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# Cytotoxic Agents of the Crinane Series of Amaryllidaceae Alkaloids

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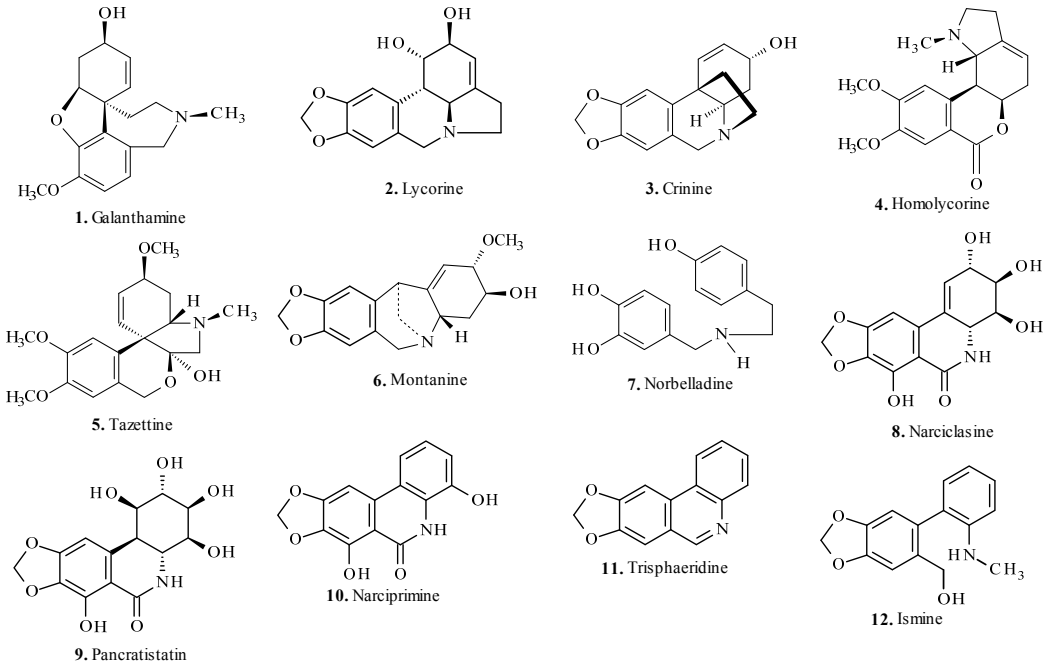
In the alkaloid galanthamine, the plant family Amaryllidaceae has endowed the pharmaceutical community with a potent and selective inhibitor of the enzyme acetylcholinestersae (AChE), of prominence in the chemotherapeutic approach towards motor neuron diseases. Following on the commercial success of this prescription drug in the treatment of Alzheimer's disease, it is anticipated that other drug candidates will in future emerge from the family. In this regard, the phenanthridones, exemplified by narciclasine and pancratistatin, of the lycorine series of Amaryllidaceae alkaloids have shown much promise as remarkably potent and selective anticancer agents, with a drug target of the series destined for the clinical market within the next decade. Given these interesting biological properties and their natural abundance, plants of the Amaryllidaceae have provided a diverse and accessible platform for phytochemical-based drug discovery. The crinane series of Amaryllidaceae alkaloids are also enriched with a significant array of biological properties. As a consequence of their close structural similarity to the anticancer agents of the lycorine series, the cytotoxic potential of crinane alkaloids has been realized through structure-activity relationship (SAR) studies involving targets of both semi-synthetic and natural origin, which has identified several members as leads with promising antiproliferative profiles. As the first of its kind, this review seeks to collate such information from the past few decades in advancing the crinane group as a viable platform for anticancer drug discovery.

**Keywords:** Alkaloids, Amaryllidaceae, Anticancer, Crinane, Cytotoxic.

Amongst the diverse array of biological properties known for the plant family Amaryllidaceae [1], two themes are recurrent: acetylcholinesterase (AChE) inhibition and cytotoxicity. Of significance in the progression of neurodegeneration associated with motor neuron diseases, the former of these has gained much prominence in both the secular and scientific media after commercialization of the Alzheimer's drug galanthamine **1** [2], derived chiefly from the Amaryllidaceous species *Galanthus nivalis* (snowdrop) and *Narcissus pseudonarcissus* (daffodil). More recently, the chemotherapeutic potential of the family has been extended to include members of the lycorine **2** series due to their potent anticancer properties [3]. Galanthamine **1**, lycorine **2** and crinine **3** are representative of the three major structural-types for these alkaloids (Scheme 1), while homolycorine **4**, tazettine **5** and montanine **6** make up the minor series of compounds discernible within the Amaryllidaceae [1c]. Other less-conspicuous members include degraded, oxidized and truncated variants such as trisphaeridine **11** and ismine **12** [1c]. Biogenetically, all of these compounds are related as a consequence of their common amino acid-derived precursor norbelladine **7** [1c].

As a distinct niche within the lycorine series, the phenanthridones exemplified by narciclasine **8** and pancratistatin **9** (Scheme 1) are known for their potent and cell line-specific anticancer activities, and are presently at various stages of development, with a clinical candidate earmarked for commercialization within the next decade [4]. Mechanistically, these phenanthridones are known to initiate cell death via the apoptotic pathway, as indicated by early activation of caspase-3 followed by flipping of phosphatidyl serine, selectively in mitochondria of cancer cells with minimal effect on normal cells [5]. Given these promising biological properties, accompanied by their synthetically-challenging molecular structures, as well as their limited availability from natural sources, these alkaloids have served to fuel sustained efforts in order to deliver a potent, selective and readily bioavailable drug target with facile synthetic access [6a-c]. Furthermore, a stronger case could be made for development of these compounds into commercially viable entities by the low interaction of narciclasine and pancratistatin with the cytochrome P450 3A4 isoenzyme, which accounts for the majority of drugs metabolized in humans [6d-f]. In addition, structure-activity relationship (SAR) studies invoking these potential drug targets have collectively facilitated the elucidation of elements of the apoptosis-inducing pharmacophore innate to these molecules [7].

Crinane alkaloids of the Amaryllidaceae, such as crinine **3** (Scheme 1), are a large and expanding group with a host of biological properties [1,8]. Structurally, they comprise the basic phenanthridine nucleus with varying degrees of oxygenation in ring-A, but usually with a methylenedioxy moiety straddling C-8 and C-9 [1c,d]. The presence of the *N*- to C-10b ethano-bridge is a diagnostic feature of their makeup, and may be either α- or β-orientated leading to two stereodefined subgroups: α- and β-crinanes (Schemes 2 and 3) [1c,d]. As a consequence, the C-10b spiro-junction may adopt one of two possible absolute configurations depending on the orientation of the ethano-bridge [1c,d]. In addition, oxygen-related substituents are usually found at various positions in ring-C and at C-11 of the bridge, while a C-1/C-2 double bond is common for many analogues of both subgroups [1c,d]. Given their close structural proximity to the potent anticancer agents of the lycorine group and their biogenesis from a common precursor, crinane compounds have recently emerged as interesting targets for cytotoxicity-based studies [1a,b]. As such, low micromolar activities in several instances were uncovered for representatives of the group, which has subsequently garnered significant interest in these alkaloids as potential leads in the chemotherapeutic approach towards cancer malignancy [1a,b].



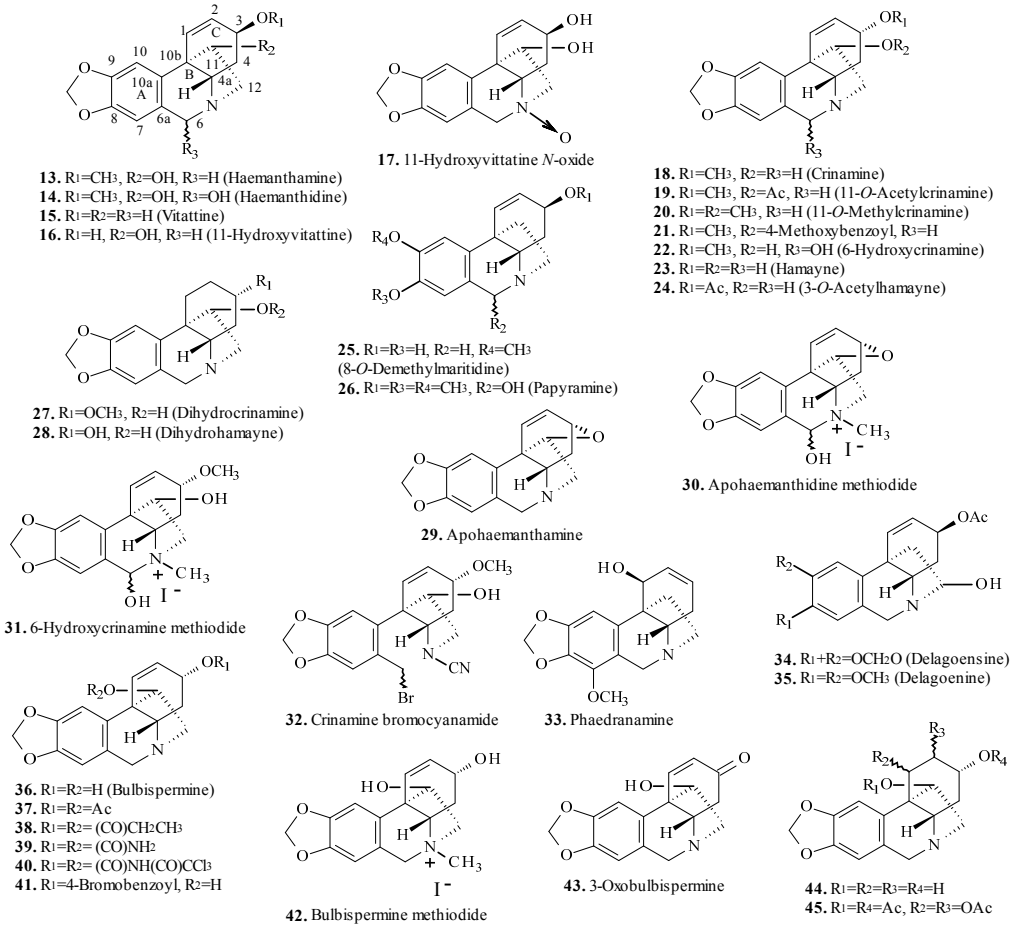

**Scheme 1**: Diverse alkaloid structures of the Amaryllidaceae, including major group representatives **1-3** as well as the common biosynthetic precursor norbelladine **7**.

While several reviews have dedicated attention to the cytotoxic properties of the lycorine series of compounds [3e,h,4d,e,6], especially the phenanthridones of the series, a comprehensive overview with the crinane series members as the focal point is absent from the literature. To this extent, we here present a detailed chronological account of such agents within the crinane series of Amaryllidaceae alkaloids, as well as their allied cytotoxic activities, taking into consideration the contributions we have made in this area over the past few years together with those of others. Core areas that will be covered include: 1) *in vitro* and *in vivo*–based studies of crinane alkaloids; 2) SAR studies utilizing both natural and synthetically-derived targets; 3) elements of the anticancer pharmacophore unraveled through these studies; and 4) mechanistic insights to the mode of operation of these cytotoxic agents.

Based on prior observations for narciclasine **8** [9a], the seminal work of Jimenez et al. (1976) [9b] sought clarity on the antiproliferative effects of a library of structurally diverse Amaryllidaceae alkaloids in cervical adenocarcinoma (HeLa) cells. Of the four crinane compounds screened (Tables 1 and 2), the α-crinane haemanthamine **13** was the most active (MIC 4 μM), while the β-crinanes crinine **3**, buphanidrine **46** and ambelline **47** exhibited no inhibitory effect on cell growth at concentrations up to 0.4 mM [9b]. It was suggested by these authors that the underlying mechanism of action of haemanthamine involved inhibition of protein synthesis by blocking the peptide bond formation step on the peptidyl transferase centre of the 60S ribosomal subunit. This dichotomy in antiproliferative activity between α- and β-crinanes is a theme commonly encountered in the literature, and will be elaborated on in several instances of this survey.

Not long after these findings, Furusawa and coworkers [10] examined various structures of the Amaryllidaceae, including several of the crinane series, for cytotoxic effects in Rauscher virus-carrying NIH/3T3 cells. During the investigation, haemanthamine **13**, crinamine **18** and 6-hydroxycrinamine **22** all exhibited minimum toxic dose (MTD) levels of 0.2 μg/mL, while crinamine bromocyanamide **32** was the next active at 5 μg/mL, and 6-hydroxycrinamine methiodide **31** was identified as the pick of these crinanes with MTD of 0.05 μg/mL. The highly strained ring system in apohaemanthidine methiodide **30** was seen to be detrimental to cytotoxicity as the compound had a MTD of 100 μg/mL, while 6-hydroxybuphanidrine methiodide **58** also exhibited the same MTD value. Again, the dichotomous behavior of α-and β-crinanes are apparent from the activity of **58** compared with the other compounds screened. Amongst the early findings on antineoplastic agents from the Amaryllidaceae, bulbs of *Amaryllis belladonna* were shown by Pettit et al. (1984) to contain two crinane compounds; ambelline **47** and undulatine **62** [11]. Of these, only ambelline was active in the murine P-388 lymphocytic leukemia assay ($ED_{50}$ 1.6 μg/mL), indicating that the compound may be more amenable towards animal models of study as it was previously shown to be inactive in human adenocarcinoma (HeLa) cells [9b].

Also in 1984, Ghosal et al. [12] isolated 1,2-epoxyambelline **61** for the first time from bulbs of *Crinum latifolium*, and subsequent cytotoxicity studies showed that at 5 μg/mL it produced moderate activation of mouse spleen lymphocytes (MSL). Furthermore, the closely-related analogue ambelline **47** in a 1:1 mixture with **61** (at 5 μg/mL) had a marked synergistic effect, producing pronounced activation of the lymphocytic cells with a stimulation index of 3.12, comparable to that of concanavalin A (stimulation index 3.23) [12]. As an extension to the work on *Crinum latifolium*, Ghosal and Singh (1986) then isolated a further two novel alkaloids crinafoline **69** and crinafolidine **71** from mature fruits of the plant [13]. Structurally, crinafoline is the 6α-hydroxy epimer corresponding to ambelline **47**, notably with the *endo* disposition for the 11-hydroxy group, while crinafolidine is the first identified natural truncated member of the crinane series, in this case with a *seco*-B-ring construction. *In vitro* studies on both compounds showed significant reductions in the viability of sarcoma 180 ascites (68.12% and 43.5% for crinafoline **69** and crinafolidine **71**, respectively) [13]. Interestingly, the B-*seco* analogue **71** was shown to be less active than the corresponding fully-cyclized crinafoline **69**, highlighting



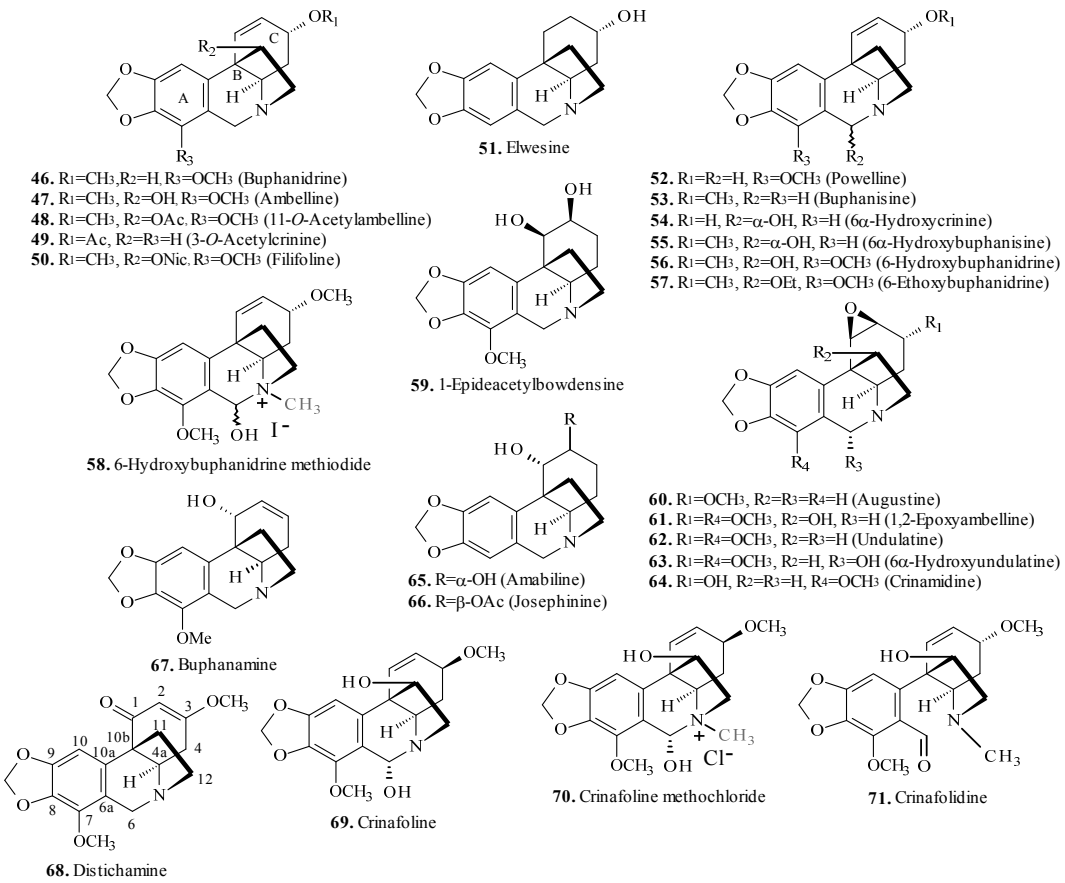

**Scheme 2**: Cytotoxic α-crinane alkaloids of the Amaryllidaceae.

the requirement of an intact phenanthridine nucleus for activity. Furthermore, quaternization of the nitrogen atom was seen to potentiate activity, as the semisynthetically-derived methochloride **70** was twice active as crinafoline **69**, with 32.31% viability shown for the sarcoma cells [13]. This work is also significant in that it represents the first demonstration of *in vivo* effects of crinane alkaloids. In this regard, compounds **69**-**71** produced marked reductions in growth of sarcoma 180 ascites tumors in mice with cell count values of 10.04 x $10^6$, 5.08 x $10^6$ and 6.14 x $10^6$ shown for the three compounds respectively, compared to untreated control animals (15.88 x $10^6$) [13]. *In vivo*, quaternization of the nitrogen atom was also seen to be ameliorative towards activity (**69** versus **70**), and **70** was identified as the choice of this set of compounds.

A few years later in 1991, Abd El Hafiz et al. [14] examined the constituents of *Crinum augustum* and *Crinum bulbispermum* for activity against human leukemic Molt 4 cells. Of the three crinanes (powelline **52**, 6α-hydroxycrinine **54** and 6α-hydroxybuphanisine **55**) tested, only **55** was moderately active, causing a steady decline (up to ~20%) in the viability of leukemia cells over the three-day treatment period at a dosage of 71 μg/mL [14] (Table 2). Since **54** and **55** are differentiated by their C-3 substituents, it may be construed that a small hydrogen bond acceptor (such as the methoxyl in **55**) as opposed to a donor-acceptor (hydroxyl) in this vicinity is essential for activity against Molt 4 cells. Substituent placement and geometry, as will become increasingly apparent during the course of this survey, have commonly been shown to have a distinctive influence on the cytotoxic abilities of crinane alkaloids.

The work of Likhitwitayawuid et al. (1993) stands out for the multi-cell line approach adopted by the authors in assessing the cytotoxic activities of alkaloid principles of *Crinum amabile* [15]. In the screen against ten cancers, including both human and animal cells, augustine **60** and crinamine **18** were prominent in most cases, with the best response ($ED_{50}$ 0.6 μg/mL) observed in human oral epidermoid carcinoma KB cells and viblastine-resistant KB cells for the two compounds, respectively (Tables 1 and 2) [15]. Augustine **60** was noticeably selective against KB cells as opposed to vinblastine-resistant KB cells ($ED_{50}$ > 20 μg/mL). Buphanisine **53** and amabiline **65** were conspicuous in that they exhibited $ED_{50}$ values > 20 μg/mL in most of the cells tested [15]. The fact that α- and β-crinane representatives were here indistinguishable based on cytotoxic outcomes suggests that there may be other more subtle features, as will be encountered later in the manuscript, attending the crinane alkaloid anticancer pharmacophore.

Soon after this, during the course of the phytochemical investigation of *Hymenocallis expansa*, Antoun et al. (1993) identified haemanthidine **14** as one of the cytotoxic constituents in the screen against eleven cancer cells [16]. Although the compound exhibited non-selective behavior, of note were the activities against prostate LNCaP ($ED_{50}$ 0.7 μg/mL) and P-388 murine lymphocytic leukemia ($ED_{50}$ 0.4 μg/mL) cells. Two years later in 1995, Lin et al. examined *Hymenocallis littoralis* and uncovered *inter alia* the presence of the crinane alkaloids crinine **3**, haemanthamine **13** and 8-*O*-demethylmaritidine **25** [17]. Of these, only haemanthamine was screened for cytotoxic activity and found to be indiscriminate in a panel comprising eleven cell lines with $ED_{50}$ values ranging




**Scheme 3**: Array of cytotoxic β-crinane members of the Amaryllidaceae.

from 0.3 to 1.3 μg/mL, but was markedly potent against HT-1080 human fibrosarcoma cells. The same year, we entered the cytotoxic evaluation arena via a study of 25 diverse alkaloid structures of the Amaryllidaceae against two human tumoral cell lines (Molt4 and HepG2) and one murine non-tumoral cell line (LMTK) [18]. Interestingly, α-crinanes in the library could be distinguished from their β-congeners based on activities against the above tumors [18]. Buphanidrine **46** and ambelline **47** exhibited low inhibitory interaction ($ED_{50}$ > 50 μg/mL) with both Molt4 and HepG2 cells [18], as also previously seen in adenocarcinoma HeLa cells [9b], while the α-crinanes, on the other hand, were markedly more responsive towards the tumor cells. As such, haemanthamine **13** and crinamine **18** both had $ED_{50}$ values of 0.5 μg/mL in LMTK cells, while papyramine **26** exhibited an $ED_{50}$ of 1.5 μg/mL in the same cell line [18].

Although no specific literature reference to cytotoxic properties of crinane compounds was found between 1996 and 1997, the period 1998 to 2000 saw three pieces of work from our groups pertaining to such activities of alkaloids of the South African Amaryllidaceae [19-21]. Firstly, while lycorine **2** was significantly active ($ED_{50}$ = 1.8 μg/mL), crinine **3** and ambelline **47** from bulbs of *Brunsvigia littoralis*, as well as their respective synthesized acetyl derivatives **49** and **48** were notably inactive ($ED_{50}$ > 100 μg/mL) against BL-6 mouse melanoma cells [19]. We next showed that 6-hydroxy-crinamine **22** from *Crinum delagoense* was active in the same cell line, in contrast to its other α-crinane co-constituents hamayne **23**, delagoensine **34** and delagoenine **35** [20]. The 12-hydroxy crinane analogues **34** and **35** are structurally unique as they have never been identified in any other species of Amaryllidaceae. Furthermore, these findings were significant in that they allowed for the first time a direct comparison of the cytotoxicities of C-6 and C-12 substituted crinanes, by which the former were seen to be preeminent. Finally, around this time *Brunsvigia radulosa* was examined for its phytochemical composition and cytotoxic ability against BL-6 cells [21]. Of the three crinanes assayed, the α-crinanes crinamine **18** ($ED_{50}$ = 1.8 μg/mL) and hamayne **23** ($ED_{50}$ = 9.4 μg/mL) were markedly more active than the β-crinane 1-epideacetylbowdensine **59** ($ED_{50}$ > 100 μg/mL). Furthermore, apohaemanthamine **29**, synthesized during the course of the study from crinamine **18** under strongly acidic conditions, exhibited low inhibitory interaction ($ED_{50}$ > 100 μg/mL) [21], which again could be explained in terms of ring strain generated through C-3 to C-11 etherification in **29**, as also seen for apohaemanthidine methiodide **30** and its low activity against NIH/3T3 cells [10].

A total of nine alkaloids were reported by Abou-Donia et al. (2002) from *Pancratium sickenbergeri* [22]. Of these, only haemanthidine **14** and 11-hydroxyvittatine **16** were assayed against the NCI 60-cell line library and shown to be inactive. The finding for haemanthidine **14** was surprising given the prior observations of Antoun et al. (1993) [16]. This anomaly was further highlighted by the investigation of Hohmann et al. [23] in the same year on alkaloid isolates of *Sprekelia formosissima* and *Hymenocallis* x *festalis*, during which haemanthamine **13** and haemanthidine **14** were noted for their pronounced antiproliferative properties in L5178 mouse lymphoma cells ($IC_{50}$s of 0.27 and 0.41 μg/mL for the two alkaloids, respectively). Equally impressive was the potency of both compounds in the multidrug resistant form of L5178 mouse

**Table 1**: Activities of α-crinane alkaloid representatives of the Amaryllidaceae in miscellaneous cancer cell line screens.

| Compound | No. | Cell line (Cytotoxicity index) [a] | | | | | | | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L6 | BL6 | KB | A549 | OE21 | Hs683 | U373 | SKMEL | B16F10 | Jurkat | HeLa | Vero | LOVO | 6T-CEM | HL-60 | HT29 | H460 | RXF393 | 5123tc | 3T3 | |
| Haemanthamine | **13** | 7.41 [b] | | 0.7 [b] | 4.5 | 6.8 | 7.0 | 3.5 [b] | 8.5 | 6.8 | 22 [d] | 4 | 32 [c] | | | active | | | | 15 | 0.2 [b] | 3e,f,9b,10,17,26,31, 33,35,36,38 |
| Haemanthidine | **14** | | | 3.6 [b] | 4.0 | 3.7 | 4.3 | 3.8 | 4.2 | 3.1 | | nd | | | | active | | | | | | 16,31,35,36 |
| Vittatine | **15** | | | | | | | | | | 70 [d] | | | | | | 21.91 | 15.88 | 29.57 | | | 27,29 |
| 11-Hydroxyvittatine | **16** | | | | | | | | | | 4 [d] | 72 [c] | 64 [c] | | | | | | | | | 3e,f |
| | **17** | >90 [b] | | >50 [b] | | | | | | | | | | | | | | | | | | 33 |
| Crinamine | **18** | | 1.8 [b] | 1.0 [b] | 15.9 [b] | | | 0.9 [b] | | | | | | 4.30 [b] | 2.82 [b] | 1.70 [b] | | | | 12.5 | 0.2 [b] | 10,15,21,26,28 |
| 11-Acetylcrinamine | **19** | | | | | | | | | | | | | | | | | | | na | | 26 |
| 11-Methylcrinamine | **20** | | | | 9.15 [b] | | | | | | | | | 81.0 [b] | 14.4 [b] | 6.16 [b] | | | | | | 28 |
| | **21** | | | | | | | | | | | | | | | | | | | na | | 26 |
| 6-Hydroxycrinamine | **22** | | active | | | | | | | | | | | | | | | | | | 0.2 [b] | 10,20 |
| Hamayne | **23** | | 9.4 [b] | | | | | | | | na | | | | | | | | | | | 21,29 |
| 3-*O*-Acetylhamayne | **24** | | | | 15.8 [b] | | | | | | | | | 5.49 [b] | 3.45 [b] | 4.27 [b] | | | | | | 28 |
| | **25** | >90 [b] | | >50 [b] | | | | | | | 4 [d] | 82 [c] | 82 [c] | | | | | | | | | 3e,f,33 |
| Dihydrocrinamine | **27** | | | | | | | | | | na | | | | | | | | | | | 29 |
| Dihydrohamayne | **28** | | | | | | | | | | active | | | | | | | | | | | 29 |
| Apohaemanthamine | **29** | | >100 [b] | | | | | | | | | | | | | | | | | | | 21 |
| | **30** | | | | | | | | | | | | | | | | | | | | 100 [b] | 10 |
| | **31** | | | | | | | | | | | | | | | | | | | | 0.05 [b] | 10 |
| | **32** | | | | | | | | | | | | | | | | | | | | 5 [b] | 10 |
| Phaedranamine | **33** | na | | | | | | | | | | | | | | | | | | | | 32 |
| Delagoensine | **34** | | na | | | | | | | | | | | | | | | | | | | 20 |
| Delagoenine | **35** | | na | | | | | | | | | | | | | | | | | | | 20 |
| Bulbispermine | **36** | | | | | | 11 | 38 | | | | 8 | | | | | | | | | | 36 |
| | **37** | | | | | | 63 | nd | | | | 90 | | | | | | | | | | 36 |
| | **38** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | **39** | | | | | | 50 | >100 | | | | 46 | | | | | | | | | | 36 |
| | **40** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | **41** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | **42** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| 3-Oxobulbispermine | **43** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | **44** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | **45** | | | | | | >100 | nd | | | | >100 | | | | | | | | | | 36 |
| | | BCA-1 | HT-1080 | LUC-1 | MEL-2 | COL-1 | KB-V1 | P-388 | A-431 | LNCaP | ZR-75-1 | L5178 | L5178mdr | Molt4 | LMTK | HepG2 | COL-2 | BC1 | HT | Lu1 | HSC-2 | |
| Haemanthamine | **13** | 0.7 [b] | 0.3 [b] | 3.6 [b] | | | 1.3 [b] | 5.0 [b] | 1.3 [b] | 0.6 [b] | 0.5 [b] | 0.27 [b] | 0.30 [b] | 1.2 [b] | 0.5 [b] | >50 [b] | 0.6 [b] | | | | active | 17,18,23,35 |
| Haemanthidine | **14** | | | | 1.9 [b] | | 8.4 [b] | 0.4 [b] | 3.0 [b] | 0.7 [b] | 2.6 [b] | 0.41 [b] | 0.40 [b] | | | | 6.0 [b] | >20 [b] | 1.6 [b] | 2.1 [b] | active | 16,23,35 |
| Crinamine | **18** | 1.4 [b] | 1.3 [b] | 1.4 [b] | 5.0 [b] | 1.0 [b] | 0.6 [b] | 0.7 [b] | 6.9 [b] | 1.5 [b] | 0.8 [b] | | | 0.5 [b] | 0.5 [b] | 10 [b] | | | | | | 15,18 |
| Papyramine | **26** | | | | | | | | | | | | | 15.8 [b] | 1.5 [b] | 17 [b] | | | | | | 18 |
| | | T98G | U87 | CEM | K562 | MCF-7 | G-361 | | | | | | SH-SY5Y | | | | | | | | | |
| Haemanthamine | **13** | 8 | 6 | 2.1 | 3.4 | 8.1 | 3.7 | | | | | | | | | | | | | | | 36,38 |
| Haemanthidine | **14** | 14 | 6 | | | | | | | | | | | | | | | | | | | 36 |
| 6-Hydroxycrinamine | **22** | | | | | | | | | | | | 54.5 | | | | | | | | | 37 |
| Bulbispermine | **36** | 9 | 9 | | | | | | | | | | | | | | | | | | | 36 |
| | **37** | 98 | 74 | | | | | | | | | | | | | | | | | | | 36 |
| | **38** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| | **39** | 91 | 15 | | | | | | | | | | | | | | | | | | | 36 |
| | **40** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| | **41** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| | **42** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| 3-Oxobulbispermine | **43** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| | **44** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |
| | **45** | >100 | >100 | | | | | | | | | | | | | | | | | | | 36 |

[a] Cytoxicity index values expressed as $ED_{50}$, $GI_{50}$, $IC_{50}$, MIC or $MTD_{50}$ in micromolar (μM) units unless otherwise stated. [b] Values are in μg/mL. [c] Indicates % cell viability. [d] Indicates % apoptosis. For compounds indicated as "active", no cytotoxicity indices were presented in the original work (na=not active, nd=not detected).

lymphoma cells, exhibiting $IC_{50}$ values of 0.3 and 0.40 μg/mL, respectively [23] (Table 1). Around this time, findings by Phan et al. (2003) revealed that while lycorine **2** was strongly active on HepG2, RD and FL cells, no such activity was detectable for the β-crinanes crinine **3** and crinamidine **64** isolated from the Vietnamese species *Crinum asiaticum* and *Crinum latifolium* [24]. Crinamidine is the

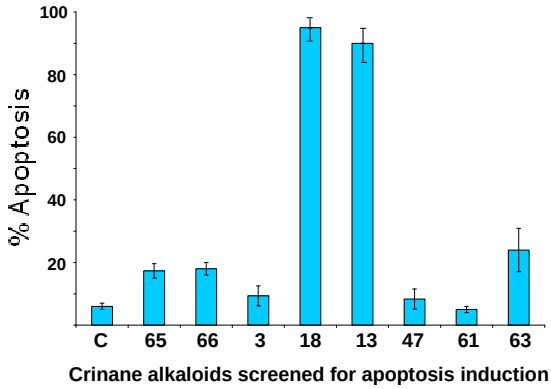



**Figure 1**: Apoptotic indices determined in rat hepatoma (5123tc) cells showing the efficacy of crinamine **18** and haemanthamine **13** at 25 □M after a 48 h treatment (after McNulty et al., 2007).[26]

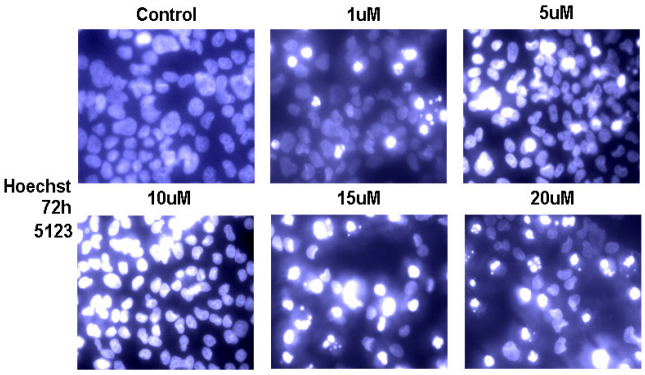



**Figure 2**: Hoechst staining of 5123tc cells 72 h after treatment with various concentrations of crinamine **18**. Apoptotic nuclei appear brighter and more fragmented than healthy nuclei (seen in control cells) (after McNulty et al., 2007).[26]

3-hydroxy analogue corresponding to undulatine **62**, previously shown to be inactive in the P-388 cell line [11]. Apart from this, during the phytochemical investigation of *Nerine filifolia*, a species of the Amaryllidaceae endemic to eastern and southeastern regions of South Africa, we identified several crinanes of the β-series, including 6α-hydroxybuphanidrine, 6α-methoxybuphanidrine, ambelline, 11-*O*-acetylambelline and filifoline [25]. Of these, only filifoline **50** was screened against rat myoblast (L6) cells and shown to be inactive [25].

The year 2007 is notable in the Amaryllidaceae alkaloid cytotoxicity calendar as it saw the first demonstration of apoptotic effects for crinane-based alkaloid structures [26]. In this regard, we examined a mini-library comprising natural and semi-synthetic derivatives of both series of crinane compounds for apoptosis-inducing effects in rat hepatoma (5123tc) cells [26]. As shown in Figure 1, the α-crinanes haemanthamine **13** and crinamine **18** were prominent amongst the other test substrates, with apoptotic indices of 90% and 95%, respectively, after a 48 h treatment at 25 μM [26]. Apoptosis induction was indicated by Hoechst staining as shown in Figure 2, by which apoptotic nuclei appeared brighter and more fragmented than healthy nuclei, in this case upon treatment with crinamine **18**. Furthermore, respective $ED_{50}$ values were established at 15 μM and 12.5 μM for haemanthamine and crinamine (Figure 3) [26]. Of the other compounds screened, only the activity of 6α-hydroxyundulatine **63** was noteworthy (25% apoptotic at the same concentration and treatment period) [26]. These results were even more impressive considering the selectivities of haemanthamine **13** and crinamine **18**, both of which were seen to exhibit negligible apoptotic effects in normal human embryonic kidney (293t) cells (Figure 4).

Apart from uncovering these novel effects in hepatoma cells, this work was also significant in shedding light on the underlying structural elements of the pharmacophore resident within these crinanes [26]. These were seen to include: 1) α-orientation for the 5,10b-ethano bridge, which renders the B,C-ring junction conformationally similar to the phenanthridone drug target pancratistatin **9**. As such, the α-crinanes crinamine and haemanthamine were clearly far superior to the other β-crinanes screened; 2) the stereochemistry at C-3 may not be significant given the relative configurations of the methoxyl group in crinamine and haemanthamine; and 3) requirement of a free hydroxyl at the C-11 position, the function of which may involve hydrogen bond donor-acceptor interactions, as acyl-derivatives (acetate and benzoate) were notably inactive [26].

However, as will be discussed later, the apoptosis-inducing pharmacophore is shown to incorporate other essential structural ingredients. The only work in 2008 on cytotoxicity studies of crinanes was that of Silva et al. [27] in which vittatine **15** from *Hippeastrum vittatum* was shown to be active against HT29, H460 and RXF393 cells with $IC_{50}$ values of 21.91, 15.88 and 29.57 μg/mL in the three cells, respectively. Interestingly, the dichloromethane and butanol extracts of the plant were markedly more active than neat vittatine **15** in all three cell lines. For example, the best $IC_{50}$ value for the butanol extract was ascertained in RXF393 cells (2.93 μg/mL), while that for the dichloromethane extract was 0.62 μg/mL in H460 cells.

The year 2009 saw a flurry of activity in the cytotoxicity field pertaining to crinane alkaloids. The first two related to major works involving a diverse Amaryllidaceae alkaloid library screen against HeLa, Vero and Jurkat cells [3e,f]. Of the crinane alkaloids evaluated (Tables 1 and 2), including 11-hydroxyvittatine **16**, 8-*O*-demethylmaritidine **25**, ambelline **47**, buphanamine **67** and buphanisine **53**, only haemanthamine **13** stood out for significantly reducing the viability of HeLa and Vero cells to 21% and 32%, respectively, over a 24 h period at a treatment dose of 25 μM [3e,f]. Furthermore, amongst these crinanes, only haemanthamine **13** was capable of apoptosis induction in Jurkat cells (22% over the same period and dosage) [3e,f]. Another important aspect of these investigations was the discovery that buphanamine **67** was the most potent of the twenty-nine compound library in the collagen type 1 invasion assay [3e,f]. At 25 μM it produced only ~2% invasion of collagen type 1 by HeLa cells [3e,f], and given its low cytotoxicity, even at relatively high dosages, it may play a significant future chemoprotective role in combination with the phenanthridone anticancer drug targets narciclasine **8** and pancratistatin **9**.

The work of Sun et al. (2009) on the chemical constituents of *Crinum asiaticum* from Yunnan province of China revealed *inter alia* the presence of the α-crinanes crinamine **18**, 11-*O*-methylcrinamine **20** and 3-*O*-acetylhamayne **24** [28]. Subsequent cytotoxicity studies in four cell lines (A549, LOVO, 6T-CEM and HL-60) showed, as expected, crinamine to be superior to the other functionalized analogues [28]. For comparison purposes, in the HL-60 cell line $IC_{50}$ values determined were 1.70, 6.16 and 4.27 μg/mL for the three compounds, respectively [28]. As mentioned above, and again seen here for this crinamine subset, the direct influence of substituents on the cytotoxic ability of crinane alkaloids is quite pronounced, and should thus guide SAR studies aimed at identifying a possible clinical candidate. Finally, the year closed out with further apoptotic studies by our research group on selected

**Table 2**: Activities of β-crinane alkaloid representatives of the Amaryllidaceae in miscellaneous cancer cell line screens.

| Compound | No. | Cell line (Cytotoxicity index) [a] | | | | | | | | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L6 | BL6 | SKW-3 | HL-60 | HL-60/Dox | MDA-MB-231 | A549 | OE21 | Hs683 | U373 | SKMEL | B16F10 | Jurkat | HeLa | Vero | 5123tc | Molt4 | LMTK | HepG2 | FI | RD | |
| Crinine | **3** | | >100 [b] | 16.95 | 20.86 | 14.04 | 68.11 | | | | | | | | na | | 9 [d] | | | na | na | na | 9b,19,24,26,34 |
| Buphanidrine | **46** | | | | | | | | | | | | | | na | | | >50 [b] | >50 [b] | >50 [b] | | | 9b,18,38 |
| Ambelline | **47** | | >100 [b] | >100 | >100 | >100 | >100 | >10 | 86 | >10 | >10 | >10 | >10 | 4 [d] | 79 [c] | 89 [c] | 8 [d] | >50 [b] | >50 [b] | >50 [b] | | | 3e,f,18,19,26,31,34 |
| 11-Acetylambelline | **48** | | >100 [b] | | | | | | | | | | | | | | | | | | | | 19 |
| 3-*O*-Acetylcrinine | **49** | | >100 [b] | | | | | | | | | | | | | | | | | | | | 19 |
| Filifoline | **50** | na | | | | | | | | | | | | | | | | | | | | | 25 |
| Elwesine | **51** | | | >100 | >100 | >100 | >100 | | | | | | | | | | | | | | | | 34 |
| Powelline | **52** | | | | | | | | | | | | | | | | | na | | | | | 14 |
| Buphanisine | **53** | | | | | | | >10 | 97 | >10 | >10 | >10 | >10 | 5 [d] | 99 [c] | 79 [c] | | | | | | | 3e,f,15,31,36 |
| 6α-Hydroxycrinine | **54** | | | | | | | | | | | | | | | | | na | | | | | 14 |
| | **55** | | | | | | | | | | | | | | | | | active | | | | | 14 |
| | **56** | | | 45.03 | 95.95 | 76.69 | >100 | | | | | | | | | | | | | | | | 34 |
| | **57** | | | 59.43 | >100 | 95.22 | >100 | | | | | | | | | | | | | | | | 34 |
| | **59** | | >100 [b] | | | | | | | | | | | | | | | | | | | | 21 |
| Augustine | **60** | | | | | | | | | | 0.6 [b] | | | | | | | | | | | | 15 |
| 1,2-Epoxyambelline | **61** | | | | | | | | | | | | | | | | 5 [d] | | | | | | 26 |
| Hydroxyundulatine | **63** | | | | | | | | | | | | | | | | 25 [d] | | | | | | 26 |
| Crinamidine | **64** | | | | | | | | | | | | | | | | | | | na | na | na | 24 |
| Amabiline | **65** | | | | | | | | | | >20 [b] | | | | | | 17 [d] | | | | | | 15,26 |
| Josephinine | **66** | | | | | | | | | | | | | | | | 18 [d] | | | | | | 26 |
| Buphanamine | **67** | | | | | | | >10 | >10 | >10 | >10 | >10 | >10 | 3 [d] | 96 [c] | 90 [c] | | | | | | | 3e,f,31,36 |
| Distichamine | **68** | | | | | | | | | | | | | | 2.2 | | | | | | | | 38 |
| | | BCA-1 | HT-1080 | LUC-1 | MEL-2 | COL-1 | KB-V1 | KB | P-388 | A-431 | LNCaP | ZR-75-1 | MSL | CEM | K562 | MCF-7 | G-361 | 3T3 | S-180 | T98G | U87 | | |
| Buphanidrine | **46** | | | | | | | | | | | | | >50 | >50 | >50 | >50 | | | | | | 38 |
| Ambelline | **47** | | | | | | | | 1.6 | | | | active | | | | | | | >100 | >100 | | 11,12,36 |
| Buphanisine | **53** | >20 [b] | >20 [b] | >20 [b] | >20 [b] | >20 [b] | 19.8 [b] | >20 [b] | >5 [b] | >20 [b] | >20 [b] | >20 [b] | | | | | | | | >100 | >100 | | 15,36 |
| | **58** | | | | | | | | | | | | | | | | | 100 | | | | | 10 |
| Augustine | **60** | 2.8 [b] | 1.2 [b] | 3.7 [b] | 3.2 [b] | 2.4 [b] | >20 [b] | 0.6 [b] | 0.6 [b] | 4.9 [b] | 1.7 [b] | 1.8 [b] | | | | | | | | | | | 15 |
| 1,2-Epoxyambelline | **61** | | | | | | | | | | | | active | | | | | | | | | | 12 |
| Undulatine | **62** | | | | | | | | na | | | | | | | | | | | | | | 11 |
| Amabiline | **65** | >20 [b] | >20 [b] | >20 [b] | >20 [b] | >20 [b] | >20 [b] | >20 [b] | >5 [b] | >20 [b] | >20 [b] | >20 [b] | | | | | | | | | | | 15 |
| Buphanamine | **67** | | | | | | | | | | | | | | | | | | | >100 | >100 | | 36 |
| Distichamine | **68** | | | | | | | | | | | | | 4.5 | 4.1 | 2.3 | 14.7 | | | | | | 38 |
| Crinafoline | **69** | | | | | | | | | | | | | | | | | | 68.12 [c] | | | | 13 |
| | **70** | | | | | | | | | | | | | | | | | | 32.31 [c] | | | | 13 |
| Crinafolidine | **71** | | | | | | | | | | | | | | | | | | 43.5 [c] | | | | 13 |

[a] Cytoxicity index values expressed as $ED_{50}$, $GI_{50}$, $IC_{50}$, MIC or $MTD_{50}$ in micromolar (μM) units unless otherwise stated. [b] Values are in μg/mL. [c] Indicates % cell viability. [d] Indicates % apoptosis. For compounds indicated as "active", no cytotoxicity indices were presented in the original work (na=not active, nd=not detected). Cell line abbreviations are as follows: A-431 (human epidermoid carcinoma), A549 (human lung carcinoma), BC1 (human breast cancer), BCA-1 (human breast cancer), B16F10 (mouse melanoma), BL6 (mouse melanoma), CEM (human lymphoblastic leukemia), COL-1 (human colon cancer), COL-2 (human colon cancer), FI (human cervical adenocarcinoma), G-361 (human melanoma), H460 (human lung carcinoma), HeLa (human cervical adenocarcinoma), HepG2 (human hepatoma), HL-60 (human promyelocytic leukemia), HL-60/Dox (human leukemia/doxorubicin resistant), Hs683 (human neuronal glioma), HSC-2 (human squamous carcinoma), HT (human sarcoma), HT29 (human colon adenocarcinoma), HT-1080 (human fibrosarcoma), Jurkat (human lymphoblast), K562 (human myelogenous leukemia), KB (human oral epidermoid carcinoma), KB-V1 (vinblastine-resistant KB), L6 (rat myoblast), L5178 (mouse lymphoma), L5178mdr (multidrug resistant L5178), LMTK (murine fibroblast), LNCaP (hormone-dependent human prostatic cancer), LOVO (human colon adenocarcinoma), Lu1 (human lung cancer), LUC-1 (human lung cancer), MCF-7 (human breast cancer), MDA-MB-231 (human adenocarcinoma), MEL-2 (human melanoma), Molt4 (human T lymphoma), MSL (mouse spleen lymphocyte), OE21 (oesophageal squamous carcinoma), P-388 (murine lymphoid neoplasm), RD (human rhabdomyosarcoma), RXF393 (human renal carcinoma), S-180 (mouse ascites tumor), SH-SY5Y (human neurobalstoma), SKMEL (human skin melanoma), SKW-3 (human T-cell leukemia), 3T3 (mouse embryonic fibroblast), 5123tc (rat hepatoma), 6T-CEM (human T-lymphoblastic leukemia), T98G (human glioblastoma), U87 (human glioblastoma), U373 (human glioblastoma astrocytoma), Vero (monkey kidney epithelial), ZR-75-1 (hormone-dependent breast cancer).

crinane alkaloids [29], based on the initial discovery for α-crinanes of the series [26]. This work is notable for demonstrating the apoptosis-inducing ability of the α-crinane vittatine **15** in Jurkat cells and for highlighting further structural features attending the crinane alkaloid pharmacophore. In this case, vittatine induced apoptosis in Jurkat cells in a dose dependent manner, notably with ~70% of cells exhibiting characteristic apoptotic morphology, as indicated by Hoechst staining, Annexin-V binding and caspase-3 activation, after 24 h with a single 20 μM dose [29]. The other pharmacophoric elements uncovered in the process include: 1) the modulatory effect of the C-1/C-2 double bond as shown by the markedly lower activities for dihydro-analogues of crinamine **18** and hamayne **23**; and 2) the presence of a small substituent at C-11 (H or OH), the function of which does not appear to involve hydrogen bonding but is subject to steric constraints alone [29].

About 90% of cancer patients die from tumor metastases [30]. Metastatic cancer cells are intrinsically resistant to apoptosis and therefore unresponsive to a large majority of anticancer drugs, most of which function through apoptosis induction [30]. Recent findings by Van Goietsenoven et al. (2010) are thus significant in

focusing on the cytotoxic abilities of diverse Amaryllidaceae alkaloids in apoptosis-resistant cancer cells [31]. Of the five crinane alkaloids evaluated, ambelline **47**, buphanamine **67** and buphanisine **53** exhibited low activities against both apoptosis-resistant (A549, OE21, U373 and SKMEL) and apoptosis-sensitive (Hs683 and B16F10) cells [31]. On the other hand, haemanthamine **13** and haemanthidine **14** were markedly active in both cell-forms ($IC_{50}$s 3.1- 8.5 μM) with the best activity seen for the latter in B16F10 cells ($IC_{50}$ = 3.1 μM) [31]. Also in 2010, phaedranamine **33**, the enantiomer of buphanamine, was isolated for the first time from the South American Amaryllidaceae species *Phaedranassa dubia* and shown to be inactive towards L6 cells ($IC_{50}$ > 300 μM) [32].

In 2011, two further Amaryllidaceous species (*Galanthus trojanus* and *Crinum zeylanicum*) were examined by our group for cytotoxic effects in various cell lines [33,34]. Firstly, 11-hydroxyvittatine-*N*-oxide **17** and 8-*O*-demethylmaritidine **25** from *Galanthus trojanus* were both weakly active in L6 cells ($IC_{50}$s > 90 μg/mL) and KB cells ($IC_{50}$s > 50 μg/mL) in contrast to the co-constituent haemanthamine which exhibited $IC_{50}$s of 7.41 and 0.97 μg/mL in the two cell lines, respectively [33]. The result for 8-*O*-demethylmaritidine is in accordance with previous findings by Lin et al. (1995), who showed that the compound was unresponsive in a multi-cell line screen [17], and thus emphasizes the significance of the A-ring attached 1,3-dioxolane moiety to the crinane anticancer pharmacophore.

Secondly, bulbs of *Crinum zeylanicum* were shown to contain *inter alia* the crinane alkaloids crinine **3**, ambelline **47**, elwesine **51**, 6-hydroxybuphanidrine **56** and 6-ethoxybuphanidrine **57**, all of the β-subgroup [34] (Table 2). In the four cell lines screened, only crinine was notably active with $IC_{50}$s of 16.95, 20.86, 14.01 and 68.11 μM in SKW-3, HL-60, HI-60/Dox and MDA-MB-231 cells, respectively [34]. The role of the double bond to the integrity of the crinane pharmacophore could be gauged directly by comparison of these responses to those of elwesine **51**, the dihydro-analogue of crinine, which exhibited $IC_{50}$s > 100 μM across the four cells [34]. Further mechanistic tests on the active compound crinine via oligonucleosomal DNA fragmentation revealed the apoptotic cell death mode as responsible for its cytotoxicity [34]. Apart from this, Jitsuno et al. (2011) showed that haemanthamine **13** and haemanthidine **14** from *Lycoris albiflora* were active against HL-60 and HSC-2 cells (Table 1) [35].

The year 2012 has already seen three detailed studies of the cytotoxic properties of crinane alkaloids [36-38]. Firstly, Luchetti et al. (2012) uncovered the cytotoxic ability of bulbispermine **36**, the 11-*endo* analogue corresponding to hamayne **23**, in various cells including apopotosis-resistant and apoptosis-sensitive forms [36]. For example, in the apoptosis-resistant line T98G the compound had a $GI_{50}$ of 9 μM, matched closely by its activity in apoptosis-sensitive HeLa cells ($GI_{50}$ of 8 μM) [36]. Further results showed as expected, the prominence of haemanthamine **13** and haemanthidine **14**, as well as the low activity of buphanamine **67**, buphanisine **53** and ambelline **47** in these cells [36]. SAR studies revealed that the semi-synthetic transformation products (**37**-**45**) of bulbispermine, with the exception of **39** ($GI_{50}$ 15 μM in U87 cells), were weakly active ($GI_{50}$s > 100 μM) in most test screens. Secondly, work by Adewusi et al. (2012) on the South African amaryllid *Boophone disticha* uncovered 6-hydroxycrinamine **22** as the cytotoxic agent in neuroblastoma (SH-SY5Y) cells with $IC_{50}$s of 54.5 and 61.7 μM, as determined by the MTT and neutral red assays, respectively [37]. Finally, we

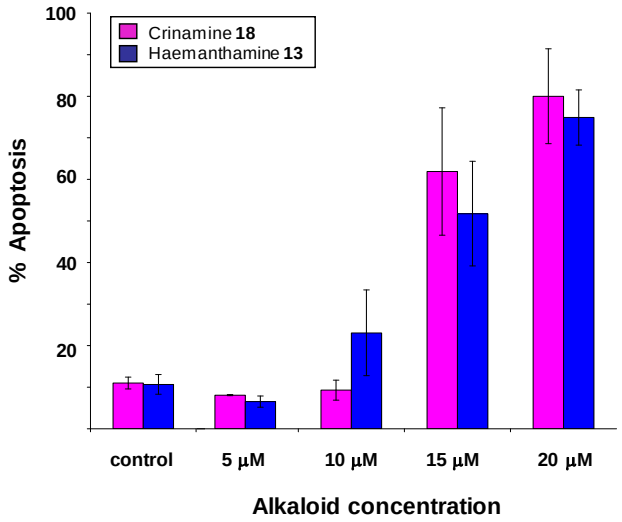



**Figure 3**: Apoptosis responses in 5123tc cells treated over 72 h with various concentrations of crinamine and haemanthamine. Effective dose ($ED_{50}$) was calculated as the concentration inducing apoptosis in 50% of cells (after McNulty et al., 2007).[26]

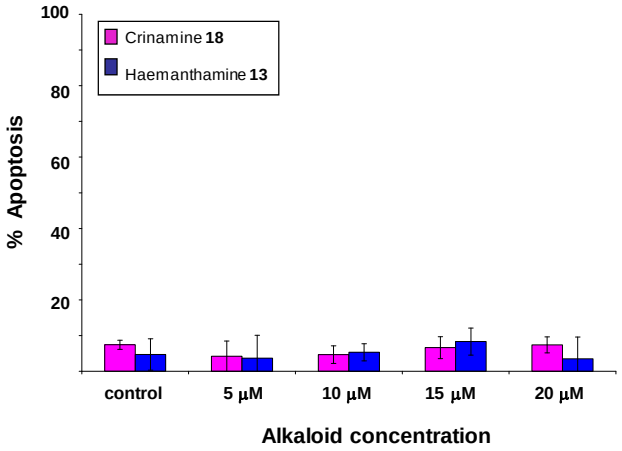



**Figure 4**: Normal human embryonic kidney (293t) cells are unaffected by treatment with various doses of crinamine and haemanthamine after 72 h (after McNulty et al., 2007).[26]

very recently revealed the antiproliferative properties of the rare Amaryllidaceae crinane constituent distichamine **68** in a mini-panel of cancer cells comprising acute lymphoblastic leukemia (CEM), chronic myelogenous leukemia (K562), breast adenocarcinoma (MCF7), malignant melanoma (G-361) and cervical adenocarcinoma (HeLa), in addition to the normal human fibroblast (BJ) cell line [38]. By means of the Calcein AM assay, distichamine **68** was seen to be active against all cancer cell lines ($IC_{50}$s 2.2-14.7 μM), with the adherent cell line HeLa shown to be the most sensitive ($IC_{50}$ 2.2 μM) to this alkaloid [38].

Mechanistic studies then invoked flow cytometry to quantify the distribution of CEM cells across the different phases of the cell cycle, and to determine the sub-$G_1$ fraction as a measure of the proportion of apoptotic cells [38]. The analysis showed that treatment with distichamine **68** increased the proportion of $G_2$/M phase cells in a dose-dependent manner, with concomitant reductions in the proportion of $G_0/G_1$ and S cells (Figure 5) [38]. In addition, the proportion of cells with sub-$G_1$ amounts of DNA (apoptotic cells) increased (up to 23.7 %) following a 24 h

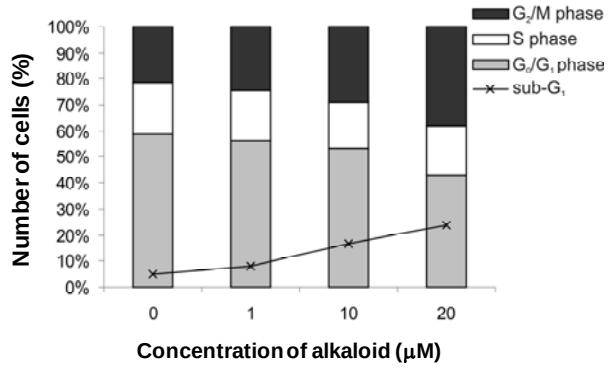



**Figure 5**: Histograms obtained by flow cytometric analysis showing the distributions of CEM cells across the $G_0/G_1$, S and $G_2/M$ phases of the cell cycle after 24 h treatment with distichamine **68**, and the sub-$G_1$ fraction of cells (relative to untreated controls). The bars show the percentage of cells in the corresponding phase (after Nair et al., 2012).[38]

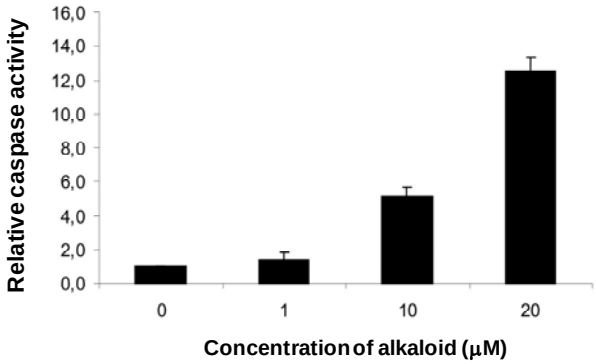



**Figure 6**: Increases in the activities of caspase-3 and 7 in CEM cells treated with distichamine **68** for 24 h, relative to untreated controls. The data shown represent averages from at least three independent experiments performed in triplicate (after Nair et al., 2012).[38]

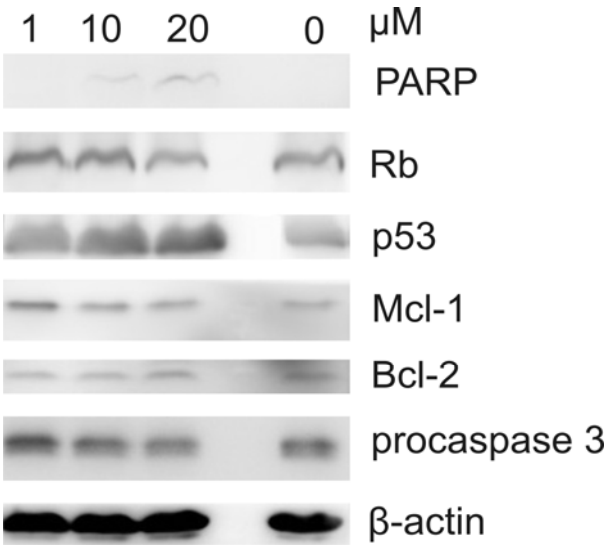



**Figure 7**: Western blot analysis of apoptosis-related proteins (PARP fragment 89 kDa, Rb, p53, Mcl-1, Bcl-2 and procaspase 3) in leukemia (CEM) cells treated with distichamine **68** compared to their expression in untreated control cells. The expression of β-actin was monitored as a protein loading control (after Nair et al., 2012).[38]

treatment with **68**, relative to that observed in untreated cells, indicating that the compound was clearly capable of cell cycle disturbance and apoptosis induction in CEM cells [38].

Furthermore, distichamine induced a 12.5-fold increase in the activity of caspase-3/7 after 24 h at the highest concentration (20 μM) compared to untreated controls (Figure 6). In addition, Western blot analysis was used to detect changes in expression of apoptosis-related proteins in CEM leukemia cells after treatment with distichamine. As shown in Figure 7, treatment with **68** (at 10 and 20 μM) induced cleavage of PARP (89 kDa fragment) after 24 h which also corresponded with decreased levels of procaspase-3. As described above, increased caspase-3 activity was observed over the same treatment period and dosage (Figure 6). Expression of the tumor suppressor protein p53 was discernible in the CEM cell line control, and **68** caused its enhanced expression after 24 h, notably at 10 and 20 μM (Figure 7). No change in the expression of the antiapoptotic protein Bcl-2 was observed, but at 20 μM, a decreased level of the antiapoptotic protein Mcl-1 was detected, indicating the onset of apoptosis. Mcl-1 is necessary for cell viability, and its decreased expression has been suggested as the cause of cell death in CEM cells [39a]. It also plays a crucial role in regulating the apoptosis of T-cells [39b].

Western blot analysis here demonstrated the dose-dependent decrease of procaspase-3 and cleavage of PARP after 24 h treatment with distichamine **68** in CEM leukemia cells (Figure 7), indicating that the compound induces caspase-3 activated apoptosis [38]. Given the unique C-ring structural formulation for distichamine, involving shift of the double bond to C-2/C-3 with concomitant vinylization of the C-3 methoxy group and oxidation at C-1, these findings point at further attendant structural features of the crinane apoptosis-inducing pharmacophore. It now appears that a $sp^2$–hybridised C-3 centre is tolerated at the active site, since most other cytotoxic crinane alkaloids possess a C-1/C-2 double bond.

Furthermore, oxygenation at C-1, as is characteristic of the anticancer phenanthridone pancratistatin **9**, was here shown to be amenable towards activity. Interestingly, in the case of distichamine, a keto-group is positioned at C-1, as a consequence of which three contiguous $sp^2$–centres are formed in ring-C, similar to that seen for the active phenanthridone narciprimine **10** [38].

In summary, a more comprehensive view has emerged of crinane alkaloids of the Amaryllidaceae as potent and selective cytotoxic agents with significant potential for preclinical development. This promising status was drawn out of multi-cell line approaches invoking targets of synthetic as well as natural origin. As such, both *in vitro* and *in vivo* models of study support the ameliorative effects of crinane compounds as anticancer agents. This survey has accounted for 54 cancer cell lines (Tables 1 and 2), including human and animal forms, against which the cytotoxic abilities of a total of 71 crinane alkaloids have been measured. As also shown in Tables 1 and 2, of the α-series, not surprisingly, haemanthamine **13**, haemanthidine **14** and crinamine **18** are the most commonly targeted agents in such studies. Within the β-series, ambelline **47** and buphanisine **53** appear routinely across most of the cell lines screened. In terms of potency, synthetic 6-hydroxycrinamine methiodide **31** is the most active crinane identified to date with a MTD of 0.05 μg/mL in NIH/3T3 cells. In animal models of study, the natural α-crinanes, haemanthamine **13**, crinamine **18** and 6-hydroxycrinamine **22** were the most active each with MTD of 0.2 μg/mL in NIH/3T3 cells. In human cancers, haemanthamine was again the most

potent compound with an $ED_{50}$ of 0.3 μg/mL in HT-1080 fibrosarcoma cells. Furthermore, selective cytotoxic abilities have been demonstrated for crinamine and haemanthamine, both of which, for example, targeted rat hepatoma (5123tc) cells as opposed to normal human embryonic kidney (293t) cells. Of the β-crinanes evaluated, augustine **60** was the most active with an $ED_{50}$ of 0.6 μg/mL in both KB (human oral epidermoid carcinoma) and U373 (human glioblastoma astrocytoma) cells. Given the close structural proximity of these alkaloids to the potent phenanthridone anticancer agents of the family, such as narciclasine and pancratistatin, as well as their common biogenetic origin, it is possible that a common, though still elusive, biological target may be operable for these agents.

Stucture-activity relationship studies, again involving natural as well as synthetically-derived targets, have provided useful insights to the structural details of the anticancer pharmacophore attending these molecules. Features that have come to light from such studies include: 1) an intact phenanthridine nucleus is essential since truncated analogues were markedly less active, as seen for crinafoline **69** against crinafolidine **71**; 2) presence of the A-ring methylenedioxyphenyl moiety since structures incorporating other substituents (as in 8-*O*-demethylmaritidine **25**) were seen to be less active; 3) B-ring modification effects such as quaternization of the nitrogen atom were shown to be deleterious; 4) small substituents with hydrogen bond donor/acceptor capability at C-11 (β to the nitrogen atom) with either *exo*- or *endo*-disposition were ameliorative (as in crinamine **18** and bulbispermine **36**, respectively), but less so for substitutions α to the nitrogen atom at C-6 (6-hydroxycrinamine **22**) or C-12 (delagoensine **34**); 5) stereochemistry at C-3 may not be significant given the relative configurations of the methoxyl group in crinamine **18** and haemanthamine **13**, as well as its planar geometry in distichamine **68**; 6) additional oxygenation of ring-A, reminiscent of the potent anticancer phenanthridones narcicalsine **8** and pancratistatin **9**, is viewed as beneficial to the pharmacophore, as shown for distichamine **68**; and 7) although active compounds were found across both α- and β-members of the series, the geometry of the 5,10b-ethano bridge appears to play a significant role given the consistent potency of the former series across most of the cell lines screened. In addition, mechanistic studies have recently uncovered the apoptotic mode of death as responsible for the cytotoxicity of several crinane compounds. These distinctive features should prove useful in guiding the development of a clinical drug target. As such, efforts to further refine the active pharmacophore associated with these molecules are an ongoing concern in our laboratories.

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