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# Structure–activity studies on acetylcholinesterase inhibition in the lycorine series of Amaryllidaceae alkaloids

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We dedicate this paper to the memory of our late friend and colleague Dr. Don Hughes

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ABSTRACT

The synthesis of differentially functionalized analogs of the Amaryllidaceae alkaloid lycorine, accessed via a concise chemoselective silylation strategy, is described uncovering two of the most potent inhibitors of acetylcholinesterase (AChE) identified to date in this series. Important elements of this novel pharmacophore were elucidated through structure–activity relationship (SAR) studies.



Alzheimer's disease (AD) is currently implicated in approximately 70% of the overall cases of dementia.[1] AD is a progressive neurodegenerative disorder, it is currently the sixth leading cause of death in the United States with the number of AD associated deaths increasing dramatically by 47.1% between 2000 and 2006.[1] Approximately 5.3 million Americans currently suffer from AD, with an estimated cost of $180 billion per annum on the health care system.[1b] Promising experimental approaches towards treatment of AD have been advanced recently involving the development of small molecules and antibodies to block the amyloid-β protein, as well as inhibition of the hyperphosphorylation and neurofibrillary tangle association of tau-protein. Experimental drugs such as rember (methylene blue), GSK2/3β inhibitors and others are at various stages of clinical development.[1b,2] Current clinical treatment of AD involves the use of reversible inhibitors of the enzyme acetylcholinesterase (AChE). In addition to the positive role of acetylcholine in neuronal transmission, AChE has been implicated in the production of amyloid fibrils, characteristic in the pathology of the brain cells of patients with AD.[3] A major focus in clinical treatment of AD therefore involves the use of reversible inhibitors of AChE including the approved drugs aricept (donepezil) **1**, rivastigmine **2** and galanthamine (reminyl) **4**, Scheme 1.

A significant number of naturally occurring compounds have been found to inhibit AChE.[4] The most significant of these are the Amaryllidaceae alkaloids galanthamine **4** and sanguinine **5**[5] as well as the Lycopodium alkaloids huperzine A (not shown) and B **3**.[6] The AChE inhibitor galanthamine hydrobromide (reminyl) is the first of the Amaryllidaceae alkaloids to be approved as a prescription drug in the treatment of AD. Although the related alkaloid sanguinine is a more potent inhibitor of AChE,[7,8] its low natural abundance has precluded its further development.

Recently, structure–activity relationships (SAR) studies was undertaken by Elgorashi et al. on AChE inhibition amongst a structurally diverse library of Amaryllidaceae alkaloids assembled from plant species endemic to the southern African region.[9a,b] The collection of natural alkaloids used in this work,[9a,b] and natural and synthetic analogs described herein are collected in Scheme 2, **6** to **34**. In addition to the confirmation of the AChE inhibitory activities of galanthamine and sanguinine, this work also revealed the naturally occurring alkaloid 1-acetyllycorine **28** to be a potent AChE inhibitor. These researchers also showed that the parent alkaloid lycorine **25** as well as 1,2-diacetyllycorine **29** and 2-acetyllycorine exhibited significantly diminished AChE inhibitory activities and ascribed the selectivity of **28** to its ability to function as a hydrogen-bond acceptor, analogous to the hydroxyl group of galanthamine. The lower AChE inhibitory activity of 1,2-diacetyllycorine **29** is difficult to explain given the narrow structural

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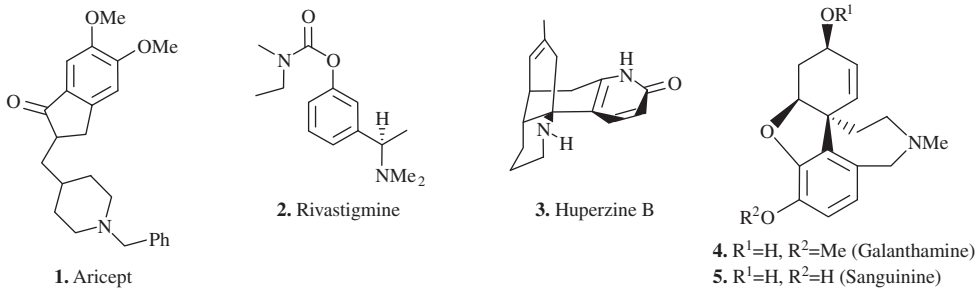
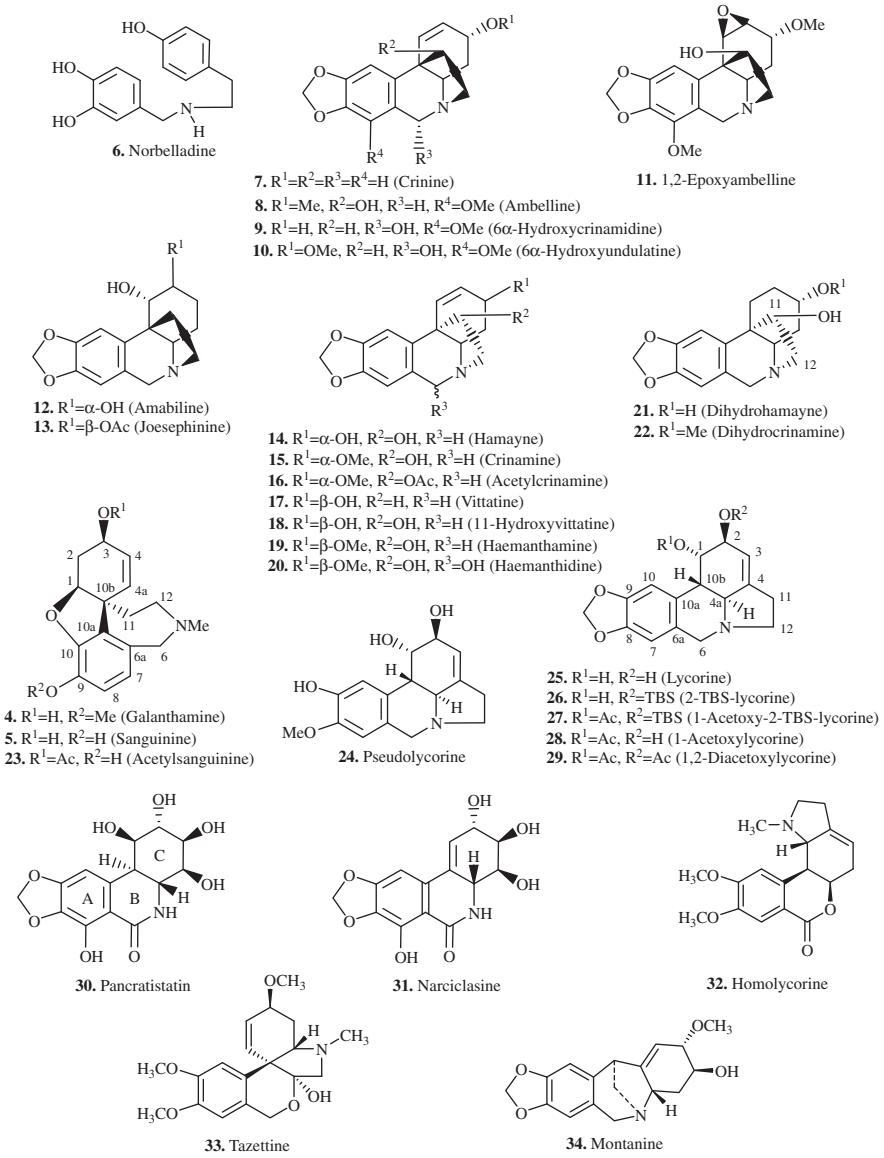



**Scheme 1.** Reversible inhibitors of AChE including the approved drugs aricept, rivastigmine and galanthamine as well as the Lycopodium alkaloid huperzine B.




**Scheme 2.** Structurally-diverse library of natural and semisynthetic alkaloids of the Amaryllidaceae screened for AChE inhibitory activity in the present study.

diversity screened within this particular sub-set of lycorine derivatives.

We have recently documented the selective anticancer activity of lycorine derivatives[10] as well as their interactions with human cytochrome P450 3A4.[11] In this work, a series of 1,2-differentially substituted lycorine derivatives were prepared and first screened for anticancer activity. While lycorine **25** (and pseudolycorine **24**) was found to be cytotoxic, substitution of either or both of the hydroxyl groups at C1 and C2 diminished this activity significantly.[10] In terms of CYP3A4 inhibitory activity, lycorine **25** was found to be inactive.[11] Substitution at C2 with the bulky, lipophilic *tert*-butyldimethylsilyl (TBS) substituent **26** introduced potent inhibition of CYP3A4 which was maintained when a *C*1-acetyl substituent was introduced **27**, while removal of the C2 substituent to

give 1-acetyllycorine **28** resulted in a 20-fold reduction in inhibitory activity.[11] Thus a small, polar H-bond acceptor at C1 of lycorine in conjunction with a lipophilic substituent at C2 are important pharmacophoric elements for the CYP3A4 inhibition.[11] The relatively high natural abundance of the alkaloid lycorine is a strong incentive to consider the development of an analog as a potentially selective AChE inhibitory lead. Cytotoxicity and inhibition of the critical enzyme CYP3A4 are two important factors that require consideration to the advancement of a selective AChE inhibitor. In this Letter, we report the preparation of several novel structural analogs of lycorine, their AChE inhibitory activity and the important finding that introduction of lipophilic substituents at C1 and C2 results in potent AChE inhibitory activity. This data, in conjunction with the lower cytotoxicity[11] and selective CYP3A4 interaction of C1/C2 substituted lycorines indicate lycorines **27** and **28** as the most potent and selective AChE inhibitors identified so far in the lycorine series.

The potency of C1 acetyl-functionalized lycorine compounds as inhibitors of AChE[9] as well as their noted lower cytotoxicity[11] prompted us to consider the semi-synthesis of further differentially substituted C1 and C2 analogs from the readily available alkaloid lycorine **25** in order to further probe the AChE pharmacophore.

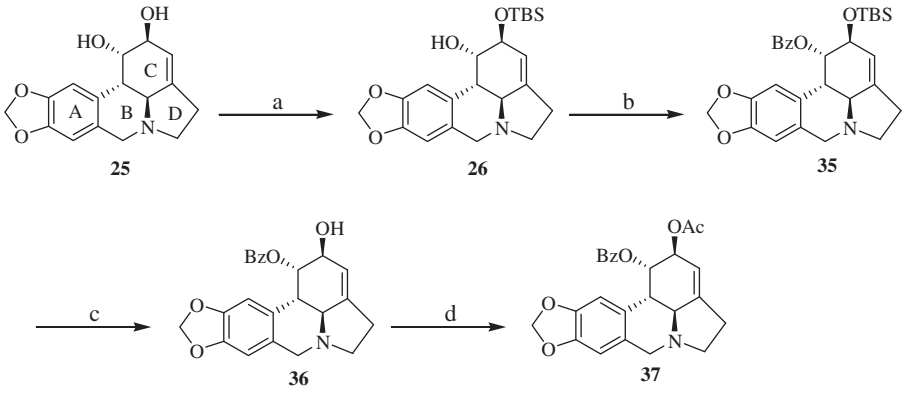
Previous studies in this regard have highlighted the difficulties attending the differential functionalization of the hydroxyl groups present within the lycorine series.[12] Work in our group has shown that highly selective mono-silylation of 1,2-diols can be achieved under certain conditions.[13] Our approach towards the selective functionalization of the 1,2-dihydroxy groups in lycorine **25** utilizes the greater reactivity of the allylic pseudoequatorial 2-hydroxy group over the axially-orientated homoallylic C1 hydroxyl, in addition to the bulkiness of the incoming *tert*-butyldimethylsilyl chloride (TBSCl) electrophile. The reaction of lycorine with TBSCl in pyridine (Scheme 3) afforded the novel monosilyl ether **26** in 56% isolated yield (81% based on recovery of lycorine). Interestingly, no bis-silylated product was detected during the course of the reaction.

Direct chemoselective access to the *tert*-butyldimethylsilyl ether **26** allowed us to introduce a benzoate group at C1 as in **35**, obtained in near quantitative yield from **26** upon benzoylation with benzoyl bromide and pyridine, and its $^1$H NMR (see Supplementary data section) spectrum showed the expected deshielding effect on H1 ($\delta$ 5.82, dd, $J$ = 3.5, 1.4 Hz). Desilylation of **35** was straightforward with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) affording 1-benzoyllycorine **36**, the proton spectrum of which indicated a slight upfield shift for H2 ($\delta$ 4.35, dd, $J$ = 3.5, 2.1 Hz) compared to **35**. Acetylation of **36** utilizing acetic anhydride and pyridine then provided in 98% isolated yield 1-benzoyl-2-acetyllycorine **37** which had the H2 resonance at $\delta$ 5.40 (dd, $J$ = 3.5, 2.1 Hz).

The entire library of compounds **4**, **5** and **7**–**37** (Schemes 2 and 3), constituted of both natural and semi-synthetic representatives of seven structural groups within the Amaryllidaceae, was then screened for AChE inhibition utilizing the standard Ellman assay.[14] It is immediately apparent that only representatives of the galanthamine and lycorine groups exhibited significant AChE inhibition (Table 1). The inhibitory activity of galanthamine **4** ($K_i$ = 0.30 μM) is striking, as noted by others.[7–9] X-ray crystallography of AChE isolated from *Torpedo californica* (*Tc*),[15] showed that the active site accommodates a catalytic triad (Ser200, His440, Glu327) located at the bottom of a deep and narrow pocket lined with aromatic residues and a subsite including Trp84 located near the bottom of the cavity. Trp84 has been identified as the binding site for acetylcholine. The structure of the complex of (−)-galanthamine and *Tc*AChE showed that galanthamine binds at the base of the active site gorge, interacting with both the acyl-binding pocket and the indole ring of Trp84.[16] While the tertiary amine group of galanthamine did not interact with Trp84, a π–π interaction was observed for the double bond of the cyclohexene ring with Trp84.[16]

Based on these remarkable findings, the search and development of synthetically-derived analogs of the drug with improved activities has gained increasing impetus.[17] Interestingly, it is noteworthy that the activity of natural products within this group, such as galanthamine, sanguinine and 11-hydroxygalanthamine are generally more potent than those of synthetic origin in terms of AChE inhibitory ability.[7–9,17–19]

As mentioned earlier, SAR[9a] followed by quantitative-structure–activity relationship (QSAR)[9b] studies by Elgorashi et al. first revealed 1-acetyllycorine **28** as a potent inhibitor of AChE while lycorine, 2-acetyllycorine, and 1,2-diacetyllycorine **29** in the same study exhibited no inhibitory activity. The AChE activity of 1-acetyllycorine **28** ($K_i$ = 0.43 μM) (Table 1), here accessed via the chemoselective silylation strategy (Scheme 3)[11] is comparable to that of galanthamine **4** ($K_i$ = 0.30 μM) and we confirm that both lycorine **25** and 1,2-diacetylycorine **29** are devoid of inhibition (Table 1). Nonetheless, silylation at C2 (Scheme 3) afforded the novel derivative **26**, which to our delight inhibited AChE at 0.86 μM, contrasting sharply with the inactive C2 acetate. With silyl ether **26** as a new lead, a mini-panel of C1 and C2 functionalized lycorine analogs was then assembled (Scheme 3) to probe this new discovery on the pharmacophore. Conversion of **26** to the C1 acetate and benzoate (both novel compounds) produced a ~2.5-fold increase in activity as seen for **27** ($K_i$ = 0.34 μM) and **35** ($K_i$ = 0.39 μM). Desilylation of the TBS group to yield the respective 1-acyl functionalities




**Scheme 3.** Chemoselective silylative discrimination of the 1,2-dihydroxy groups in lycorine. Reagents and conditions: (a) TBSCl, pyridine, rt, 12 h, 56%; (b) BzCl, py, DCM, rt, 3 h, 96%; (c) TBAF, THF, rt, 1 h, 95%; (d) $Ac_2O$, py, DCM, rt, 3 h, 98%.

**Table 1**
Inhibitory activity against the biotransformation of acetylthiocholine by eel acetylcholinesterase (AChE)

| Compound | $K_i$[a] (μM) |
|---|---|
| Galanthamine **4** | 0.30 (±0.06) |
| Crinine **7** | na |
| Crinamine **15** | na |
| Pseudolycorine **24** | na |
| Lycorine **25** | na |
| **26** | 0.86 (±0.03) |
| **27** | 0.34 (±0.08) |
| **28** | 0.43 (±0.02) |
| **29** | na |
| Homolycorine **32** | na |
| Tazettine **33** | na |
| Montanine **32** | na |
| **35** | 0.39 (±0.03) |
| **36** | 0.54 (±0.03) |
| **37** | 0.97 (±0.10) |

[a] Values are means of three experiments, standard error is given in parentheses (na = not active at 10 μM).

effected a slight decrease in activity as evident for acetate **28** ($K_i$ = 0.43 μM) and benzoate **36** ($K_i$ = 0.54 μM). Acylation then afforded diacetate **29**, which was inactive, and 1-benzoate-2-acetate **37** ($K_i$ = 0.97 μM), respectively. This AChE inhibitory pattern of activity within the lycorine series of compounds (Table 1) highlights several core features of this fascinating pharmacophore. An intact pyrrolo-phenanthridine nucleus appears to be necessary for high potency. Recent work identified semi-synthetic N-alkylated ring D *seco*-lycorine analogs, possessing a planar dihydrophenanthridine nucleus, as inhibitors of AChE, however the most potent of these exhibited an $IC_{50}$ value of 2.66 μM.[18] This is in stark contrast to the natural phenanthridine variants pancratistatin **30** and narciclasine **31**, the most potent anticancer agents within the Amaryllidaceae,[20] shown here to have no observable effect on AChE. Aromatization of ring C is seen to potentiate AChE inhibition, compared to the partially saturated lycorine **25** precursor, which was demonstrated for natural, aromatized ring C lycorine variants such as assoanine ($IC_{50}$ 3.87 μM).[8] This enhanced activity was rationalized in terms of the planarity generated through aromatization of ring C. Modification of ring A is seen to have a negligible effect as both pseudolycorine **24** and lycorine **25** are inactive (at 10 μM) (Table 1). The dramatic spike in activity in going from lycorine to analog **26** ($K_i$ = 0.86 μM) suggests that the C2 area of the molecule must occupy a bulky, lipophilic pocket within the active site to accommodate the TBS group. This substitution contrasts sharply with the inactive 2-acetyllycorine,[9] suggesting that a small, polar H-bond acceptor at C2 may impede inhibition. Acylation at C1 had a pronounced effect on inhibition (Table 1), affording compounds **27** ($K_i$ = 0.34 μM) and **35** ($K_i$ = 0.39 μM), which are now advanced as the most potent inhibitors of AChE identified to date within the lycorine series. Superposition of minimum-energy conformations of galanthamine **4** and 1-acetyllycorine **28** indicated that the acetate and nitrogen atom of the later superimpose on the hydroxyl group and the nitrogen atom of galanthamine, respectively.[9b] Therefore, it is possible that the 1-acetyl group may be involved in hydrogen bonding in a manner similar to that elegantly demonstrated for the hydroxyl of galanthamine complexed within *Tc*AChE.[16] Benzoate **35** may similarly also be involved in hydrogen bonding via the carbonyl, in addition to π–π interactions. Superposition models also showed partial overlap for the methoxyl group of galanthamine and the methylenedioxy of the lycorine pharmacophore.[9b] The methoxyl group of galanthamine is known to occupy the acetyl-binding pocket in acetylcholine.[16] The drop in activity (~1.3-fold) observed upon desilylation to compounds **28** and **36**, respectively, endorses the silylative lipophilic effect observed for **26**, **27** and **35**. Acylation at C-2 had a pronounced effect on inhibition since diacetate **29** was void of activity, and **37** ($K_i$ = 0.97 μM) was almost half as potent as the hydroxy benzoate **36**. These results also vindicate the requirement for a lipophilic interaction in the C2 area of the molecule.

In conclusion, a more comprehensive view has emerged from this SAR study of the lycorine pharmacophore as a potent inhibitor of AChE. The screen of a library of Amaryllidaceae alkaloids comprising seven different structural-types here showed the galanthamine and lycorine groups, as noted by others,[7–9] to be superior at AChE inhibition. A chemoselective silylation strategy was employed to install the TBS group at C2, providing access to new C1 and C2 functionalized analogs in the lycorine subset. A pronounced spike in activity is observed on proceeding from the inactive parent lycorine **24** to the 2-TBS analog **26** ($K_i$ = 0.86 μM), which is rationalized in terms of lipophilic binding at the enzyme active site. Acylation (acetylation or benzoylation) of **26** then afforded **27** ($K_i$ = 0.34 μM) and **35** ($K_i$ = 0.39 μM) respectively, which are here uncovered as the most potent inhibitors of AChE to date within the lycorine series. The lipophilic effect of the C2 silyl group again becomes apparent upon desilylation to the alcohols **28** ($K_i$ = 0.43 μM) and **36** ($K_i$ = 0.54 μM) and subsequent acetylation to compounds **29** and **37**, seen to be the least efficacious of the series. While the similarities between the galanthamine and lycorine pharmacophores as discussed above are striking, it is speculated here and elsewhere[9b] that they may have similar interactions at the enzyme active site. More tangible evidence for this is required, such as has been afforded by X-ray studies of galanthamine complexation within *Tc*AChE.[16] The effect of lipophilic substitution at C2 and polar H-bond acceptor groups at C1 is additive in that the most potent analogs (**27** and **35**) have both of these functionalities. These spectacular findings should be of value in the design of other novel derivatives and provide direction in the development of a selective AChE clinical candidate from the lycorine series. Further refinement of the lycorine AChE inhibitory pharmacophore, in conjunction with cytotoxicity studies and mapping the CYP3A4 interactions of these new compounds, is currently in progress in our laboratories.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.130.

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