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Structure-activity studies on the lycorine pharmacophore: A potent inducer of apoptosis in human leukemia cells

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ABSTRACT

The direct chemoselective differential functionalization of the ring-C hydroxyl groups present in the Amaryllidaceae alkaloid lycorine is described allowing for selective manipulation of the 1,2-hydroxyl groups. A mini-library comprised of synthetic and natural lycorane alkaloids was prepared and their apoptosis-inducing activity investigated in human leukemia (Jurkat) cells. Further insights into the nature of this interesting apoptosis-inducing pharmacophore are described, including the requirement of both free hydroxyl groups in ring-C.

1. Introduction

Three major structurally distinct classes of Amaryllidaceae alkaloids are recognized as the lycorane, galanthamine or crinane types (Jin, 2005), while several other less common structural variants are also known (Kornienko and Evidente, 2008). These structural types are related as a consequence of their common biogenesis from the amino acid-derived precursor norbelladine **1** (Scheme 1). Representatives from each of the major series include lycorine **2**, galanthamine **3** and crinamine **4**, respectively, illustrating the remarkable structural diversity that Nature has evolved from **1**. The plethora of biological properties exhibited by these compounds is not surprisingly as equally diverse (Bastida et al., 2006). Of the galanthamine class, galanthamine **3** itself is the first of the Amaryllidaceae alkaloids to be approved as a pharmaceutical in the treatment of a human disease. Its remarkable ability to reversibly inhibit acetylcholinesterase, of relevance in Alzheimer's and other neuro-degenerative diseases, has very recently led to its approval as a prescription drug under the generic name Reminyl (Houghton et al., 2006).

Alkaloids of the crinane series have been shown to exhibit a range of biological activities (Tram et al., 2002). For example, crinamine **4** is known to be cytotoxic to the malarial parasite and to a series of tumor cell lines (Likhitwitayawuid et al., 1993), while the activity of 6-hydroxycrinamine against mouse melanoma cells has been documented (Nair et al., 1998) and haemanthamine (the C-3 epimer of crinamine) is known to inhibit protein synthesis and have anti-proliferative action (Jimenez et al., 1976; Hohmann et al., 2002). We recently demonstrated the potent apoptosis-inducing ability of the α-ethano bridged crinane alkaloids crinamine **4** and the C-3 epimeric haemanthamine. These compounds were shown to be significantly more active than the β-ethano bridged compounds (McNulty et al., 2007a, 2009) demonstrating the critical structural requirements in the vicinity of the C-ring. In the lycorane series, pancratistatin **5**, and its analogues such as narciclasine (Kornienko and Evidente, 2008), are known for their potent and selective anticancer properties as well as their synthetically challenging structures. Synthetic efforts in this area involving a complementary pair approach and pharmacophore minimization (McNulty and Mo, 1998; McNulty et al., 2001, 2005, 2007b, 2008; Rinner and Hudlicky, 2005) disclosed the strict requirements of the free hydroxyl groups in ring-C of the compounds for potent apoptosis-inducing activity. We have shown that pancratistatin induces apoptosis selectively in cancer cells with minimal effect on normal cells and that mitochondria are the site of action (McLachlan et al., 2005). Evidence for the apoptotic mode of death in cancer cells was related to early activation of caspase-3 followed by flipping of phosphatidyl serine (Kekre et al., 2005).

The interesting structural requirements for apoptosis-inducing activity in the pancratistatin-type of lycorane derivative drew our attention to alkaloids of the lycorine subclass. Lycorine **2** is

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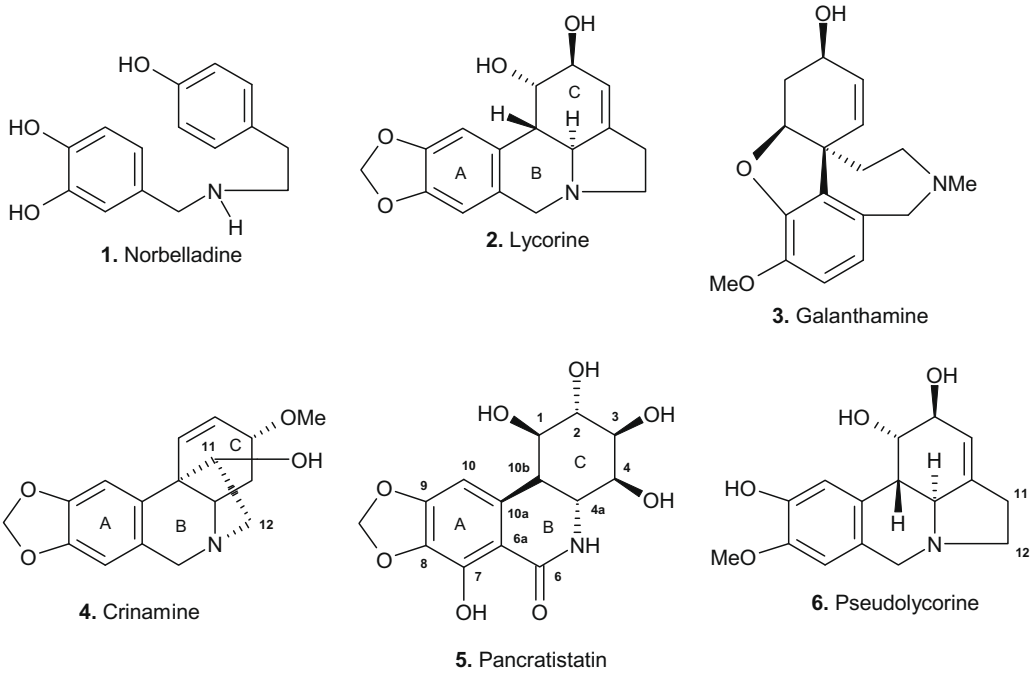

Scheme 1. Structurally diverse alkaloid representatives of the Amaryllidaceae.

by far the most common alkaloid within the Amaryllidaceae family. This compound exhibits a vast array of biological properties. It is active against poliovirus (Hwang et al., 2008), vaccinia smallpox virus (Deng et al., 2007) and SARS-associated coronavirus (Li and Tan, 2005). Furthermore, it exhibits antifungal activity against *Saccharomyces cerevisiae* (Del Giudice et al., 2005), is fatal to the protozoan parasite *Trypanosoma brucei* (Mackey et al., 2006) and is more potent than indomethacin as an anti-inflammatory agent (Citoglu et al., 1998). In addition, lycorine **2** has been shown to have insect antifeedant activity (Evidente et al., 1986), inhibit ascorbic acid biosynthesis (Arrigoni et al., 1975) and inhibit the enzyme acetylcholinesterase of significance in motor neuron disease (Lopez et al., 2002). The fact that it has an inhibitory effect towards cell division and cell elongation in plants suggests that it may inhibit either (or both) RNA and protein synthesis (De Leo et al., 1973; Jimenez et al., 1976). As a potential chemotherapeutic, it has shown most promise as an antiproliferative agent of a number of cancer cell lines (Likhitwitayawuid et al., 1993). Investigation into the *in vitro* mode of action in a leukemia (HL-60) cell line model established that lycorine **2** suppressed tumor cell growth and reduced cell survival via cell cycle arrest and induction of apoptosis (Liu et al., 2004). Further investigation of the effect of lycorine **2** on severe combined immuno-deficiency (SCID) mice inoculated with HL-60 cells showed that it decreased tumor cell growth and increased survival rates with no observable adverse effects on treated animals (Liu et al., 2007). Arrest of cell cycle progression and apoptosis induction was also the demonstrated mechanism of action of lycorine **2** on multiple myeloma (KM3) cells (Li et al., 2007).

Motivated by these interesting and diverse biological properties, we decided to investigate structure-activity studies in the lycorine-series in order to further refine some of the pharmacophoric requirements. Herein we describe a concise, chemo- and regioselective three-step synthesis of 1-acetyllycorine **9** from lycorine **2** utilizing the reactivity of the allylic pseudoequatorial C-2 hydroxy group. This selective functionalization allowed us to prepare a mini-library of lycorine derivatives analogues **7–10** (Scheme 2). Investigations on the apoptosis-inducing properties of six derivatives showed that only lycorine **2** and pseudolycorine **6** were able to induce apoptosis in human leukemia (Jurkat) cells at the micromolar level.

2. Results and discussion

Previous studies have highlighted the difficulties attending the differential functionalization of the hydroxyl groups present within the lycorine series (Takeda et al., 1958). Previous work in our group has shown that highly selective mono-silylation of 1,2-diols can be achieved under certain conditions (McNulty and Mao, 2002). The reaction of lycorine **2** with *tert*-butyldimethylsilyl chloride (TBSCl) and pyridine in dichloromethane (DCM) (Scheme 2) was therefore investigated. To our delight, this reaction afforded the novel monosilyl ether **7** (in 82% yield), most likely due to the greater reactivity of the allylic pseudoequatorial 2-hydroxy group over the axially-orientated C-1 hydroxyl. The ^{1}H NMR spectrum of **7** (see Section 3) had the H-2 signal at δ 4.29 (dd), upfield-shifted from δ 5.37 (dd) where it resonates for lycorine (**2**) while COSY contours were established between H-2 and both H-1 (δ 4.43, dd) and H-3 (δ 5.42, dd). In addition, three-bond HMBC showed H-2 to be correlated to both C-4 (δ 142.01, s) and C-10b (δ 41.20, d).

Direct access to **7** allowed us to prepare the new allylic acetate **8**, which was obtained in near quantitative yield from silyl ether **7** upon acetylation with acetic anhydride and pyridine, and its ^{1}H NMR spectrum showed the expected deshielding effect on H-1 (δ 5.56, dd). Desilylation of **8** proceeded smoothly with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) to give 1-acetyllycorine **9**, the proton spectrum of which indicated a slight upfield shift for H-2 (δ 4.15, dd) compared to **8**, whose spectroscopic data (mpt, α_D, IR, ^{1}H NMR, ^{13}C NMR) was similar to literature values (Kobayashi et al., 1984). Acetylation of **9** then provided in 97% isolated yield 1,2-diacetyllycorine **10** which had the H-2 signal at δ 5.25 (dd) and its spectroscopic data were in close agreement with documented values (Kobayashi et al., 1984, Campbell et al., 1998).

The minipanel of synthetic compounds **7–10** together with natural products lycorine **2** and pseudolycorine **6** were then screened for their ability to induce apoptosis in human leukemia (Jurkat)