Translational Oncology
www.transonc.com

Volume 1 Number 1 March 2008 pp. 1–13 1

Review Article

Amaryllidaceae Isocarbostyril Alkaloids and Their Derivatives as Promising Antitumor Agents

Laurent Ingrassia*, Florence Lefranc[†,‡], Véronique Mathieu[‡], Francis Darro* and Robert Kiss[‡]

*Unibioscreen SA, Avenue Joseph Wybran, B-1070 Brussels, Belgium; [†]Service de Neurochirurgie, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik, 808, B-1070 Brussels, Belgium; [‡]Laboratoire de Toxicologie, Institut de Pharmacie, Université Libre de Bruxelles, Campus de la Plaine CP205/1, Boulevard du Triomphe, B-1050 Brussels, Belgium

Abstract

This review covers the isolation, total synthesis, biologic activity, and more particularly the *in vitro* and *in vivo* antitumor activities of naturally occurring isocarbostyril alkaloids from the Amaryllidaceae family. Starting from these natural products, new derivatives have been synthesized to explore structure–activity relationships within the chemical class and to obtain potential candidates for preclinical development. This approach appears to be capable of providing novel promising anticancer agents.

Translational Oncology (2008) 1, 1–13

Introduction

Natural products have played a highly significant role in the discovery and development of new drugs for the treatment of human diseases [1]. This is particularly evident in the cancer field, where more than 60% of drugs are of natural origin [2]. Plants from the Amaryllidaceae family have long been known for their medicinal and toxic properties. Indeed even in ancient Greece, the oil from *Narcissus* species was already being used successfully for the treatment of cancer [3]. Consequently, efforts have been made to isolate the active ingredients responsible for this antitumor activity. Some 48 alkaloids bearing a variety of carbon skeletons have been isolated from *Narcissus* species [4]. One small group of these alkaloids does not contain basic nitrogen atoms and is represented by hydroxylated benzophenanthridone [5] or isoquinolinone types of structure. The most widely known compounds [5] of this group are narciclasine **1**, lycoricidine **2**, and pancratistatin **3** (Figure 1), and the most frequently used term in the literature to describe this group of alkaloids is the isocarbostyrils [6,7].

All these natural products have demonstrated potent *in vitro* cytotoxicity against cancer cell lines [7] and potent *in vivo* antitumor activity against mouse M-5076 sarcoma and P388 leukemia [7]. Therefore, this family as a whole seems of interest as a potential source of new lead structures for the development of a future generation of anticancer drugs.

This review focuses on the isocarbostyril alkaloid family, with a special emphasis on the isolation and synthesis of these natural products and their hemi- and fully synthetic derivatives and their *in vitro* and *in vivo* antitumor activity. Finally, recent advances in understanding the mechanism of action of these antitumoral molecules as well as their original role in the control of the plant growth will be discussed.

Isolation of Amaryllidaceae Isocarbostyril Alkaloids

Narciclasine and Natural Derivatives

Narciclasine **1**, also known as lycoricidinol given it is the 7-hydroxy derivative of lycoricidine **2** (Figure 1), was the first alkaloid of the class isolated in 1967 from *Narcissus* (Amaryllidaceae species) bulbs based on its inhibition of the growth of wheat grain radicles [8]. The typical procedure for the isolation of this compound involves ethanolic extraction of fresh daffodil bulbs. The alcohol is then evaporated to leave an aqueous residue, which is itself extracted with dichloromethane to remove nonpolar contaminants. Narciclasine is then extracted from the residual aqueous phase with ethyl acetate. After evaporation of the solvent, the residue is dissolved in ethanol, and after that, solid deposit was further purified by column chromatography [8,9]. Narciclasine is

Address all correspondence to: Robert Kiss, Laboratoire de Toxicologie, Institut de Pharmacie, Université Libre de Bruxelles, Campus de la Plaine CP205/1, Boulevard du Triomphe, B-1050, Brussels, Belgium. E-mail: rkiss@ulb.ac.be
Received 29 January 2008; Revised 29 January 2008; Accepted 29 January 2008

DOI 10.1593/tlo.08100

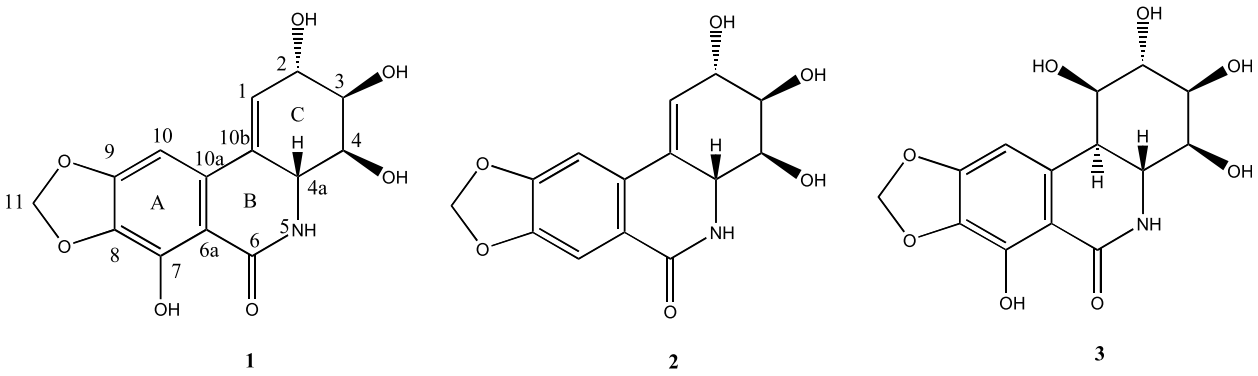


Figure 1. Chemical structures of narciclasine, lycoricidine and pancratistatin.

obtained as a pure product after alcohol or acetic acid crystallization (as light yellowish needles) of the eluted fraction containing the compound. The structure of narciclasine was elucidated in 1970 by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR, mass spectral, infrared, and elemental analyses and was further characterized with respect to optical rotation [9,10].

One of the most widely reported sources of narciclasine is the bulbs of *Narcissus* species [4,8,11]. Thirty-two species and varieties of this genus have been examined for narciclasine content, which varies from 30 to 200 mg/kg fresh bulbs. The typical content in this genus is 100 to 120 mg/kg (Table 1) and may vary during the year. For example, a content of 200 mg/kg was exceptionally found in bulbs of *Narcissus incomparabilis* Mill. Var. Helios at the flowering stage in March (Table 1). The occurrence of narciclasine was also investigated in others species of Amaryllidaceae, including *Lycoris radiata* [12,13], *Haemanthus* [6,11], *Hymenocallis* [7,11,14,15], *Brachystola magna* [16], *Galanthus* [11], *Leucojum* [11], *Pancratium maritimum* [11], *Sprekelia formosissima* [11], *Sternbergia lutea* [11], and *Vallota speciosa* [11]. The content of narciclasine in these species does not exceed 50 mg/kg fresh bulbs (Table 1). Narciclasine was exceptionally found in high concentrations (~135 mg/kg) in *Zephyranthes flava*, a tropical and subtropical plant cultivated in India (Table 1) [6].

However, in general and based on analyses to date, *Narcissus* bulbs are the best and easiest to obtain source of narciclasine, given that these plants are relatively common and can grow in many countries with different climates.

Table 1. Occurrence of Narciclasine **1**, Lycoricidine **2**, and Pancratistatin **3** (in mg/kg of Bulbs or Seeds) in Some Amaryllidaceae Species.

Amaryllidaceae Species	Compounds		
	1	**2**	**3**
N. imcomparabilis	200 in March 100-120 in November	NI	NI
Narcissus "Carlton"	100-120	NI	NI
H. kalbreyeri	45	17	157
Z. flava	135	NI	165
P. maritimum	50	NI	6
L. radiata	4.2	3.2	NI
Ismene × "Sulfur Queen"	NI	32	NI
H. littoralis	5	118	100-150 from Hawaii 22 from Arizona
B. magna	1.5	NI	2.5
Z. carinata	NI	NI	7.5

NI indicates not isolated.

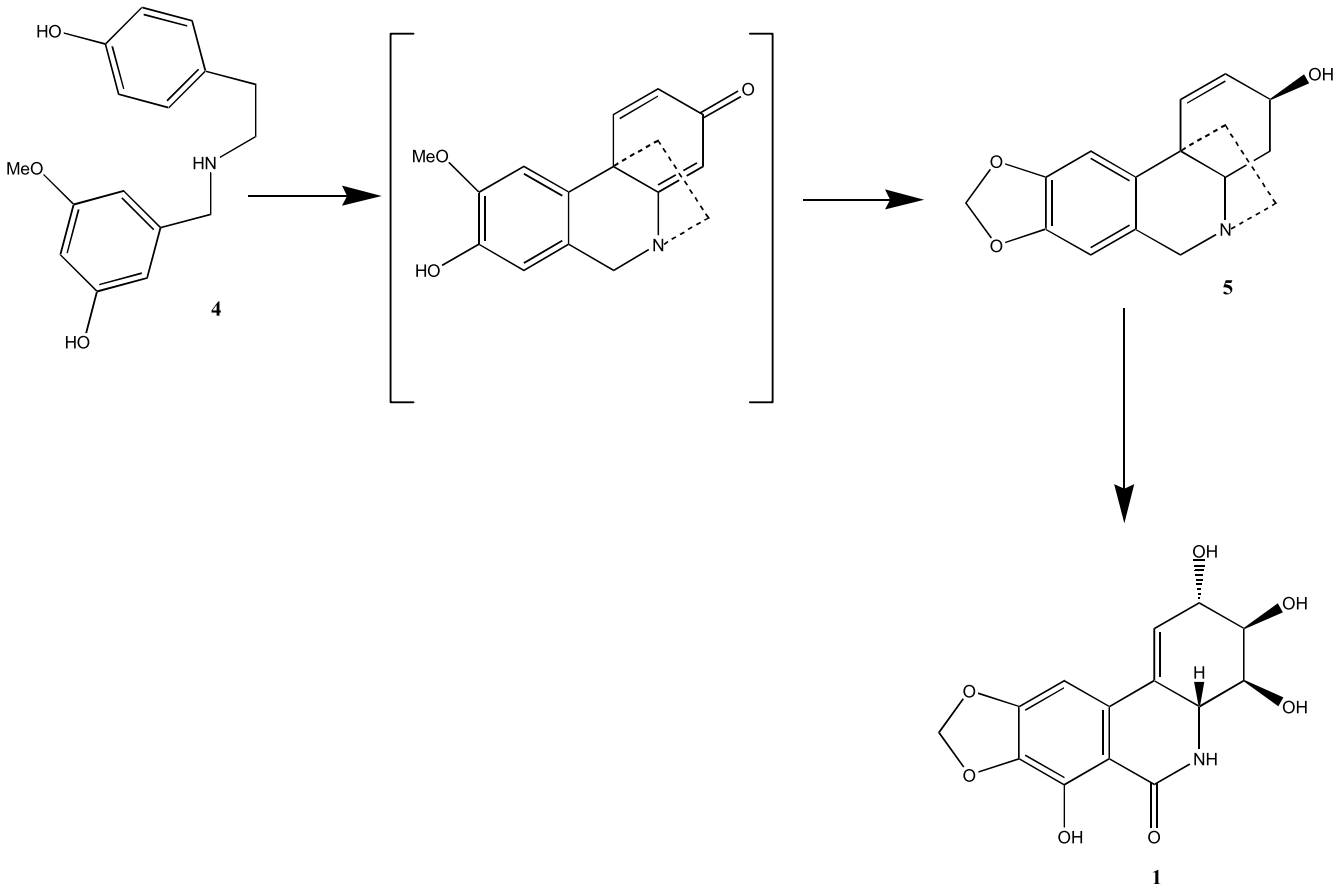
The biosynthesis of these Amaryllidaceae alkaloids in plants was deciphered in 1971 [17]. Narciclasine is synthesized from *O*-methylnorbelladine **4** by *para–para* phenol coupling to obtain vittatine **5** as an intermediate (Scheme 1) [17,18]. Subsequent elimination of two carbon atoms and hydroxylations of compound **5** then leads to narciclasine (Scheme 1).

In 1985, the first biosynthetic derivative of narciclasine, compound **6** (Figure 2), was isolated in good quantities from *Haemanthus kalbreyeri* [19] and *Z. flava* [20] (concentrations of 78 and 165 mg/kg, respectively), two tropical and subtropical Amaryllidaceae species. So this natural product was named kalbreclasine, and it is the 2-*O*-β-D-glucopyranoside derivative of narciclasine. This compound, whose structure was fully elucidated by spectroscopic and chemical methods, provoked a significant activation of splenic lymphocytes *in vitro*, a feature characteristic of immunostimulants [19]. Another β-D-glucoside derivative of narciclasine **7** (Figure 2) was isolated in 1991 in trace amounts from *P. maritimum* (~3.5 mg/kg) [21]. Currently, the last known natural derivative of narciclasine (Figure 2) isolated is *trans*-dihydronarciclasine **8**, which is extracted at low levels (11 mg/kg) from *Zephyranthes candida* [22].

Lycoricidine and Natural Derivatives

7-Deoxynarciclasine **2** (Figure 1), more generally known as lycoricidine, was first discovered in *L. radiata* in 1968 [12]. The product was isolated from methanol extracts of the plant's bulbs by a similar procedure to that used for narciclasine. Lycoricidine, which was obtained only at low levels (~3 mg/kg; Table 1), was found similar to narciclasine to be a plant growth inhibitor [12]. The structure of lycoricidine was fully established particularly by ^{1}H NMR and ^{13}C NMR and by chemical reactions [12,23]. Lycoricidine and narciclasine were shown in 1983 to have a potent insecticidal action, in addition to reducing feeding activity in the larvae of certain butterfly species [13].

Lycoricidine was also isolated in low quantities (~17 mg/kg) from *H. kalbreyeri* [6] and in moderate quantities (~32 mg/kg) from *Ismene* × "Sulphur Queen" [15]. The best sources of lycoricidine are bulbs of *Hymenocallis littoralis* collected from the wild in Hawaii as these yield in the order of 118 mg/kg [14,24]. However, if this rare tropical plant is cultivated in Arizona, the content of lycoricidine is much reduced, varies during the year, and only a maximum of 15 mg/kg can be isolated even in the peak month of October [14]. This difference in the content to the native Hawaiian bulb could be explained by an Arizona climate that is much hotter, drier, and sunnier [14].



Scheme 1. The biosynthesis of narciclasine.

To date, only one natural derivative of lycoricidine **9** (Figure 3) has been isolated from *H. littoralis* [14,23,25] (the tropical spider lily) and *Hymenocallis caribaea* [7]. Compound **9** (7-deoxy-*trans*-dihydronarciclasine or alternatively *trans*-dihydro lycoricidine) is a *trans*-dihydro derivative of lycoricidine in which the two ring cycles are *trans* linked. *Hymenocallis littoralis* contains a maximum of 16 mg/kg of **9** when sampled in October [14], whereas *H. caribaea* contains a maximum of only 3.8 mg/kg. The structure has been fully elucidated by spectroscopic and chemical methods [7,25].

Pancratistatin and Natural Derivatives

In 1984, pancratistatin **3** (Figure 1) was first discovered in a Hawaiian species originally designated as *Pancratium littorale*, but later reclassified as *H. littoralis* [26]. The bulb section of this native Hawaiian plant was extracted with dichloromethane–methanol–water and the extract was concentrated to obtain an aqueous phase. Organic compounds were extracted with *n*-butanol and purification of the resulting crude product was undertaken using gel permeation chromatography (Sephadex LH-20) and precipitation/crystallization to give the pure natural product **3** [26]. This experimental procedure is very effective for laboratory-scale isolation of pancratistatin, but for large-scale production, the cost is too high. Another procedure was then developed to avoid the use of gel permeation, but this approach remains more complicated than the isolation of narciclasine [14]. The structure of pancratistatin was fully elucidated by ^{1}H NMR and ^{13}C NMR, mass spectrometry, infrared, and elemental analyses [24,26] and was corroborated by an X-ray crystal structure determination of the hemisynthesized 7-methoxy derivative [26]. As with narciclasine and lycoricidine, pancratistatin has been found to exhibit strong *in vitro* RNA antiviral activity [27]. *In vivo*, pancratistatin increased survival by 100% when it was used to treat mice infected with Japanese encephalitis [27]. Furthermore, this product showed activity against two other RNA-containing flaviviruses and bunyaviruses, namely Punta Toro and Rift Valley fever [27]. Pancratistatin and lycoricidine also showed an *in vitro* antiparasitic effect against a microsporidium causing infections in humans [28].

The rare tropical bulbs of *H. littoralis* seem to be a good source of pancratistatin as it can be isolated from these in the order of 100 to 150 mg/kg when bulbs are obtained from the wild in Hawaii (Table 1) [14,24]. However, the compound has to be commercially extracted from field- and greenhouse-grown bulbs or from tissue cultures cultivated, for example, in Arizona, which generate lower levels of pancratistatin (a maximum of 22 mg/kg) even in the peak month of October (Table 1) [14]. After October, when the bulb becomes dormant, levels of pancratistatin drop to only 4 mg/kg by May [14]. Field-grown bulbs, which show monthly changes in pancratistatin content, generate somewhat smaller amounts (2-5 mg/kg) compared to those grown in greenhouse cultivated over the same period [14]. The occurrence of pancratistatin has also been investigated in other Amaryllidaceae, including several species of *Hymenocallis* [15], *B. magna* [16], and *P. maritimum* [15]. The pancratistatin content of these species does not exceed 29 mg/kg of fresh bulbs. Pancratistatin was exceptionally found in good quantity in *Z. flava* [20] and *H. kalbreyeri* [6] (Table 1), two tropical and subtropical plants cultivated in India. Consequently and to date, the best sources of pancratistatin are rare, tropical, and subtropical bulbs of *Z. flava*, *H. kalbreyeri*, and *H. littoralis* when these plants are cultivated in their native region.

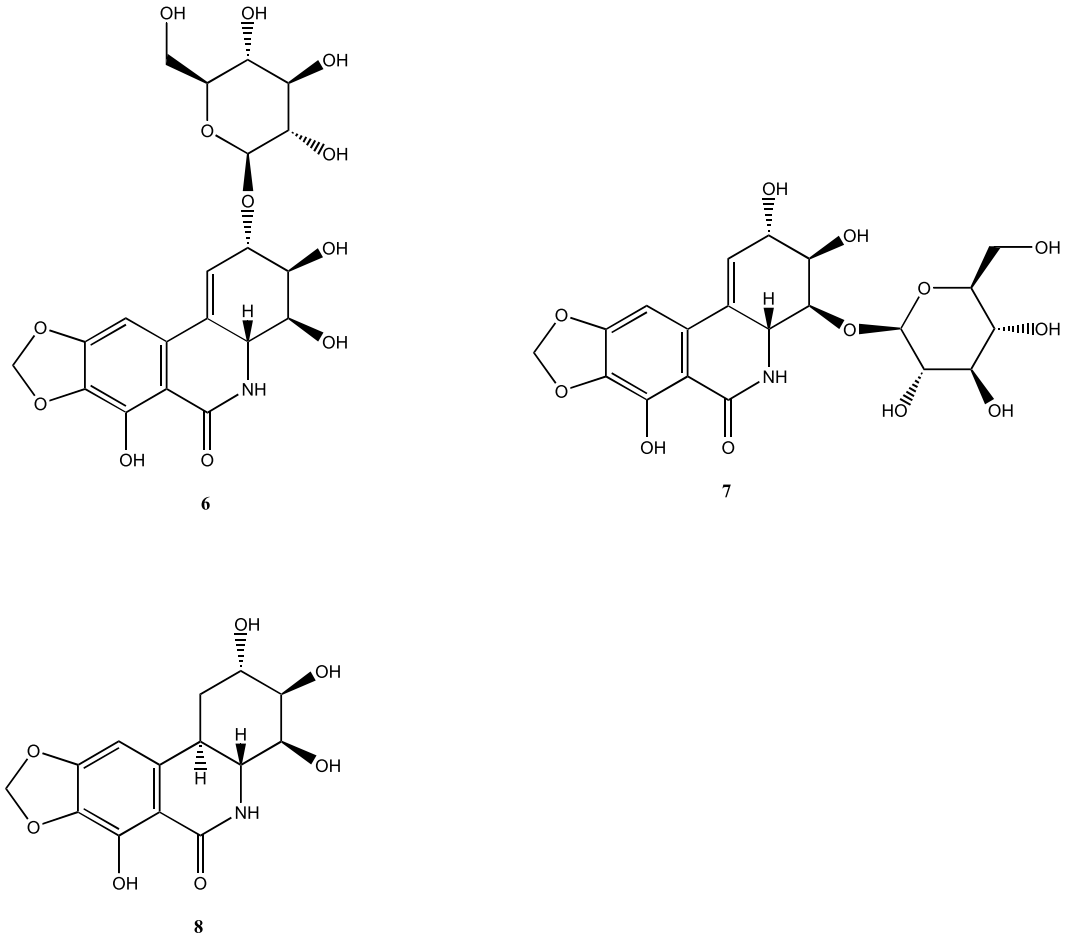


Figure 2. Natural derivatives of narciclasine.

The first natural derivative of pancratistatin, compound **10** (Figure 4), was isolated from *H. kalbreyeri* in 1989 [6]. This 2-*O*-β-D-glucopyranoside derivative, pancratiside, was found to promote the germination of seeds and the growth of roots similar to kalbreclasine **6** but unlike its aglycone derivatives [6]. 7-Deoxy-pancratistatin **12** (Figure 4) was also discovered in the same plant in small amounts (~30 mg/kg) [6]. The structures of these two alkaloids were established by comprehensive spectral analyses and chemical transformations [6]. Another glycoside derivative, telastaside **11** (Figure 4), was also isolated from this family of plants [29]. In 1998, two new derivatives of pancratistatin **13** and **14** (Figure 4) were extracted in moderate amounts (24 and 44 mg/kg, respectively) from *Zephyranthes carinata* [30]. Key substituents in these two molecules were identified as a 3-hydroxy-butyryl group for compound **13** and as a 3-*O*-glucosylated butyryl group for derivative **14** [30]. The structures of these two natural products were established by 1D and 2D NMR spectral analyses [30].

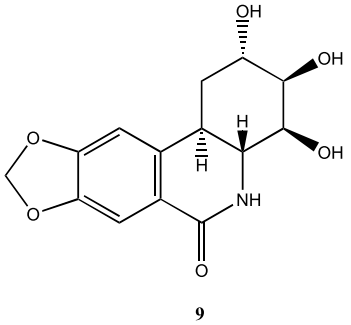


Figure 3. Chemical structure of the only one isolated natural derivative of lycoricidine.

Synthesis of Isocarbostyril Alkaloids

In general, these alkaloids are isolated only in moderate quantities from natural sources. As they have revealed promising antitumor activities (Sections 4 and 5), there is a strong need to develop total syntheses of these alkaloids and their derivatives to obtain them more easily and in appreciable quantities. There are more than 25 references on this subject revealing these syntheses to be complex multistep processes. The full synthesis of these alkaloids has been reviewed on several occasions [31,32], and the focus here will be on representative syntheses for narciclasine, lycoricidine, and pancratistatin.

Narciclasine and Lycoricidine

One of the shortest synthetic routes for narciclasine and lycoricidine was established by Rinner and Hudlicky [31] and Hudlicky et al. [32]. The key product of this synthesis was the cyclohexadiene diol **16** (Scheme 2) obtained by the whole-cell fermentation of 1,3-dibromobenzene **15** with recombinant *Escherichia coli* JM0109 [33]. This diol **16** was then protected by an acetonide group, and the resulting product subject to a hetero–Diels-Alder reaction to generate

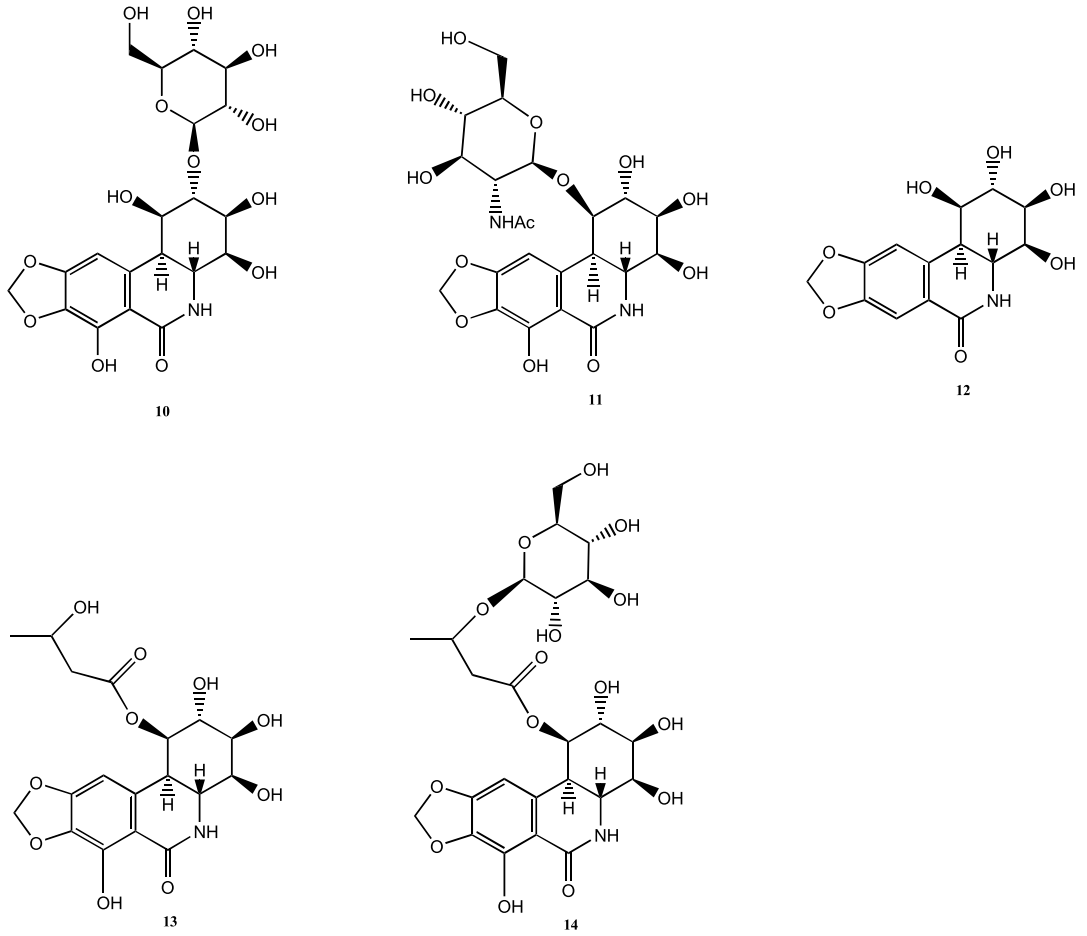
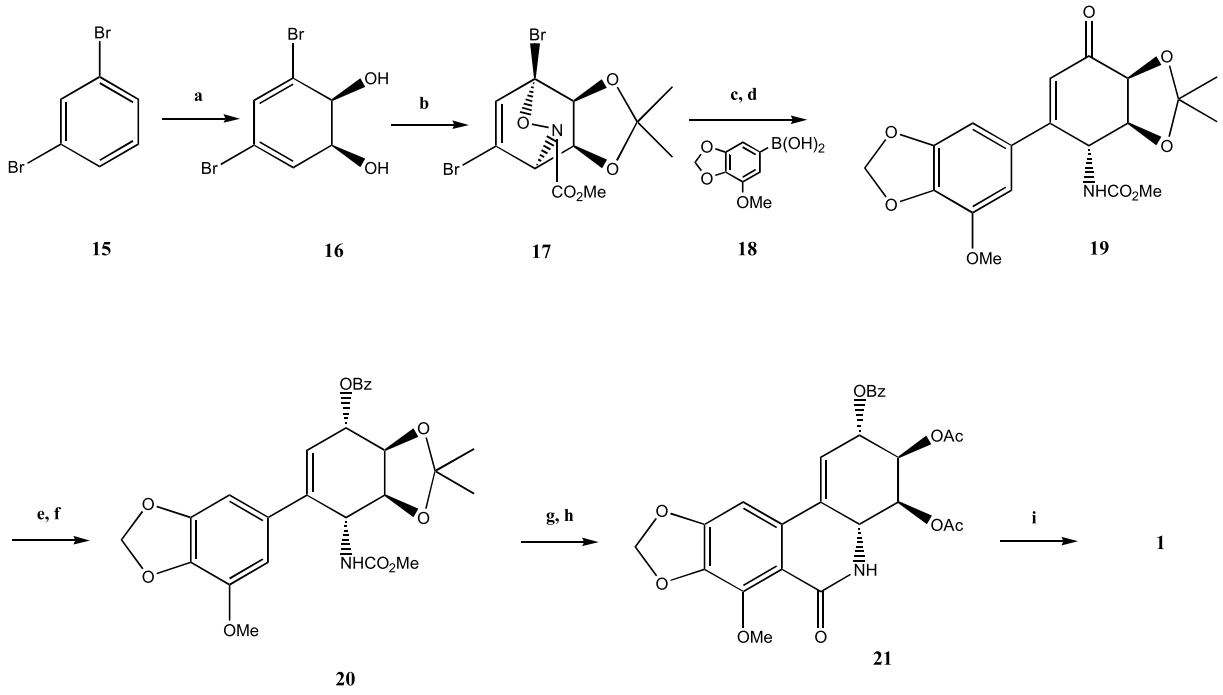


Figure 4. Chemical structures of natural derivatives of pancratistatin.



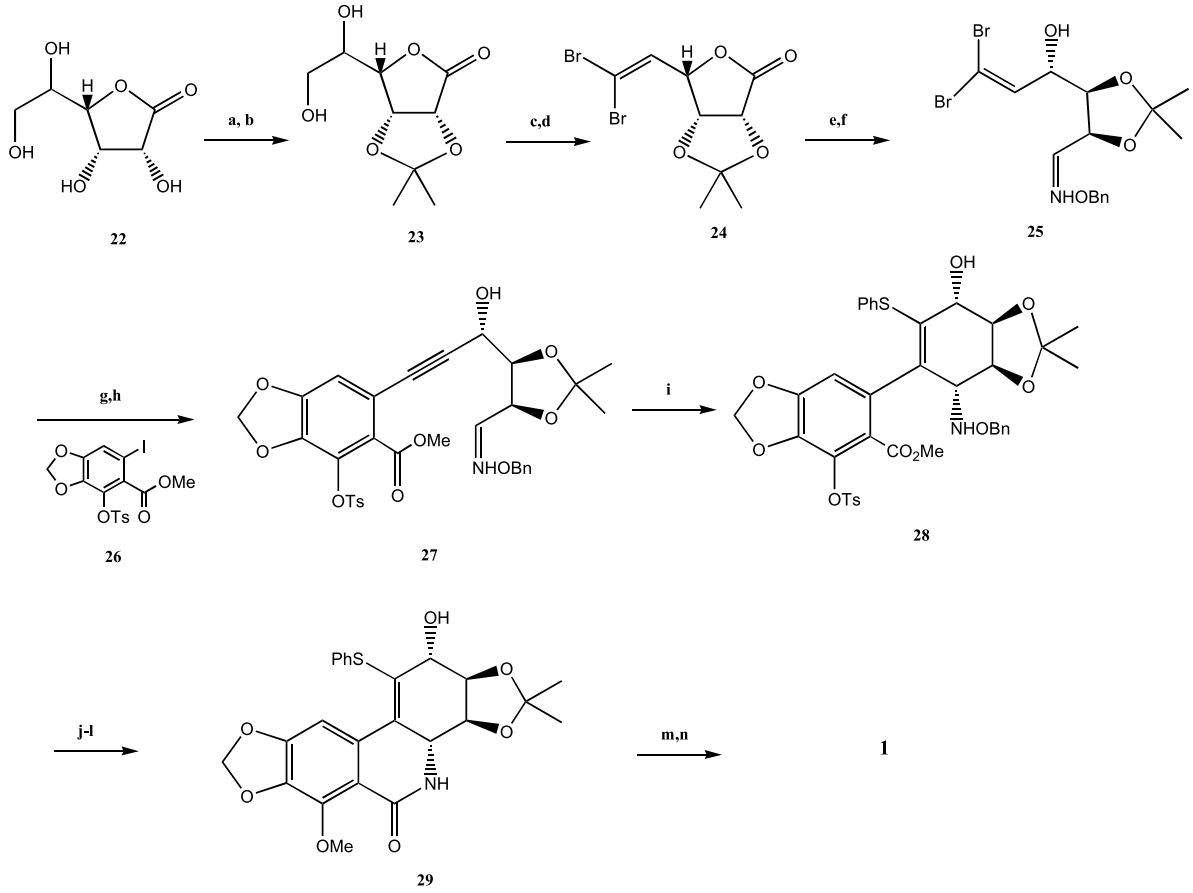
Scheme 2. **a**: *E. coli* JM109 (pDTG601A), 4 g/L. **b**: i) DMP, acetone, *p*-TsOH, r.t.; ii) $NHCO_2Me$, $NaIO_4$, r.t., 70%. **c**: borate **18**, $Pd(PPh_3)_4$, aq Na_2CO_3, PhH, reflux, 30%. **d**: TTMSS, AIBN, PhH, reflux, 80%. **e**: $NaBH_4$, $CeCl_3$, MeOH, 0°C, 80%. **f**: BzOH, Bu_3P, DEAD, THF, r.t., 65%. **g**: i) Dowex 50X8-100, MeOH, r.t.; ii) Ac_2O, pyridine, DMAP, r.t., 70%. **h**: Tf_2O, DMAP, CH_2Cl_2, 0°C, 40%. **i**: i) Amberlyst A21, MeOH; ii) LiCl, DMF, 120°C, 20%.

oxazine **17**. A Suzuki coupling of one of the bromines on **17** with the boronic acid derivative **18** afforded the desired coupled product, which was treated with a silylsilane derivative to form the ketone **19**. The reduction of the ketone function was followed by a Mitsunobu reaction to obtain benzoate **20**. After replacement of the acetonide by acetates, a modified Bischler–Napieralski ring-closure gave the protected product **21**. Cleavage of the phenolic methyl function and removal of protecting groups afforded narciclasine **1** in 12 steps with an overall yield of 0.5% from the cyclohexadiene diol **16**. The synthesis of lycoricidine **2** was realized in nine steps (30% overall yield) by the same method using a cyclohexadiene diol such as **16** obtained by biooxidation with the same bacteria *E. coli* [34].

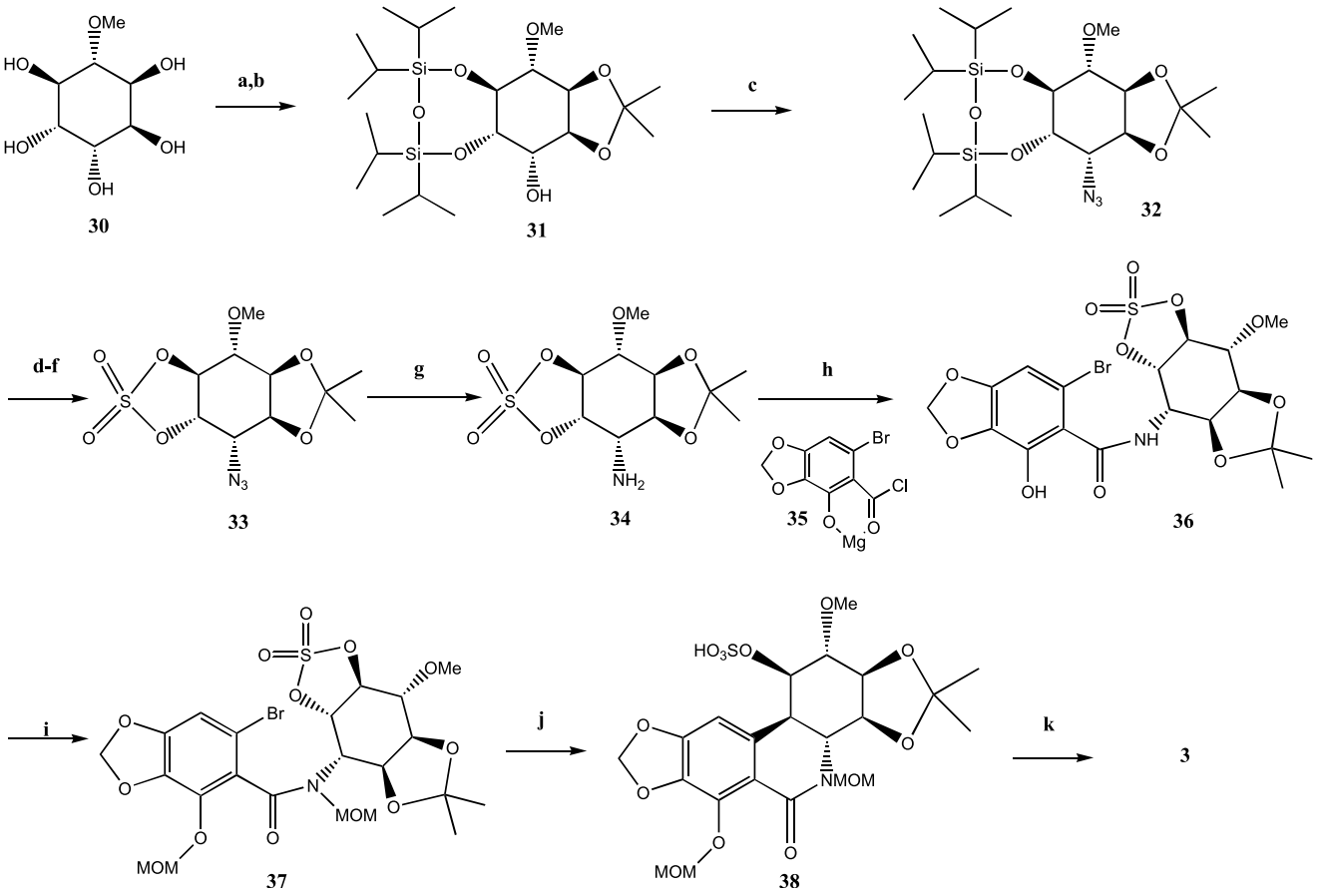
A second method of synthesis used for narciclasine and lycoricidine was established in 1999 by Keck as reviewed in the work of Rinner and Hudlicky [31]. The starting material of these total syntheses is the commercially available D-gulonolactone **22** that fits correctly with three of the four stereocenters of these isocarbostyril products (Scheme 3). After 2,3-acetonide protection, compound **23** was obtained in two steps. Oxidation of **23** followed by a Corey–Fuchs reaction of this latter product gave dibromide **24**. The lactone function of **24** was then converted to the *O*-benzyl amine derivative **25**. A Pd-catalyzed coupling reaction of **25** with compound **26** provided the alkyne **27**. The radical cyclization of this latter product afforded compound **28** with an 88% yield. Cleavage of the tosyl group in **28** and methylation of the phenolic hydroxyl functionality allowed aluminum-catalyzed cyclization to deliver the protected product **29**. Deprotection of the thioether and removal of acetonide by acid treatment afforded narciclasine **1** in 14 steps from D-gulonolactone with an overall yield of 16%. This method of synthesis was also used for lycoricidine in 11 steps with an overall yield of 27% starting from D-gulonolactone **22** and the appropriate aromatic analog of **26** [31].

Pancratistatin

Danishefsky et al., as reviewed in the work of Rinner and Hudlicky [31], reported in 1989 the first total synthesis of racemic pancratistatin. Thereafter, numerous research groups published the stereoselective synthesis of pancratistatin. These syntheses were previously reviewed [31] and include those of Tian et al. [35], Doyle et al. [36], Trost and Pulley [37], Rigby et al. [38], Magnus and Sebhat [39], and Ko et al. [40]. Another interesting synthesis of pancratistatin was reported in 2006 by Li et al. [41] and started from pinitol **30** whose stereogenic centers exactly match those of the C-ring of pancratistatin (Scheme 4). Protection of the diol functions of compound **30** gave compound **31**. The free hydroxyl of this was subsequently substituted by an azide to give **32**. After removal of the silyl function, a cyclic sulfate was installed to obtain product **33**. The Staudinger reaction gave the free amine **34** from azide **33**. The coupling reaction between **34** and **35** gave compound **36** with a moderate yield. Methocymethyl protection of both



Scheme 3. **a**: DMP, *p*-TsOH, DMF, 79%. **b**: AcOH, H_2O, THF, 79%. **c**: $NaIO_4$, CH_2Cl_2. **d**: CBr_4, PPh_3, Et_3N, 80% (more than two steps). **e**: L-Selectride, Et_2O, −78°C. **f**: $HCl{\cdot}H_2NOBn$, pyridine, 90% (more than two steps). **g**: *n*-BuLi, Et_2O, −90°C, 93%. **h**: $Pd(OAc)_2$, PPh_3, CuI, Et_3N, **26**, THF, 89%. **i**: PhSH, hν, toluene, 27°C, 88%. **j**: SmI_2, THF, H_2O, 0°C, 94%. **k**: MeI, K_2CO_3, DMF, 96%. **l**: Me_3Al, THF, −15°C to 65°C, 72%. **m**: SmI_2, MeOH, THF, 0°C, 87%. **n**: TFA, 0°C, 89%.



Scheme 4. **a**: $TIPDSCl_2$, imidazole, DMAP, DMF, 24%. **b**: DMP, *p*-TsOH, acetone, 81%. **c**: PPh_3, DEAD, CH_3SO_3H, CH_2Cl_2, 0°C to r.t. then NaN_3, DMF, 60°C, 72%. **d**: TBAF, THF, 0°C to r.t., 100%. **e**: $SOCl_2$, Et_3N, CH_2Cl_2, 0°C. **f**: $NaIO_4$, $RuCl_3$, aq CH_3CN, 87% (more than two steps). **g**: PPh_3, aq THF, 0°C to r.t., 94%. **h**: Et_2O, **35**, 0°C, 64%. **i**: K_2CO_3, MOMCl, DMF, 84%. **j**: *t*-BuLi, $CeCl_3$, ultrasound, THF, −78°C to r.t., 72%. **k**: BBr_3, CH_2Cl_2, −78°C to 0°C, 1 hour then MeOH, −78°C to 0°C, 2 hours, 52%.

the amide and the free phenol gave compound **37**. Treatment of this latter product with *t*-BuLi followed by addition of cerium chloride gave compound **38**. Full deprotection of **38** by BBr_3 and methanol afforded pancratistatin **3** in 12 steps from commercially available pinitol with an overall yield of 2.3%.

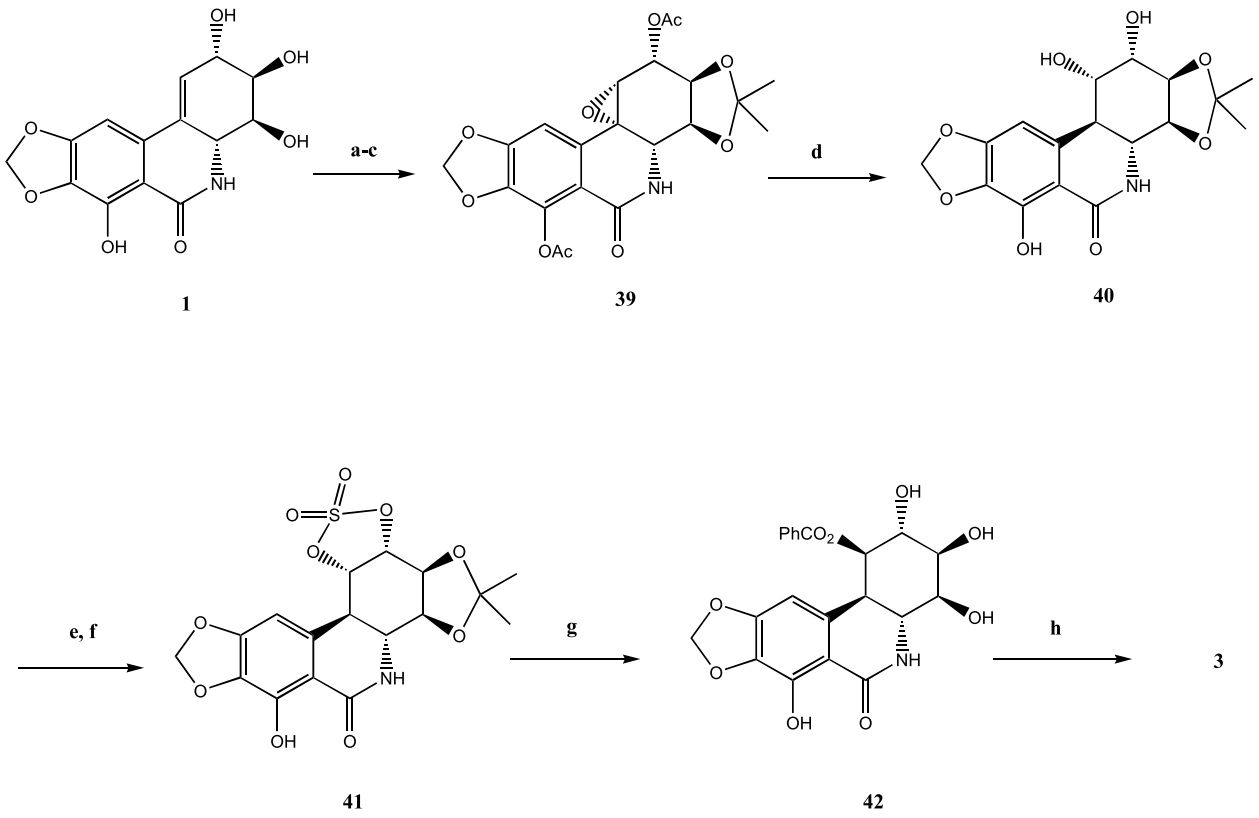
Given the higher availability of narciclasine in extracts of *Narcissus* species (up to 200 mg/kg), Pettit et al. [42,43] have developed and patented a second process to synthesize pancratistatin from Amaryllidaceae-derived narciclasine. The hydroxyl functions of narciclasine are first protected and the olefin function oxidized to give epoxide **39** (Scheme 5). Hydrogenation of this latter product and subsequent saponification yields a mixture of four compounds, one of which is the desired diol **40**. Activation of this diol with thionyl chloride and oxidation of the epimeric sulfites give the cyclic sulfate **41**. Nucleophilic opening of compound **41** and hydrolysis of the sulfate and acetonide functions affords the benzoate product **42**. Deprotection of the benzoyl group at C-1 position gives pancratistatin **3** in 10 steps and an overall yield of 3.6% from narciclasine **1**.

In Vitro Antitumor Activity

Narciclasine, Lycoricidine, Pancratistatin, and Natural Derivatives

The cytotoxicity of these isocarbostyril alkaloids was first demonstrated in basic assays some 30 years ago [12,44] and later confirmed in standard MTT colorimetric assays on diverse human tumor cell lines by the National Cancer Institute (NCI) [7,23,45,46].

Narciclasine **1** exhibits *in vitro* potent cytotoxicity against human cancer cell lines and murine P388 leukemia cells (Table 2) [45,46]. The cytotoxicity of this product was evaluated in 60 cancer cell lines by the NCI, and the mean IC_{50} value (the concentration which inhibits overall cell growth in culture by 50%) was 0.046 μM [45]. With respect to narciclasine, lycoricidine **2** demonstrates cytotoxic activity against these 60 cancer cell lines, which is ~10 times weaker (mean IC_{50} = 0.33 μM), and pancratistatin **3** an activity ~5 times weaker (IC_{50} = 0.26 μM) [46]. The observed cytotoxicity of these three isocarbostyrils against murine P388 leukemia cell line is similar but results against human cancer cell lines (Table 2) confirm those found by the NCI for lycoricidine but not for pancratistatin [23,45]. Against the murine P388 leukemia cell line, dehydronarciclasine **8** showed more potent cytotoxicity than narciclasine (Table 2), but in the 60 cancer cell line panel, the cytotoxicity was of the same order of magnitude with a mean IC_{50} of 0.053 μM [23,45,46]. Dehydrolycoricidine **9** revealed the same level of cytotoxicity as lycoricidine (Table 2) [23]. These results with compounds **8** and **9** revealed that the double bond between the C-1 and C-10b is not essential for the cytotoxicity of these isocarbostyril alkaloids. For glycoside derivatives **6**, **7**, **10**, and **11** (Figures 2 and 4), there are no data in the literature on the cytotoxicity measured by MTT assays against cancer cells. However, for glucoside derivative **7**, a potato disc cytotoxicity assay has revealed an activity very similar to



Scheme 5. **a**: DMF, DMP, *p*-TsOH, 97%. **b**: Ac_2O, pyridine, 81%. **c**: *m*-CPBA, phosphate buffer, CH_2Cl_2, H_2O, 52%. **d**: i) H_2, Pd/C 10%; ii) K_2CO_3, MeOH, H_2O, 28%. **e**: $SOCl_2$, Et_3N, THF; **f**: $RuCl_3{\cdot}3H_2O$, $NaIO_4$, MeCN, CCl_4, H_2O, 47% (more than two steps). **g**: i) $PhCO_2H$, $CsCO_3$, DMF; ii) THF, H_2O, H_2SO_4, 74%. **h**: K_2CO_3, MeOH, 75%.

narciclasine [21]. For 7-deoxy pancratistatin **12**, only two cancer cell lines have been tested and this product demonstrated a 10- to 20-fold lower cytotoxic activity than pancratistatin (Table 2) [47]. It can be concluded that the phenolic hydroxyl group of compounds **1** and **3** contributes to the greater (≥10-fold) activity of these products compared to that of their congeners **12** and **2**.

The hydroxybutyril derivative of pancratistatin **13** gave two-fold better cytotoxicity than pancratistatin against P388 leukemia cells [30]. This better activity was then confirmed by the NCI in the 60–cancer cell line panel with a mean IC_{50} of 0.037 μM [46]. The glucoside derivative **14** revealed the same order of cytotoxicity as pancratistatin against P388 leukemia cells [30].

Fully and Hemisynthesized Derivatives

A series of derivatives and analogs have been synthesized by full or hemisynthesis to obtain more active and/or soluble products (Figure 5) that could be potentially selected for preclinical development as anticancer agents.

Only one compound that has been synthesized, a derivative of narciclasine (Scheme 5) [42,43], has been found to be more active than pancratistatin. This 1-benzoate pancratistatin derivative **42**, phenpanstatin, has revealed a cytotoxicity 10 times higher than pancratistatin **3** against a panel of human cancer cell lines (Tables 2 and 3) [45].

Naturally occurring isocarbostyril alkaloids such as pancratistatin have low aqueous solubility (~50 μg/ml) [45]. This has limited their clinical evaluation in intravenous formulations. To increase aqueous solubility, phosphate prodrugs, which release the active drug through the action of cellular phosphatases, have been synthesized [23,45,48]. Several positions (4-OH, 7-OH, and 3,4-OH) on the natural isocarbostyril alkaloids were used to obtain 3,4-cyclic phosphate derivatives **43**, **44**, **45**, **50** (7-*O*-phosphate pancratistatin), **51** (4-*O*-phosphate pancratistatin), **52**, **53** (narcistatin), and **54** (Figure 5) [23,45,48]. These

Table 2. *In Vitro* Cytotoxic-Related Antitumor Effects (IC_{50} Values in μM)* of Certain Naturally Occurring Isocarbostyril Alkaloids [23,44].

Compounds	Cancer Cell Lines						
	Leukemia P388	Pancreas BXPC-3	Breast MCF-7	CNS SF268	Lung-NSC NCI-H460	Colon KM20L2	Prostate Du-145
1	0.042	0.011	0.010	0.010	0.027	0.011	0.011
2	0.065	0.24	0.16	0.41	0.18	0.29	0.17
3	0.052	0.061	0.071	0.043	0.098	0.077	0.046
8	0.0078	0.039	0.017	0.065	0.030	0.049	0.021
9	0.099	0.16	0.12	0.20	0.15	0.17	0.14
12	1.42	NT	NT	NT	NT	0.71	NT

*Data are initially given in μg/ml [50% effective dose (ED_{50}) or 50% growth inhibition (GI_{50})].
NT indicates not tested.

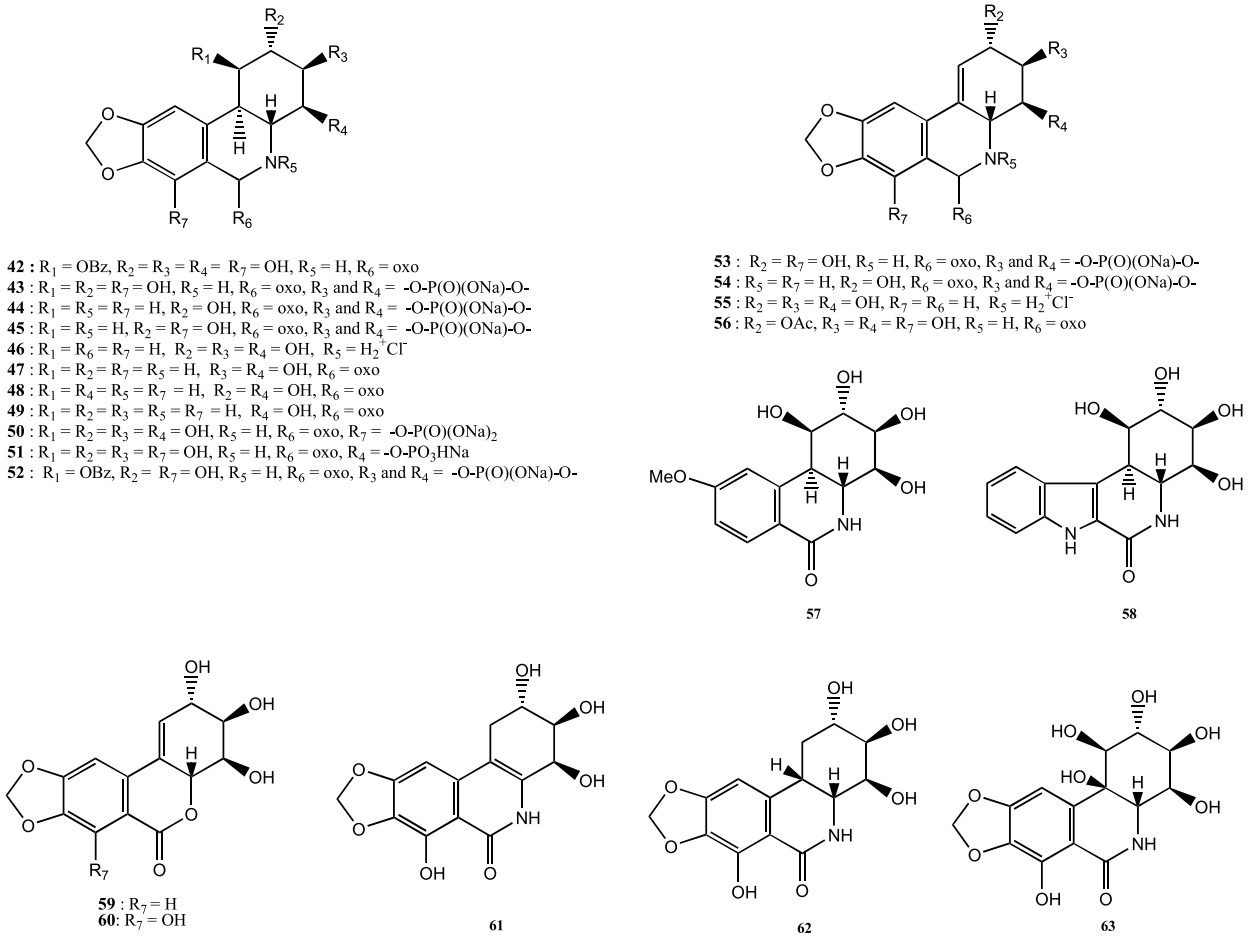


Figure 5. Chemical structures of fully and hemisynthesized isocarbostyril derivatives.

phosphate derivatives had considerably improved aqueous solubility, notably the sodium salt of narcistatin **53** (60 mg/ml) and have been duly patented [49–52]. All phosphate derivatives have markedly lower cytotoxicity *in vitro* compared to their active parent compound (Tables 2 and 3) [23,45,48]. However, as indicated above, hydrolysis of the phosphate groups to release the active isocarbostyril is expected to be very effective *in vivo*. *In vivo* antitumor evaluations will be undertaken shortly to validate this concept of water-soluble phosphate prodrugs [23].

Table 3. *In Vitro* Cytotoxic-Related Antitumor Effects (IC_{50} Values in μM)* of Fully and Hemisynthetic Isocarbostyril Alkaloid Derivatives.

Compounds	Cancer Cell Lines						
	Leukemia P388	Pancreas BXPC-3	Breast MCF-7	CNS SF268	Lung-NSC NCI-H460	Colon KM20L2	Prostate Du-145
42	0.0037	0.0044	0.00072	0.0013	0.00023	0.00086	0.00049
43	8.1	8.1	7.1	7.1	9.3	9	5.6
44	4.2	> 26.5	19.1	> 26.5	> 26.5	> 26.5	> 26.5
45	2.2	14.2	11.7	22.4	19.8	24.4	13.2
46	10.5	4.4	5.4	5.1	2.5	5.4	2.2
47	5	> 36.1	> 36.1	> 36.1	> 36.1	> 36.1	> 36.1
48	1.6	NT	NT	NT	NT	NT	NT
49	153.6	NT	NT	NT	NT	NT	NT
50	0.53	0.44	0.44	0.18	0.42	0.38	0.058
51	0.042	0.42	0.42	0.28	0.89	0.56	2.2
52	0.12	0.49	0.08	0.33	0.056	0.25	0.25
53	0.031	0.18	0.15	0.12	0.15	0.15	0.08
54	4.5	14.1	10.7	16.8	12.5	14.9	10.4
55	22.6	14	10.8	9.2	10.8	> 32	> 10.2
56	< 0.03	0.78	0.72	0.57	0.96	0.54	0.78
57	14.6	16.6	14.9	11.2	9.5	12.2	8.8
58	60.2	> 32.9	> 32.9	NT	NT	> 32.9	NT

*Data are initially given in μg/ml (ED_{50} or GI_{50}).
NT indicates not tested.

To explore the structure–activity relationship (Figure 5 and Tables 2 and 3) of these isocarbostyril alkaloids, several other key positions in the molecule were modified. One modification involved the reduction of the lactam carbonyl group. For this purpose, 2-acetate lycoricidine **56** (Figure 5) was synthesized as an intermediate, but this modification was found to have a low impact on cytotoxicity. In contrast, compounds **46** and **55** (Figure 5) were found to be inactive against the panel of human cancer cell lines (Table 3) [25]. Fully synthetic compounds **59** and **60** (Figure 5) in which the lactam carbonyl was also replaced by a lactone function, which is considered an analog [5], showed no significant cytotoxic activity against L1210 murine leukemia cells [5]. All these modulations appear to indicate that the lactam carbonyl function is essential for cytotoxic activity.

Three deoxy-analogs of these isocarbostyril alkaloids **47**, **48**, and **49** (Figure 5) were synthesized to determine the minimum structural pharmacophore [53,54]. These three analogs showed weak antitumor activity (Table 3) and so revealed that the C-2, C-3, and C-4 hydroxyl groups were essential for the cytotoxic activity and can be considered as a minimum pharmacophore [53,54].

Another modulation was the B/C ring junction stereochemistry (Figure 1) and was evaluated through the synthesis of derivatives **61**, **62**, and **63** (Figure 5) [7,55]. For the isonarciclasine **61**, the B/C ring junction is planar because a double bond connects the two rings. A *cis*–B/C ring junction was obtained in products **62** and **63** after hydrogenation or oxidation of the double bond of narciclasine [7,22]. All these products showed weaker cytotoxicity than the parent natural compounds. Compound **61** gave an IC_{50} in the order of 12 μM and **62** an IC_{50} of ~4 μM [7]. The cytotoxicity of compound **63** against P388 murine leukemia and several human cancer cell lines was found to be about 1000 times less potent than pancratistatin [55]. All these results appear to confirm the need for a *trans*–B/C ring junction in these compounds to maintain potent cytotoxicity.

Modulation of the benzodioxole moiety of pancratistatin was investigated through the full synthesis of analogs **57** and **58** (Figure 5) [47,54]. The methoxy analog **57** and the β-carboline-1-one derivative **58** were revealed to be 100-fold less cytotoxic than the corresponding natural product [47,54]. These results show that an intact methylenedioxyphenyl or benzodioxole functionality is essential for significant cancer cell cytotoxicity in this class of natural compounds.

Table 4. National Cancer Institute Pancratistatin (A) and Narciclasine (B) *In Vivo* Antitumor Activities in a Range of Different Mouse Tumor Models.

(A) Pancratistatin, mg/kg

T/C Survival	B16 Melanoma	L1210 Leukemia	P388 Leukemia	M5076 Sarcoma	M5076 Sarcoma
Tumor Site Inoculation	i.p.	i.p.	i.p.	i.p.	s.c.
0.18	NT	NT	113%	153%	101%
0.25	NT	NT	NT	NT	106%
0.37	NT	NT	122%	NT	NT
0.38	NT	120%	119%	NT	NT
0.5	NT	NT	NT	NT	112%
0.75	NT	122%	139%	159%	106%
0.78	NT	NT	133%	132%	NT
1	NT	NT	NT	NT	109%
1.5	100%	121%	142%	166%	111%
1.56	108%	131%	144%	129%	NT
2	NT	NT	NT	NT	112%
3	103%	133%	161%	184%	125%
3.12	129%	131%	151%	143%	NT
4	NT	NT	NT	NT	113%
6	114%	NT	165%	177%	126%
6.25	110%	NT	173%	152%	NT
8	NT	NT	NT	NT	121%
12	NT	NT	156%	144%	NT
12.5	NT	NT	203%	NT	NT
24	NT	NT	NT	Toxic	NT
25	Toxic	Toxic	Toxic	NT	NT

(B) Narciclasine, mg/kg

T/C Survival	B16 Melanoma	L1210 Leukemia	P388 Leukemia	M5076 Sarcoma	Lewis Lung Carcinoma
Site of Inoculation	i.p.	i.p.	i.p.	i.p.	i.v.
0.62	115%	110%	110%	NT	93%
1.25	111%	108%	129%	NT	109%
2.5	109%	113%	162%	NT	117%
3	NT	NT	NT	133%	NT
5	123%	115%	100%	NT	NT
6	NT	NT	NT	160%	NT
10	NT	NT	87%	NT	NT
12.5	NT	NT	NT	NT	NT

All compounds were administrated intraperitoneally.
i.p. indicates intraperitoneal; *i.v.*, intravenous; *NT*, not tested; *s.c.*, subcutaneous.

In Vivo Antitumor Activity

Pancratistatin and narciclasine have proven to be effective in murine P388 lymphocytic leukemia and M-5076 ovary sarcoma models *in vivo* [24]. Table 4 (A and B) shows the *in vivo* survival values from the NCI database [46] for the antitumor activities of pancratistatin (A) and narciclasine (B) in different mouse models. The *T/C* index represents the percentage survival ratio of drug-treated (*T*) compared to untreated (*C*) mice. A *T/C* value >125% indicates that drug treatment significantly enhances the survival of the tumor-bearing mice.

The promising antineoplastic activity of pancratistatin has led to its preclinical development and selection as an important synthetic starting point for a growing number of research groups. Pancratistatin is particularly attractive for clinical development because of its potent activity against experimental melanoma and ovary carcinoma. Unfortunately, pancratistatin exhibits very low water solubility (53 μg/ml) and this property has complicated its formulation for intravenous administration. Although the solubility of this isocarbostyril can be increased in organic solvents, such as dimethylformamide and dimethylsulfoxide, and lower boiling aliphatic alcohols, their use as formulation components is not desirable.

Mechanism of Action

Little is known regarding the anticancer mechanism of action of this family of compounds. Narciclasine and lycoricidine were first identified as plant growth inhibitors [8,12]. In plants, there is clear evidence that narciclasine inhibits the synthesis of proteins and the development of chloroplasts. Additionally, the compound isolated from the mucilage of *Narcissus* bulbs showed inhibitory effects on the growth and plastid development in excised radish cotyledons [56]. Narciclasine (0.1 μM) starts to show inhibitory effects on plant isocitrate lyase and hydroxypyruvate reductase activities after 24 hours of incubation in light. When the concentration is increased to 10 μM, the activities of both enzymes are completely inhibited [56]. From ultrastructure studies, 1 μM narciclasine markedly prevents the degradation of protein bodies and lipid bodies, as well as totally preventing chloroplast formation in excised radish cotyledons [56]. Visible symptoms of tepal senescence in cut *Iris* × "Hollandica" (cv. Blue Magic) flowers are delayed by placing one cut daffodil flower (*Narcissus pseudonarcissus*, cv. "Carlton") in the same vase

[57]. Addition of mucilage, exuded by daffodil stems to the vase water has the same effect as the flowering daffodil stem. The active compound in the mucilage was identified as narciclasine. This delay of senescence by narciclasine correlates with a delayed increase in protease activity and marked reduction in maximum protease activity [57]. Narciclasine, although not affecting *in vitro* protease activity, is known to inhibit protein synthesis at the ribosomal level. Narciclasine was also originally described as an antimitotic substance displaying colchicine-like effects [8]. It was also found to be an inhibitor of peptide bond formation in eukaryotic ribosomes given its ability to bind to the 60-S ribosomal subunit and more precisely to the peptidyltransferase center [58,59]. Furthermore, unlike many other anticancer drugs, narciclasine has been found not to interact or form a complex with DNA [60]. More recently, McLachlan et al. [61] demonstrated that pancratistatin, whose chemical structure is very close to that of narciclasine, induced rapid apoptosis in neuroblastoma cells accompanied by disruption of the mitochondrial membrane potential. Additionally, a decrease in ATP synthesis and an increase in the production of reactive oxygen species, indicative of a dysfunction of the mitochondrial respiratory chain, were observed in intact mitochondria incubated with the molecule [61]. These changes were not observed in normal fibroblasts [61,62] and normal endothelial cells [63]. Caspase-3 activation and exposure of phosphatidyl serine on the outer leaflet of the plasma membrane were earlier events than the generation of reactive oxygen species and DNA fragmentation observed following pancratistatin treatment of cancer cells [64]. By investigating isolated mitochondria, McLachlan et al. [61] demonstrated that pancratistatin acted directly on mitochondria and confirmed that this effect was selective for mitochondria isolated from cancer cells. Quite recently, Griffin et al. [62] undertook a synthetic structure–activity relationship evaluation, which indicated that the minimum cytotoxic pharmacophore of pancratistatin comprises the transfused B/C ring system containing the 2,3,4-triol unit in the C-ring. The results of their study further indicated that the phenanthridone skeleton in natural Amaryllidaceae alkaloids may be a significant common element for selectivity against cancer cells [62]. McNulty et al. [65] have previously demonstrated that a 2,3,4-triol–functionalized ring-C is required as the minimum pharmacophoric element for potent anticancer activity of pancratistatin.

We have recently reported that narciclasine shows potent *in vitro* cytotoxic activity against six different human cancer cell lines. In contrast, normal human lung fibroblasts appear to be markedly less sensitive to the molecule with a mean cytotoxic IC_{50} value (the concentration that reduces by 50% the number of viable cells after 3 days of treatment) of 7.5 μM compared to 0.03 μM for the six human cancer cell lines [66]. The cytotoxic activity of narciclasine was similar in the six cancer cell lines investigated, a feature consistent with NCI data that revealed a mean IC_{50} value of 0.046 μM for the compound across a panel of 60 cancer cell lines, including doxorubicin-resistant cancer cells [46]. Additionally, based on comparative evaluations assessing morphologic changes, after internucleosomal DNA fragmentation or the externalization of phosphatidyl serine in normal fibroblasts and in human cancer cells, the proapoptotic effects of narciclasine at concentrations up to 1 μM were confined to cancer cells [66]. These results collectively emphasize the marked selectivity of narciclasine for cancer cells, as also demonstrated by Griffin et al. [62] for pancratistatin. We have shown that the narciclasine-induced apoptosis-mediated cytotoxic effects in human cancer cells not evident in normal fibroblasts is paralleled by the triggering of the activation of the initiator caspases of the death receptor pathway (caspase-8 and -10), at least in human MCF-7 breast and PC-3 prostate carcinoma cells. This is a feature not observed in normal human fibroblasts [66]. Indeed, formation of the Fas and DR4 death-inducing signaling complex was clearly evidenced in MCF-7 and PC-3 cancer cells [66]. Additionally, in these two cancer cell lines, caspase-8 was found to interact with the Fas and DR4 receptors on narciclasine treatment. However, after this initial step, narciclasine-induced downstream apoptotic pathways in MCF-7 cells diverged from those found in PC-3 cells, where caspase-8 directly activated effector caspases such as caspase-3 in the absence of any further release of mitochondrial proapoptotic effectors [66]. In contrast, in MCF-7 cells, the apoptotic process was found to require an amplification step that is mitochondria-dependent with Bid processing, release of cytochrome *c* and caspase-9 activation [66].

We have postulated that the high selectivity of narciclasine for cancer cells might be linked at least in part to this activation of the death receptor pathway. Normal human fibroblasts appear approximately 250-fold less sensitive to the cytotoxic effects of narciclasine, which does not induce apoptosis in these normal fibroblasts probably due to the absence of death receptor pathway activation [67–69]. Moreover, it has been suggested that Amaryllidaceae alkaloids, such as narciclasine, have a modulating activity on inflammatory reactions by inhibiting tumor necrosis factor-alpha production in stimulated macrophages [70]. Inflammation has been implicated in the development of many human epithelial cancers, including those of the stomach, lung, colon, and prostate, and tumor necrosis factor-alpha is a potent pleiotropic, proinflammatory cytokine produced by many cells in response to injury and inflammation [71].

Conclusions

Since the discovery of narciclasine, the first member of isocarbostyril class of alkaloids in 1967, the search for new natural compounds from the Amaryllidaceae plant family has resulted in the isolation of other isocarbostyrils such as lycoricidine and pancratistatin.

To date, for the synthesis of novel isocarbostyril derivatives for the potential discovery and development of new anticancer drug candidates, the best starting material for these products is narciclasine, which can be isolated from *Narcissus* species with a good yield. The total synthesis of these products is still very complex and requires too many steps to be developed commercially for the synthesis and anticancer testing of novel isocarbostyrils.

The biologic activities of isocarbostyril alkaloids include excellent *in vitro* and *in vivo* cytotoxicity against many tumor cell lines and high selectivity for cancer cells *versus* normal cells. This has encouraged several research groups to identify new derivatives or analogs of these isocarbostyril alkaloids for preclinical development. It is to be hoped that continuing interest in structure modulation of these isocarbostyril alkaloids will provide novel promising anticancer agents.

References

[1] Cragg GM, Newman DJ, and Snader KM (1997). Natural products in drug discovery and development. *J Nat Prod* **60** (1), 52–60.

[2] Newman DJ, Cragg GM, and Snader KM (2003). Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* **66** (7), 1022–1037.

[3] Hartwell JL (1967). Plants used against cancer. A survey. *Lloydia* **30** (4), 379–436.

[4] Weniger B, Italiano L, Beck JP, Bastida J, Bergoñon S, Codina C, Lobstein A, and Anton R (1995). Cytotoxic activity of Amaryllidaceae alkaloids. *Planta Med* **61** (1), 77–79.

[5] Ibn-Ahmed S, Khaldi M, Chrétien F, and Chapleur Y (2004). A short route to enantiomerically pure benzophenanthridone skeleton: synthesis of lactone analogues of narciclasine and lycoricidine. *J Org Chem* **69** (20), 6722–6731.

[6] Ghosal S, Singh S, Kumar Y, and Srivastava S (1989). Isocarbostyril alkaloids from *Haemanthus kalbreyeri*. *Phytochemistry* **28** (2), 611–613.

[7] Pettit GR, Pettit GR III, Backhaus RA, Boyd MR, and Meerow AW (1993). Antineoplastic agents: 256. Cell growth inhibitory isocarbostyrils from *Hymenocallis*. *J Nat Prod* **56** (10), 1682–1687.

[8] Ceriotti G (1967). Narciclasine: an antimitotic substance from *Narcissus* bulbs. *Nature* **213** (5076), 595–596.

[9] Piozzi F, Fuganti C, Mondelli R, and Ceriotti G (1968). Narciclasine and narciprimine. *Tetrahedron* **24** (11), 1119–1131.

[10] Mondon A and Krohn K (1970). Revision der Narciprimin-Struktur. *Chem Ber* **103** (9), 2729–2743.

[11] Piozzi F, Marino L, Fuganti C, and Di Martino A (1969). Occurrence of nonbasic metabolites in Amaryllidaceae. *Phytochemistry* **8** (9), 1745–1748.

[12] Okamoto T, Torii Y, and Isogai Y (1968). Lycoricidinol and lycoricidine, new plant-growth regulators in the bulbs of *Lycoris radiata* herb. *Chem Pharm Bull* **16** (9), 1860–1864.

[13] Numata A, Takemura T, Ohbayashi H, Katsuno T, Yamamoto K, Sato K, and Kobayashi S (1983). Antifeedants for the larvae of the yellow butterfly, *Eurema hecabe mandarina*, in *Lycoris radiata*. *Chem Pharm Bull* **31** (6), 2146–2149.

[14] Pettit GR, Pettit GR III, Backhaus RA, and Boettner FE (1995). Antineoplastic agents: 294. Variations in the formation of pancratistatin and related isocarbostyrils in *Hymenocallis littoralis*. *J Nat Prod* **58** (1), 37–43.

[15] Pettit GR, Pettit GR III, Groszek G, Backhaus RA, Doubek DL, Barr R, and Meerow AW (1995). Antineoplastic agents: 301. An investigation of the Amaryllidaceae genus *Hymenocallis*. *J Nat Prod* **58** (5), 756–759.

[16] Pettit GR, Meng Y, Herald DL, Knight JC, and Day JF (2005). Antineoplastic agents: 553. The Texas grasshopper *Brachystola magna*. *J Nat Prod* **68** (8), 1256–1258.

[17] Fuganti C, Staunton J, and Battersby AR (1971). The biosynthesis of narciclasine. *J Chem Soc D: Chem Commun* **19**, 1154–1155.

[18] Fuganti C and Mazza M (1972). The absolute configuration of narciclasine: a biosynthetic approach. *J Chem Soc Chem Commun* **4**, 239.

[19] Ghosal S, Lochan R, Ashutosh Kumar Y, and Srivastava RS (1985). Alkaloids of *Haemanthus kalbreyeri*. *Phytochemistry* **24** (8), 1825–1828.

[20] Ghosal S, Singh SK, and Srivastava RS (1986). Alkaloids of *Zephyranthes flava*. *Phytochemistry* **25** (8), 1975–1978.

[21] Abou-Donia AH, De Giulio A, Evidente A, Gaber M, Habib AA, Lanzetta R, and Seif El Din AA (1991). Narciclasine-4-*O*-β-D-glucopyranoside, a glucosyloxy amidic phenanthridone derivative from *Pancratium maritimum*. *Phytochemistry* **30** (10), 3445–3448.

[22] Pettit GR, Cragg GM, Singh SB, Duke JA, and Doubek DL (1990). Antineoplastic agents: 162. *Zephyranthes candida*. *J Nat Prod* **53** (1), 176–178.

[23] Pettit GR and Melody N (2005). Antineoplastic agents: 527. Synthesis of 7-deoxynarcistatin, 7-deoxy-*trans*-dihydronarcistatin, and *trans*-dihydronarcistatin 1. *J Nat Prod* **68** (2), 207–211.

[24] Pettit GR, Gaddamidi V, Herald DL, Singh SB, Cragg GM, Schmidt JM, Boettner FE, Williams M, and Sagawa Y (1986). Antineoplastic agents: 120. *Pancratium littorale*. *J Nat Prod* **49** (6), 995–1002.

[25] Pettit GR, Eastham SA, Melody N, Orr B, Herald DL, McGregor J, Knight JC, Doubek DL, Pettit GR III, Garner LC, et al. (2006). Isolation and structural modification of 7-deoxynarciclasine and 7-deoxy-*trans*-dihydronarciclasine. *J Nat Prod* **69** (1), 7–13.

[26] Pettit GR, Gaddamidi V, Cragg GM, Herald DL, and Sagawa Y (1984). Isolation and structure of pancratistatin. *J Chem Soc Chem Commun* **24**, 1693–1694.

[27] Gabrielsen B, Monath TP, Huggins JW, Kefauver DF, Pettit GR, Groszek G, Hollingshead M, Kirsi JJ, Shannon WM, Schubert EM, et al. (1992). Antiviral (RNA) activity of selected Amaryllidaceae isoquinoline constituents and synthesis of related substances. *J Nat Prod* **55** (11), 1569–1581.

[28] Ouarzane-Amara M, Franetich JF, Mazier D, Pettit GR, Meijer L, Doerig C, and Desportes-Livage I (2001). *In vitro* activities of two antimitotic compounds, pancratistatin and 7-deoxynarciclasine, against *Encephalitozoon intestinalis*, a microsporidium causing infections in humans. *Antimicrob Agents Chemother* **45** (12), 3409–3415.

[29] Ghosal S, Datta K, Singh SK, and Kumar Y (1990). Telastaside a stress-related alkaloid-conjugate from *Polytela gloriosa*, an insect feeding on Amaryllidaceae. *J Chem Res* **10**, 334–335.

[30] Kojima K, Mutsuga M, Inoue M, and Ogihara Y (1998). Two alkaloids from *Zephyranthes carinata*. *Phytochemistry* **48** (7), 1199–1202.

[31] Rinner U and Hudlicky T (2005). Synthesis of Amaryllidaceae constituents—an update. *Synlett* **3**, 365–387.

[32] Hudlicky T, Rinner U, Gonzales D, Akgun H, Schilling S, Siengalewicz P, Martinot TA, and Pettit GR (2002). Total synthesis and biological evaluation of Amaryllidaceae alkaloids: narciclasine, *ent*-7-deoxypancratistatin, regioisomer of 7-deoxypancratistatin, 10b-*epi*-deoxypancratistatin, and truncated derivatives. *J Org Chem* **67** (25), 8726–8743.

[33] Gibson DT, Hensley M, Yoshioka H, and Mabry TJ (1970). Formation of (+)-*cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene by *Pseudomonas putida*. *Biochemistry* **9** (7), 1626–1630.

[34] Hudlicky T and Olivo HF (1992). A short synthesis of (+)-lycoricidine. *J Am Chem Soc* **114** (24), 9694–9696.

[35] Tian X, Hudlicky T, and Königsberger K (1995). First total synthesis of (+)-pancratistatin: an usual set of problems. *J Am Chem Soc* **117** (12), 3643–3644.

[36] Doyle TJ, Hendrix M, VanDerveer D, Javanmard S, and Haseltine JA (1997). Convergent synthesis of (+)-pancratistatin based on intramolecular electrophilic aromatic substitution. *Tetrahedron* **53** (32), 11153–11170.

[37] Trost BM and Pulley SR (1995). Asymmetric synthesis of (+)-pancratistatin. *J Am Chem Soc* **117** (40), 10143–10144.

[38] Rigby JH, Maharoof USM, and Mateo ME (2000). Studies on the narciclasine alkaloids: total synthesis of (+)-narciclasine and (+)-pancratistatin. *J Am Chem Soc* **122** (28), 6624–6628.

[39] Magnus P and Sebhat IK (1998). Application of the β-azidonation reaction to the synthesis of the antitumor alkaloid (+)-pancratistatin. *Tetrahedron* **54** (51), 15509–15524.

[40] Ko H, Kim E, Park JE, Kim D, and Kim S (2004). Total synthesis of pancratistatin relying on the [3,3]-sigmatropic rearrangement. *J Org Chem* **69** (1), 112–121.

[41] Li M, Wu A, and Zhou P (2006). A concise synthesis of (+)-pancratistatin using pinitol as a chiral building block. *Tetrahedron Lett* **47** (22), 3707–3710.

[42] Pettit GR, Melody N, and Herald DL (2001). Antineoplastic agents: 450. Synthesis of (+)-pancratistatin from (+)-narciclasine as relay. *J Org Chem* **66** (8), 2583–2587.

[43] Pettit GR and Melody N (2002). *Synthesis of pancratistatin. WIPO patent WO0250023* [June 27].

[44] Mondon A and Krohn K (1975). Zur kenntris des narciclasins. *Chem Ber* **108** (2), 445–463.

[45] Pettit GR, Melody N, and Herald DL (2004). Antineoplastic agents: 511. Direct phosphorylation of phenpanstatin and pancratistatin. *J Nat Prod* **67** (3), 322–327.

[46] NCI database. Available at: http://dtp.nci.nih.gov/.

[47] Rinner U, Hudlicky T, Gordon H, and Pettit GR (2004). A β-carboline-1-one mimic of the anticancer Amaryllidaceae constituent pancratistatin: synthesis and biological evaluation. *Angew Chem Int Ed Engl* **43** (40), 5342–5346.

[48] Pettit GR, Melody N, Simpson M, Thompson M, Herald DL, and Knight JC (2003). Antineoplastic agents: 500. Narcistatin. *J Nat Prod* **66** (1), 92–96.

[49] Pettit GR, Orr B, and Ducki S (2002). *Synthesis of pancratistatin prodrugs. WIPO patent WO02085848* [October 31].

[50] Pettit GR and Melody N (2006). *Pancratistatin cyclic phosphate prodrugs an phenpanstatin cyclic phosphate prodrugs. US Patent US2006128668* [June 15].

[51] Pettit GR and Melody N (2004). *Narcistatin prodrugs. WIPO patent WO2004052298* [June 24].

[52] Pettit GR and Melody N (July 20, 2006). *Synthesis of sodium narcistatin and related compounds. WIPO patent WO2006076726.*

[53] McNulty J, Mao J, Gibe R, Mo R, Wolf S, Pettit GR, Herald DL, and Boyd MR (2001). Studies directed towards the refinement of the pancratistatin cytotoxic pharmacophore. *Bioorg Med Chem Lett* **11** (2), 169–172.

[54] Rinner U, Hillebrenner HL, Adams DR, Hudlicky T, and Pettit GR (2004). Synthesis and biological activity of some structural modifications of pancratistatin. *Bioorg Med Chem Lett* **14** (11), 2911–2915.

[55] Pettit GR, Melody N, O'Sullivan M, Thompson MA, Herald DL, and Coates B (1994). Synthesis of 10b-*R*-hydroxy-pancratistatin via narciclasine. *J Chem Soc Chem Commun* **23**, 2725–2726.

[56] Bi Y, Guo J, Zhang L, and Wong Y (2003). Changes in some enzymes of microbodies and plastid development in excised radish cotyledons: effect of narciclasine. *J Plant Physiol* **160** (9), 1041–1049.

[57] Van Doorn WG, Sinz A, and Tomassen MM (2004). Daffodil flowers delay senescence in cut Iris flowers. *Phytochemistry* **65** (5), 571–577.

[58] Jimenez A, Santos A, Alonso G, and Vazquez D (1976). Inhibitors of protein synthesis in eukaryotic cells. Comparative effects of some Amaryllidaceae alkaloids. *Biochim Biophys Acta* **425** (3), 342–348.

[59] Baez A and Vazquez D (1978). Binding of [^{3}H]narciclasine to eukaryotic ribosomes. A study on a structure–activity relationship. *Biochim Biophys Acta* **518** (1), 95–103.

[60] Dall'acqua F and Rodighiero C (1977). Investigations on the mechanism of action of narciclasine. *Farmaco* **32** (1), 67–75.

[61] McLachlan A, Kekre N, McNulty J, and Pandey S (2005). Pancratistatin: a natural anti-cancer compound that targets mitochondria specifically in cancer cells to induce apoptosis. *Apoptosis* **10** (3), 619–630.

[62] Griffin C, Sharda N, Sood D, Nair J, McNulty J, and Pandey S (2007). Selective cytotoxicity of pancratistatin-related natural Amaryllidaceae alkaloids: evaluation of the activity of two new compounds. *Cancer Cell Int* **7**, 10.

[63] Pandey S, Kekre N, Naderi J, and McNulty J (2005). Induction of apoptotic cell death specifically in rat and human cancer cells by pancratistatin. *Artif Cells Blood Substit Immobil Biotechnol* **33** (3), 279–295.

[64] Kekre N, Griffin C, McNulty J, and Pandey S (2005). Pancratistatin causes early activation of caspase-3 and the flipping of phosphatidyl serine followed by rapid apoptosis specifically in human lymphoma cells. *Cancer Chemother Pharmacol* **56** (1), 29–38.

[65] McNulty J, Larichev V, and Pandey S (2005). A synthesis of 3-deoxydihydrolycoricidine: refinement of a structurally minimum pancratistatin pharmacophore. *Bioorg Med Chem Lett* **15** (23), 5315–5318.

[66] Dumont P, Ingrassia L, Rouzeau S, Ribaucour F, Thomas S, Roland I, Darro F, Lefranc F, and Kiss R (2007). The Amaryllidaceae isocarbostyril narciclasine induces apoptosis by activation of the death receptor and/or mitochondrial pathways in cancer cells but not in normal fibroblasts. *Neoplasia* **9** (9), 766–776.

[67] Zhang XD, Nguyen T, Thomas WD, Sanders JE, and Hersey P (2000). Mechanisms of resistance of normal cells to TRAIL induced apoptosis vary between different cell types. *FEBS Lett* **482** (3), 193–199.

[68] Zhu H, Ling W, Hu B, Su Y, Qiu S, Xiao W, and Qi Y (2006). Adenovirus E1A reverses the resistance of normal primary human lung fibroblast cells to TRAIL through DR5 upregulation and caspase 8–dependent pathway. *Cancer Biol Ther* **5** (2), 180–188.

[69] Tanaka T, Yoshimi M, Maeyama T, Hagimoto N, Kuwano K, and Hara N (2002). Resistance to Fas-mediated apoptosis in human lung fibroblast. *Eur Respir J* **20** (5), 359–368.

[70] Yui S, Mikami M, Mikami Y, Sashida Y, and Yamazaki M (2001). Inhibition effect of Amaryllidaceae alkaloids, lycorine and lycoricidinol on macrophage TNF-alpha production. *Yakugaku Zasshi* **121** (2), 167–171.

[71] Babbar N and Casero RA Jr (2006). Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. *Cancer Res* **66** (23), 11125–11130.