Bioorganic & Medicinal Chemistry Letters 19 (2009) 3233–3237

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Selective cytochrome P450 3A4 inhibitory activity of Amaryllidaceae alkaloids

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ARTICLE INFO

Article history:
Received 26 March 2009
Revised 16 April 2009
Accepted 21 April 2009
Available online 24 April 2009

Keywords:
Cytochrome P450 3A4
CYP3A4
Amaryllidaceae alkaloid
Anticancer agent
Pancratistatin
Narciclasine
Galanthamine

ABSTRACT

A library of natural and semi-synthetic Amaryllidaceae alkaloids was screened for cytochrome P450 3A4 (CYP3A4) inhibitory activity. Of the crinane, lycorane and galanthamine representatives examined two semi-synthetic silylated lycorane analogues, accessed via a chemoselective silylation strategy from lycorine, and the natural compound narciclasine exhibited low micromolar activities. Important pharmacological features uncovered include the lack of CYP3A4 inhibitory activity seen for galanthamine and the selective activity that is seen with narciclasine over pancratistatin.

The plant family Amaryllidaceae produces an impressive assortment of structurally-diverse alkaloids, all of which are biogenetically related to the common amino acid-derived precursor norbelladine **1** (Scheme 1). Three major groups of compounds within the family are identified as the crinane, galanthamine and lycorane groups, represented by crinine **2**, galanthamine **16** and lycorine **19** respectively, although at least six other minor groups are known to occur.[1] Perhaps as impressive as this structural diversity is the broad spectrum of biological properties that has been demonstrated by such alkaloids. Taken together, these two attributes have fuelled the advancement of Amaryllidaceae alkaloids and their synthetic analogues as potential therapeutics.[2,3] The alkaloid galanthamine **19**, under the generic name Reminyl, is the first of the Amaryllidaceae alkaloids to be approved as a prescription drug in the treatment of Alzheimer's disease.[4]

A lot of attention has focussed recently on the anticancer alkaloids within the lycorane series including lycorine **19**, and particularly pancratistatin **25** and narciclasine **26**.[5–7] This recent surge of interest resulted from the disclosure of the potent, cancer cell-line selective cytotoxicity seen with pancratistatin.[6] Similar selectivity has also now been demonstrated with narciclasine,[5] although both alkaloids have been known to share a common pharmacophore for some time.[7h] Lycorine **19**, the most abundant of the Amaryllidaceae alkaloids, is known for its wide array of biological activities.[8] It has been shown to be an effective antiviral, antifungal, antiparasitic, anti-inflammatory and insect antifeedant agent.[8a–g] It is also known to inhibit ascorbic acid biosynthesis, acetylcholinesterase and RNA and protein synthesis.[8h–l,9] Alkaloids of the crinane series have also been shown to exhibit a range of biological activities,[10a] including antimalarial[8l] and antiproliferative[10b,c] action as well as protein synthesis inhibition.[8k] We have recently distinguished α-crinane analogues from their β-relatives as potent initiators of apoptosis in rat liver hepatoma (5123tc) cells.[11,9]

Although these anticancer compounds have been widely studied for therapeutic effects, and it seems likely that further clinical candidates will evolve, very little is known regarding their interaction with the cytochrome P450 drug metabolizing enzymes. Humans possess 57 putatively functional genes that encode for cytochromes P450. Cytochromes P450 are heme-containing mono-oxygenase enzymes that are primarily expressed on smooth ER membranes of hepatocytes and by cells along the intestinal tract mucosal surface.[12] They are involved in the detoxification of a wide variety of xenobiotics such as drugs, biogenic amines from food sources, environmental toxins, and chemical carcinogens, and in the oxidation of steroids, fatty acids, prostaglandins, leukotrienes, and fat-soluble vitamins.[13,14] The CYP3A subfamily comprises about 30% of the total liver cytochrome P450 enzyme pool in humans, and the isoenzyme CYP3A4 accounts for approximately 60% of drugs metabolized.[13] In addition, an estimated 70% of total CYP protein in the small intestinal epithelium is formed by this isoenzyme.[14]

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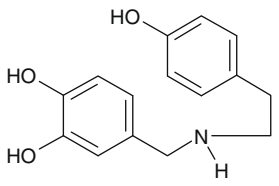
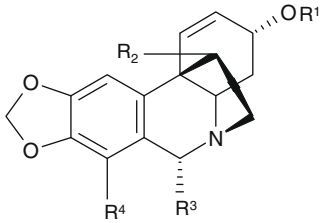
doi:10.1016/j.bmcl.2009.04.086



1. Norbelladine

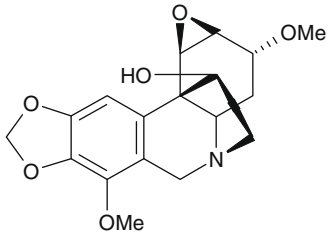


2. $R^1=R^2=R^3=R^4=H$ (Crinine)

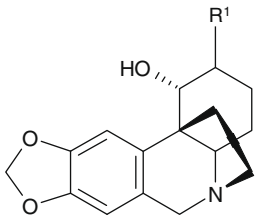
3. $R^1=Me$, $R^2=OH$, $R^3=H$, $R^4=OMe$ (Ambelline)

4. $R^1=H$, $R^2=H$, $R^3=OH$, $R^4=OMe$ (6α-Hydroxycrinamidine)

5. $R^1=OMe$, $R^2=H$, $R^3=OH$, $R^4=OMe$ (6α-Hydroxyundulatine)

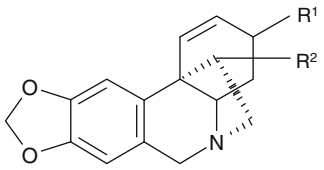


6. 1,2-Epoxyambelline



7. R^1=α-OH (Amabiline)

8. R^1=β-OAc (Joesephinine)



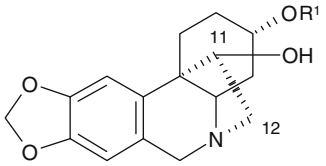
9. R^1=α-OH, R^2=OH (Hamayine)

10. R^1=α-OMe, R^2=OH (Crinamine)

11. R^1=α-OMe, R^2=OAc (Acetylcrinamine)

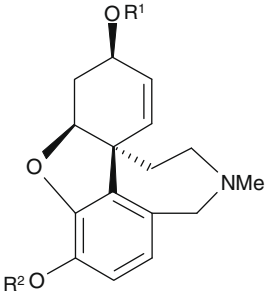
12. R^1=β-OH, R^2=H (Vittatine)

13. R^1=β-OMe, R^2=OH (Haemanthamine)



14. R^1=H (Dihydrohamayine)

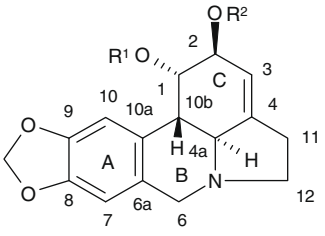
15. R^1=Me (Dihydrocrinamine)



16. R^1=H, R^2=Me (Galanthamine)

17. R^1=H, R^2=H (Sanguinine)

18. R^1=Ac, R^2=H (Acetylsanguinine)



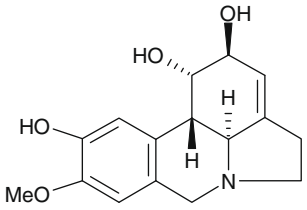
19. R^1=H, R^2=H (Lycorine)

20. R^1=H, R^2=TBS (2-TBS-lycorine)

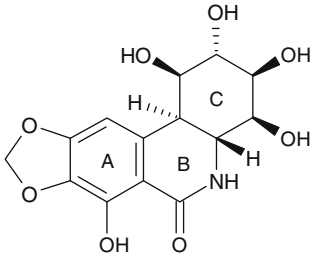
21. R^1=Ac, R^2=TBS (1-Acetoxy-2-TBS-lycorine)

22. R^1=Ac, R^2=H (1-Acetoxylycorine)

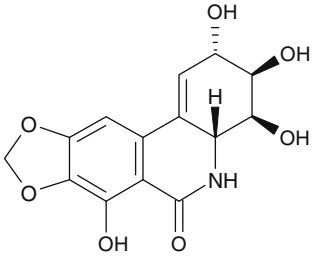
23. R^1=Ac, R^2=Ac (1,2-Diacetoxylycorine)



24. Pseudolycorine



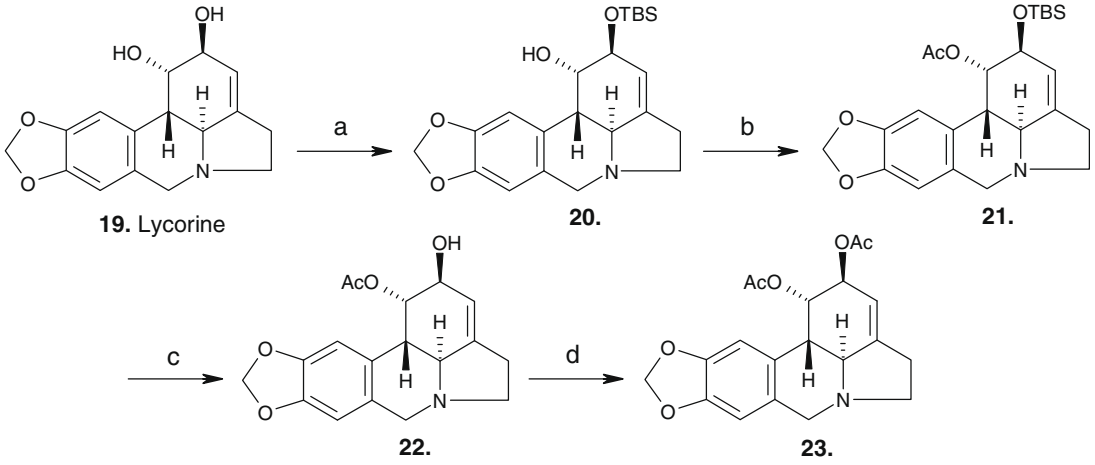
25. Pancratistatin



26. Narciclasine

Scheme 1. Library of natural and semisynthetic alkaloids of the Amaryllidaceae screened for CYP3A4 inhibitory activity.

It is widely known that co-administration of multiple CYP3A4 substrates, inducers or inhibitors, including compounds from food sources, may alter pharmacokinetic and pharmacodynamic parameters of many commonly prescribed drugs. Given the pharmaceutical potential of the anticancer Amaryllidaceae alkaloids, it is most surprising that no cytochrome P450 activity data for these compounds is available in the literature. Furthermore, the acceptance of the role of reactive oxygen species (ROS) and free radical reactions in a broad range of pathological processes has prompted the search for molecules with antioxidative properties as potentially useful therapeutic agents. A few recent reports[15] allude to the antioxidant properties of Amaryllidaceae alkaloids, especially



Scheme 2. Chemoselective silylative discrimination of the 1,2-dihydroxy groups in lycorine. Reagents and conditions: (a) TBSCl, py, DCM, rt, 6 h, 82%; (b) Ac_2O, py, DCM, rt, 3 h, 95%; (c) TBAF, THF, rt, 1 h, 96%; (d) Ac_2O, py, DCM, rt, 3 h, 97%.

of galanthamine **16**, as potent scavengers of reactive oxygen species. In this Letter, we report the screening of a diverse Amaryllidaceae alkaloid library (Scheme 1) for CYP3A4 inhibitory activity. Of the compounds screened, two semisynthetic lycorine derivatives (**20** and **21**), accessed via chemoselective allylic hydroxy silylation, and the natural compound narciclasine **26** exhibited low micromolar (0.21–0.63 μM) inhibitory activity against CYP3A4.

The alkaloid library (Scheme 1, **2–26**) was assembled from compounds previously isolated and characterized.[11,9,16] Semisynthetic analogues (**11, 14, 15, 20, 21, 22, 23**) were prepared according to methods highlighted in the accompanying Supplementary data section of this Letter. Direct access to the novel monosubstituted silyl ether **20** (obtained in 82% yield) is noteworthy and was gained via the selective silylation reaction of lycorine **19** with *t*-butyldimethylsilyl chloride (TBSCl) and pyridine in dichloromethane (DCM) (Scheme 2). Chemoselectivity in this silylation is 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 **20** (see Supplementary data) had the H2 signal at δ 4.29 (dd), upfield-shifted from δ 5.37 (dd) where it occurs in lycorine, while COSY contours were established between H2 and both H1 (δ 4.43, dd) and H3 (δ 5.42, dd). In addition, a three-bond HMBC correlated H2 both to C4 (δ 142.01, s) and C10b (δ 41.20, d). Acetylation of **20** with acetic anhydride in pyridine afforded the new allylic silyl acetate **21**, which was obtained in near quantitative yield. The ^{1}H NMR spectrum of **21** showed the expected deshielding effect on H1 (δ 5.56, dd). Desilylation of **21** proceeded smoothly with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) to give 1-acetoxylycorine **22**, the proton spectrum of which indicated a slight upfield shift for H2 (δ 4.15, dd) compared to **21**, whose spectroscopic data (mp, α_D, IR, ^{1}H NMR, ^{13}C NMR) was similar to literature values.[17] Acetylation of **22** then provided in 97% isolated yield 1,2-diacetoxylycorine **23** which had the H-2 signal at δ 5.25 (dd). The remainder of its spectroscopic data were in close agreement with documented values.[16a] The selective manipulation of the hydroxyl residues in lycorine thus allowed for the synthesis of a range of hydroxyl-differentiated patterns **20** to **23** in ring C.

The entire library of alkaloids **2–26** was screened for CYP3A4 inhibitory activity[18] via kinetic monitoring of the conversion of 7-benzyloxyquinoline (BQ) to 7-hydroxyquinoline (HQ) by fluorometric measurement of emission at 538 nm after excitation at 410 nm (see Supplementary data), utilizing ketoconazole as control (Table 1). Interestingly, no activity was observed for any of the crinane (**2–15**) or galanthamine group of compounds (**16–18**). In the lycorane series, neither lycorine **19** nor its biosynthetically-related analogue pseudolycorine **24** showed any activity. A remarkable spike in activity is observed in going from lycorine **19** to the silyl ether **20** (K_i 0.26 μM) and upon acetylation to the silyl acetate **21** (K_i 0.21 μM) inhibition is virtually unchanged. Cleavage of the silyl group from **21** resulted in a ~30-fold drop in activity for **22** with K_i 7.6 μM, while activity dropped even further with the introduction of an additional acetate group as seen in compound **23** (K_i 11 μM).

Table 1
Inhibitory activity against the biotransformation of 7-benzyloxyquinoline by cDNA expressed human CYP3A4

Compound	K_i (μM)	pK_i[a] (M)
16	na	
19	na	
20	0.26	6.6 (±0.1)
21	0.21	6.8 (±0.2)
22	7.6	5.1 (±0.1)
23	11	5.0 (±0.1)
25	na	
26	0.63	6.2 (±0.2)
Ketoconazole	0.024	7.6 (±0.02)

[a] Values are means of three experiments, standard error is given in parentheses (na = not active).

More importantly, the natural compound narciclasine **26** exhibited a K_i of 0.63 μM and is therefore a potent inhibitor of CYP3A4. In contrast, pancratistatin **25** did not exhibit any CYP3A4 inhibition up to >10 μM. Narciclasine, although less active than the synthetic silyl acetate **21**, is a stronger inhibitor than some well known natural inhibitors of 3A4 such as naringenin (IC_{50} 87 μM), curcumin (IC_{50} 16 μM) and resveratrol (IC_{50} 4 μM). In this context, we note that some of the furanocoumarins present in grapefruit have set the benchmark for CYP3A4 inhibition by natural compounds with IC_{50}s as low as 3 nM.[19] Given the now intense research activity with anticancer lycorane-type alkaloids,[5,6] the inactivity of pancratistatin and parent lycorines in contrast to the potent CYP3A4 activity seen exclusively with narciclasine has important pharmacological consequences that will require appropriate consideration in furthering a clinical candidate.

From the data described above, two features appear to be relevant in eliciting CYP3A4 activity. First of all, the structural similarity of pancratistatin **25** and narciclasine **26** imply that the C1–C10b olefin is required for this activity. This is not surprising given the role of P450 enzymes in oxidizing olefinic substrates.[13] In contrast,

the C3–C4 bond in lycorine results in no CYP3A4 inhibition. There is considerable ring C conformational modulation in going from a C1–C10b (narciclasine) to C3–C4 (**20–23**) double bond, favouring a distorted chair conformation for ring C in lycorine, which is further restricted by the N5–C4 ethyl tether. Thus, double bonds can be tolerated in ring C but not at the C1–C10b position. We speculate that this selective inhibition may be due to the trapping of a peroxy intermediate at the benzylic position of narciclasine upon reaction at C1 with the cytochrome. Secondly, while the parent alkaloid lycorine **19** is inactive, the potency of C2–C3 lipophilic derivatives point to the interaction of the top portion of these alkaloids with a lipophilic binding site in the enzyme. The introduction of a bulky lipophilic TBS group onto C2 results in the potent CYP3A4 inhibitor **20**. A small, polar group appears to be important at C1, in conjunction with the lipophilic group at C2, for potent CYP3A4 inhibition as the simple acetate **23** is significantly less active. Removal of the bulky lipophilic TBS group from the C2-hydroxyl of **21** gave acetate **22**, which was also significantly less active. Thus a bulky lipophilic group is required at C2 for optimal CYP3A4 activity. This is evident for **20** and **21** compared to **22** and **23**. Polar functional groups are also seen to be detrimental to CYP3A4 inhibition in considering lycorine **19**, pseudolycorine **24** and pancratistatin **25**. All of these derivatives contain polar 1,2-dihydroxy substituents, and all were seen to be inactive. Overall, these results appear to indicate that a lipophilic interaction at the C2 region of these alkaloids with a binding pocket in the enzyme is important for cytochrome inhibition. Thus the exclusion of a C1–10b olefin and the avoidance of bulky, lipophilic substituents around C2 are important structural features to be considered in avoiding interactions with CYP3A4.

Perhaps as striking as the remarkable activities of **20** and **21** is the complete lack of CYP3A4 inhibition seen with the current Alzheimer's drug galanthamine **16** and the potent anticancer agent pancratistatin **25**, in contrast to the inhibition that is seen with narciclasine **26**. The lack of such inhibition enhances the status of **16** and **25** as biologically privileged chemotherapeutic molecules and is worthy of further comment.

Galanthamine **16** is known to be slowly metabolized by the CYP3A4 and CYP2D6 isoenzymes through demethylation and subsequent transformation to the glucuronide of sanguinine **17**.[20] While both **16** and **17** are potent inhibitors of acetylcholinesterase (AChE), neither was seen to be an inhibitor of the CYP3A4 as described herein. This is interesting as galanthamine has recently been shown to function as an antioxidant in addition to an inhibitor of AChE and associated nicotinic acetylcholine receptors.[20] Thus this antioxidant activity is now seen to be selective. Although oxidation reactions are crucial for life, they can also be damaging hence organisms maintain a complex balance of antioxidants (glutathione, vitamin C, and vitamin E) as well as enzymes such as catalase, superoxide dismutase and various peroxidases.[21] Low levels of antioxidants, or inhibition of the antioxidant producing pathways may lead to oxidative stress. As oxidative stress may be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied particularly as treatments for stroke and neurodegenerative diseases.[22] Recent work[15b,c] has elegantly described the antioxidative properties of galanthamine and the resulting enhancement of its neuroprotective capability by lowering exposure to oxidative injury. Direct evidence for this was provided by an in vitro model study of H_2O_2-induced oxidative stress where galanthamine reduced the release of reactive oxygen species, prevented reduction in mitochondrial membrane potential and caused significant inhibition of H_2O_2-induced nitrite generation; features employed to ascertain oxidative injury that accompanies Alzheimer's disease.[15b,c] These striking antioxidative properties, in conjunction with the lack of CYP3A4 inhibition uncovered here for the first time, elevates the status of galanthamine **16** further as a privileged selective drug candidate in the treatment of Alzheimer's disease.

Finally, intense interest in the anticancer drug pancratistatin **25** has been maintained over the last two decades in view of the potent[7h] and selective[6] cytotoxicity displayed to certain tumor cells. More recently, this interest has turned to the related compound narciclasine **26** which shares a common pharmacophore with pancratistatin,[7h] but which is more abundant in nature and structurally simpler.[5] The present results demonstrating potent CYP3A4 activity for narciclasine and the lack of such activity for pancratistatin re-elevates the status of pancratistatin as a selective anticancer agent. This work draws attention to the presence of a C1–C10b double-bond and a lipophilic substituent at the C2 position as important contributing features for CYP3A4 inhibition.

Conclusion: This manuscript reports the screening of a diverse library of Amaryllidaceae alkaloids for cytochrome 3A4 inhibitory activity. Of the compounds examined, neither the crinane nor galanthamine groups exhibited any inhibitory activity. In the lycorane series, the natural compound narciclasine as well as two synthetic silylated lycorine analogues, accessed through chemoselective silylation, exhibited low micromolar activities. The presence of a C1–C10b double bond and lipophilic substitution at C2 are shown to be important factors in CYP3A4 activity and thus represent features to be avoided in the design of pharmacologically selective anticancer agents in the lycorane series. The lack of CYP3A4 inhibitory activity of the Alzheimer's drug galanthamine and the potential anticancer drug pancratistatin is viewed as bolstering their utility as chemotherapeutic agents. These results also draw attention to potential difficulties with the investigational anticancer agent narciclasine **26** in terms of the potent CYP3A4 inhibitory activity that is demonstrated herein.

Acknowledgements

We thank NSERC and McMaster University for financial support of this work. We are grateful to Dr. D. Hughes for obtaining high field NMR data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.086.

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