia,* and *Zephyranthes*[10]. Pancratistatin is a close analog of narciclasine, isolated for the first time in 1984 from *Hymenocallis littoralis* [49]. Further, it has been isolated from *Zephyranthes flava* and *Haemanthus kalbreyeri*[141]. The structure of pancratistatin differs from that of narciclasine with the presence of a hydroxyl group in the position C1 and the absence of a double bond between C1 and C10b.

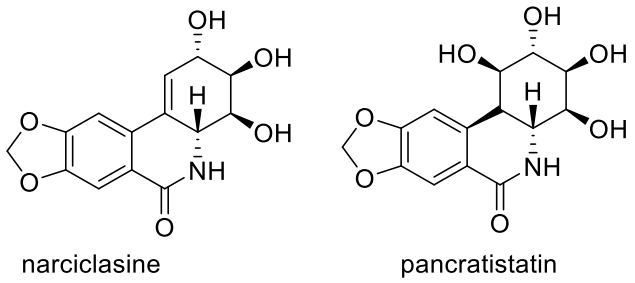


Fig. 13. Structure of narciclasine and pancratistatin

The anticancer potential of narciclasine is well documented[142]. The molecular mechanism involves inhibition of eukaryotic ribosomal protein synthesis through direct interaction with the 60S ribosome subunit. Narciclasine blocks the formation of peptide bonds by preventing the binding of the 3′-terminal ends of the donor peptide to the peptidyl transferase center. Narciclasine showed

promising anticancer activity against human glioblastoma multiforme (GBM) tumors in preclinical animal models, significantly reduced the growth of GBM tumors, and extended the survival time of immunodeficient mice with xenografts of GBM tumors in their brain[143]. In addition, it was found to trigger actin stress fibers formation through activation of the small GTPase RhoA which is involved in cell migration, cell morphology, protein synthesis, and cell death[142]. The activation of RhoA eventually led to the formation of F-actin stress fibers through the Rhokinase/LIM kinase/cofilin pathway. The increased generation of stress fibers was speculated to be the basis for the inhibition of cytokinesis, as well as for the decreased migratory capacity of glioblastoma cells. Evaluation of growth inhibitory properties has shown that narciclasine reduced cancer cell proliferation and migration at concentrations >1 μM. Another possible mechanism is targeting the eukaryotic translation elongation factor eEF1A[105].

Table 9. Antiproliferative activity of narciclasine type alkaloids against cancer cell lines

Cell lines	Cytotoxic activity of narciclasine type Amaryllidaceae alkaloids, IC_{50} (μM)		Ref.
	Narciclasine	Pancratistatin	
A549	0.03	nm	[107]
BxPC3	0.011	0.061	[141]
DU145	0.011	0.046	[141]
HL-60	0.018	nm	[83]
HSC-2	0.05	nm	[83]
KM20L2	0.011	0.077	[141]
LoVo	0.03	nm	[106]
MCF-7	0.01	0.07	[141]
NCI-H460	0.027	0.098	[141]
PC-3	0.028	nm	[106]
P388	0.042	0.052	[141]
SF268	0.010	0.043	[141]
U373	0.029	nm	[106]

Cell line abbreviations are as follows: A549 (lung carcinoma), BxPC3 (pancreas carcinoma), DU145 (prostate carcinoma), HL-60 (promyelocytic leukemia), HSC-2, KM20L2 (colon carcinoma), LoVo (colorectal carcinoma), MCF-7 (breast cancer), NCI-H460 (lung carcinoma), PC-3 (prostate carcinoma), P388 (leukemia), SF268 (astrocytoma), U373 (glioblastoma astrocytoma); nm stands for not measured.

The binding of narciclasine to eEF1A was predicted by molecular docking analysis and was proven in a cell-free system with recombinant human eEF1A. *In vitro* antiproliferative activities of naciclasine and pancratistatin against various human cancer lines are summarized in Table 9.

The promising antiproliferative activity of pancratistatin has been demonstrated against various human cancer cell lines (Table 9). There are several suggestive mechanisms of inducing cancer cell apoptosis, such as caspase-3 activation, the flipping of the phosphatidylserine-rich inner leaflet to the outer leaflet, the production of reactive oxygen species, and loss of mitochondrial membrane potential[144]. Furthermore, pancratistatin promotes permeabilization of the mitochondrial membrane, which causes apoptosis by releasing caspase activators and causes the loss of essential mitochondrial function. Furthermore, it has the ability to induce apoptosis in skin carcinoma and human teratocarcinoma NT2 cell lines without adverse effect on normal human fibroblasts[145].

Other biological activities of narciclasine and pancratistatin, reported in the literature, are anti-inflammatory, antiviral, and anticholinesterase activity [83, 142]. Narciclasine showed potential anti-inflammatory activity by suppressing of the production of TNF-α in LPS-activated murine macrophages. Narciclasine also exhibited potential antiviral activity against various flaviviruses such as Japanese encephalitis, yellow fever, and dengue fever. Furthermore, it can reduce Aβ production in the brain of AD and cross the blood-brain barrier, which is a characteristic often lacking in many drugs[142]. Pancratistatin showed strong *in vitro* RNA antiviral activity against Japanese encephalitis, flaviviruses, and bunyaviruses[146].

3.6. Genus *Narcissus* L.: occurrence, classification, ethnobotany

The genus *Narcissus* L. belongs to the Amaryllidaceae family and contains monocotyledonous, bulbous, flowering plants. Linnaeus identified this genus in 1753 in the book *Species Plantarum*[147]. Plants belonging to this genus are exclusively studied for their content of AAs. Approximately 40 wild species and around 100 cultivars of the *Narcissus* genus have been studied in relation to the presence of alkaloids. More than 100 alkaloids have been isolated from the genus *Narcissus* L. The plants of *Narcissus* L. are commonly known as Daffodil/Narcissus/Jonquil. They are perennial bulbous plants which are cultivated for ornamental purposes and for the perfume industries. These plants are widely distributed throughout the Mediterranean region, southern France, Italy, and the Balkans. The bulbs are usually cultivated during the winter season. *Narcissus* L. is classified into eleven sections that comprise 80-100 species[148]. Genus *Narcissus* has an important place in

traditional medicine. In the fourth century BC, a Greek physician used the oil of *Narcissus poeticus* to treat uterine tumors, and this practice continued until the first and second centuries AD[149]. The traditional use of daffodil was also found in Arabian, North Africa, Central America, and Chinese medicine during middle age[11]. Many species can be hybridized, and thus a huge number of hybridized cultivars have been developed. Remarkably, some hybridized cultivars are now used as a source for galanthamine production. More than 27000 *Narcissus* cultivars have been registered in the international daffodil registry[19]. The cultivar Carlton is a popular cultivar containing a high concentration of alkaloid constituents and is used for commercial isolation of galanthamine. A short botanical description of phytochemically investigated *Narcissus* species is summarized in the following sections.

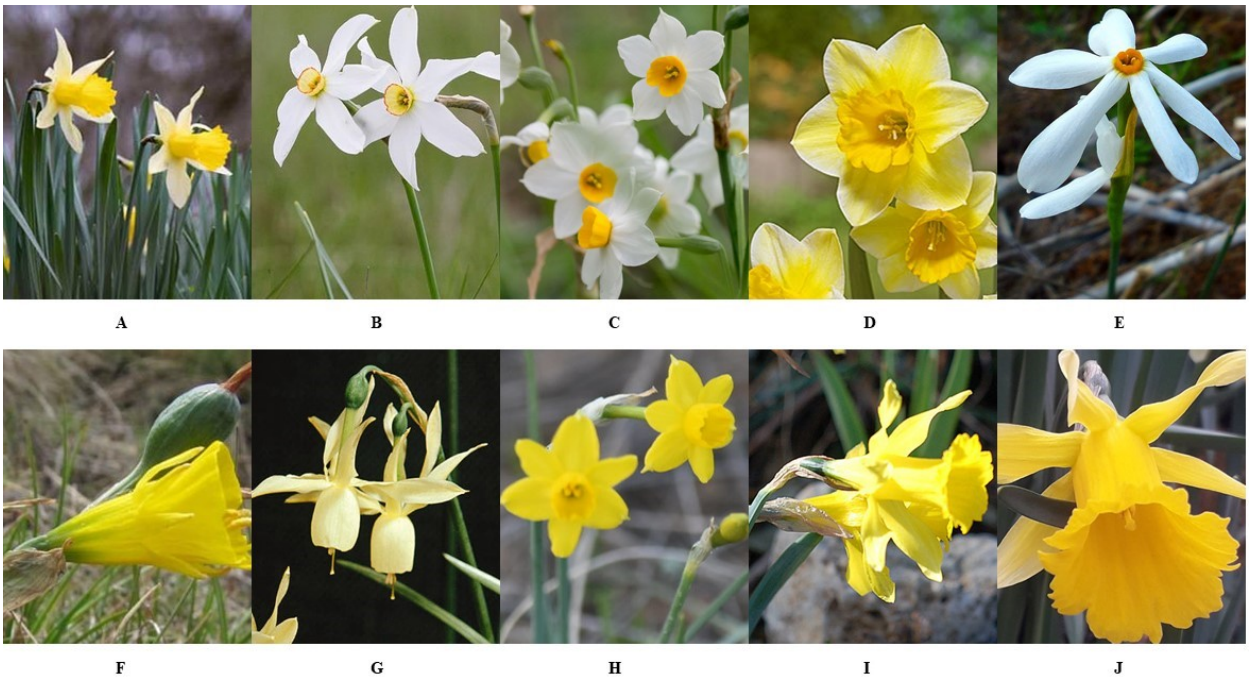
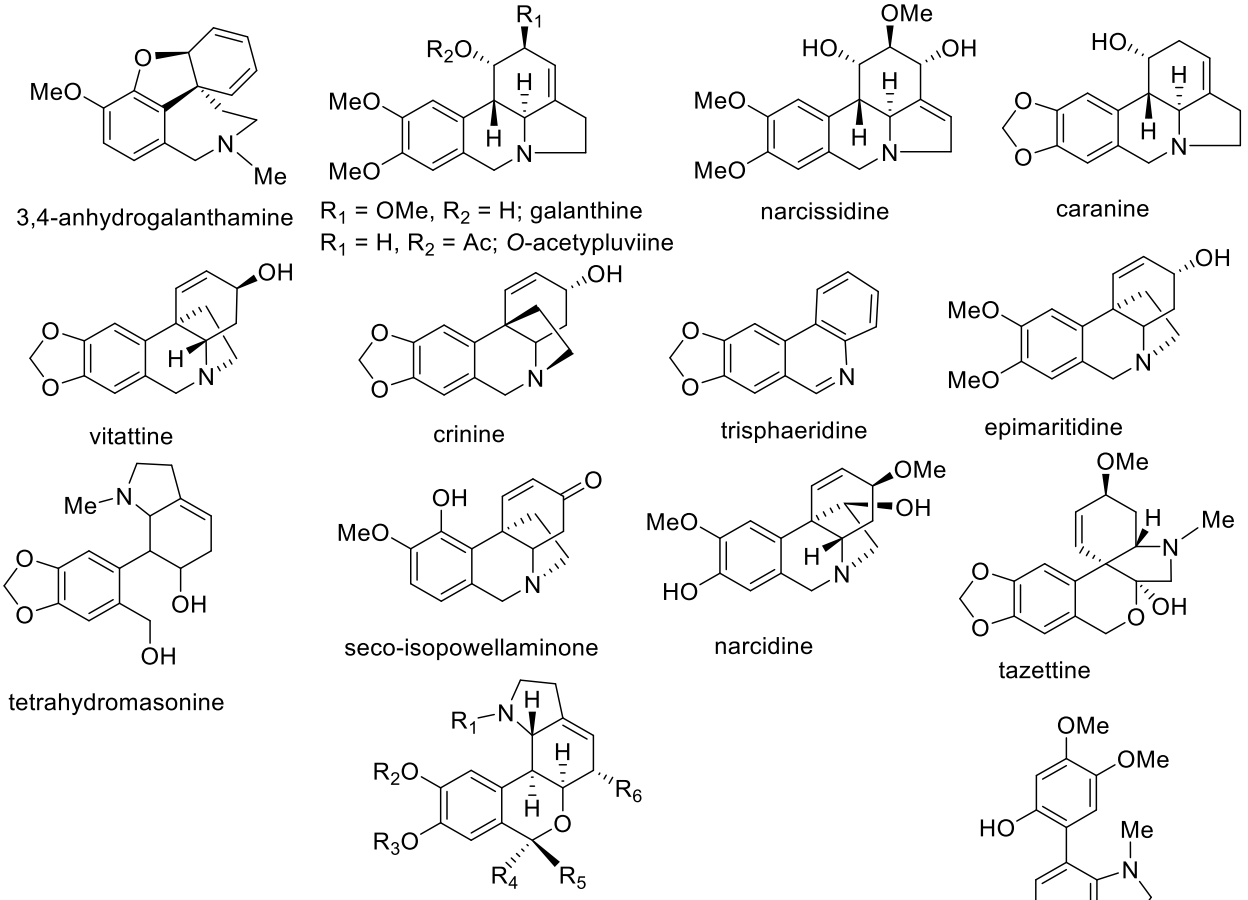


Fig. 14. Selected species from the genus *Narcissus* - A (*Narcissus pseudonarcissus)*[150], B *(Narcissus poeticus)*[151], C (*Narcissus tazetta)*[152], D *(Narcissus jonquilla)*[153], *E (Narcissus serotinus)*[154], *F (Narcissus bulbocodium)*[155], *G (Narcissus triandrus)*[156], *H (Narcissus assouanus)*[157], *I (Narcissus. bujei)*[158], J (*Narcissus confusus*)[159].

3.6.1. *Narcissus pseudonarcissus*[160]

Narcissus pseudonarcissus (Fig. 14A) is a perennial bulbous plant, mainly cultivated as an ornamental plant, native to Spain, Portugal, Germany, northern England, and Wales. It has a small trumpet flower surrounded with pale yellow petals. Flowers blooms in the mid-spring. The plants grow up to 35 cm long. They are easily grown in average, medium moisture, well-drained soils in full-sun atmosphere[150]. The alkaloids of galanthamine-, haemanthamine-, and lycoramine-types

have been found as major components, while crinine-, lycorenine- and lycorine-type AAs are present in lower concentrations. Newly discovered carltonine- and narcikachnine–types of AAs (Fig. 8) have been described for the first time in the plant of *N*arcissus *pesudonarcissus* cv. Carlton and *Narcissus pseudonarcissus* cv. Dutch Master[13, 63, 161]. Examples of AAs isolated from *Narcissus pseudonarcissus* are shown in Fig. 15.

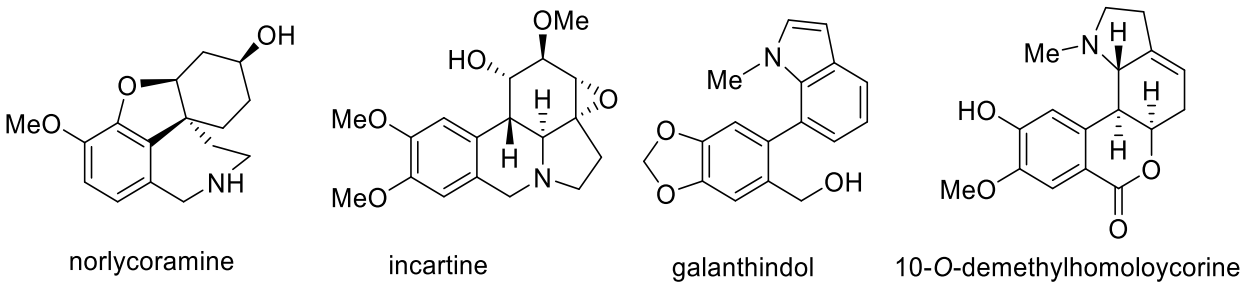


R_1 = Me, R_2 + R_3 = -CH_2-, R_4 = OH, R_5 = H, R_6 = H; oduline
R_1 = Me, R_2 + R_3 = -CH_2-, R_4 = OMe, R_5 = H, R_6 = H; 7-*O*-methyloduline
R_1 = Me, R_2 = Me, R3 = Me, R_4 = OH, R_5 = H, R_6 = H; lycorenine
R_1 = Me, R_2 = Me, R_3 = Me, R_4 = OMe, R_5 = H, R_6 = H; 7-*O*-methyllycorenine
R_1 = Me, R_2 = Me, R_3 = Me, R_4 = OEt, R_5 = H, R_6 = H; 7-*O*-ethyllycorenine
R_1 = Me, R_2 = H, R_3 = Me, R_4 = OMe, R_5 = H, R_6 = H; 9-*O*-demethyl-7-*O*-methyllycorine
R_1 = Me, R_2 = Me, R_3 = Me, R_4 + R_5 = O, R_6 = H; homolycorine
R_1 = Me, R_2 = Me, R_3 = H, R_4 + R_5 = O, R_6 = H; 8-*O*-demethylhomolycorine
R_1 = Me, R_2 = H, R_3 = Me, R_4 + R_5 = O, R_6 = H 9-*O*-demethylhomolycorine
R_1 = Me, R_2 + R_3 = -CH_2-, R_4 + R_5 = O, R_6 = OH; hippeastrine
R_1 = Me, R_2 + R_3 = -CH_2-, R_4 + R_5 = O, R_6 = H; masonine
R_1 = H, R_2 + R_3 = -CH_2-, R_4 + R_5 = O, R_6 = H; *N*-demethylmasonine

Fig. 15. Selected alkaloids isolated from the cultivars of *Narcissus pseudonarcissus*[13, 19, 63, 162, 163]

3.6.2. *Narcissus poeticus*[164]

Narcissus poeticus is the oldest daffodil that is cultivated as a source of alkaloids for pharmacy, fragrance, and ornamental purposes (Fig. 14B). Plants of this species are widely distributed in the alpine areas of southern Europe, Spain and Italy. The plant grows mainly at medium-altitude and on high mountains, in cold, moist, and semi-shaded habitats. It is a wild, bulbous, herbaceous plant, 20 to 30 cm in height; the leaves are radical, green, long, and narrow along with extreme fragrance, blooming from April to May; the flowers are white with a short corona of a light yellow and reddish edge[32, 151]. Alkaloids of crinine-, haemanthamine-, galanthamine-, lycorine-, homolycorine- and tazettine-types have been detected in various cultivars of *Narcissus poeticus* [165]. Examples of AAs isolated from *Narcissus poeticus* are shown in Fig. 16.



norlycoramine incartine galanthindol 10-*O*-demethylhomoloycorine

Fig. 16. Selected Amaryllidaceae alkaloids isolated from *Narcissus poeticus*

3.6.3. *Narcissus tazetta*[166]

It is a bulbous perennial plant cultivated for ornamental purposes and essential oil (Fig. 14C). Plants of this species are widely distributed throughout the Mediterranean regions from Portugal to Turkey; they are also found in the Middle East, Central Asia, Australia, Mexico, and the USA. Flower blooms in early spring[152, 167]. Phytochemical studies of *Narcissus tazetta* indicated the presence of a higher concentration of AAs of lycorine-, homolycorine-, haemanthamine-, tazettine- and galanthamine-type. Alkaloids buphanisine, ismine, pancratinine C, ungeremine, zefbetaine, and some *N*-oxides such as lycorine-*N*-oxide, pseudolycorine-*N*,β-oxide, and homolycorine-*N*-oxide are present in trace amounts[167, 168]. Examples of AAs isolated from *Narcissus tazetta* are shown in Fig. 17.

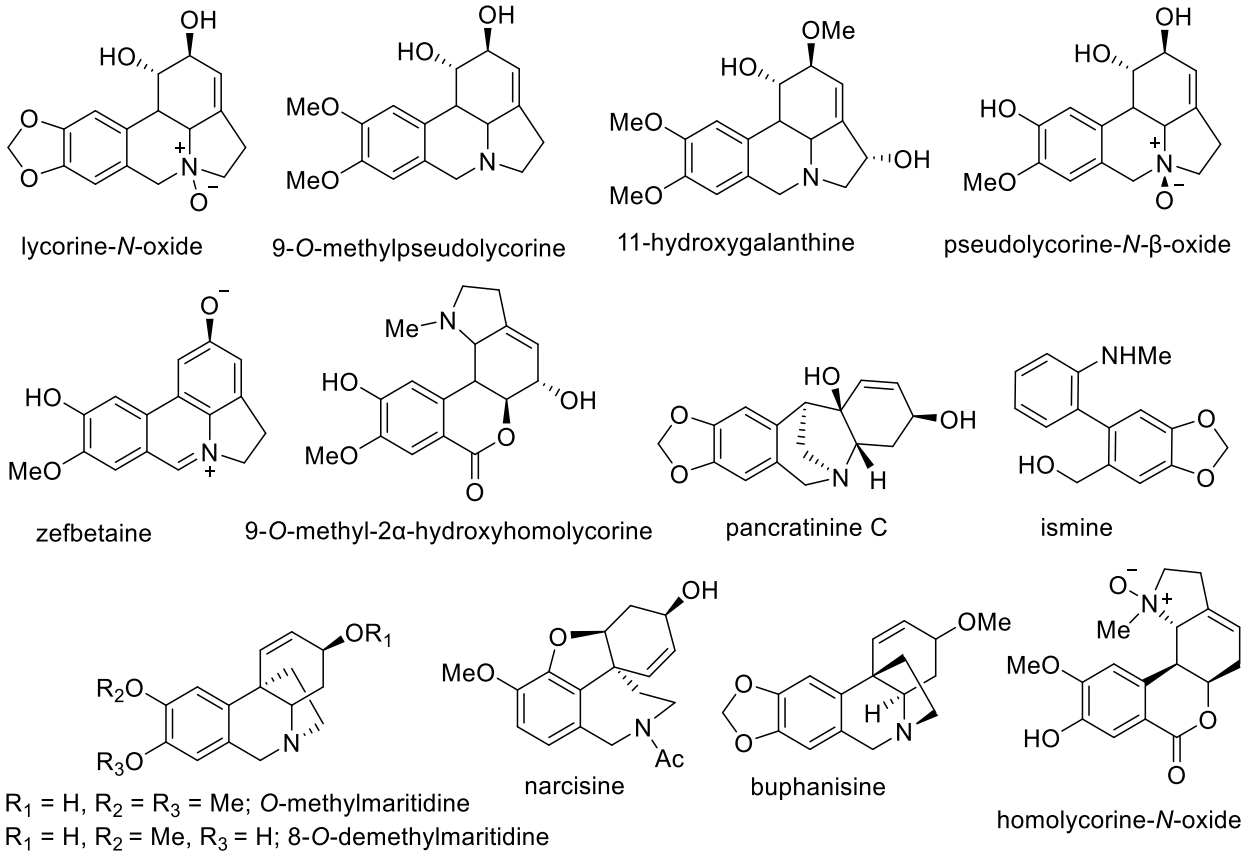
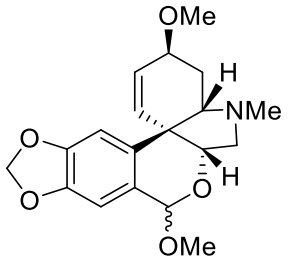


Fig. 17. Alkaloids isolated from *Narcissus tazetta*

3.6.4. Narcissus jonquilla[169]

Narcissus jonquilla (Fig. 14D) is commonly known as jonquil. It is a perennial, bulbous, and late-flowering species. Flowers bloom during the spring season. The plants are native in Spain and Portugal. Flowers are golden yellow, and leaves are narrow, rush-like, dark green[153]. The plants have been cultivated mainly for essential oils used in the perfume industry. Phytochemical studies of *Narcissus jonquilla* indicated the presence of galanthamine-, lycoramine-, tazettine-, haemanthamine-, narciclasine-, and lycorine-type AAs[161, 170]. An interesting alkaloid named jonqualline has been isolated from this species. This compound demonstrated promising anticancer activity against drug-resistant lung cancer cells within initial biological studies[171, 172] (Fig. 18).



jonqualline

Fig. 18. Structure of jonqualline isolated from *Narcissus jonquilla*

3.6.5. Narcissus serotinus[173]

It is an autumn flowering species (Fig. 14E), distributed throughout Europe, including Portugal, Spain, Greece, Croatia, and on the Mediterranean coast, Algeria, Libya, and coastal Morocco. It grows near coastal areas with a height of 5 inches. The flowers are bright white, cuspidate, slightly reflexed tepals and a minuscule dark orange cup less than a tenth of an inch wide[154]. Phytochemical studies of *Narcissus serotinus* indicate presence of 1-*O*-(3′-acetoxybutanoyl)lycorine, 1-*O*-(3′-hydroxybutanoyl)lycorine, lycorine, galanthine, assoanine, hippeastrine, narseronine, 3-*O*-methylnarcissidine, 1-*O*-acetyl-3-*O*-methylnarcissidine, 1-*O*-acetyl-3-*O*-methyl-6-oxonarcissidine, 2-methoxypratosine, 11-hydroxygalanthine, 2-*O*-methylclivonine, narseronine, galanthine, incartine, and masonine [174, 175]. The natural existence of butanoyl-moiety in lycorine-type compounds such as 1-*O*-(3′-acetoxybutanoyl)lycorine, and 1-*O*-(3′-hydroxybutanoyl)lycorine should be re-investigated because when butanol is used in the isolation process, it can lead to the formation of isolation artefacts[10, 176]. Examples of AAs isolated from *Narcissus serotinus* are shown in Fig. 19.

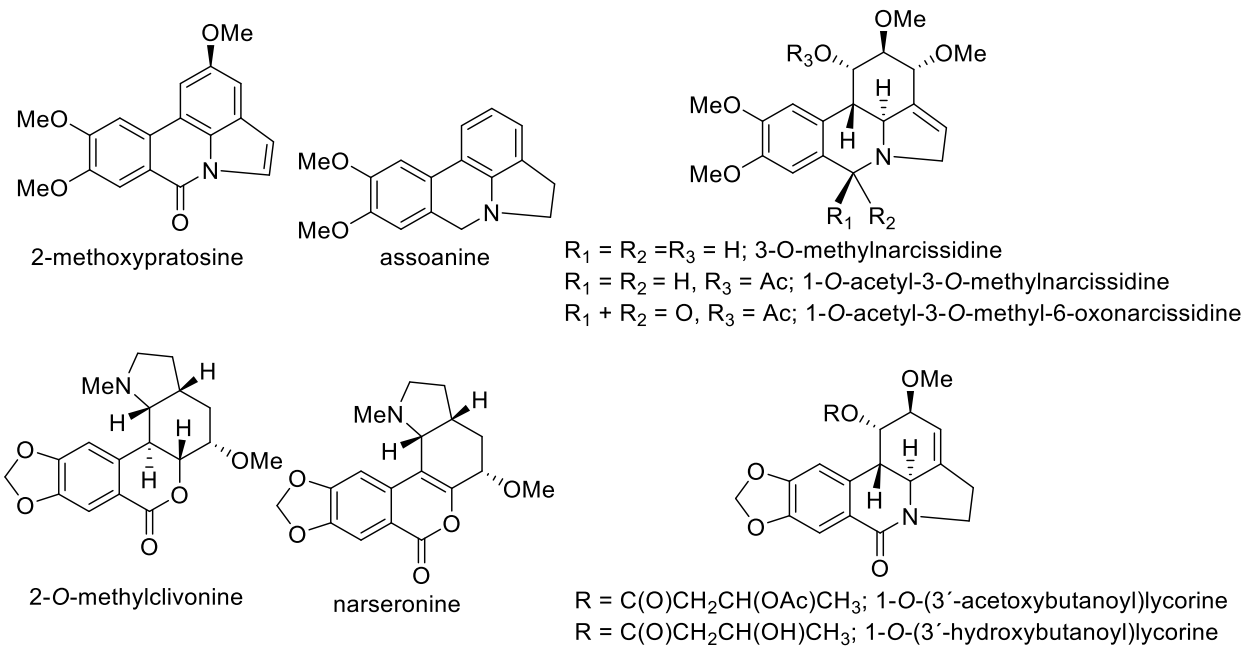


Fig. 19. Examples of Amaryllidaceae alkaloids from *Narcissus serotius*

3.6.6. *Narcissus bulbocodium*[177]

Narcissus bulbocodium (Fig. 14F) is a perennial bulbous plant. The flowers bloom in the middle of spring. It is widely distributed in western France, Spain, Portugal, and Morocco. A minimal number of phytochemical studies have been carried out on this plant. Interestingly, the alkaloid profile has been dominated by tyramine-type protoalkaloids (a side product of AAs biosynthesis), mainly methyltyramine and tyramine[170]. The traces of the lycorine-, homolycorine-, galanthamine-haemanthamine-, and tazettine-types have also been identified in this plant.

3.6.7. *Narcissus triandrus*[178]

It is a perennial bulbous plant native to France, Spain, and Portugal. The plant grows up to 15–35 cm long, 2 to 3 cm small to medium-size with delicate looking white flowers per stem that bloom in mid to late spring (Fig. 14G). The flowering season of these plants is relatively long. In the coastal area, begins in February, while at higher altitudes in May [156]. Phytochemical investigation of *Narcissus triandrus* indicated the presence of mesembrine-type alkaloids. The presence of mesembrine type alkaloids is usually concentrated in the plants of the family Aizoaceae[49]. Other Amaryllidaceae plants such as *Hymenocallis arenicola*, *Crinum oliganthum*, and *Nerine sarniensis* are also reported to contain mesembrine-type alkaloids[42]. Alkaloids mesembrine, mesembrenol, mesembrenone, 6-epimesembrenol, 2-oxoepimesembranol, 4′-*O*-demethylmesembrenone, 2-

oxomesembrenone, and 7,7a-dehydromesembrenone have been isolated from *Narcissus triandrus*[179, 180]. Furthermore, some varieties of *Narcissus triandrus,* namely Thalia and Tresamble, contain a high concentration of narciclasine, while margetine has been found in a minor quantity[181]. Examples of selected mesembrine type alkaloids are displayed in Fig. 20.

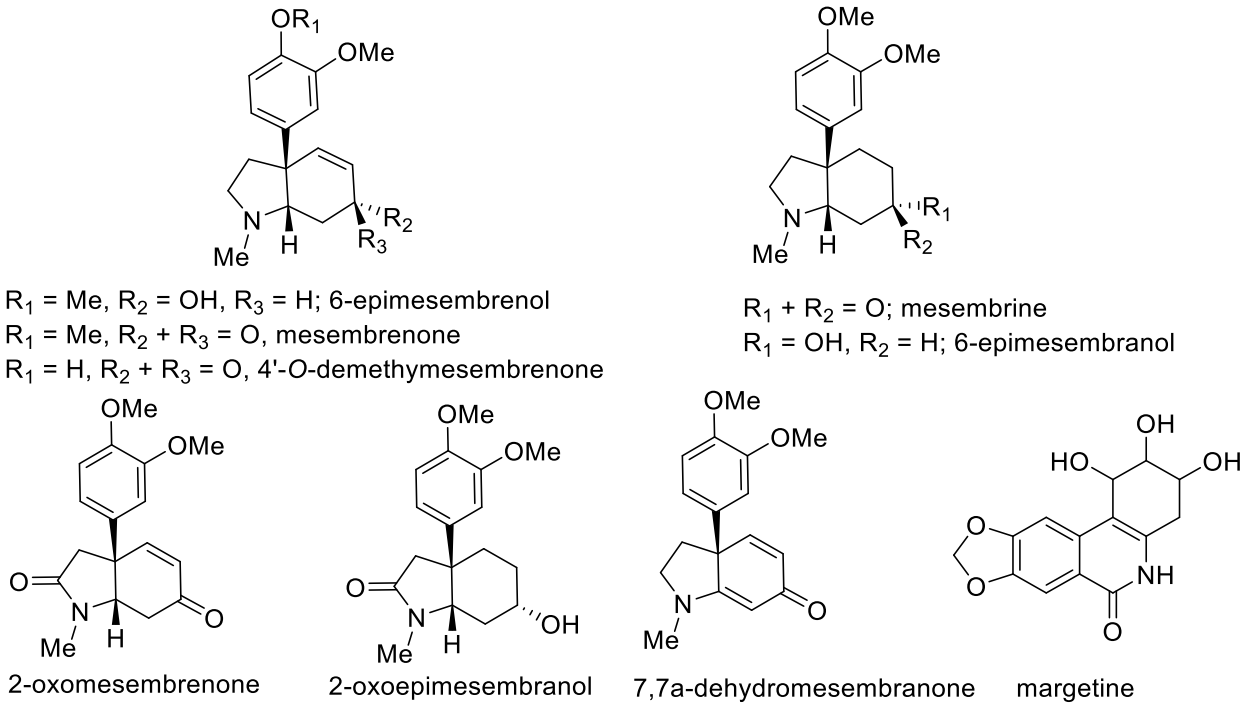
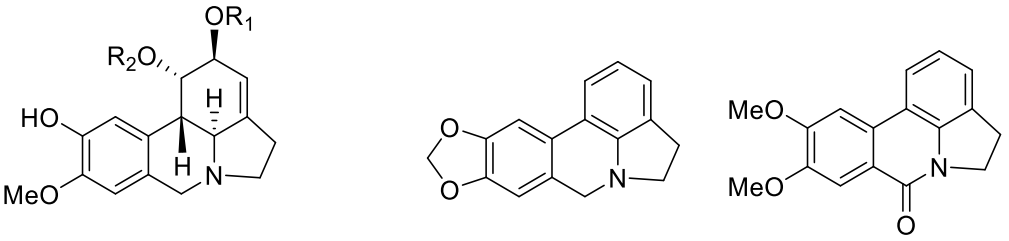


Fig. 20. Examples of alkaloids isolated from *Narcissus triandrus*

3.6.8. *Narcissus assoanus*[182]

Narcissus assoanus is a perennial bulbous herb with a height of 10 to 25 cm long (Fig. 14H). The flowers are individual, or in pairs, fragrant, and the petals are significantly long, cylindrical, and yellow. The flowers bloom in March and April. Plants are widely distributed throughout the western Mediterranean, France, and Spain[157]. The alkaloids lycorine, pseudolycorine, anhydrolycorine, and acetylpseudolycorine have been identified as major constituents of *N. assoanus*[183] (Fig. 18). The phytochemical study conducted by Viladomat described four alkaloids and two rare phenanthridine alkaloids named assoanine and oxoassoanine[184]. Examples of AAs isolated from *Narcissus assoanus* are shown in Fig. 21.



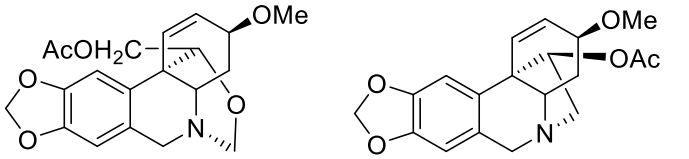
R_1 = H, R_2 = Ac; 1-*O*-acetylpsudolycorine — anhydrolycorine — oxoassoanine

R_1 = Ac, R_2 = H; 2-*O*-acetylpseudolycorine

Fig. 21. Alkaloid constituents isolated from *Narcissus assoanus*

3.6.9. *Narcissus bujei*[185]

Narcissus bujei (Fig. 14I) is considered as an endangered species distributed throughout Spain[158]. A minimal number of phytochemical studies have been carried out on this plant. The alkaloids of homolycorine-, haemanthamine- and tazettine-types are abundant in this plant. In addition, alkaloids *O*-methyllycorenine, 11-*O*-acetylhaemanthamine, and bujeine have been also isolated from *Narcissus bujei*[186]. Examples of AAs isolated from *Narcissus bujei* are shown in Fig. 22.

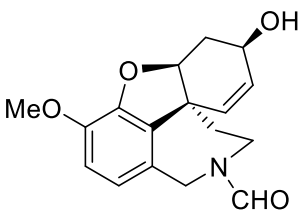


bujeine — 11-*O*-acetylhaemanthamine

Fig. 22. Examples of alkaloids isolated from *Narcissus bujei*

3.6.10. *Narcissus confusus*[187]

It is a large bulbous plant with a height of 45 cm (Fig. 14J), widely distributed throughout the Mediterranean region. Flowers blooms from March to April[159]. This plant is found to be rich in galanthamine content (0.1% fresh weight of bulbs). The alkaloids haemanthamine, galanthamine, pretazettine, homolycorine, and 9-*O*-demethylhomolycorine have been isolated from *Narcissus confusus*[188, 189]. In this species, an uncommon *N*-formyl derivative of galanthamine named *N*-formylgalanthamine has been identified[190].



N-formylgalanthamine

Fig. 23. An unusual alkaloid isolated from *Narcissus confusus*

3.6.11. Other species of the genus *Narcissus* and their phytochemistry

Over the last few decades, many phytochemical studies have been performed on some minor species of the genus *Narcissus* L. Several interesting and unusual alkaloids have been isolated (Fig. 24). The alkaloids lycorine, 1-*O*-acetyllycorine, sternbergine, 2-*O*-acetylpseudolycorine, 1-*O*-acetylpseudolycorine, isopseudolycorine, and 1-*O*-acetylisolycorine have been isolated from *Narcissus requienii*[191]. An uncommon alkaloid dubiusine has been isolated from the aerial parts of *Narcissus dubius*[192], which is a rare species native to Spain. Phytochemical study of *Narcissus radinganorum,* a rare endemic species found in the Iberian Peninsula, indicated the presence of homolycorine type alkaloids, including 8-*O*-demethylhomolycorine 9-*O*-demethylmaritidine[193]. *Narcissus tortifolius* is a minor species, and its phytochemical study resulted in the isolation of galanthamine, homolycorine, 8-*O*-demethylhomolycorine, 9-*O*-demethyl-2α-hydroxyhomolycorine, and dubiusine[194]. Alkaloids bicolorine, 5,6-dihydrobicolorine, oxoassoanine-*N*-oxide, pretazettine, 9-*O*-demethylhomolycorine, and 3-epimacronine have been isolated from *Narcissus bicolor* [195]. Phytochemical investigation of *Narcissus obesus* resulted in the isolation of bicolorine, 5,6-dihydrobicolorine, epimacronine, galanthamine, pretazettine, and haemanthamine together with a rare alkaloid obesine [195]. A phytochemical study of *Narcissus vasconicus* led to the isolation of 8-*O*-acetylhomolycorine and vasconine[196]. The alkaloids epinorgalanthamine, epinorlycoramine, and norgalanthamine have been isolated from *Narcissus leonensis*[197]. Tortusine is a quaternary phenantridinium alkaloid isolated from *Narcissus tortuosus*[198]. The alkaloids pancracine, pseudolycorine, vasconine, ungeremine, 8-*O*-demethylhomolycorine, cherylline, and nangustine have been isolated from *Narcissus angustifolius* subsp. *transcarpathicus*[104]. Examples of AAs isolated from different species of the genus *Narcissus* L. are displayed in Fig. 24.

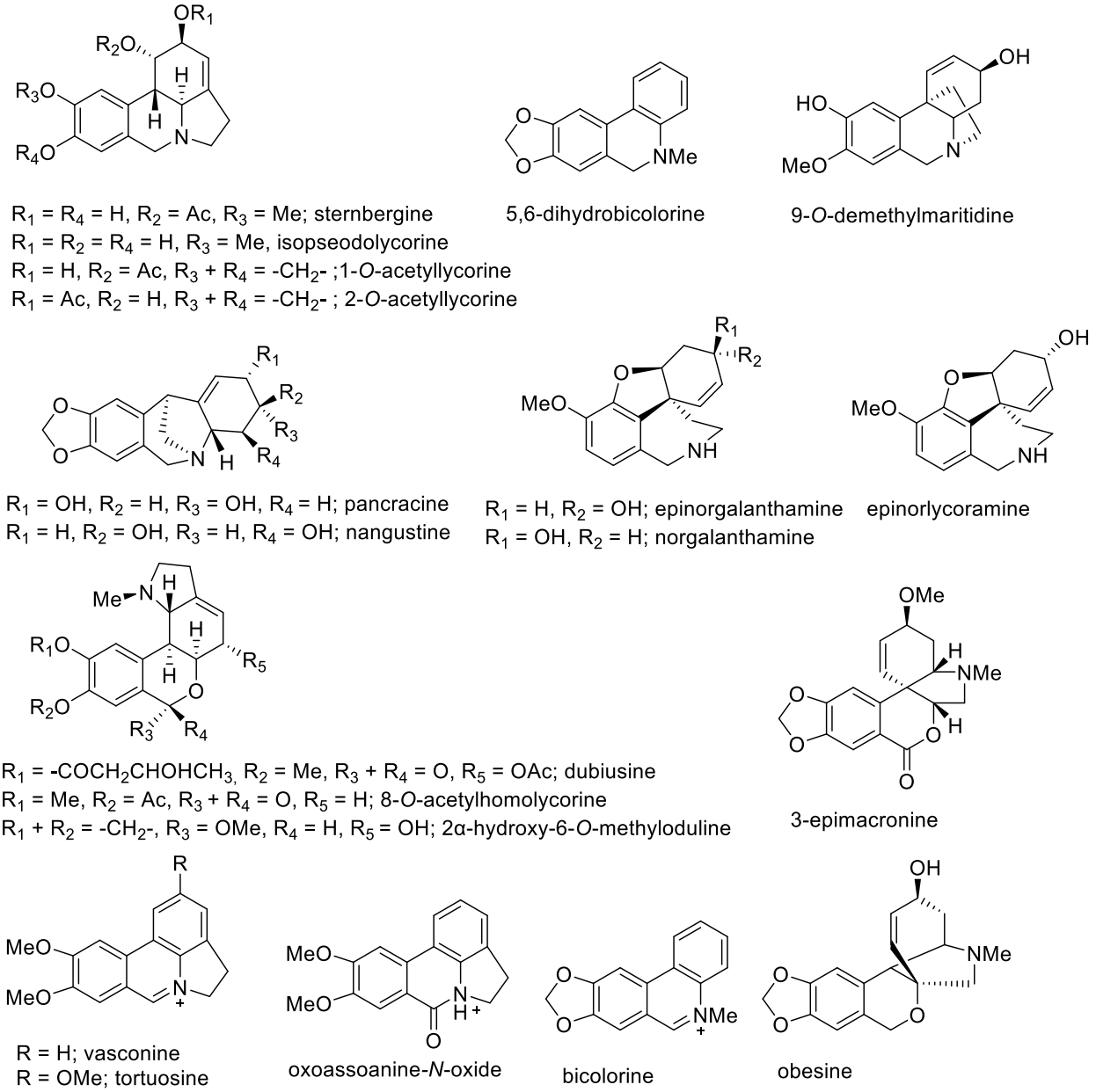


Fig. 24. Examples of Amaryllidaceae alkaloids isolated from different species of the genus *Narcissus* L.

3.7. Alzheimer's disease: etiology, hypothesis, and popular targets

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease, clinically manifested by dementia and generally diagnosed between 60 and 70 years. The number of patients with dementia is increasing rapidly, and is predicted to increase to 150 million by 2050[199]. The reason for AD pathology is still unclear, but several hypothesis have been proposed, including the cholinergic hypothesis, amyloid-β (Aβ) production, hyperphosphorylation of tau protein, imbalance of metal ions, and oxidative stress[16]. Currently, there is no cure, but patients are frequently treated with

drugs to relieve and manage symptoms of AD. The FDA has approved four cholinesterase inhibitors donepezil (Aricept®), galanthamine (Reminyl®), rivastigmine (Exelon®), tacrine (Cognex®), *N*-methyl-D-aspartate receptor antagonist (memantine) and fixed-combination of donepezil and memantine (approved in 2014) for the treatment of AD[200, 201]. However, tacrine is no longer marketed due to its poor tolerability and unfavorable pharmacological profile. Recently, the FDA approved a drug named aducanumab in June 2021 under the brand name Aduhelm for AD treatment. It is a human IgG1 monoclonal antibody, that should be able to reduce Aβ plaques in the brain[202]. The following section discusses the important hypothesis of AD pathology and possible drug targets.

3.7.1. Cholinergic hypothesis

The cholinergic hypothesis is the oldest hypothesis of AD pathology. The degradation of the neurotransmitter acetylcholine (ACh) in the basal forehead is responsible for the loss of neurotransmission in the cerebral cortex. As a result of this process, there is a significant deterioration in cognitive function within in AD patients. Generally, ACh can be decomposed by the enzymes AChE and BuChE in the neocortex and hippocampus of the brain[203]. In the normal brain, nerve impales are transported through the fusion of ACh-containing vesicles and the membrane of presynaptic neurons. Subsequently, ACh is released into the synaptic cleft and binds to cholinergic receptors in the postsynaptic neuron. Thus, transmission of nerve impulses to maintain pulsatile cholinergic stimulation. AChE is a serine hydrolase enzyme that hydrolyzes ACh into choline and acetate. Furthermore, choline enters the cycle as a precursor to produce ACh[204]. The AChE has two forms, namely G1 and G4. G4 is the major form, its level increase during AD progression[205]. Recent studies have emphasized that ACh can be hydrolyzed by another enzyme BuChE, which is available mainly in glial cells[206-208]. The function of BuChE has received enormous interest from the scientific community due to its potential role not only in the progression of AD, but also in the multifunctional aspect in different disease progression[204, 209]. BuChE functions are enhanced by 40–90% in the later stage of AD brain[210]. Furthermore, some studies indicated that cholinesterase enzymes play a role in Aβ aggregation and maturation to oligomers, fibrils, and plaques[210].

3.7.2. Amyloid β hypothesis: formation of Aβ and its aggregation

Over the past few decades, the amyloid β (Aβ) hypothesis is one of the most discussed causes of AD. Aβ is derived from a transmembrane protein named amyloid precursor protein (AAP). APP has a crucial role in the development of the central nervous system (CNS). The proteolytic cleaves of APP occur by either amylogenic or nonamylogenic pathway. The nonamylogenic cleavage is initiated with the membrane-bound α-secretase enzyme and release soluble sAPPα fragment[211]. Amylogenic cleavage of APP initiated by β-secretase (BACE1)[212]. Thus, sAPPβ and C99 fragment are produced. Subsequentially, membrane-bound C99 fragment is sliced by γ-secretase which generates the intracellular domain of APP and Aβ peptide[213]. Aβ consists of 38-43 amino acids. The most represented peptides are Aβ1-40 and Aβ1-42. Further, these monomeric Aβ peptides are aggregated to form neurotoxic Aβ oligomers. The massive accumulation and polymerization of Aβ oligomers are responsible for the activation of the cascade which generates oxidative stress and disrupts the level of Ca^{+} within the cells. In the course of AD progression, the extracellular accumulation of Aβ occur in the microglia cells which are the major immune cells in CNS. Aβ1-42 activates the formation of plaques and Aβ1-40 store them simultaneously or with a delay. Thus, cellular integrity is disrupted by massive accumulation of Aβ oligomers. Furthermore, Aβ itself causes oxidative damage and interrupt the function of mitochondria. Additionally, presenilin (PS1) is another important protein responsible for Aβ1-42 production. Mutation in the PS1 protein increases γ-secretase activity and is associated with the production of Aβ42[211, 214].

3.7.3. Tau hypothesis: formation of neurofibrillary tangle

The tau proteins are found in neurons under normal conditions. It is a microtubular-associated protein tau (MAPT) which is responsible for tubulin assembly, stabilization, and affecting transport in axons[215]. In the course of AD progression, neurofibrillary tangles (NTFs) have been formed in the intracellular space which consists of the accumulation of paired helical filaments[216]. Hyperphosphorylated tau is the main component of these filaments. Cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β) are the key factors of tau hyperphosphorylation[217]. Recent studies showed that the activity of GSK3β has increased when massive amount of Aβ accumulates in the extracellular space. Overexpression of GSK-3β results in memory impairment, tau hyperphosphorylation, increased Aβ production, and local plaque-associated microglial-mediated inflammatory responses[218]. Experimental findings support the

hypothesis that just oligomers are the toxic forms of the tau protein showing during the AD[219]. Tau mutations near the C-terminal region have disrupted the ability of tau to bind with microtubules and interrupted microtubule regulation. Therefore, the microtubule structure has collapsed along with the disruption of many cellular processes ranging from protein trafficking to overall cellular morphology, leading to cell death[220, 221].

3.7.4. Hypothesis based on inflammation

Neuroinflammation is frequently observed in postmortem tissue from AD patients[220, 222]. Several epidemiological studies from 1990 suggested that the risk of AD development has reduced to 50% among patients who used non-steroidal anti-inflammatory drugs for a long time[223]. Resident immune cells in the CNS named microglia have been activated due to the massive accumulation of Aβ toxic species. Subsequently, some pro-inflammatory cytokines have been released, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor α (TNF α), interleukin-8 (IL-8), macrophage inflammatory protein-1 α (MIP-1α), and monocyte chemoattractant protein-1 (MCP-1) along with various toxic products such as reactive oxygen species, nitric oxide, and cytokines. The study showed that increased levels of Aβ proteins were observed in the patients suffering with head trauma and elevated levels of IL-1, which is responsible for the accumulation of neurotoxic Aβ peptides[224]. In addition, an increased level of other cytokines such as IL-1β and IL-6 is responsible for the activation of CDK5[225]. Neuroinflammation observed in AD appears to play a primary role in exacerbating the Aβ burden and tau hyperphosphorylation[222].

3.7.5. Prolyloligopeptidase

Prolyloligopeptidase (POP) is a serine protease that cleaves peptide bonds on the carboxyl side of proline in peptides with a relatively small molecular mass. POP is generally expressed in the regions of the brain like hippocampus, hypothalamus, amygdala, cortex, and striatum. It involves processing some memory enhancing neurotransmitters such as oxytocin, thyrotropin-releasing hormone, angiotensin, arginine-vasopressin, α-melanocyte-stimulating hormone, luteinizing hormone-releasing hormone, substance P, and neurotensin[226]. It can play a vital role in learning and memory, which further influences social behavior, emotions, stress, and blood pressure[13, 227]. POP is currently considered as a potential therapeutic target for modulating many diseases such as schizophrenia, bipolar affective disorder, Parkinson's disease, and AD[227]. The imbalance of the POP enzyme has been frequently observed in patients with neurodegenerative diseases, and their

inhibition is found to be neuroprotective. Under pathological conditions, POP could be involved in the processing of the C-terminal region of APP. Therefore, the production of neurotoxic Aβ peptides is increased. Specific inhibitors of POP have been reported to suppress the production of Aβ toxic forms and in the mouse model, accelerated by senescence [228, 229].

3.7.6. Monoamine oxidase

Monoamine oxidase (MAO) is a monoamine neurotransmitter, catalyzes the oxidative deamination of biogenic and xenobiotic amines. MAOs plays an important role in the metabolism of neuroactive and vasoactive amines in the CNS and peripheral tissues. The lower level of MAO has been found in many pathological processes such as neurodegeneration and depression[230]. Studies have shown that activated MAO present in the brain of AD is considered as a potential biomarker for the diagnosis of disease. MAO has been established to be a marker of oxidative stress, which is linked to the production of reactive oxygen species and other molecules that cause oxidative stress, and further neuronal damage and neurodegeneration has occurred. Therefore, excessive MAO activity contributes to neurodegeneration in AD[231, 232]. There are two isoenzymes of MAO that have been found in humans, MAO-A and MAO-B. Activated MAO-B leads to cognitive dysfunction, destruction of cholinergic neurons, contributes to the formation of Aβ plaques, and is associated with the formation of NFTs[16]. MAO inhibitors have demonstrated neuroprotective effects related to oxidative stress, which are desirable properties for the development of multitarget drugs for AD.

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

The summarized results of the submitted dissertation were collected during the academic years 2018 – 2021. The results obtained were achieved during the doctoral study in the Department of Pharmacognosy and Pharmaceutical Botany (Faculty of Pharmacy in Hradec Králové, Charles University). I am an author and co-author of eight articles published in IF journal, seven of them are original work and one is a review on the biological activity of isoquinoline alkaloids in connection with AD. My work focuses on complete chromatographic studies and the isolation of Amaryllidaceae alkaloids from *Narcissus pseudonarcissus* cv. Carlton. The study continued with the preparation of compounds structurally inspired by minor Amaryllidaceae alkaloids. Compounds have been tested for biological activities related to the potential treatment of neurodegenerative diseases or mycobacterial diseases.

4.1. Amaryllidaceae alkaloids of belladine-type from *Narcissus pseudonarcissus* cv. Carlton as new selective inhibitors of butyrylcholinesterase[13]

Narcissus pseudonarcissus cv. Carlton is alkaloid containing plant species under the Amaryllidaceae family. Based on preliminary screening, this cultivar has been selected for a detailed phytochemical study. The cultivar Carlton is cultivated for the commercial isolation of galanthamine, because of its high concentration in the bulbs, large bulb size, and their availability in large amount. Galanthamine is reported to be the major alkaloid of *Narcissus pseudonarcissus*, followed by haemanthamine. Thirteen known (**1-12,** and **16**) and three new AAs (**13-15**) were isolated using common chromatographic methods from 30 kg of fresh bulbs of *Narcissus pseudonarcissus* cv. Carlton. The compounds were identified by MS, ESI-HRMS, 1D- and 2D-NMR spectroscopic methods and by comparison of the obtained data with the literature. These techniques led to the identification of lycosinine B (**1**) trisphaeridine (**2**), 3,4-anhydrogalanthamine (**3**), oduline (**4**), masonine (**5**), galanthamine (**6**), galanthine (**7**), lycorenine (**8**), lycoramine (**9**), homolycorine (**10**), haemanthamine (**11**), vittatine (**12**), carltonine A (**13**), carltonine B (**14**), carltonine C (**15**). and 9-O-demethylhomolycorine (**16**). The isolated alkaloids belong to the galanthindole (**1**), narciclasine (**2**), galanthamine (**3, 6, 9**), homolycorine (**4, 5, 8, 10, 16**), lycorine (**7**), and haemanthamine (**11, 12**) structural types; newly isolated alkaloids **13-15** belong to the belladine-type of AAs. This structural type was later named the carltonine type. It differs from the norbelladine-type with the presence of two or more atoms of nitrogen instead of one, and in the structure, we can find two overlapping fragments: belladine and lycosinine (Fig. 25). All isolated compounds that had not been previously studied for their inhibition potential of cholinesterases and which were obtained in sufficient amounts were screened for their *h*AChE/*h*BuChE and POP inhibition potency. The results are summarized in Table 10. Moreover, *in vitro* data were justified by docking studies proposing the orientation of the top-ranked ligands within the *h*BuChE gorge.

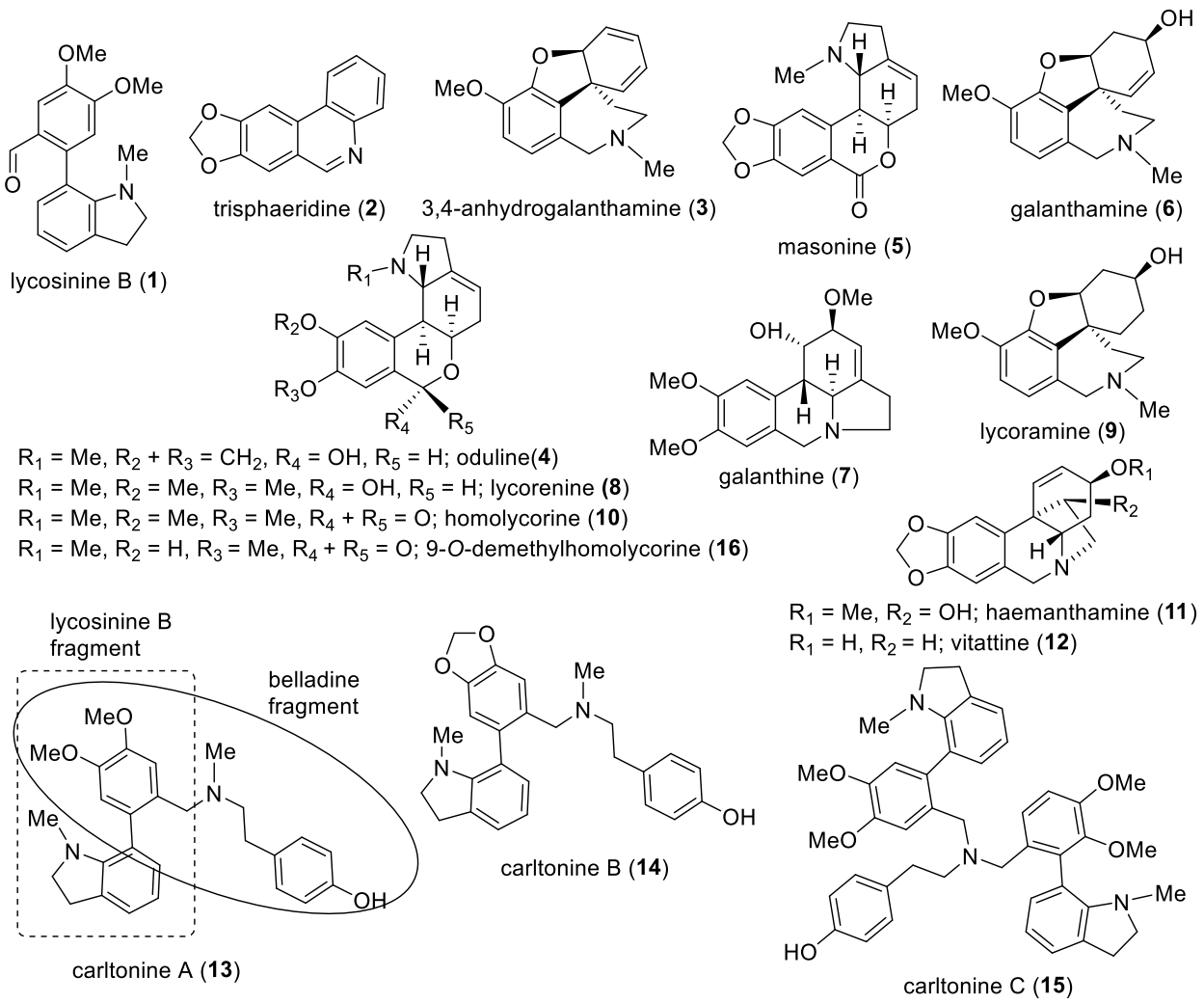


Fig. 25. Isolated alkaloids from *Narcissus pseudonarcissus* cv. Carlton

All newly isolated carltonine-type alkaloids (**13, 14,** and **15**) demonstrated promising inhibition activity toward *h*BuChE (IC_{50} = 0.91 ± 0.02 μM, 0.031 ± 0.001 μM, and 14.8 ± 1.1 μM, respectively). Carltonine A and carltonine B have the same structural skeleton, they differ only in the positions C-5′ and C-6′, respectively. Carltonine C has an additional lycosinine fragment in its structure. The presence of 1,3-dioxalone ring in carltonine B appears to be responsible for a 30-fold higher potency toward *h*BuChE inhibition activity with outstanding selectivity index value greater than 100 in compared to carltonine A.

Table 10. *In vitro* results of *h*AChE, *h*BuChE and POP inhibition of selected AAs isolated from *Narcissus pseudonarcissus* cv. Carlton

Compound	% inhibition *h*AChE ± SEM[a]	*h*AChE IC_{50} ±SEM (μM)[b]	% inhibition *h*BuChE ± SEM[a]	*h*BuChE IC_{50} ±SEM (μM)[b]	SI for *h*BuChE[c]	POP IC_{50} ± SD (μM)[b]
Lycosinine B (**1**)	28 ± 1	> 100	42 ± 1	> 100	nc	258 ± 14
Trispheridine (**2**)	6 ± 1	> 100	13 ± 1	> 100	nc	nm
3,4-Anhydrogalanthamine (**3**)	4 ± 0	> 100	28 ± 1	> 100	nc	nm
Carltonine A (**13**)	2 ± 0	> 100	98 ± 1	0.91 ± 0.02	>110	143 ± 12
Carltonine B (**14**)	40 ± 1	> 100	99 ± 1	0.031 ± 0.001	>3,226	nm
Carltonine C (**15**)	9 ± 0	> 100	78 ± 1	14.8 ± 1.1	>7	nm
Galanthamine[d]	nm	1.72 ± 0.12	nm	42 ± 1	0.04	nm
Eserine[d]	nm	0.063 ± 0.005	nm	0.13 ± 0.01	0.48	nm
Berberine[d]	nm	0.72 ± 0.11	nm	31 ± 4	0.02	142 ± 21

[a] Tested at 100 μM compound concentration; [b]Compound concentration required to decrease enzyme activity by 50%; the values are the mean values ± standard deviations (SD) of three independent measurements, each performed in triplicate; [c]Selectivity index (SI) for *h*BuChE is determined as ratio *h*AChE IC_{50}/*h*BuChE IC_{50}; [d]Reference compounds; nc stand for not calculated; nm stands for not measured.

The novel AAs have structural similarities with 6-*O*-demethylbelladine and 4′-*O*-demethylbelladine, alkaloids previously isolated from *Nerine bowdenii*[72]. Neither of these alkaloids has substitution at position C-7′ and differs from each other by the absence of one methoxy group (Fig 26). 4′-*O*-Demethylbelladine (IC_{50} = 30.7 ± 4.0 μM) displayed slightly better *in vitro* inhibition activity of *h*BuChE compared to galanthamine (IC_{50} = 42 ± 1 μM). On the other hand, compounds isolated within this study are more than 30 to 100 times more potent *h*BuChE inhibitors, producing a new structural lead scaffold, that can be used in AD research. Since some of the alkaloids were isolated only on a small scale, only two (**1** and **13**) were tested for POP inhibition. Carltonine A demonstrated POP inhibition in the same range as berberine (Table 10).

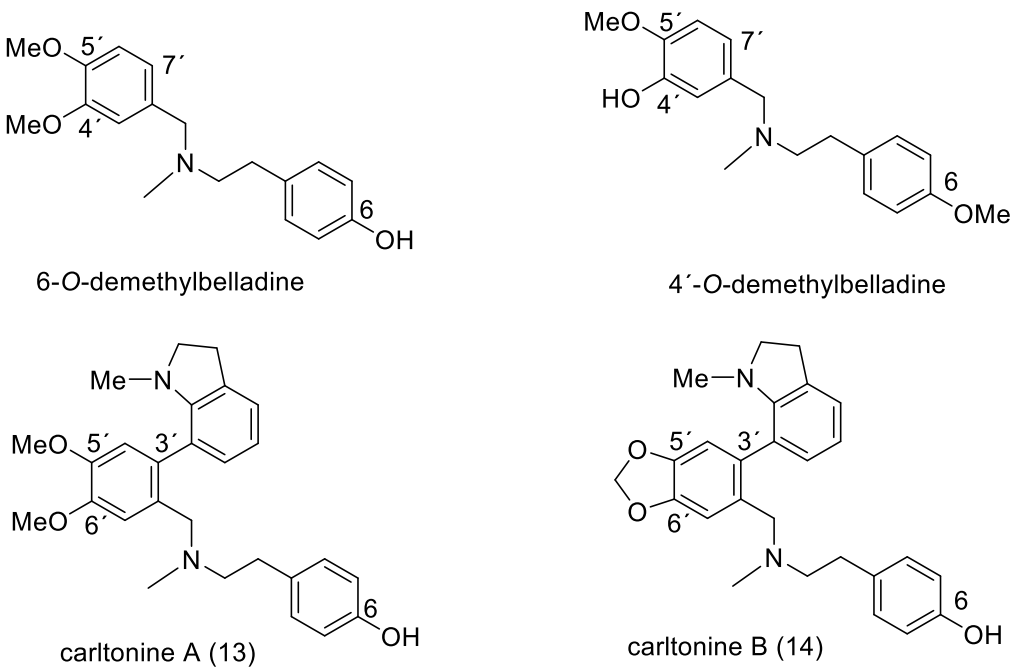


Fig. 26. Structures of newly isolated (**13** and **14**) and recently reported belladine-type AAs 6-*O*-demethylbelladine and 4′-*O*-demethylbelladine

Molecular docking studies were carried out to understand the fundamental interactions of carltonine A and carltonine B within the *h*BuChE active site (PDB ID: 4BDS), to gain more in-depth view of the overall ligand-enzyme complex, predict more bioactive conformers based on their docking energy score, and their topology within *h*BuChE (Fig. 27 and Fig. 28). The estimated binding scores of both compounds were calculated −10.6 kcal/mol and −10.9 kcal/mol. The *N*-methylindoline moiety of both compounds occupies the vicinity of the catalytic triad with a T-shaped π-π interaction close to Phe329 (5.0 Å). In carltonine B, 2H-1,3-benzodioxole moiety shows T-shaped π-π stacking (4.8 Å). Overall docking experiment indicates that inhibition potency could be attributed to the presence of the 2H-1,3-benzodioxole moiety for its extended aromatic properties. The new compounds have exerted an interesting biological profile that deserves further lead-optimization. The next step will be the development of an appropriate synthetic route leading to carltonine derivatives with the follow-up preparation of semi-synthetic derivatives.

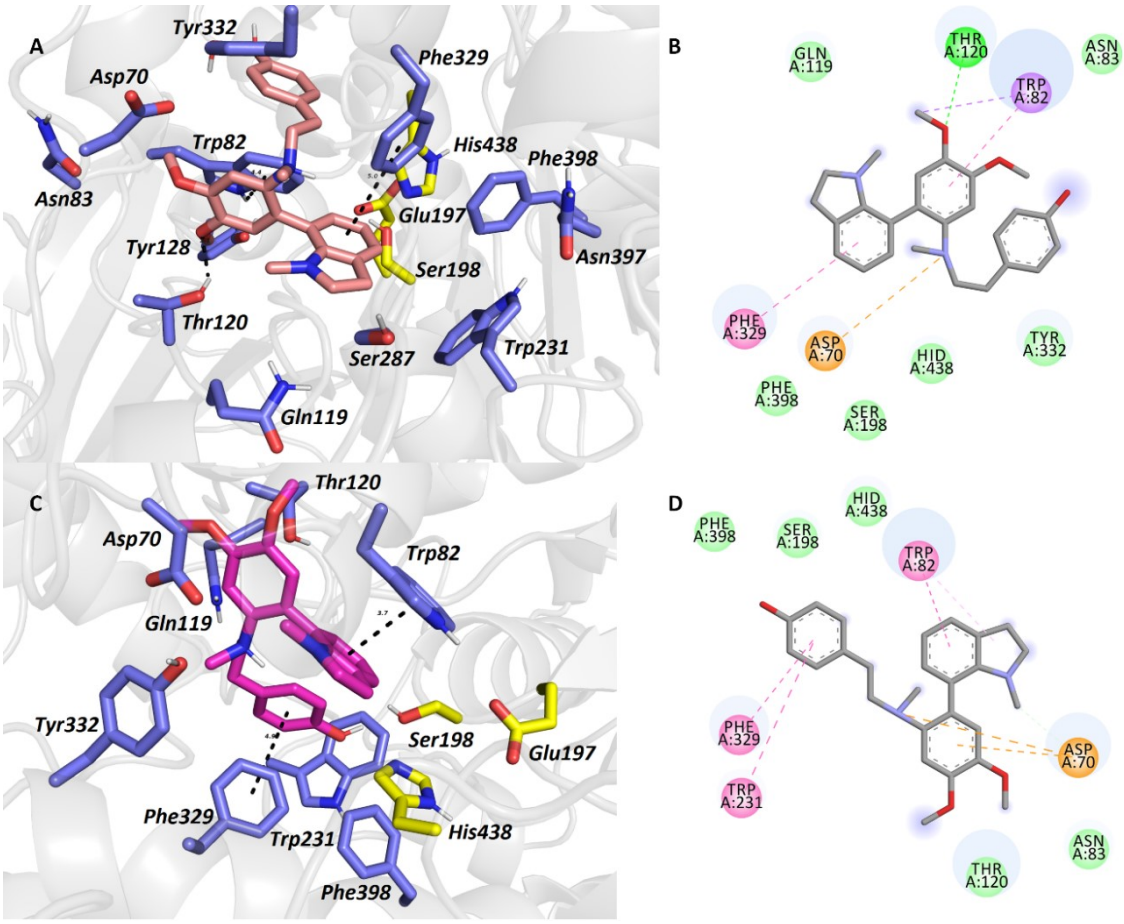


Fig. 27. *h*BuChE active site in complex with (*R*)-**13** (in salmon, A and B) and (*S*)-**13** (in purple, C and D) *pseudo*-enantiomers. In 2D diagrams (B and D), crucial amino acid residues are displayed in different colours depending on the nature of the interaction (e.g., purple for π-π, orange for anion-π, dark green for van der Waals contact, and light green for conventional hydrogen bond).

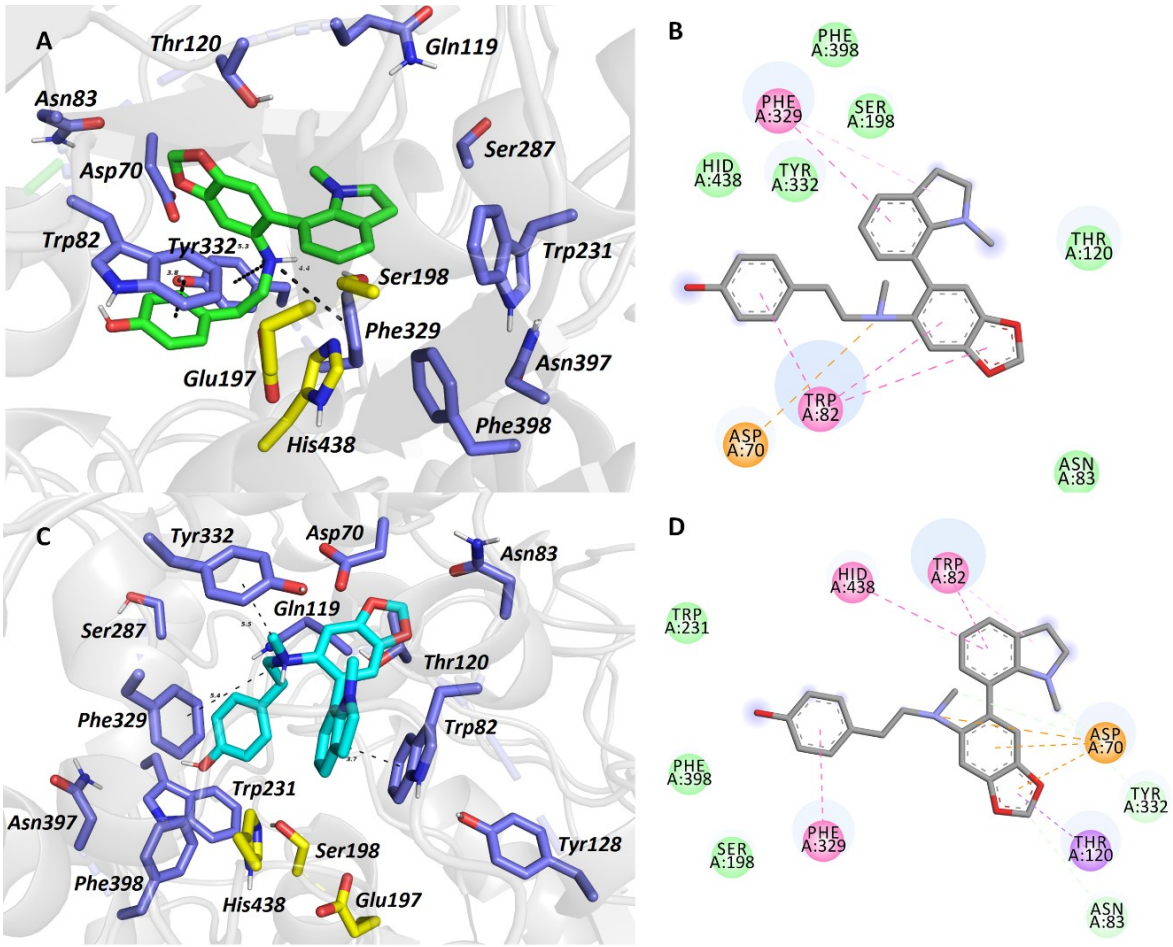


Fig. 28. *h*BuChE active site in complex with (*R*)-**14** (in green, A and B) and (*S*)-**14** (in light-blue, C and D) *pseudo*-enantiomers. In 2D diagrams (B and D), crucial amino acid residues are displayed in different colours depending on the nature of the interaction (e.g., purple for π-π, orange for anion-π, dark green for van der Waals contact, light green for conventional hydrogen bond).

4.2. Structure elucidation and cholinesterase inhibition activity of two new minor Amaryllidaceae alkaloids[63]

The continuation of the phytochemical study of *Narcissus pseudonarcissus* cv. Carlton led to the isolation of the next new AAs of narcikachnine-type, which has been named narciabduliine. This compound has been reported together with another new AA isolated from *Hippeastrum* × *hybridum* cv. Ferrari (Fig. 21) named 9-*O*-demethyllycorenine[233]. Because this alkaloid has been isolated by another Ph.D. student (dr. Latifah Al Shammari), the summary of this article will be concentrated only on narcibduliine. It has been isolated from the mother liquor (150 mg) of fraction VI using preparative TLC. Its chemical structure has been identified using 1D-, and 2D-NMR, CD, and HRMS techniques, and by comparison with data from the literature of other AAs of narcikachnine-type. It has been screened for its *h*AChE/*h*BuChE inhibition potency. Interestingly, this compound showed balanced inhibition activity against *h*AChE/*h*BuChE with IC_{50} values 3.24 ± 0.73 μM for *h*AChE, and 3.44 ± 0.02 μM for *h*BuChE, respectively. The closest match in the structure of narciabduliine and its biological activity was observed for narcieliine, recently isolated from *Zephyranthes citrina*[36]. Three methoxy groups of the 1,2,3,4,5-pentasubstituted benzene ring are present in the structure of the narcieliine, while narciabduliine contains one methoxy and one hydroxy group in 1,2,4,5-tetrasubstituted ring. These small changes in the structure are responsible for an increase in *h*AChE, and a small decline in *h*BuChE inhibition activity. However, other AAs of this group contain a benzo[d][1,3]dioxol moiety instead, and showed weak inhibition activity towards *h*AChE/*h*BuChE (Fig. 29).

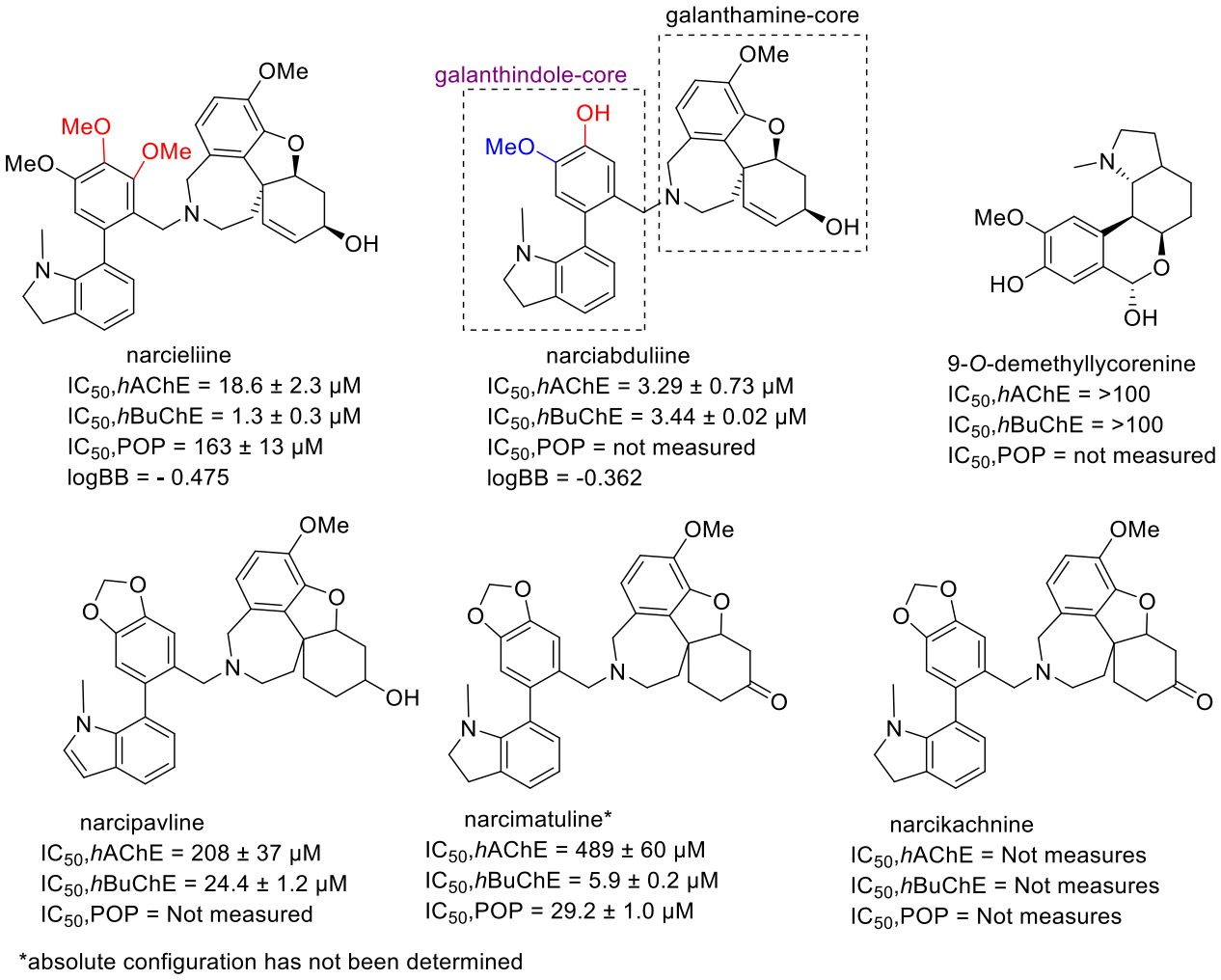


Fig. 29. Amaryllidaceae alkaloids isolated from *Narcissus pseudonarcissus* cv. Carlton and *Hippeastrum* × *hybridum* cv. Ferrari

A molecular modeling study of narciabduliine has been performed to determine the binding mode on the active side of the enzyme *h*AChE/*h*BuChE (Fig 30). The galanthindole moiety is bound to the catalytic anionic site (CAS), while the galanthindole core is lodged peripherally. The overall topology of narciabduliine in *h*AChE shares a high similarity to that of galanthamine, but the galanthindole fragment allowed the compound to be spanning into the peripheral anionic site (PAS) of the enzyme. On the other hand, the ligand adopted an inverse accommodation in the active site of *h*BuChE compared to that observed for the narciabduliine-*h*AChE complex. The critical interactions for the galanthindole moiety within the CAS region of *h*BuChE can be defined as follows: (i) distorted π–π stacking between the 1-methyl-2,3-dihydro-1Hindole moiety of narciabduliine and Trp82 (4.6 Å), (ii) hydrogen bond between oxygen from the methoxy group and hydrogen in the 1-methyl-2,3-dihydro-1H-indole moiety, (iii) two hydrogen bonds between the

phenolic hydroxyl functionality of narciabduliine and the glycine residues 116 and 117 (2.7 Å and 2.7 Å), and (iv) plausible hydrogen contact with Ser198 (3.1 Å) from the catalytic triad.

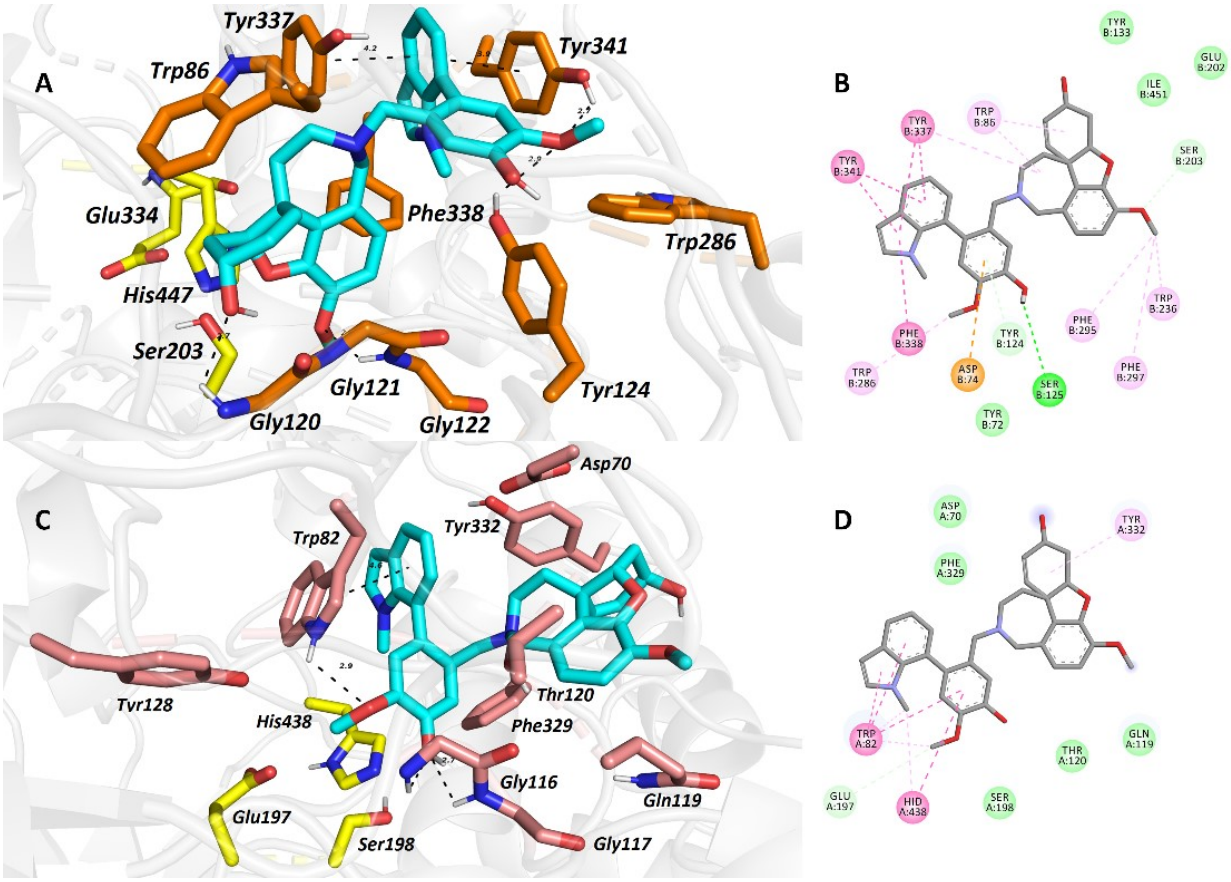


Fig. 30. The top-scored docking poses of narciabduliine at the active sites of *h*AChE (A, B; PDB ID: 4EY6) and *h*BuChE (C, D; PDB ID: 4BDS)

The galanthamine fragment has less impact on the overall ligand anchoring. The availability of narciabduliine in the CNS has been implicit by calculating the logBB value, which predicts the logarithmic ratio between the concentration of a compound in the brain (C_{brain}) and blood (C_{blood}). Narciabduliine has a logBB value of -0.362 which indicates the ability to reach the target area in the CNS. In conclusion, it can be deduced that narciabduliine represents an interesting structural scaffold of AAs with promising cholinesterase inhibition potential.

4.3. Amaryllidaceae alkaloids of norbelladine-type as inspiration for development of highly selective butyrylcholinesterase inhibitors: synthesis, biological activity evaluation, and docking studies[234]

This study was inspired by the structure of newly described AAs carltonine A and B isolated from *Narcissus pseudonarcissus* cv. Carlton, which demonstrated strong selective *h*BuChE inhibition potency[13]. Unfortunately, these compounds are present in plants only in trace concentration, therefore, we decided to synthesize a pilot series of compounds (**1-20**) that preserve some of the crucial structural requirements of carltonine A/B, that are plausibly responsible for the high inhibition activity of *h*BuChE, i.e. the 4-[2-(benzylamino)ethyl]phenol moiety. We also modified other molecular regions to elucidate detailed SAR study. Specifically, we were interested in a) the role of the secondary or tertiary amino group (the presence of allyl group), b) the etherification of the phenolic hydroxyl group in aromatic ring B, and c) position of alkoxy or aryloxy substituents in benzene ring A (Fig. 31), all concerning the cholinesterase inhibitory activity. All developed compounds have been screened for their *in vitro h*AChE/*h*BuChE inhibition activity and selected compounds also for their POP inhibition potency (Table 11). The hit compound **1** was synthesized from commercially available *O*-benzylvanillin and tyramine with excellent yield (95%). Different structural modifications were explored using the condensation of *O*-benzylvanillin, *O*-benzylisovanillin, and 3-ethoxy-4-methoxybenzaldehyde with primary amines such as tyramine, 2-phenylethan-1-amine, and 2-(4-methoxyphenyl)ethan-1-amine, to elucidate the SAR of the synthesized compounds (Fig. 32). The conditions of the reductive amination furnished the desired secondary amines **2, 7, 8, 11, 12, 15** and **18** in good yields. Subsequent reaction of the prepared compounds with an excess of allyl bromide provided the corresponding *N*-allyl derivatives **3, 4, 9, 10, 13, 14, 16,** and **19,** respectively. In addition to this allylation, compound **18** also underwent another alkylation to give the *N*-benzyl derivative **20.** Further nucleophilic substitution with additional allyl bromide was performed on the phenolic group of compounds **3, 4** and **16** to obtain allyl ethers **5, 6** and **17**.

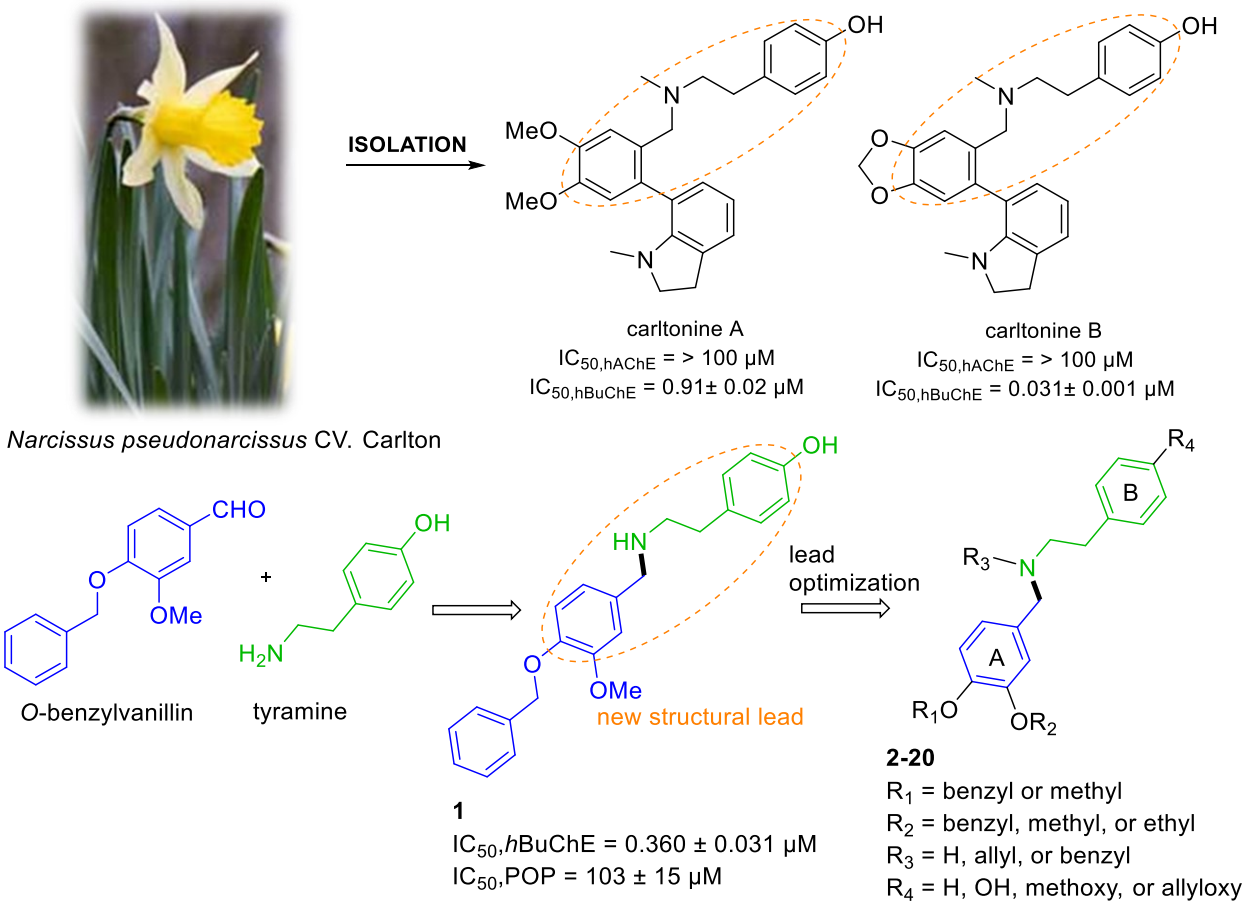


Fig. 31. Design of novel selective butyrylcholinesterase inhibitors derived from hit compound **1**

All developed compounds (**1-20**) were screened for *h*AChE/*h*BuChE inhibition potency according to a slightly modified Ellman's method for the determination of their IC_{50} values. All compounds have shown only weak to moderate *h*AChE inhibition potency (IC_{50} > 20 µM) and were selective toward *h*BuChE. Indeed, all the novel compounds revealed *h*BuChE inhibition potency in the micromolar to low nanomolar range. Compound **5** was the only one to show no inhibition potency against either enzyme. Compound **6**, originating from *O*-benzylisovanillin and tyramine with two allyl substitutions, was the most pronounced *h*BuChE inhibitor (IC_{50} = 72 ± 5 nM). Most strikingly, **6** emerged as the most selective *h*BuChE with a SI value of almost 1400. Surprisingly, its close topological derivative **5** was completely inactive in the *h*BuChE assay. The inhibition ability of compound **1** (IC_{50} = 0.36 ± 0.03 µM) has been gradually decreased by *O*-allyl/*N*-allyl substitution, which is observed in compounds **3** (IC_{50} = 0.61 ± 0.04 µM) and **5** (IC_{50} > 100 µM). The inhibition potency associated with the *h*BuChE enzyme is gradually reduced by structural modifications in

position C-4′ of compound **1** with hydroxy/methoxy substitution, for example, compound **7** (1.28 ± 0.05 μM) and **11** (2.39 ± 0.27 μM), respectively, were the inhibition potency.

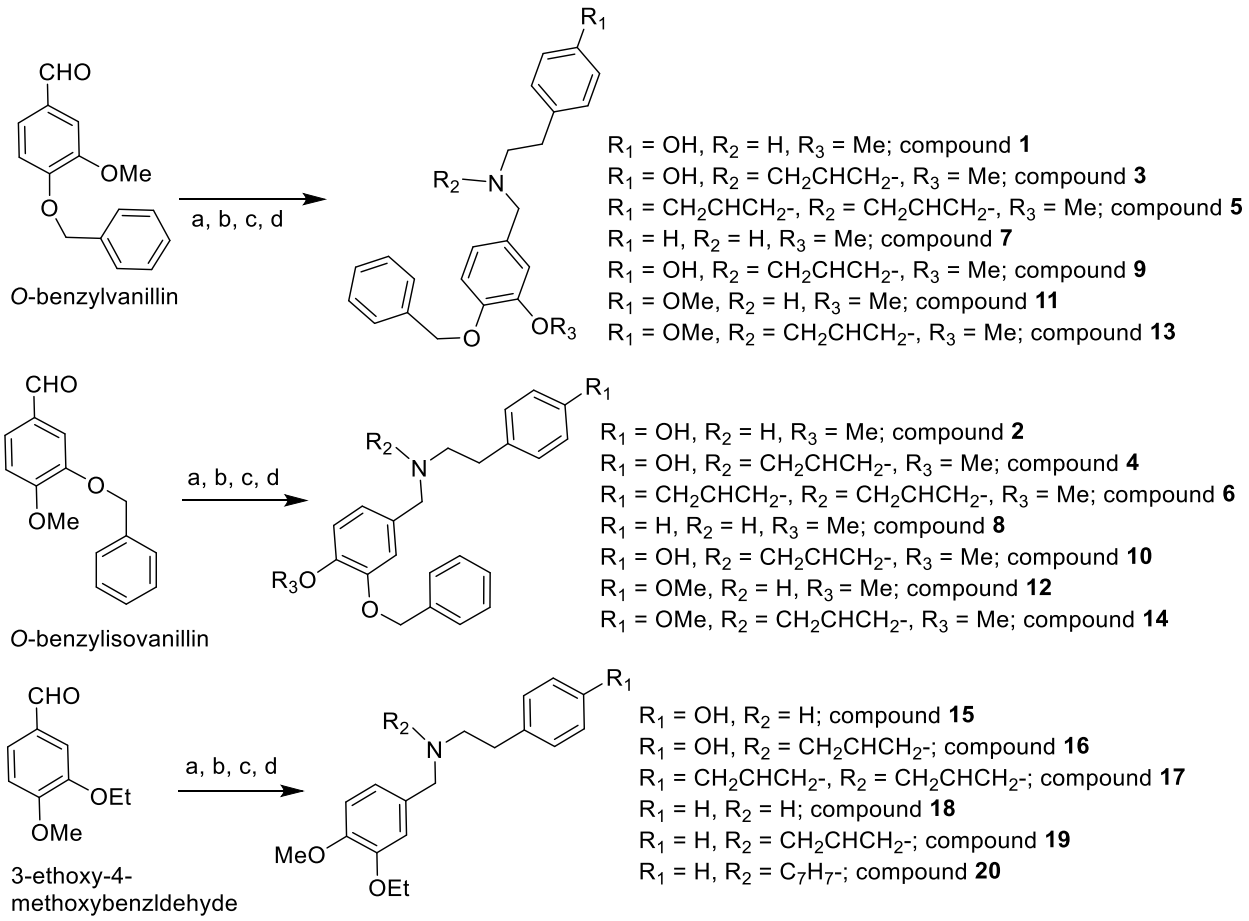


Fig. **32**. Synthesis of novel *h*BuChE inhibitors; starting from *O*-benzylvanillin/*O*-benzylisovanillin/3-ethoxy-4-methoxybenzaldehyde; reagents and conditions: a: 1) tyramine, MeOH; 2) NaBH4, rt, 3 h; b: allyl bromide (1.3 eq.), NaH (1.2 eq.), THF; c: 1) 2-phenylethan-1-amine, MeOH; 2) NaBH4, rt, 3 h; d: 1) 2-(-4-methoxyphenyl)ethan-1-amine/ benzyl bromide, MeOH; 2) NaBH4, rt, 3 h

A similar effect was observed in the series of compounds derived from *O*-benzylisovanillin (compounds **2**, **8** and **12** with IC_{50} values of IC_{50} = 0.29 ± 0.02 μM, 1.10 ± 0.05 μM, and 1.12 ± 0.11 μM, respectively). In the series derived from 3-ethoxy-4-methoxybenzaldehyde (**15-20)**, compound **20** (IC_{50} = 0.69 ± 0.03 μM) was classified as the highest ranked *h*BuChE inhibitor. The selected compounds were screened for POP inhibition activity. Unfortunately, the low solubility of the compounds tested in the buffer allowed the determination of IC_{50} only for compound **1**. This compound showed slightly lower POP inhibition potency (IC_{50} = 186 ± 14 μM) in compared to used standard POP inhibitor berberine (IC_{50} = 142 ± 21 μM). The results of the *in vitro* *h*AChE/*h*BuChE, and POP activities of the developed compounds are summarized in Table 11.

Table 11: *In vitro h*AChE/*h*BuChE, and POP inhibition of synthetic compounds and calculation of the BBB score.

Compound	%inhibition *h*AChE ± SEM[a]	IC_{50}, *h*AChE ± SEM (μM)[b]	% inhibition *h*BuChE ± SEM[a]	IC_{50}, *h*BuChE ± SEM (μM)[b]	SI for *h*BuChE[c]	IC_{50}, POP ± SEM (μM)[b]	BBB score[d]
1	30.4 ± 2.1	> 100	98.7 ± 0.3	0.36 ± 0.03	> 277	103 ± 15	4.53
2	35.8 ± 1.2	> 100	97.7 ± 0.5	0.29 ± 0.02	> 348	> 79[f]	4.53
3	20.8 ± 0.9	> 100	96.8 ± 1.1	0.61 ± 0.04	> 163	> 200[f]	4.79
4	45.2 ± 2.4	> 100	97.9 ± 0.6	0.25 ± 0.01	> 394	> 79[f]	4.79
5	3.4 ± 0.5	> 100	38.9 ± 0.9	> 100	-	n.s.	4.87
6	10.1 ± 0.6	> 100	98.6 ± 0.9	**0.07 ± 0.01**	> 1,389	> 79[f]	4.87
7	23.4 ± 2.5	> 100	94.5 ± 0.9	1.28 ± 0.05	> 78	n.s.	5.15
8	12.6 ± 0.5	> 100	96.6 ± 0.4	1.10 ± 0.05	> 90	n.s.	5.15
9	18.8 ± 1.9	> 100	74.9 ± 2.4	5.19 ± 0.28	> 19	n.s.	5.04
10	72.4 ± 1.1	21.5 ± 0.6	92.0 ± 2.4	1.17 ± 0.04	18	n.s.	5.04
11	27.9 ± 0.7	> 100	93.5 ± 0.3	2.39 ± 0.27	> 41	n.s.	4.87
12	0.0 ± 0.0	> 100	94.6 ± 0.6	1.12 ± 0.11	> 89	n.s.	4.87
13	32.7 ± 1.6	> 100	90.9 ± 1.5	2.72 ± 0.50	> 37	n.s.	4.96
14	60.9 ± 0.4	37.7 ± 1.7	95.8 ± 0.9	0.38 ± 0.01	98	> 200[f]	4.96
15	25.8 ± 1.3	> 100	75.3 ± 0.6	15.06 ± 2.34	> 6	n.s.	4.80
16	28.3 ± 1.1	> 100	91.7 ± 0.4	1.21 ± 0.08	> 82	n.s.	5.21
17	29.3 ± 3.9	> 100	77.0 ± 1.0	9.89 ± 1.37	10	n.s.	5.39
18	0.0 ± 0.0	> 100	60.0 ± 1.6	41.1 ± 2.6	> 2	n.s.	5.53
19	5.9 ± 2.1	> 100	80.2 ± 0.2	4.63 ± 0.48	> 22	n.s.	5.60
20	49.5 ± 0.8	> 100	82.5 ± 1.0	0.69 ± 0.03	> 145	n.s.	5.13
galanthamine[e]	98.8 ± 1.1	2.0 ± 0.1	68.2 ± 1.2	29.31 ± 3.49	0.07	n.s.	5.01
eserine[e]	99.8 ± 0.6	0.20 ± 0.01	99.9 ± 0.5	0.30 ± 0.01	0.67	n.s.	5.02
berberine[e]	-	-	-	-	-	194 ± 14	n.s.
chlorothiazide[e]	-	-	-	-	-	-	2.14
promazine[e]	-	-	-	-	-	-	5.64

[a]Tested at 100 μM compound concentration; [b]Compound concentration required to decrease enzyme activity by 50%; the values are the mean ± SEM of three independent measurements, each performed in triplicate; [c]Selectivity index for *h*BuChE is determined as ratio *h*AChE IC_{50}/*h*BuChE $IC_{50;}$ [d]calculated using BBB score [235]; [e]Reference compound; [f]Due to low solubility of compounds in buffer, the presented values correspond to the highest tested concentration; n.s. stands for not studied

Because brain exposure is a critical factor in the design of novel AD drugs, we have applied *in silico* calculation of the so-called blood-brain barrier (BBB) score. All compounds with BBB score

values greater than 4.0 are assumed to enter the CNS area. All studied compounds (**1-20**) can reach in the target area.

The most active compound **6** was subjected to enzyme kinetics analysis to investigate the binding mode and compound **6** was identified as reversible inhibitor of the *h*BuChE enzyme. Furthermore, *in silico* experiments concluded that the higher inhibition ability of compound **6** is due to the nicely fil benzyloxy group in the active gorge and makes crucial interactions with amino acid residues. Compound **6** was also subjected to analysis of cytotoxicity in the human neuroblastoma SH-SY5Y cell line and the inhibition potency of monoamine oxidase (MAO) was further determined. The compound was completely devoid of cytotoxicity and demonstrated only weak MAO-A and MAO-B inhibition activity.

4.4. Semisynthetic derivatives of selected Amaryllidaceae alkaloids as a new class of antimycobacterial agents[17]

The current study was inspired by previously reported structural modification of haemanthamine, ambelline, and vittatine[233, 236, 237], which showed promising inhibition potency of cholinesterases. Therefore, some of the previously synthesized and newly synthesized derivatives of AAs were studied in terms of their antimycobacterial activity. Within study, nineteen derivatives of galanthamine (**1a**-**1s**), seven derivatives of 3-*O*-methylpancracine (**3a**-**3g**), two derivatives of vittatine (**4a**-**4b**) and two derivatives of maritidine (**5a**-**5b**) were tested for their antimycobacterial potential. Derivatives of 3-*O*-methylpancracine, vittatine, and maritidine have been synthesized by my colleagues, summary of this article focus only on galanthamine derivatives (Fig 33). Nineteen new derivatives (**1a**-**1s**) of galanthamine (**1**) have been prepared by derivatization of the free hydroxyl group at position C-6 with yield >52% (Fig 33). Their structures were determined by MS, HRMS and 1D- and 2D-NMR spectroscopic techniques.

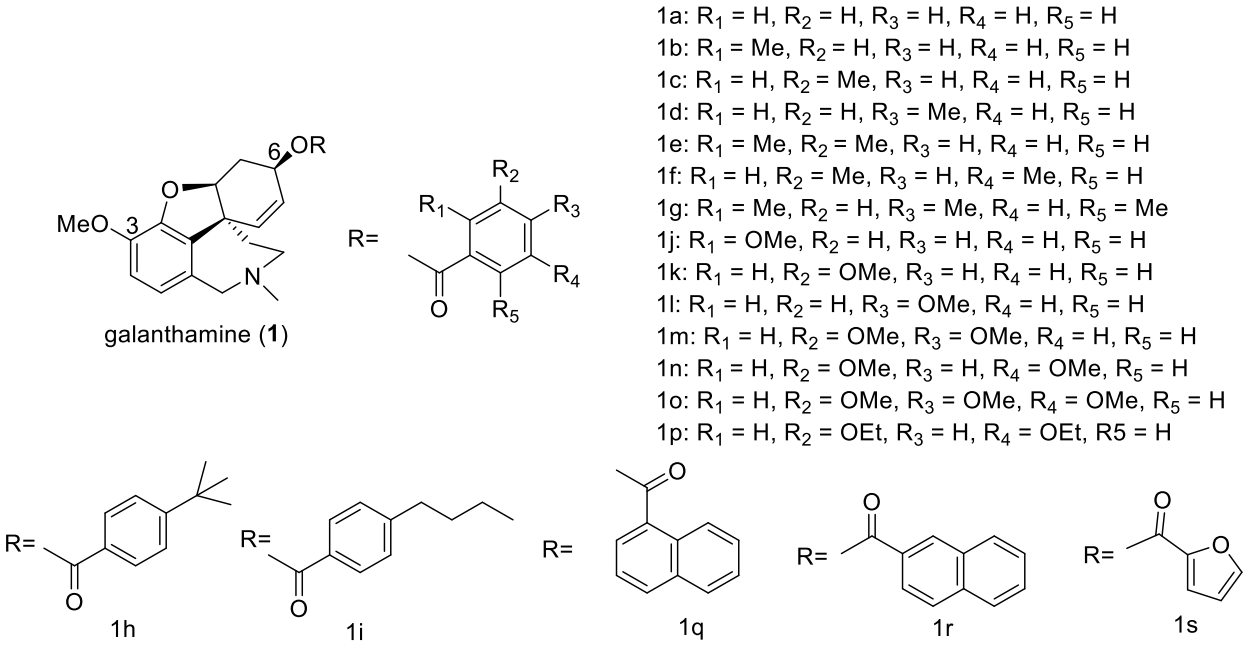


Fig. 33. Galanthamine derivatives (**1a**-**1s**); reagents and conditions; starting from galanthamine. Corresponding acyl chloride (2-4 eq.), 4-dimethylaminopyridine (catalytic amount), pyridine, 80ºC, 24h, rt.

Galanthamine derivatives (**1a**-**1s**) were tested for their antimycobacterial activity against three different *Mycobacterium* strains: *Mycobacterium tuberculosis* H37Ra, *Mycobacterium aurum,* and *Mycobacterium smegmatis*. The most potential antimycobacterial activity was demonstrated by derivatives with isobutyl- (**1h**) or butyl-chain (**1i**) in the *para* position on the benzene ring. The carbon chain present in the *para* position of the benzene ring is responsible for a substantial increase of antimicrobial activity. Both compounds showed activity against all studied strains with MICs values of 3.125-7.81 µg/mL and 1.56-7.81 µg/mL, respectively. *Mycobacterium tuberculosis* H37Ra was the most sensitive strain, which showed activity with MIC 3.125 µg/mL (6.9 µM) for 6-*O*-(4-tert-butylbenzoyl)galanthamine (**1h**), and 1.56 µg/mL (3.5 µM) for 6-*O*-(4-butylbenzoyl)galanthamine (**1i**). The shortening of the hydrocarbon chain in the *para* position on the aromatic ring was associated with a decrease in antimycobacterial activity as in 6-*O*-(4-methylbenzoyl)galanthamine (**1d**, MIC = 15.625 µg/mL; 35.4 µM). Therefore, it can be deduced that a longer chain in position *para* on the aromatic ring is connected with a better antimycobacterial activity. Furthermore, naphthoyl derivatives of galanthamine (**1q** and **1r**) have also shown interesting activity against all studied *Mycobacterium* strains with MICs of 6.25-7.81 µg/mL for 6-*O*-(1-naphthoyl)galanthamine (**1q**), and 1.98-7.81 µg/mL for 6-*O*-(2-naphthoyl)galanthamine (**1r**).

Furthermore, the most active derivatives were evaluated for *in vitro* cytotoxicity in hepatocellular carcinoma cells (HepG2) by using MTT assay, which allowed calculation of selectivity indexes (SI), as the ratio of $IC_{50,\ HepG2}$ to MIC of *Mycobacterium tuberculosis* H37Ra. A SI value greater than 10 indicates more acceptable toxicity, because antitubercular drugs are known to have a risk of hepatotoxicity. Active compounds were screened at an initial concentration of 50 μM, and the IC_{50} values were subsequently determined. The most active derivatives in the antimycobacterial assay (**1i** and **1r**) have shown cytotoxicity with IC_{50} values of 14.7 ± 1.6 μM and 21.2 ± 3.8 μM, reaching SI values of 4.20 and 5.17, respectively, which can result in a potential risk of hepatotoxicity. Therefore, a subsequent structural optimization is required.

4.5. Recent progress on biological activity of Amaryllidaceae and further isoquinoline alkaloids in connection with Alzheimer's Disease[16]

This review summarizes recent progress on AAs and additional isoquinoline alkaloids (IAs), and biological activities related to AD reported between 2010 and 2021. For the determination of biological activity, different research groups used various types of enzymes within biological assays, e.g. electric eel acetylcholinesterase (*Ee*AChE), human acetylcholinesterase (*h*AChE), mouse brain acetylcholinesterase, equine serum BuChE (*Eq*BuChE), and human BuChE (*h*BuChE). It is necessary to mention that the use of different types of enzymes can result in dramatic differences in the obtained IC_{50} values. For example, acetylcaranine has been observed to have interesting activity in *Ee*AChE (IC_{50} = 11.7 ± 0.7 μM), but it demonstrated weak inhibition activity when *h*AChE has been used (IC_{50} = 443.7 ± 62.4 μM)[238]. A similar phenomenon has been observed for 1-*O*-acetyllycorine. It was reported as a strong inhibitor when *Ee*AChE (IC_{50} = 0.96 ± 0.04 μM) has been used in the assay, but inactive against *h*AChE (IC_{50} = > 1000 μM)[72, 80]. The best inhibition potency of AChE among recently isolated AAs has been demonstrated by *N*-norgalanthamine (IC_{50}, *Ee*AChE = 2.76 ± 0.56 μM) and 11-hydroxygalanthamine (IC_{50}, *Ee*AChE = 3.04 ± 0.61 μM), which were isolated from *Pancratium maritimum* and *Lycoris longituba*, respectively. Interesting inhibition activity of AChE has been shown by *N*-methylcrinasiadine (*Ee*AChE, IC_{50} = 4.23 ± 1.13 μM), which has been isolated from *Lycoris longituba* (Fig 33). Carltonine A and B demonstrated the strongest inhibition activity against *h*BuChE enzymes with an IC_{50} value of 0.913 ± 0.020 μM and 0.031 ± 0.001 μM respectively (Table 10). As mentioned previously, they have been isolated from *Narcissus pseudonarcissus* cv. Carlton. Carltonine A also

showed POP inhibition ability with IC_{50} value of 143 ± 21 μM, which has similar value as berberine (IC_{50} = 142 ± 21 μM), a recognized natural POP inhibitor[13]. Furthermore, a narcikachnine-type alkaloid, narciabduliine isolated from *Narcissus paseudonarcissus* cv. Carlton showed dual inhibition ability against *h*AChE (IC_{50} = 3.29 ± 0.73 μM) and *h*BuChE (IC_{50} = 3.44 ± 0.02 μM) (Fig 29)[63]. The strongest *h*BuChE inhibition ability among all narcikachnine-type AAs has been shown by narcieliine (IC_{50} = 1.3 ± 0.3 μM), which was isolated from *Zephyranthes citrina* (Fig 29)[36]. Furthermore, this compound was able to inhibit POP (IC_{50} = 163 ± 13 μM). Narcimatuline isolated from *Narcissus pseudonarcissus* cv. Dutch Master has been reported as a multitarget candidate[19, 239]. It showed inhibition activity towards *h*BuChE (IC_{50} = 5.9 ± 0.2 μM), GSK-3β (IC_{50} = 20.7 ± 2.4 μM), and POP (IC_{50} = 29.2 ± 1.0 μM) (Fig 28). Furthermore, compounds of lycorine-, and homolycorine-type, namely: caranine, 9-*O*-demethylgalanthine, masonine, and 9-*O*-demethylhomolycorine, showed interesting GSK-3β inhibition activity with IC_{50} values of 30.75 ± 0.04 μM, 50.9 ± 8.9 μM, 27.81 ± 0.05 μM, and 30.01 ± 0.04 μM, respectively[239]. 9-*O*-Demethylgalanthine also showed POP inhibition activity (IC_{50} = 150 ± 20 μM)[240].

Several IAs with interesting biological activities have been isolated in the past decades. Examples of IAs with biological activity connected with AD are showed in Fig. 34. Alkaloids, nitidine and avicine isolated from *Zanthoxylum rigidum* and chelerythrine from *Chelidonium majus,* have been reported as a multitarget candidates with promising inhibition activity against *h*AChE (IC_{50}, 0.52 ± 0.05 μM, 1.25 ± 0.09 μM, and 1.54 ± 0.07 μM), *Ee*BuChE (0.88 ± 0.08 μM, 5.73 ± 0.60 μM, and 6.33 ± 0.93 μM), and MAO-A (IC_{50}, 0.41 ± 0.02 μM, 1.89 ± 0.17 μM, and 0.55 ± 0.04 μM), respectively[16]. Furthermore, these compounds showed significant $A\beta_{1\text{-}42}$ anti-aggregation activity with IC_{50} values of 5.56 ± 0.94 μM, 1.89 ± 0.40 μM, and 4.20 ± 0.43 μM, respectively[16]. From kinetic studies, it has been revealed that avicine and nitidine are reversible-mixed inhibitors of both cholinesterases. The chelerythrine and 6-ethoxydihydrochelerythrine alkaloids 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. Sanguinarine isolated from *Corydalis saxicola* demonstrated *Ee*AChE inhibition activity with an IC_{50} value of 1.93 ± 0.01 μM[241]. 6-Ethoxydihydrosanguinarine isolated from *Chelidonium majus* inhibited both *h*AChE/*h*BuChE enzymes with an IC_{50} value of 3.25 ± 0.24 μM and 4.51 ± 0.31 μM[16, 242]. A bisbenzylisoquinoline alkaloid aromoline showed potential activity against the *h*BuChE enzyme with an IC_{50} value of 0.82 ± 0.10 μM[16]. Aromoline also exhibited a POP inhibition activity of IC_{50} 189 ± 32 μM, which is the same as the activity of berberine. Overall,

the presented review showed that AAs and IAs are compounds with promising neuropharmacological properties for further exploration and optimization of structure as multi-target directed drugs for AD treatment.

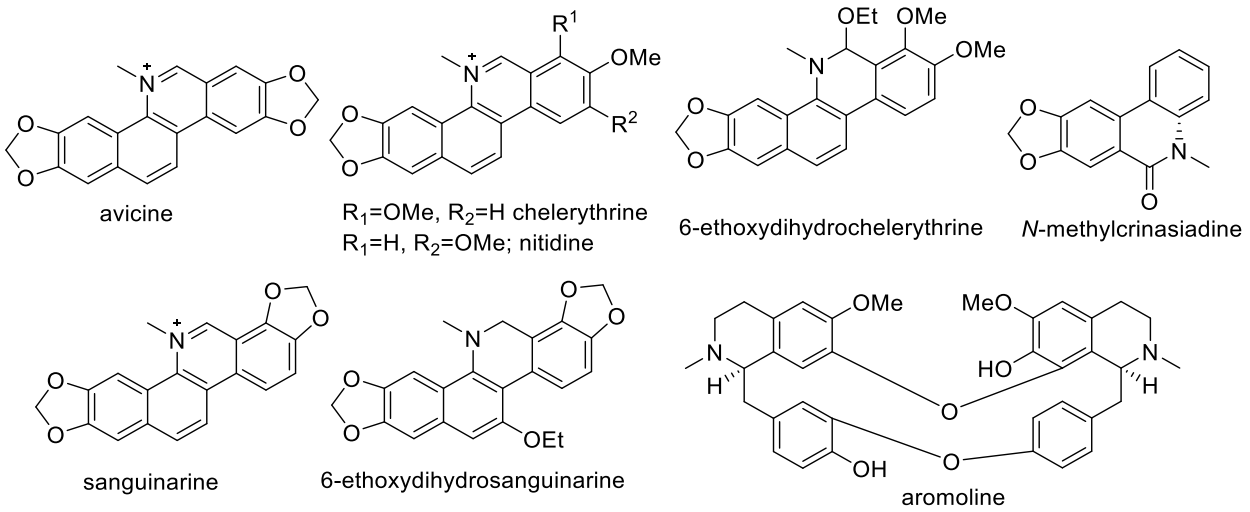


Fig. 34. Examples of isoquinoline-type alkaloids isolated from natural plants

5. CONCLUSION

Plants of the genus *Narcissus* (Amaryllidaceae) have been shown to be a promising source of Amaryllidaceae alkaloids, which are derived from amino acid tyrosine within the norbelladine pathway. Many species of *Narcissus* have been widely used as primary health remedies by indigenous peoples for many centuries[13, 19, 21]. AAs research is focused mainly on their isolation from natural sources, identification, and subsequent screening of a wide range of biological activities, such as inhibition of enzymes related to AD (*h*AChE, *h*BuChE, POP, GSK3β, and BACE1), oncological disease (cytotoxicity on panel of cancerous cell lines), microbial diseases, etc. Galanthamine is the well-known AA, which is still used in the therapy of AD as a long-acting, selective, reversible, and competitive inhibitor of *h*AChE. This alkaloid was approved by the FDA in 2001 under the commercial name Reminyl® for the treatment of mild to moderate stages of AD[69]. In many places in Europe, galanthamine is commercially isolated from the plant of *Narcissus pseudonarcissus* cv. Carlton. [13, 63].

In this Commentary, we emphasize the isolation and identification of alkaloids from the *Narcissus pseudonarcissus* cv. Carlton (30 kg of fresh bulbs). Four new and thirteen known alkaloids have been isolated, and their structures have been identified by spectroscopic techniques (1D, 2D-NMR, CD, and HRMS) and compared with the data from the literature. Three novel belladine-type alkaloids named carltonine A, B, and C have been isolated. In the structure of carltonine A and B, the lycosinine fragment is embedded with 4-hydroxyphenethylamine, while carltonine C has an additional lycosinine fragment in its structure. A novel heterodimeric AA of narcikachnine-type named narciabduliine has been also isolated from this plant. This structure consists of a galanthamine moiety combined with a galanthindole core. All alkaloids isolated in adequate amounts were tested for biological activity related to AD (inhibition activity against the *h*AChE, *h*BuChE, and POP enzymes). Carltonine A and B alkaloids exhibited selective *h*BuChE inhibition potency with IC_{50} values of 913 ± 20 nM and 31 ± 1 nM, respectively[13]. Carltonine C showed only moderate inhibition activity of *h*BuChE with an IC_{50} value of 14.8 ± 1.1 μM. In addition, carltonine A showed the POP inhibition potency with IC_{50} value of 143 ± 12 μM, which is the same as berberine (a recognized natural POP inhibitor)[13]. The heterodimer alkaloid narciabduliine inhibited both *h*AChE and *h*BuChE with IC_{50} values of 3.24 ± 0.73 μM, and 3.44 ± 0.02 μM[63]. Carltonine-

type alkaloids are present in plant materials in a trace amount. For this reason, alkaloids were taken as inspiration for the development of a pilot series of synthetic derivatives (**1-20)**, and their biological activity related to AD was evaluated (*in vitro h*AChE, *h*BuChE, and POP inhibition). Twenty derivatives have been synthesized from commercially available *O*-benzylvanillin, *O*-benzylisovanillin, and 3-ethoxy-4-methoxybenzaldehyde, attached to different amins (tyramine/2-(4-methoxyphenyl)ethan-1-amine/2-phenylethan-1-amine). The compound **6** exhibited the strongest inhibition potency against *h*BuChE with IC_{50} value of 72 nM[234]. Moreover, compound **6** was subjected to enzyme kinetic analysis and an *in silico* study*,* which concluded that it bind to the active site of *h*BuChE in reversible mode. The greater inhibition ability was attributed to composition of its benzyloxy group and subsequent revealing several crucial interactions with the enzyme. In addition, CNS availability was calculated by applying the BBB score and assuming that compounds can pass through the BBB.

A large amount of galanthamine (26.0 g) has been isolated from *Narcissus pseudonarcissus* cv. Carlton, which allowed us to prepare semi-synthetic derivatives by substituting its free hydroxyl group in position C-6. Their synthetic preparation has been inspired by our previous work[236, 237]. All prepared derivatives were evaluated for anticholinesterases activity, but all were found to be inactive. Therefore, their initial screening of antimycobacterial activity against three different *Mycobacterium* strains (*Mycobacterium tuberculosis H37Ra*, *Mycobacterium aurum,* and *Mycobacterium smegmatis)* was carried out. Interestingly, all compounds showed significant antimycobacterial activity against all studied *Mycobacterium* strains (MIC = 1.56-62.5 µg/mL). *Mycobacterium tuberculosis* H37Ra was the most sensitive strain. The strongest activity demonstrated by 6-*O*-(4-butylbenzoyl)galanthamine with and 6-*O*-(2-naphthoyl)galanthamine with MIC values of 1.56 µg/mL and 1.98 µg/mL, respectively. The *in vitro* cytotoxicity of the compounds was tested in hepatocellular carcinoma cells (HepG2) using MTT assay. Both analogues were found to be a potential risk of hepatotoxicity. Further optimization of the structure is required to improve antibacterial activity and reduce cytotoxicity.

It can be concluded that *Narcissus pseudonarcissus* cv. Carlton is a rich source of AAs with various important biological activities and is promising for further phytochemical investigations, which could lead to the isolation of new structure types of AAs. Moreover, a large quantity of galanthamine and haemanthamine have been isolated from this plant, which allows us to develop further semisynthetic analogs for detailed structural activity relationship study.

6. ABSTRACT

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Department of Pharmacognosy and Pharmaceutical Botany

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Title of Doctoral Thesis: Amaryllidaceae alkaloids of the genus *Narcissus* and their biological activity.

Narcissus pseudonarcissus cv. Carlton has been chosen for the phytochemical investigation based on the screening study. An alkaloidal extract of 485 g has been obtained from 30 kg of fresh bulbs. Repeated liquid-liquid extraction gave 187 g of concentrated crude extract, which was separated by column chromatography (CC, Al_2O_3; 5800 g), followed by repetitive CC, preparative TLC, and crystallization. Thirteen previously described Amaryllidaceae alkaloids were obtained along with four novel compounds named carltonine A, B, C, and narciabduliine. All compounds were identified and characterized by spectrometric techniques (1D and 2D NMR, CD, and HRMS) and by comparison with data from the literature. Alkaloids isolated in sufficient amounts were used for further evaluation of their inhibition activity against human acetylcholinesterase (*h*AChE), butyrylcholinesterase (*h*BuChE) and prolyloligopeptidase (POP). Carltonine A and B demonstrated promising inhibition activity against the *h*BuChE enzyme with IC_{50} values of 913 ± 20 nM and 31 ± 1 nM, respectively. Both alkaloids showed excellent selectivity profile against *h*BChE. Moreover, carltonine A showed the ability to inhibit POP (IC_{50} = 143 ± 12 μM) at the same extent as berberine. New narcikachnine-type alkaloid narciabduliine demonstrated balanced inhibition activity against *h*AChE and *h*BuChE with IC_{50} values of 3.24 ± 0.73 μM and 3.44 ± 0.02 μM, respectively.

As a part of ongoing studies, pilot series of compounds, structurally inspired by carltonine A, and B (**1**-**20**) was developed. Newly synthesized compounds were tested for their *h*AChE/*h*BuChE inhibition activity. Seven compounds (**1**-**4, 6, 14,** and **20**) demonstrated *h*BuChE inhibition activity with IC_{50} values lower than 1 μM. Compound **6** was the strongest *h*BuChE inhibitor with an IC_{50} value of 0.07 ± 0.01 μM. The binding mode of *h*BuChE inhibition of compound **6** was inspected

by using enzyme kinetic analysis in tandem with molecular dynamic simulation. Furthermore, the CNS availability of **6** was predicted by calculating their BBB score.

Keywords: *Narcissus pseudonarcissus* cv. Carlton, Amaryllidaceae, alkaloids, biological activity, acetylcholinesterase, butyrylcholinesterase, prolyloligopeptidase.

7. ABSTRAKT

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Školitel: Prof. Ing. Lucie Cahlíková, Ph.D.

Název disertační práce: Amaryllidaceae alkaloidy rodu *Narcissus* a jejich biologická aktivita.

Narcissus pseudonarcissus cv. Carlton byl vybrán pro fytochemický výzkum na základě screeningové studie. Z 30 kg čerstvých cibulí byl připraven surový alkaloidní extrakt (485 g). Opakovaná extrakce kapalina-kapalina poskytla 187 g koncentrovaného alkaloidního extraktu, který byl následně separovan sloupcovou chromatografií (CC, Al_2O_3; 5800 g), opakovanou CC, preparativní TLC, a krystalizací. Bylo izolováno třináct dříve popsaných alkaloidů spolu se čtyřmi novým sloučeninami, které byly pojmenované karltonin A, B, C a narciabduliin. Všechny sloučeniny byly identifikovány a charakterizovány spektrometrickými technikami (1D a 2D NMR, CD a HRMS) a srovnáním s údaji v literatuře. Alkaloidy izolované v dostatečném množství byly použity pro screeningové testování jejich inhibiční aktivity vůči lidské acetylcholinesteráze (*h*AChE), butyrylcholinesteráze (*h*BuChE) a prolyloligopeptidáze (POP). Alkaloidy karltonin A a B prokázaly slibnou inhibiční aktivitu vůči enzymu *h*BuChE s hodnotami IC_{50} 913 ± 20 nM a 31 ± 1 nM, v daném pořadí. Oba alkaloidy vykazovaly výraznou selektivitu vůči *h*BuChE. Karltonin A inhiboval i POP (IC_{50} = 143 ± 12 μM). Nový alkaloid narcikachninového typu narciabduliin prokázal vyváženou inhibiční aktivitu proti *h*AChE a *h*BuChE s hodnotami IC_{50} 3.24 ± 0.73 μM a 3.44 ± 0.02 μM.

V rámci navazujících studií byla připravená pilotní série sloučenin strukturně inspirovaných karltoninem A a B (**1**-**20**). Nově syntetizované sloučeniny byly testovány na jejich inhibiční aktivitu vůči *h*AChE/hBuChE. Sedm sloučenin (**1**-**4, 6, 14** a **20**) vykázalo inhibiční aktivitu *h*BuChE s hodnotami IC_{50} nižšími než 1 uM. Sloučenina **6** byla nejsilnějším inhibitorem *h*BuChE s hodnotou IC_{50} 0.07 ± 0.01 uM. Kromě toho byla dostupnost CNS **6** zhodnocena výpočtem jejich skóre BBB.

Klíčová slova: *Narcissus pseudonarcissus* cv. Carlton, Amaryllidaceae, alkaloidy, biologická aktivita, acetylcholinesteráza, butyrylcholinesteráza, prolyloligopeptidase.

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} = 4.411

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

Author's contribution: Preparation of all galanthamine derivatives and writing of their synthesis procedure. Collection of results for the supplementary materials. Reading of final manuscript.

P2. 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} = 4.411

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

Author's contributions: Participation in the literature search of the selected topics. Data collection and evaluation of the draft manuscript. Reading of final manuscript.

P3. 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} = 5.923

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

Author's contribution: Preparation of all derivatives and writing their synthesis procedure. Preparation of derivatives for the biological assay. Data collection from biological analysis, preparation data for supplementary materials, and preparation of them for the daft manuscript. Reading of the final manuscript.

P4. Maříková, J.; **Mamun, A.A**.; Shammari, L.A.; Korábečný, J.; Kučera, T.; Hulcová, D.; Kuneš, J.; Malaník, M.; Vašková, M.; Kohelová, E.; Nováková, L.; Cahlíková, L.; Pour, M. Structure elucidation and cholinesterase inhibition activity of two new minor Amaryllidaceae alkaloids. *Molecules* 2021, 26 (5), 1279. $IF_{2021} = 4.411$

Full text: https://doi.org/10.3390/molecules26051279

Author's contribution: Preparation of crude extraction from the plant. Complete column chromatography and isolation of the alkaloid narciabduliine. Preparation of the alkaloid for the biological study and writing of its isolation process. Reading of the final manuscript.

P5. Mamun A.A.; Maříková, J.; Hulcová, D.; Janoušek, J.; Šafratová, M.; Nováková L.; Kučera, T.; Hrabinová, M.; Kuneš, J.; Korábečný, J.; Cahlíková, L. Amaryllidaceae alkaloids of belladine-type from *Narcissus pseudonarcissus* cv. Carlton as new selective inhibitors of butyrylcholinesterase. *Biomolecules* 2020, 10(5), 800. $IF_{2020} = 4.879$

Full text: https://doi.org/10.3390/biom10050800

Author's contribution: Preparation of the crude extract from the plant. Complete column chromatography, and isolation of all alkaloids. Preparation of alkaloids for the biological study and writing of isolation process. Reading of the final manuscript.

8.2. Publications not included in the dissertation

P6. Peřinová, R.; Maafi, N.; Korábečný, J.; Kohelová, E.; Simone, A.D.; **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.

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

Author's contribution: Preparation of haemanthamine derivatives and writing of their synthesis procedure. Collection of data for supplementary material. Reading of the final manuscript.

P7. Maříková, J.; Ritomská, A.; Korábečný, J.; Peřinová, R.; **Mamun, A.A.**; Kučera, T.; Kohelová E.; Hulcová, D.; Kobrlová, T.; Kuneš, J.; Nováková, L.; Cahlíková L. Aromatic esters of the crinane Amaryllidaceae alkaloid ambelline as selective inhibitors of butyrylcholinesterase. *J. Nat. Prod.* 2020*,* 83, 5, 1359–1367.

Full text: https://doi.org/10.1021/acs.jnatprod.9b00561

Author's contribution: Preparation of semisynthetic derivatives from crinane type Amaryllidaceae alkaloids and writing of their synthesis procedure. Data collection for supplementary material. Reading of the final manuscript.

P8. Shammari, L.A.; **Mamun, A.A.**; Koutová, D.; Majorošová, M.; Hulcová, D.; Šafratová, M.; Breiterová, K.; Maříková, J.; Havelek, R.; Cahlíková, L. Alkaloid Profiling of *Hippeastrum* cultivars by GC-MS, isolation of Amaryllidaceae alkaloids and evaluation of their cytotoxicity. *Rec. Nat. Prod*. 2019, 14, 154-159.

Full text: https://doi.org/10.25135/rnp.147.19.06.1302

Author's contribution: Participation in the literature search of the selected topics. Data collection and evaluation for the draft manuscript. Reading of the final manuscript.

8.3. Conference

8.3.1. Lectures

L1. Mamun, A. A., Cahlíková, L., Hulcová, D., Maříková, J. New Amaryllidaceae alkaloids from *Narcissus pseudonarcissus* cv. Carlton as inspiration for the development of new drugs for Alzheimer′s disease, 11th Postgraduate and 9th Postdoc Conference (Online), Charles University, Faculty of Pharmacy in Hradec Kralove, Czech Republic, 27th and 28th January 2021.

L2. Mamun, A. A., Cahlíková, L., Maříková, J. Biological evaluation of alkaloids isolated from *Narcissus* cv. Carlton and antiproliferative potential of their semisynthetic derivatives, 10th postgraduate and postdoc conference, Charles University, Faculty of Pharmacy in Hradec Kralove, Czech Republic, 22nd and 23rd January 2020.

L3. Mamun, A. A., Cahlíková, L., Maříková, J. Derivatives of Amaryllidaceae alkaloids isolated from *Narcissus* cv. Carlton and their biological activity, 9th postgraduate and 7th postdoc conference, Charles University, Faculty of Pharmacy in Hradec Kralove, Czech Republic, 23rd and 24th January 2019.

L4. Mamun, A. A., Cahlíková, L., Maříková, J. Phytochemical study of Amaryllidaceae alkaloids from *Narcissus* cv. Carlton and their biological activity, 8th postgraduate and 6th postdoc conference, Charles University, Faculty of Pharmacy in Hradec Kralove, Czech Republic, 24th and 25th January 2018.

L5. Mamun, A. A., Cahlíková, L. Maříková, J. Structural diversity and pharmacological activity of Amaryllidaceae alkaloids from *Narcissus* cv. Carlton, 47th Conference on Synthesis and Analysis of Drugs, University of Veterinary and Pharmaceutical Science, Brno, Czech Republic, 12th - 14th September 2018.

8.3.2. Poster

P. Mamun, A. A., Cahlíková, L., Maříková, J. Structural diversity and pharmacological activity of Amaryllidaceae alkaloids from *Narcissus* cv. Carlton, 47th Conference on Synthesis and Analysis of Drugs, University of Veterinary and Pharmaceutical Science, Brno, Czech Republic, 12th - 14th September 2018.

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