ALKALOIDS FROM THE *HIPPEASTRUM* GENUS: CHEMISTRY AND BIOLOGICAL ACTIVITY

Jean Paulo de Andrade[A], Natalia Belén Pigni[A], Laura Torras-Claveria[A], Ying Guo[A], Strahil Berkov[B], Ricardo Reyes-Chilpa[C], Abdelaziz El Amrani[D], José Angelo S. Zuanazzi[E], Carles Codina[A], Francesc Viladomat[A], Jaume Bastida[A*]

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ABSTRACT

In recent years alkaloids from the genus *Hippeastrum* have been shown to exhibit a broad spectrum of biological activities, including antiparasitic, antiproliferative, apoptosis-induced, psychopharmacological, acetylcholinesterase-inhibitory, among others. This work presents a brief chemical and biological review of the alkaloids found in the genus *Hippeastrum*. *www.relaquim.com*

Keywords: Amaryllidaceae, "hippeastroid" clade, *Hippeastrum*, montanine, candimine, 11*β*-hydroxygalanthamine.

RESUMEN

En los últimos años, los alcaloides aislados del género *Hippeastrum* han mostrado un amplio espectro de actividades incluyendo, entre otras, la antiparasitaria, antiproliferativas, inductoras de apoptosis, psicofarmacológicas y como inhibidores de la acetilcolinesterasa. En este trabajo se presenta una breve revisión química y biológica de los alcaloides del género *Hippeastrum*. *www.relaquim.com*

Palabras clave: Amaryllidaceae, clado "hippeastroid", *Hippeastrum*, montanina, candimina, 11*β*-hidroxigalantamina.

[a]Departament de Productes Naturals, Biologia Vegetal i Edafologia. Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, España
[b]AgroBioInstitute, 8 Dragan Tzankov Blvd., Sofia, 1164, Bulgaria
[c]Instituto de Química, Universidad Nacional Autónoma de México. Circuito Exterior s/n. Ciudad Universitaria, Coyoacán, 04510, México DF
[d]Faculté des Sciences Aîn –Chock, Laboratoire de Synthèse, Extraction et Etude Physico-Chimique des Molécules Organiques, BP5366, Mâarif – Casablanca, Morocco
[e]Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000, Porto Alegre, Brasil
*Corresponding author. Tel.: +34 934020268; fax: +34 934029043. E-mail address: jaumebastida@ub.edu (J. Bastida).

1. INTRODUCTION

Hippeastrum is a well-known ornamental Amaryllidaceae genus from South America, particularly Brazil. The Amaryllidaceae family is one of the 20 most important alkaloid-containing plant families, comprising about 1100 perennial bulbous species classified in 85 genera. A particular characteristic of Amaryllidaceae plants is the consistent presence of a large, exclusive and still expanding group of isoquinoline alkaloids, the majority of which are not known to occur in any other plant family (Bastida *et al.*, 2006).

Their highly particular skeleton arrangements and broad spectrum of biological activities have prompted numerous chemical and pharmacological studies of this group of alkaloids. As an example, the well-known Amaryllidaceae alkaloid galanthamine (**50**) is a long-acting, selective, reversible and competitive inhibitor of the acetylcholinesterase enzyme (Thomsen *et al.*, 1998) as well as acting as an allosterically potentiating ligand in nicotinic acetylcholine receptors (Maelicke *et al.*, 2001). Due to these attributes, galanthamine (**50**) is one of the most important drugs used for the clinical management of Alzheimer's disease (AD) and is also useful in poliomyelitis and other neurological diseases. It is marketed as a hydrobromide salt under the name of Razadyne® (formerly Reminyl®). As a result of these and other activities demonstrated by the other skeleton-types (da Silva *et al.*, 2006; McNulty *et al.*, 2007; Giordani *et al.*, 2010a, 2011a), plants from the Amaryllidaceae family are currently seen as an important source of new and bioactive molecules.

The Amaryllidaceae are found mainly in the Southern Hemisphere, especially in South Africa and South America, which are considered to be the primary and secondary centers of diversification, respectively, of this family (Ito *et al.*, 1999). Recent nrDNA ITS sequence studies have divided the American Amaryllidaceae species in Andean tetraploid and extra-Andean "hippeastroid" clades. In addition, a probable Brazilian origin of the *Hippeastrum* genus has been accepted, based on its nrDNA ITS sequences (Meerow *et al.*, 2000). The *Hippeastrum* genus comprises approximately 70 species (Judd *et al.*, 1999), 34 being found in Brazil with 22 endemics (Dutilh, 2010). Although few of them have been studied to date, compounds with remarkable biological activity have been isolated in *Hippeastrum* species. Presented here is a brief overview of the phytochemical and biological studies of the *Hippeastrum* genus up to May, 2012.

2. GEOGRAPHICAL DISTRIBUTION, TAXONOMICAL ASPECTS

The *Hippeastrum* genus is distributed from Mexico and the West Indies to Argentina, the majority in eastern Brazil, the Peruvian Andes and Bolivia. It basically consists of large herbs of annual leaves, mostly hysteranthous, sessile, rarely persistent, and subpetiolate. Generally, the leaves are more than 2 cm wide. The scape is hollow with 2 free bracts. The flowers (2-13) are usually large and mostly purple or red. They are funnelform, zygomorphic, declinate, usually with a short tube and paraperigonal fibriae or with a callose ridge present at the throat. The stamens are fasciculate and declinate-ascendent. The stigma is trifid or shortly 3-lobed. The seeds are dry, flattened, obliquely winged or irregularly discoid, hardly ever turgid and globose or subglobose, with a brown or black phytomelanous testa (Dahlgren *et al.*, 1985; Meerow and Snijman, 1998).

A diploidism of 2n=22 is characteristic of the *Hippeastrum* genus, which is inarguably monophyletic with the exception of a single species, *Hippeastrum blumenavium*. This was first described as *Griffinia blumenavia* Koch and Bouche ex Carr and further studies are required to clarify its correct position (Meerow *et al.*, 2000).

The beauty of their flowers has led to numerous *Hippeastrum* species being grown as ornamentals after hybridization (Meerow and Snijman, 1998), although in horticultural circles the use of the name "*Amaryllis*" for this genus persists (Meerow *et al*, 1997).

3. BIOSYNTHESIS AND STRUCTURAL TYPES OF AMARYLLIDACEAE ALKALOIDS

As mentioned above, the consistent presence of an exclusive group of isoquinoline alkaloids is the outstanding feature of the Amaryllidaceae plant species. Amaryllidaceae alkaloids are formed biogenetically by intramolecular oxidative coupling of the key intermediate *O*-methylnorbelladine, derived from the amino acids L-phenylalanine and L-tyrosine (Bastida *et al.*, 2006). Most of them can be classified into nine skeleton-types (Figure 1), namely lycorine, crinine, haemanthamine, narciclasine, galanthamine, tazettine, homolycorine, montanine and norbelladine (Bastida *et al.*, 2006). *Ortho-para'* phenol oxidative coupling of the precursor *O*-methylnorbelladine results in the formation of a lycorine-type skeleton, from which homolycorine-type compounds proceed. *Para-para'* phenol oxidative coupling leads to the formation of crinine, haemanthamine, tazettine, narciclasine and montanine structures. The galanthamine-type skeleton is the only one that originates from *para-ortho'* phenol oxidative coupling (Bastida *et al.*, 2006). In the present review, the numbering system according to Ghosal *et al.* (1985) has been adopted for the structures (Figure 1).

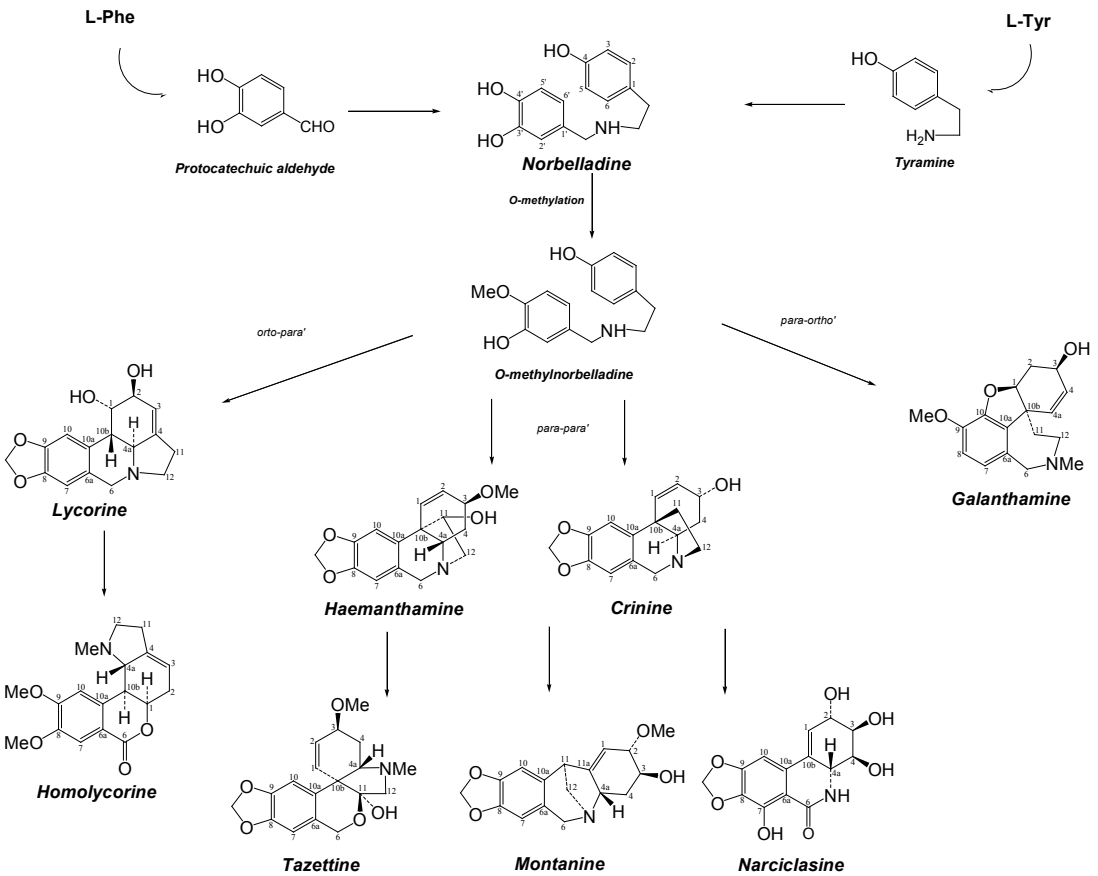

Figure 1. Biosynthetic pathway of the main skeleton-type found in the genus *Hippeastrum*.

Some new structural subgroups have been proposed recently (Ünver, 2007). Graciline and plicamine-type alkaloids have been found in species of *Galanthus*, *Cyrthanthus* and *Narcissus* (Ünver *et al.*, 1999; Brine *et al.*, 2002; de Andrade *et al.*, 2012). The biogenetic pathway of gracilines possibly originates from the 6-hydroxy derivatives of haemanthamine-type alkaloids (Noyan *et al.*, 1998), while plicamine-type alkaloids most probably proceed from the tazettine-type skeleton, considering their structural similarities. Augustamine-type alkaloids represent a very rare structure found in *Crinum* species (Ali *et al.*, 1983; Machocho *et al.*, 2004). Galanthindole (**62**) is another example of an unusual compound isolated from the *Galanthus* genus and also found in *Hippeastrum* genus. It has been classified as a new skeleton-type (Ünver *et al.*, 2003), although the possibility that it is an artifact from the homolycorine series should be considered. Another uncommon alkaloid found in *Hippeastrum* was a simple carboline, isolated from *Hippeastrum vittatum* (Youssef, 2001).

A few alkaloids commonly found in other plant families have also been described in Amaryllidaceae plants, for example, the mesembrane-type alkaloids, which were isolated in *Narcissus* species (Bastida *et al.*, 2006) despite being typical of the genus *Sceletium* (Aizoaceae). Phtalideisoquinoline-, benzyltetrahydroisoquinoline- and aporphine-type alkaloids were found in *Galanthus trojanus* (Kaya *et al.*, 2004, 2011), being most commonly associated with Papaveraceae and Fumariaceae. Tyramine-type protoalkaloids, which are biosynthesized in Poaceae, Cactaceae, some algae and fungi, have also been found in *Galanthus* and *Leucojum* species (Berkov *et al.*, 2008, 2011a, 2011b). However, it should be borne in mind that these unusual alkaloids have always been isolated together with typical Amaryllidaceae alkaloids. To date, nearly 500 alkaloids have been isolated from amaryllidaceous plants (Zhong, 2005).

4. DISTRIBUTION OF ALKALOIDS IN THE GENUS *HIPPEASTRUM*

Phytochemical studies of the genus *Hippeastrum*, as well as of other genera of the Amaryllidaceae family, started in the early 1950s. The alkaloids reported in the genus *Hippeastrum* are summarized in Table 1 and their respective structures are shown in Figure 2. The first phytochemical study was described with varieties of *H. vittatum*, which yielded the alkaloids tazettine (**39**) and lycorine (**1**) (Boit, 1954). Two years later, a new phytochemical study of the same species yielded the alkaloids haemanthamine (**26**), homolycorine (**14**), hippeastrine (**15**), and vittatine (**27**), as well as tazettine (**39**) and lycorine (**1**) (Boit, 1956). In 1957, a study of *H. bifidum* only yielded lycorine (**1**) (Boit and Döpke, 1957). One year later, galanthamine (**50**) was found for the first time in a *Hippeastrum* species, specifically in *H. rutilum*, although it was isolated as a minor compound (Boit *et al.*, 1958). The work carried out in the 1950s and 60s was notable for the isolation of montanine (**33**) in *H. aulicum* along with some crinine-type representatives (Boit and Döpke, 1959). The main research on the genus in these two decades can be found by searching for the authors Boit, HG and Döpke, W.

There was little phytochemical research on *Hippeastrum* species between the 1970s and 1990s. An interesting study was carried out with *H. vittatum* grown in Egypt in different years, which allowed the elucidation of the alkaloids pancracine (**32**) (formerly hippagine) and hippadine (**8**) (El Mohgazi *et al.*, 1975; Ali *et al.*, 1981, 1984). A phytochemical study of *H. añañuca* from Chile yielded a new alkaloid but with undefined stereochemistry (Pacheco *et al.*, 1978). Quirion *et al.*, (1991) isolated the new compound 3-*O*-acetylnarcissidine (**11**) from *H. puniceum*, and Döpke *et al.*, (1995a) isolated a new phenantridone alkaloid named phamine (**48**) from *H. equestre*. Several known alkaloids were also isolated

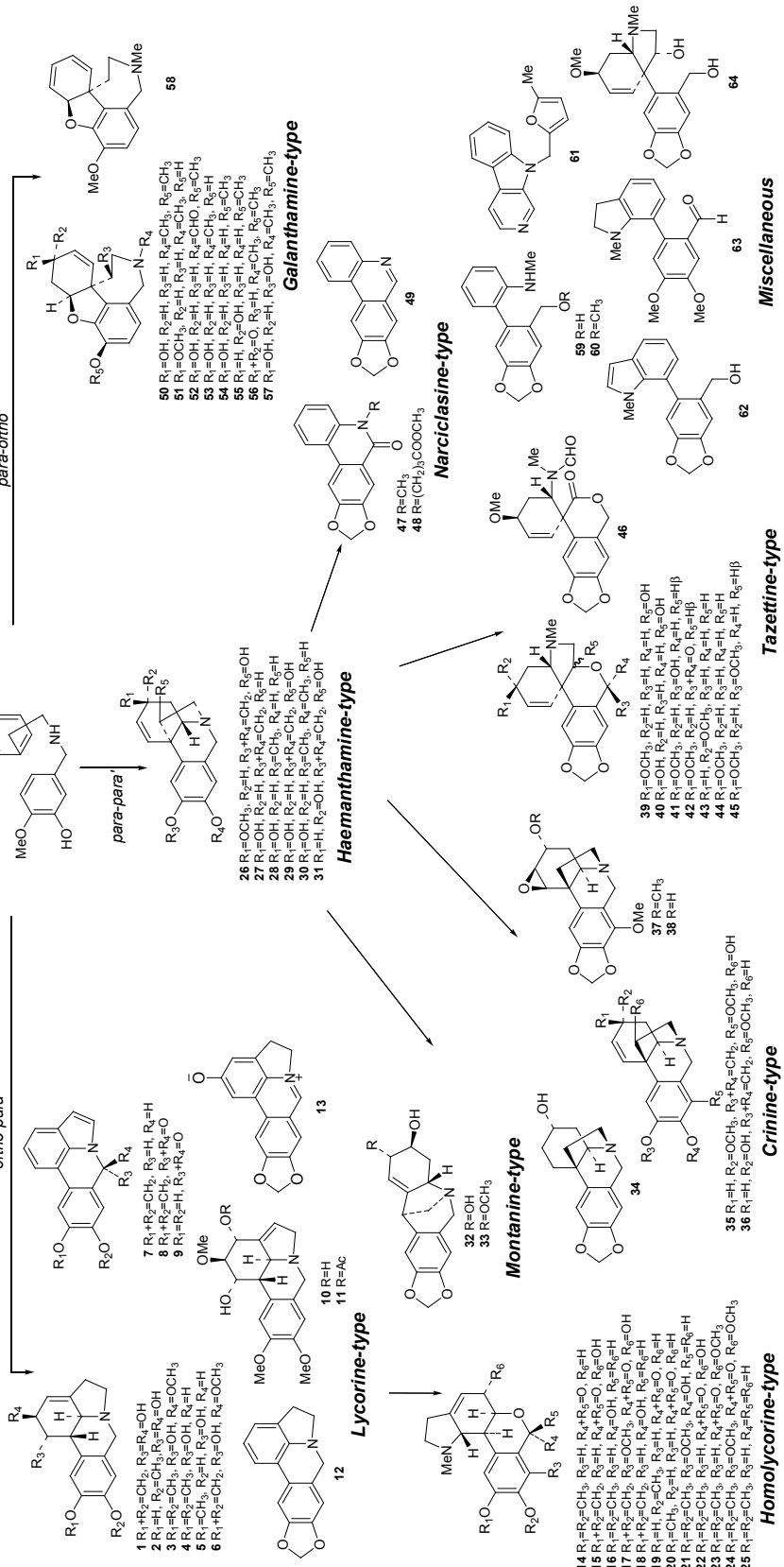

Figure 2. Structure of the alkaloids reported in the genus *Hippeastrum*.

Table 1. Alkaloids reported in the genus *Hippeastrum*.

	H. vittatum	*H. bifidum*	*H. rutilum*	*H. rachyandrum*	*H. aulicum*	*H. hybrids**	*H. candidum*	*H. johnsonii*	*H. añañuca*	*H. bicolor*	*H. equestre*	*H. puniceum*	*H. solandriflorum*	*H. glaucescens*	*H. morelianum*	*H. psittacinum*	*H. striatum*	*H. santacatarina*	*H. papilio*	*H. breviflorum*
Lycorine-type																				
Lycorine (**1**)	1,2,12,29,30	3	4,5	5	6	7,8,21	8	10	14	16	11,17,18,22,23		24	28		29	29	29,33		
Pseudolycorine (**2**)					6			10			11									
Galanthine (**3**)					6	7,8					17,18									
Pluviine (**4**)						7														
Norpluviine (**5**)					6															
Hippamine (**6**)						8														
11,12-Dehydroanhydrolycorine (**7**)																29		29		29
Hippadine (**8**)	12,15																			
Hippacine (**9**)	9,12,13																			
Narcissidine (**10**)				5	6	7,8														
3-*O*-Acetylnarcissidine (**11**)												20								
Anhydrolycorine (**12**)	29																	29		
Ungeremine (**13**)													24							
Homolycorine-type																				
Homolycorine (**14**)	2		4,5						14											
Hippeastrine (**15**)	2,12		4,5			7,8,21					17,18,22,23			29		29,31				
Lycorenine (**16**)				5																
Candimine (**17**)							8								29,32					
Oduline (**18**)						9														
9-*O*-Demethylhomolycorine (**19**)											25									
8-*O*-Demethylhomolycorine (**20**)																29				29
Nerinine (**21**)															29					
2-Hydroxyhomolycorine (**22**)															29					
2-Methoxyhomolycorine (**23**)															29					
2α,7-Dimethoxyhomolycorine (**24**)															29,32					
Deoxylycorenine (**25**)																				29
Haemanthamine-type																				
Haemanthamine (**26**)	2		4,5			7,8,21				16	18				32			29	29,34	
Vittatine (**27**)	2,12,13,29,30					21						20	24					29	29,34	29
8-*O*-Demethylmaritidine (**28**)	29																		29,34	
11-Hydroxyvittatine (**29**)						21					25	20							29,34	
Maritidine (**30**)									14											
Hamayne (**31**)													24		32			29		
Montanine-type																				
Pancracine (former hippagine) (**32**)	12,19,29					21														
Montanine (**33**)	29,30				6	21														

	H. vittatum	*H. bifidum*	*H. rutilum*	*H. brachyandrum*	*H. aulicum*	*H. hybrids**	*H. candidum*	*H. johnsonii*	*H. añañuca*	*H. bicolor*	*H. equestre*	*H. puniceum*	*H. solandriflorum*	*H. glaucescens*	*H. morelianum*	*H. psittacinum*	*H. striatum*	*H. santacatarina*	*H. papilio*	*H. breviflorum*
Crinine-type																				
Crinidine (**34**)						8														
Ambelline (**35**)				5	6	7														
Powelline (**36**)						7,8														
Undulatine (**37**)				5																
Crinamidine (**38**)				5		7														
Tazettine-type																				
Tazettine (**39**)	1,2, 12					8,21		10			11,17,18 22,23,26			28,29	29,32	29		29	29	29
3-*O*-demethyltazettine (**40**)											26									
Pretazettine (**41**)											25			28	32	31				
3-*Epi*-macronine (**42**)											25			29	29,32	29		29		29
3-*Epi*-deoxytazettine (**43**)														29	29	29				29
Deoxytazettine (**44**)														29	29	29		29		29
6-Methoxypretazettine (**45**)															29	29				29
Tazettamide (**46**)																29				
Narciclasine-type																				
N-Methylcrinasiadine (**47**)											22									
Phamine (**48**)											22,23									
Trisphaeridine (**49**)											22			29	29,32	29	29	29	29	29
Galanthamine-type																				
Galanthamine (**50**)	29		4,5			7,8					18			29	29	29	29	29	29,34	
Chlidanthine (**51**)					6															
N-Formylnorgalanthamine (**52**)														29						
Sanguinine (**53**)														29					29	
N-Demethylgalanthamine (**54**)														29						
3-*Epi*-norgalanthamine (**55**)														29						
Narwedine (**56**)														29					29,34	
11β-hydroxygalanthamine (**57**)																			29,34	
Anhydrogalanthamine (**58**)														29						
Miscellaneous																				
Ismine (**59**)	27										25		24		29	29				29
O-Methylismine (**60**)	27																			
Vittacarboline (**61**)	27																			
Galanthindole (**62**)															29	29				29
Lycosinine B (**63**)																				29
Egonine (**64**)											26									

1) Boit, 1954; 2) Boit, 1956; 3) Boit and Döpke, 1957; 4) Boit et al., 1958; 5) Boit and Döpke, 1959; 6) Boit and Döpke, 1960a; 7) Boit and Döpke, 1960b; 8) Döpke, 1962; 9) Döpke and Bienert, 1966; 10) Rao et al., 1971; 11) Rao and Vimaladevi, 1972; 12) El Mohgazi et al., 1975; 13) El Mohgazi and Ali, 1976; 14) Pacheco et al., 1978; 15) Ali et al., 1981; 16) Sepúlveda et al., 1982, 17) Alam and Murav'eva, 1982; 18) Murav'eva and Alam, 1982; 19) Ali et al., 1984; 20) Quirion et al., 1991; 21) Mügge et al., 1994; 22) Döpke et al., 1995b; 23) Döpke et al, 1995a; 24) Bastida et al., 1996; 25) Pham et al., 1997; 26) Pham et al., 1999; 27) Youssef, 2001; 28) Hofmann et al., 2003; 29) de Andrade et al., Personal communication, 12 June 2012; 30) da Silva et al., 2008; 31) Pagliosa et al., 2010; 32) Giordani et al., 2011b; 33) Giordani et al., 2011a; 34) de Andrade et al., 2011. * mixture of hybrids from *Hippeastrum.*

from *H. equestre* and submitted to circular dichroism studies (Wagner *et al.*, 1996). A few years later, *H. equestre* yielded another new alkaloid, egonine (**64**) (Pham *et al.*, 1999). This structure has been related as a typical *Sceletium* mesembrine-type alkaloid (Aizoaceae), although its similarity with tazettine-type skeleton should be considered.

Phytochemical studies of *H. vittatum* flowers in 2001 yielded a representative alkaloid of the carboline group named vittacarboline (**61**), as well as the new alkaloid *O*-methylismine (**60**) (Youssef, 2001). A rapid phytochemical study of *H. glaucescens* provided lycorine (**1**), pretazettine (**41**) and tazettine (**39**), but not all alkaloid fractions were studied (Hoffman *et al.*, 2003). In the last decade, most *Hippeastrum* studies have been focused on the biological activity of alkaloids isolated from the genus, although the new alkaloids 2α,7-dimethoxyhomolycorine (**24**) and 11β-hydroxygalanthamine (**57**) found in *H. morelianum* and *H. papilio*, respectively, should be mentioned (Giordani *et al.*, 2011b, de Andrade *et al.*, 2011).

In the last decade, the GC-MS technique has proved to be very effective for rapid separation and identification of complex mixtures of Amaryllidaceae alkaloids obtained from low mass samples (Kreh *et al.*, 1995, Berkov *et al.*, 2008, 2011a). The high resolution ability of the capillary column and numerous EI-MS spectra available in the literature allow the identification and quantification of known Amaryllidaceae alkaloids, avoiding time-consuming and laborious isolation procedures. This technique has been much applied with the genera *Pancratium*, *Galanthus*, *Leucojum* and *Narcissus* (Kreh *et al.*, 1995; Torras-Claveria *et al.*, 2010; Berkov *et al.*, 2011b; de Andrade *et al.*, 2012). The only study applying GC-MS in the genus *Hippeastrum,* carried out with species from South Brazil, identified more compounds than had been isolated previously and two species were found to produce significant levels of galanthamine (**50**) (de Andrade *et al.*, Personal communication, 12 June 2012).

To the best of our knowledge, 19 species, including hybrids, from the genus *Hippeastrum* have been phytochemically studied to date. Sixty-four different alkaloids with defined structures have been isolated, while fourteen remain undefined (Boit and Döpke, 1959, 1960a, 1960b; Pacheco *et al.*, 1978; de Andrade *et al.*, Personal communication, 12 June 2012). Table 1 and Figure 2 summarize the alkaloids found in the genus *Hippeastrum.*

5. BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF THE ALKALOIDS FOUND IN *HIPPEASTRUM*

Like most Amaryllidaceae alkaloids, the compounds found in the genus *Hippeastrum* have been little evaluated for their biological activity. However, some of them have demonstrated a broad spectrum of interesting properties.

5.1. *Ortho-para'* phenolic coupling

5.1.1 Lycorine-type

Lycorine (**1**) is probably the most frequent occurring alkaloid in Amaryllidaceae plants and has been found in almost all *Hippeastrum* species. This compound possesses a vast array of biological properties, being reported as a potent inhibitor of ascorbic acid synthesis, cell growth and division, and organogenesis in higher plants, algae and yeasts, inhibiting the cell cycle during the interphase (Bastida *et al.*, 2006). Additionally, lycorine (**1**) exhibits antiviral, anti-inflammatory, antifungal and antiprotozoan activities (Çitoğlu *et al.*, 1998; McNulty *et al.*, 2009; Giordani *et al.*, 2010b, 2011a). Lycorine (**1**) has also been shown to have insect antifeedant activity (Evidente *et al.*, 1986), as does 3-*O*-acetylnarcissidine (**11**), isolated from *H. puniceum*, which is particularly active against the polyphagous

insect *Spodora littoralis* but not against the olphage *Leptinotarsa decemlineata* (Santana *et al.*, 2008).

As a potential chemotherapeutic drug, lycorine (**1**) has been studied as an antiproliferative agent against a number of cancer cell lines (Likhitwitayawuid *et al.*, 1993; McNulty *et al.*, 2009). The *in vitro* mode of action in a model HL-60 leukemia cell line is associated with suppressing tumor cell growth and reducing cell survival via cell cycle arrest and induction of apoptosis. Furthermore, lycorine (**1**) was able to decrease tumor cell growth and increase survival rates with no observable adverse effects in treated animals, thus being a good candidate for a therapeutic agent against leukaemia (Liu *et al.*, 2004, 2007; Liu *et al.*, 2009).

Lycorine (**1**) isolated from *H. santacatarina* showed remarkable inhibitory activity of the enzymes NTPDase and ecto-5'-nucleo- tidase from *Trichomonas vaginalis*, which contributes to an increased susceptibility of this parasite to the host immune response (Giordani *et al.*, 2010b). Lycorine (**1**) was also demonstrated to have anti-*T. vaginalis* activity, involving a mechanism of cell death induction associated with paraptosis rather than the apoptosis observed in tumor cells. This mechanism also differs from the one associated with other pro-apoptotic compounds tested against *T. vaginalis* such as staurosporine, doxorubicin, etoposide and methyl jasmonate. The authors have called for additional molecular studies for a better characterization of the different cell death mechanisms (Giordani *et al.*, 2011a). Lycorine (**1**) has also been tested *in vitro* against human immunodeficiency virus type 1 (HIV-1), results of antiviral showed low inhibition of the replication of HIV-1(NL4-3) with an EC_{50}> 0.5 μg/ml with infected lymphoid MT-4 human cells (Reyes-Chilpa *et al.*, 2011).

Compared to other lycorine-type alkaloids, anhydrolycorine (**12**) showed a greater ability to inhibit ascorbic acid synthesis (Evidente *et al.*, 1986). Analgesic, hypotensive and antiparasitic activities have been reported for galanthine (**3**). Ungeremine (**13**) has shown acetylcholinesterase inhibitory activity (Bastida *et al.*, 2006). In summary, the lycorine skeleton-type is a promising target for further biological assessments.

5.1.2 Homolycorine-type

Homolycorine (**14**), 8-*O*-demethylhomolycorine (**20**) and hippeastrine (**15**) are well-known cytotoxic alkaloids. Homolycorine (**14**) has also shown high antiretroviral activity, while hippeastrine (**15**) is active against *Herpes simplex* type 1. Homolycorine (**14**) and 8-*O*-demethylhomolycorine (**20**) have a hypotensive effect on normotensive rats. In addition, hippeastrine (**15**) shows antifungal activity against *Candida albicans* and also possesses a weak insect antifeedant activity (Bastida *et al.*, 2006). Candimine (**17**), first found in *H. candidum* (Döpke, 1962), has been tested against *Trichomonas vaginalis* and found to inhibit the *T. vaginalis* enzymes NTPDase and ecto-5'-nucleotidase to a greater extent than lycorine (**1**) (Giordani *et al.*, 2010b). Candimine (**17**) was also active against *T. vaginalis*, apparently inducing cell death by paraptosis, as in the case of lycorine (**1**) (Giordani *et al.*, 2010a). Homolycorine (**14**) and 8-*O*-demethylhomolycorine (**20**) were tested against the parasitic protozoa *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense*, *Leishmania donovani* and *Plasmodium falciparum* but showed no significant activity (de Andrade *et al.*, 2012). However, the bioactivity of most homolycorine-type alkaloids is largely unknown.

5.2. *Para-para'* phenolic coupling

5.2.1. Haemanthamine-type

Haemanthamine (**26**), as well as crinamine, has proven to be a potent inducer of apoptosis in tumor cells at micromolar concentrations (McNulty *et al.*, 2007). This

compound also possesses antimalarial activity against strains of chloroquine-sensitive *Plasmodium falciparum*, hypotensive effects and antiretroviral activity (Bastida *et al.*, 2006; Kaya *et al.*, 2011). Vittatine (**27**), isolated from *H. vittatum*, and maritidine (**30**), have shown cytotoxic activity against HT29 colon adenocarcinoma, lung carcinoma and RXF393 renal cell carcinoma (Bastida *et al.*, 2006; da Silva *et al.*, 2008). Vittatine (**27**) also showed antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, as well as 11-hydroxyvittatine (**29**) (Kornienko and Evidente, 2008).

5.2.2. Crinine-type

The alkaloids crinine, 6-hydroxybuphanidrine and 6-ethoxybuphanidrine showed antiproliferative effects against human tumor cell lines, crinine being the most active (Berkov *et al.*, 2011c). A comparative study of skeleton-types concluded that the crinine-type alkaloid buphanamine was the most promising, since it showed important anti-proliferative effects and was well tolerated even at high concentration (Evidente *et al.*, 2009). Further evaluations are needed to gain more insight into the biological activity of the crinine-type skeleton.

5.2.3. Tazettine-type

The alkaloids 3-*epi*-macronine (**42**) and tazettine (**39**) showed moderate cytotoxic activity. Tazettine (**39**) is an isolation artefact of chemically labile pretazettine (**41**) (de Andrade *et al.*, 2012), the latter being far more interesting due to its antiviral and anticancer activities (Bastida *et al.*, 2006). Pretazettine (**41**) shows cytotoxicity against fibroblastic LMTK cell lines and inhibits HeLa cell growth, being therapeutically effective against advanced Rauscher leukemia, Ehrlich ascites carcinoma, spontaneous AKR lymphocytic leukemia, and Lewis lung carcinoma (Bastida *et al.*, 2006). Pretazettine (**41**) isolated from *H. psittacinum* was tested for its ability to inhibit the AChE enzyme but showed no significant result (Pagliosa *et al.*, 2010).

5.2.4. Montanine-type

This group has very few representatives. The alkaloids montanine (**33**) and pancracine (**32**) have been isolated in different periods from *Hippeastrum* species growing in Europe and South America, such as *H. vittatum*. In recent work montanine (**33**) showed anxiolytic-, antidepressant- and anticonvulsant-like effects in mice (da Silva *et al.*, 2006). Montanine (**33**) and vittatine (**27**) were also submitted to an antiproliferative study, the former showing the highest level of cytotoxicity (da Silva *et al.*, 2008). Furthermore, montanine (**33**) significantly inhibited AChE activity at concentrations of 1 milimolar, and 500 and 100 micromolar using the Ellman method (Pagliosa *et al.*, 2010). Pancracine (**32**) showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as weak activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Plasmodium falciparum* (Bastida *et al.*, 2006). The montanine-type skeleton represents one of the most interesting alkaloids for biological evaluations due to its remarkable and broad spectrum of activities.

5.2.5. Narciclasine-type

Trisphaeridine (**49**) has a high retroviral activity but a low therapeutic index (Bastida *et al.*, 2006). Narciclasine and pancratistatin are the most studied alkaloids of this group but they have never been found in the *Hippeastrum* genus. Both compounds show strong antimitotic and antitumoral activities (Bastida *et al.*, 2006). No biological evaluation of the alkaloids *N*-methylcrinasiadine (**47**) and phamine (**48**) has been carried out to date.

5.3. *Para-ortho'* phenolic coupling

5.3.1. Galanthamine-type

Galanthamine (**50**) is a long-acting, selective, reversible and competitive inhibitor of

acetylcholinesterase (AChE) and an allosteric modulator of the neuronal nicotinic receptor for acetylcholine. Its action increases acetylcholine levels, thus facilitating cholinergic synapses and helping in the management of patients suffering certain stages of AD (Maelicke *et al.*, 2001; Bastida *et al.*, 2006; Heinrich and Teoh, 2004). Galanthamine (**50**), therefore, is the most studied Amaryllidaceae alkaloid in terms of biological activity, clinical response, tolerance and safety, being marketed as a hydrobromide salt under the name of Razadine®, formerly Reminyl®. Galanthamine (**50**) has superior pharmacological profiles and higher tolerance than the original AChE inhibitors physostigmine or tacrine (Grutzendler and Morris, 2001).

After the therapeutic success of galanthamine (**50**), the search for new AChE inhibitors has intensified. *Epi*-galanthamine, with a hydroxyl group at the *α*-position, and narwedine (**56**), with a keto group at C3, are also active AChE inhibitors, but about 130-times less powerful than galanthamine (**50**) (Thomsen *et al.*, 1998). The loss of the methyl group at the *N* atom, as in *N*-demethylgalanthamine (**54**), decreases the activity 10-fold. The alkaloids habranthine and its new epimer 11*β*-hydroxygalathamine (**57**), isolated from *H. papilio*, which shows a hydroxyl-substituent at C11, were both also *ca.* 10-times less active than galanthamine (**50**) (López *et al.*, 2002; de Andrade *et al.*, 2011). Hydrogenation of the C4-C4a double bond, as in lycoramine, results in a complete loss of AChE inhibitory activity (López *et al.*, 2002).

On the other hand, sanguinine (**53**), which has a hydroxyl group at C9 instead of a methoxyl group, is *ca.* 10 times more active than galanthamine (**50**). Recently, *N*-alkylated galanthamine derivatives were isolated from *Leucojum* species and were also *ca.* 10 times more active than galanthamine (**50**). It has been suggested that these naturally occurring AChE inhibitors can act as ecological pesticides, since the AChE-inhibitory activity of synthetic pesticides, such as phospho-organic derivatives, is non-reversible (Houghton *et al.*, 2006).

Galanthamine (**50**) has also been tested *in vitro* against human immunodeficiency virus type 1 (HIV-1), results of antiviral assays indicated that galanthamine (**50**), as well as its structural isomer chlidanthine (**51**) and galanthamine *N*-oxide, did not showed inhibition of the replication of HIV-1(NL4-3) with infected lymphoid MT-4 human cells, but they were also not toxic to non infected cells showing $EC_{50,}$ and CC_{50} > 20 μg/ml), respectively (Reyes-Chilpa *et al.*, 2011). The galanthamine-type skeleton is currently the most studied group in terms of biological activity.

5.4. Miscellaneous

Ismine (**59**) shows a significant hypotensive effect on rats and cytotoxicity against Molt 4 lymphoid and LMTK fibroblastic cell lines (Bastida *et al.*, 2006). Recently, extracts from *H. breviflorum* showing different ratios between lycosinine B (**63**) and lycorine (**1**) by HPLC demonstrated significant anti-*Trichomonas vaginalis* activity (Vieira *et al.*, 2011). To date, the alkaloids vittacarboline (**61**), galanthindole (**62**) and *O*-methylismine (**60**) have not been biologically evaluated.

6. CONCLUSION

Over the last 50 years, the bulbous genus *Hippeastrum* has yielded 64 different alkaloids, together with others whose structures remain undefined. Further studies on the isolation of these compounds are called for, especially after recent biological studies showing their significant antiparasitic, psychopharmacological and AChE-inhibitory activities. Notably, some *Hippeastrum* species are able to produce a high level of galanthamine (**50**), comparable with species

of other genera currently being used for the commercial production of this alkaloid. The lack of biological activity shown by most of the alkaloids found in the *Hippeastrum* genus may be due to the small amounts isolated. Consequently, their synthesis or *in silico* studies will facilitate further bioactivity assessment.

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