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Pyridine alkaloids with activity in the central nervous system

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ABSTRACT

This review discusses all pyridine alkaloids with CNS activity, their therapeutic potential, and the interesting array of sources whence they originate.

1. Introduction

Central Nervous System (CNS) disease and disorders encompass a vast range of pathologies that includes neurodegenerative disease (e.g. Alzheimer’s and Parkinson’s), psychiatric conditions (e.g. anxiety, depression and psychosis), epilepsy, multiple sclerosis, neuropathic pain, autism and many more. 1–9 The disease burden from CNS disorders is enormous. Studies have revealed that neurological disorders were the leading cause of disability-adjusted life years (DALYs; ~280 million) and the second leading cause of deaths (~9.0 million) globally. 10 The absolute number of deaths and DALYs from all CNS-related diseases between 1990 and 2016 have increased by 39% and 15%, respectively. 10 Dementia is one of the largest contributors to neurological DALYs (~10.4%), with at least 50 million people believed to be living with a form of dementia. 10 In 2017, it was estimated that ~792 million people worldwide lived with a form of mental and/or behavioural illness. 11–13 As the global population surges, the prevalence of CNS-related disease will inevitably increase and as a result, there is a pressing need to develop more effective treatment strategies. 10–13

Modern medicine has famously relied on natural product-based therapeutics to treat CNS disorders due to the intimate relationship between natural products and the human brain. A recent report estimates that ~84% of approved drugs for the treatment of CNS diseases are natural products or natural product inspired, and 400 clinically approved CNS drugs can be traced back to 20 natural product scaffolds. 14 Alkaloids are particularly well represented in this list; famous examples used clinically include morphine, atropine, physostigmine, papaverine and galantamine.

Pyridines are privileged scaffolds in medicinal chemistry 15 and the nitrogen atom in pyridine plays a crucial role in the pharmacological profile of many drugs that contain this heterocycle. 16 In this account, all pyridine alkaloids that are active in the CNS are detailed, including the array of terrestrial and marine sources from whence they originate, their bioactivity and in some cases, their use as clinically approved therapies. All pyridines, pyridones and pyridiniums are presented, but their benzofused (e.g. quinolines) and saturated variants (e.g. piperidines) are not covered herein. Pyridine alkaloids with CNS activity have been isolated from plants, fungi, bacteria, amphibian and marine sources, and some are present in a wide variety of life forms. This review has been structured along these lines accordingly.

2. Plant-derived

2.1. Nicotine

Tobacco is the dried leaves of Nicotiana tabacum, a plant belonging to the Solanaceae (nightshade) family. 17–20 The use of N. tabacum by indigenous American Indians dates back ~8000 years, where the plant was smoked in pipe ceremonies for therapeutic and ritualistic purposes. 20 The use of Nicotiana plants was revealed to English explorers in 1565 and they began growing the plants commercially in 1612 in what is now Virginia (USA). 20 Tobacco consumption was first introduced to Europe in the late 16th century for both recreational and medical use, including the treatment of fatigue, abscesses, external wounds, nasal blockages and syphilis. 17–20 In 1828, the major component responsible for the psychopharmacological response to tobacco use, nicotine

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activation of nAChRs located pre-synaptically on the glutamatergic
nergic neurons and thus elevates dopamine levels. The nicotine-NMDA receptor interaction is indeed important for the
administered, the effects of nicotine were attenuated, inferring that
dizocilpine (a non-competitive NMDA receptor antagonist) was co-
with nicotine use.

The effects of nicotine include heightened arousal, reduced stress and anxiety, energy
ingress and enhanced pain thresholds. Nicotine has also been
to improve learning, problem-solving ability, reaction time, se-
ctive attention and vigilance in those performing repetitive tasks. These positive reinforcing effects induced from acute nicotine admin-
istration are critical factors in tobacco addiction. Nicotine binds to
nicotinic acetylcholine receptors (nAChRs), a group of cationic ligand-
gated ion channels found in both the peripheral nervous system (PNS)
and CNS. There are three nAChR subtypes in the mammalian brain
(α4β2, α3β4- and α7-nAChRs); the most predominant subtype in
humans, α4β2-nAChR, is where nicotine displays the highest binding
affinity (K_i < 1 nM), and is a full agonist at this site. The binding
affinity for nicotine at the α3β4-nAChR (K_i = 530 nM) and the α7-nAChR
(K_i = 6290 nM) are much weaker than at the α4β2-nAChR. Nicotine
binds as a full agonist, opening the ion channels and stimulating cation
influx (e.g. sodium, potassium and calcium) to induce the release of
multiple neurotransmitters including dopamine, serotonin (5-HT),
norepinephrine, acetylcholine (ACH), γ-aminobutyric acid (GABA),
β-endorphins and glutamate into the mesolimbic area, the corpus
striatum and the frontal cortex. In particular, the release of dopa-
mine from the dopaminergic presynaptic terminals in a concentration dependent
manner. Both mecamylamine and dihydro-β-erythroidine (nonselective
nAChR antagonists) inhibited the [3H]-overflow effect of nicotine
(<100 µM) on rat striatal slices with preloaded [3H]-dopamine. However, the inhibitory activities of both nAChR antagonists were not observed when the nicotine concentration was increased (>100 µM), suggesting a nAChR-mediated mechanism was involved. Effect of (−)-nicotine on dopamine release was greater than the (+)-enantiomer at concentrations 1, 10 and 100 µM, indicating the mediated nAChR subtype is more sensitive to the (−)-enantiomer. Subsequently, Papke and co-workers demonstrated that nicotine is more potent at the rat α7-nAChR (EC50 = 17.4 µmol/L) than at the α4β2-nAChR (EC50 = 375 µmol/L) and α3β4-nAChR (EC50 = 614 µmol/L) subtype receptors. These findings imply that nicotine contributes to the neuropharmacological
effects of tobacco smoking via a nicotinic receptor stimulation.

2.2. Nornicotine

Nornicotine (Fig. 2) is a minor peripheral metabolite of nicotine in
various mammal species (e.g. humans, monkeys and rodents). This pyridine alkaloid possesses a demethylated pyrrolidine ring and was extracted from N. glutinosa by Ehrenstein in 1931. Subsequent phytochemical studies revealed that nornicotine is also present in many
Nicotiana species and is one of the three most abundant minor alkaloids
produced in N. tabacum, alongside anabasine (Fig. 3) and anatabine
(Fig. 4). Another of the three abundant minor alkaloids found in
Anabasis aphylla (Nonselec-
tive nAChR antagonists) inhibited the [3H]-overflow effect of nicotine
(<100 µM) on rat striatal slices with preloaded [3H]-dopamine.

Nicotine displays significant agonist properties at nAChR sub-
types in the CNS. However, co-workers have demonstrated that nornicotine evokes the release of dopamine by stimulating nAChRs from the dopaminergic presynaptic terminals in a concentration dependent
manner. Both mecamylamine and dihydro-β-erythroidine (nonselective
nAChR antagonists) inhibited the [3H]-overflow effect of nicotine
(<100 µM) on rat striatal slices with preloaded [3H]-dopamine. However, the inhibitory activities of both nAChR antagonists were not observed when the nicotine concentration was increased (>100 µM), suggesting a nAChR-mediated mechanism was involved. Effect of (−)-nicotine on dopamine release was greater than the (+)-enantiomer at concentrations 1, 10 and 100 µM, indicating the mediated nAChR subtype is more sensitive to the (−)-enantiomer. Subsequently, Papke and co-workers demonstrated that nicotine is more potent at the rat α7-nAChR (EC50 = 17.4 µmol/L) than at the α4β2-nAChR (EC50 = 375 µmol/L) and α3β4-nAChR (EC50 = 614 µmol/L) subtype receptors. These findings imply that nicotine contributes to the neuropharmacological
effects of tobacco smoking via a nicotinic receptor stimulation.

2.3. Anabasine

Another of the three abundant minor alkaloids found in Nicotiana
plants, anabasine (Fig. 3), was first isolated from the toxic Asian plant
Anabasis aphylla by Orechoff and Menschikoff in 1931, its
crystal structure was established by Smith in the same year through
total synthesis. Anabasine, a pyridine-piperidine alkaloid structurally
related to nicotine, occurs as a racemic mixture in Nicotiana plants and is
the predominant alkaloid in N. glauca (also known as Tree Tobacco).
leaves; small traces of anabasine have also been detected in hop-lonemertines, Messor and Aphaenogaster ants. Stereochemical integrity is an important pharmacological factor in determining specific biological activities of chiral natural products, and enantiomers often exhibit significant differences in their biological properties. The binding affinities (Ki) and the agonist potencies (EC50) of the two anabasine enantiomers at rat α4β2- and α7-nAChRs were examined. Using nicotine (Ki = 0.0056 μM; EC50 = 19 μM) as a comparison, in vitro experiments have demonstrated that (−)-anabasine is a more potent agonist at the α7-nAChR (Ki = 0.39 μM; EC50 = 18 μM) than at the α4β2-receptor (Ki = 1.1 μM; EC50 > 30 μM); however, (−)-anabasine binds more selectively at the α4β2-nAChR (Ki = 0.91 μM) than at the α7-receptor (Ki = 3.7 μM). Subsequent toxicity analysis showed that (−)-anabasine (LD50 = 11 mg/kg) is more toxic than (−)-anabasine (LD50 = 16 mg/kg), implying that the stereocherny affects both pharmacological activities and lethality.

### 2.4. Anatabine

Anatabine (Fig. 4) is the second most abundant alkaloid (~4%) present in Nicotiana species. This alkaloid was first isolated from the leaves of N. tabacum by Spath and Kesztler in 1937, who also established the chemical structure through total synthesis. Anatabine is a pyridine-dihydropyridine structurally related to anabasine. The binding affinities (Ki) of (−)- and (+)-anatabine were evaluated at rat α4β2-nAChR by displacement of [3H]-cytisine radioligand binding using anabasine and nicotine as comparisons. Kem and co-workers demonstrated that (−)-anatabine (Ki = 119 nM) exhibited a higher binding affinity than (−)-anabasine (Ki = 249 nM), (~−8- and 4-fold more potent than anabasine (Ki = 910 nM) respectively; the nicotine Ki value was found to be ~2 nM. In the same study, the agonist potency (EC50) and ACh stimulation efficacies (I_max) of both anatabine enantiomers at human α4β2- and α7-nAChRs were also examined. At the α4β2-subtype receptor, (−)-anatabine (EC50 = 2.65 μM; I_max = 43.2%) was ~2-fold more efficacious than (−)-anabasine (EC50 = 0.74 μM, I_max = 25.0%); much higher efficacies were observed at the α7-receptors for both (−)-anabasine (EC50 = 69.7 μM; I_max = 113%) and (−)-anatabine (EC50 = 51.8 μM; I_max = 105%). These findings suggest that anatabine is a selective and potent ligand at α7-nAChRs. Recent publications have reported that anatabine also lowers the production of β-amyloid in human brain cells and enhances memory and attention dysfunction in rats, suggesting that anatabine may represent a potential therapeutic candidate for dementia disorders such as Alzheimer’s disease (AD).

### 2.5. N-Methylanatabine

An N-methylated anatabine isomer, N-methylanatabine (Fig. 5), was isolated from the leaves of N. tabacum by Spath and Kesztler in 1937; its chemical structure was affirmed through total synthesis in the same study. Although structurally similar to nicotine and anabasine, studies on the biological activities of N-methylanatabine are scarce in the current literature database. The interactions of N-methylanatabine and monoamine oxidase (MAO) A and B were evaluated by Castagnoli and co-workers; the group established that N-methylanatabine did not produce any significant changes of MAO-A and B activities in rat brain upon administration (data not shown). In 2020, McHugh and co-workers re-examined the therapeutic potential of N-methylanatabine through an electrophysiological characterization test using Xenopus oocytes expressing the human α4β2-nAChRs. The agonist potency (EC50) and the maximal receptor response (I_max) of N-methylanatabine (EC50 = 6.2 μM; I_max = 26%) at the human α4β2-receptor were ~8- and 6-fold less active than nicotine (EC50 = 0.8 μM; I_max = 159%) respectively.

### 2.6. Cotinine

Cotinine (Fig. 6), often used as a biomarker for tobacco exposure, is the major peripheral metabolite of nicotine with a half-life of 19–24 h in many animal species (including humans). Between 70% and 80% of the nicotine consumed by humans is oxidized to cotinine by cytochrome P450 2A6 (CYP2A6) and cytoplasmic aldehyde oxidase. The chemical structure of cotinine was initially proposed by Pinner and later confirmed by Frankenburg and Vaitkeunas in 1957 through oxidative degradation. Subsequent pharmacological investigations revealed that cotinine is also present in Nicotiana plants (e.g. N. tabacum) and Duboisia homewoodii. Several reviews and studies on the pharmacological properties of cotinine are available.

Cotinine crosses the blood brain barrier and acts as a low affinity nAChR agonist at brain receptors. Vainio and co-workers demonstrated that cotinine displays weak binding affinity (Ki) and agonist potency (EC50) at rat brain nAChRs labelled with [3H]-epibatidine when compared with nicotine. Cotinine (Ki = 3.0 μM; EC50 = 21 μM) was ~270-fold less active than nicotine (Ki = 11 nM; EC50 = 77 nM) at competing for nAChRs binding sites with [3H]-epibatidine (250 μM) from rat frontal cortex; a similar pattern was observed in rat hippocampus cells (data not shown). These findings indicated that cotinine exhibits weak binding potency to nAChRs in the CNS and mediates its various pharmacological effects upon voluntary nicotine administration.

In a subsequent study, the specific receptor subtype that cotinine primarily acts on was examined by Terry Jr and co-workers. More than seventy neurotransmitter receptors, transporters and enzymes (including dopamine D1-4, adrenergic α1-2, GABA A,B, glutamate, histamine H1-3, muscarinic acetylcholine receptor M1-5, opioid, acetylcholine1-2, Ca/K/Na channels, nitric oxide, Bradykinin, neurokinin and acetylcholinesterase) were screened; it was found that cotinine was relatively inactive (~50% inhibition at 10 μM) across a wide range of pharmacological targets. However, cotinine (1 μM) significantly enhanced the responses evoked by low concentrations of ACh (~40 μM) in Xenopus oocytes expressing the human α7-nAChRs, inferring an interaction of cotinine with the α7-receptor. Subsequent behavioural studies also showed that cotinine (1.0–10 mg/kg) increased the
expansion time in rats when it was co-administered (intraperitoneal injection) with donepezil (0.5 mg/kg). Although completely inactive if given alone, cotinine could be considered an adjunctive therapeutic agent to improve the effective dose of cholinergic medications (e.g. donepezil) commonly used for AD and other memory disorders. Echeverria and co-workers demonstrated that cotinine (5 mg/kg) enhances extinction of a contextual fear memory upon acute administration in rats by at least 20%, suggesting the potential therapeutic value for memory improvement and post-traumatic stress disorder (PTSD). Working memory performance and depressive behaviours were improved when cotinine (0.03–10 mg/kg) was administered in normal and MK801- (an NMDA receptor antagonist used to mimic psychotic symptoms) impaired animal models (e.g. rats and monkeys), thus implicating cotinine as a potential therapeutic agent for attention deficit hyperactivity disorder (ADHD) and neuropsychiatric disorders (e.g. anxiety, depression and psychosis). It was also shown that cotinine is efficacious in treating dementia-related memory impairments; cotinine (0.1 µM) reduces β-amyloid (Aβ) neurotoxicity in primary cortical neurons and prevents working memory loss by decreasing the Aβ aggregation and plaque deposition in memory impaired rats. These findings indicate that the neuroprotectivity and the absence of toxicity exhibited by cotinine is due to its agonist property at the α7-nAChRs. Cotinine (1 µM–3 mM) has also been shown to evoke the release of dopamine by stimulating the α7-nAChRs in a calcium-dependent manner in rat striatum; the levels of serotonin and noradrenaline in rat brains increased when cotinine (2 mg/kg) was given in repeated doses, suggesting potential use as an antidepressant agent through activation of α7-nAChRs. Taken together, cotinine displays a safer therapeutic profile than nicotine due to its much longer half-life and a lower risk of abuse.

2.7. DINIC

In a phytochemical study attempting to discover new minor tobacco alkaloids in Nicotiana plants, a nicotine derivative consisting two 1-methyl-2-pyrrolidinyl moieties attached to a central pyridine scaffold was isolated from dried N. tabacum roots by Crooks and co-workers; structural elucidation was affirmed by total synthesis and this 3,5-dinitocotine alkaloid was consequently named DINIC (Fig. 7) due to the presence of the two N-methylpyrroolidine rings. The pharmacological activities of DINIC was investigated by evaluating its ability to displace the binding of [3H]nicotine and [3H]-methyllycaconitine at rat α4β2- and α7-nAChRs respectively. DINIC displayed potent binding affinity (Ki = 1.18 µM) and inhibited the effect of [3H]nicotine at the α4β2- and α7-nAChRs in the [3H]-dopamine release assay (<64% inhibition at 100 nM); no inhibition on [3H]-methyllycaconitine binding at the α7-nAChR was observed, indicating that DINIC is selective for the α4β2-receptor.

2.8. Metanicotine

Metanicotine (Fig. 8), also known as Rivacantine, TC-2403 and RJR-2403, is a nicotine alkaloid examined as a potential therapeutic candidate for the treatment of neurodegenerative disorders, including AD. The chemical structure of metanicotine was first assigned by Pinner in 1895 through the degradation of nicotine. In 1953, Wahl reported the natural occurrence of metanicotine as the biological degradation of nicotine upon its isolation from fermented tobacco. Subsequent phytochemical studies revealed that metanicotine is also present in Solanaceae plants (e.g. N. tabacum and D. hopwoodii) and tobacco smoke.

The pharmacological properties of metanicotine in the CNS were characterized by Bencherif and co-workers in 1996. The in vitro receptor binding studies using [3H]-nicotine radioligand displacement showed a high binding affinity exhibited by metanicotine at the α4β2-nAChRs in rat brains (Ki = 26 nM); at other receptor sites (i.e. angiotensin II, cholecystokinin A3, endothelin ET A, muscarinic acetylcholine receptor M1, histamine H3, bradykinin B2, leukotriene B4, neurokinin NK1, phenylcyclidine, neuropeptide Y2, thromboxane A2, dopamine D3-receptor, NMDA, 5-HT1A, and sodium channel 2), metanicotine was a poor competitive inhibitor and failed to displace [3H]-nicotine ligand (IC50 > 10 µM). The potency (EC50) and efficacy (Emax) of metanicotine in evoking [3H]-[3H]-nicotine release from rat thalamic synaptosomes (EC50 = 732 nM; Emax = 79%) was slightly less active than nicotine (EC50 = 591 nM; Emax = 87%) when compared with the full agonist tetramethylammonium at 300 µM. The ability of metanicotine to induce dopamine release from rat striatal synaptosomes (EC50 = 1.2 µM; Emax = 81%) is equally efficacious and potent as ABT-418 (EC50 = 1.1 µM; Emax = 91%), a neuroprotective anxiolytic agent used in the treatment of AD and ADHD; but ~10-fold less potent than nicotine (EC50 = 100 nM; Emax = 113%).

In the subsequent in vivo study, metanicotine (3.6 µmol/kg; subcutaneous administration) significantly increased the levels of ACh, dopamine, norepinephrine and serotonin in rat cortex by 190%, 150%, 150% and 170% respectively. Acute toxicity studies indicated that metanicotine (0.6 µmol/kg; subcutaneous injection) significantly reversed the amnesic effects induced by scopolamine (0.5 µmol/kg; subcutaneous injection) in rats by ~50%; oral administration of metanicotine (0.3–3.0 µmol/kg) decreased the memecylamine-induced amnesia by 40–50%; both long- (reference) and short-term (working) memory of rats whose forebrain cholinergic projection system impaired by ibotenic acid (10 mg/mL) were improved upon metanicotine administration (0.36, 0.72 and 1.4 µmol/kg) by ~2- to 8-fold. Acute toxicity studies indicated that metanicotine is much less toxic than nicotine after single or repeated doses in rats and dogs (LD50 = 1.8 mg/kg). These preclinical studies suggested that metanicotine is a potent and selective α4β2-nAChR agonist; its safer physiological and more desired behavioural profiles prompted further investigation.

Metanicotine (as RJR-2403) was originally developed as an orally available medication for the treatment of neurodegenerative disorders (e.g. AD) by RJ Reynolds Tobacco Co.
2.11. Cytisine and its derivatives

2.10. Anabasamine

An alkaloid named anabasamine (Fig. 10), possessing a 2,3’-bipyrindyl scaffold bonded to a piperidine ring, was isolated from the seeds of *Anabasis aphylla* (Central Asian shrub) by Mukhamedzhanov and co-workers in 1967. In a subsequent phytochemical study investigating the inhibition of cholinesterases, anabasamine was found to exhibit weak but selective anti-acetylcholinesterase properties. The binding affinity ($K_i$) of anabasamine at human erythrocyte acetylcholinesterase was ~8.6-fold more effective than at horse blood serum butryrylcholinesterase (51 µM vs 440 µM).

2.11. Cytisine and its derivatives

Plants belonging to the *Leguminosae* family, such as *Cytisus, Laburnum* and *Sophora*, have been used in traditional medicine for hundreds of years. American Indians are known to have consumed the seeds of *L. anagyroides* (also known as *Cytisus laburnum*) for their purgative and emetic effects during rituals; traditional European medicine used alcoholic extracts of *Cytisus* plants for constipation, migraine and insomnia; the leaves of *L. anagyroides* were used as tobacco substitute during World War II. In several phytochemical studies examining the biological active secondary metabolite of *L. anagyroides*, the aqueous extract of the seeds was found to contain cytisine (Fig. 11), a quinolizidine alkaloid fused to a bispidine ring with absolute configuration later assigned as 1R,5S through stereoselective total synthesis. Subsequent isolation studies have demonstrated that cytisine is present in multiple genera of the *Leguminosae* family, and is most abundant in the seeds of these plants (between 59% and 80%). Pure cytisine has been used as a respiratory analeptic, diuretic and an insecticide in Europe. Several detailed reviews on the biological properties of cytisine and its therapeutic applications are available.

The binding affinity ($K_i$) and the maximum number of binding sites ($B_{max}$) of cytisine at nAChRs have been examined in whole rat brains. Cytisine has a significantly higher binding affinity ($K_i = 0.145$ nM) when compared with nicotine ($K_i = 0.89$ nM); with a similar binding density between the two compounds ($B_{max} = 99.1$ and 114.5 fmol/mg, respectively). Cytisine displays a million-fold binding specificity for nAChRs ($K_i = 0.16$ nM) over muscarinic acetylcholine receptors ($K_i > 400$ µM). Cytisine binds with high density in the thalamus of both rat and human brain, where the α4β2-subtype is the predominant nAChRs. Cytisine was subsequently shown to selectively bind at the α4β2-nAChR ($K_i = 0.17$ nM) in rat brain cells, with ~6-fold greater specificity than nicotine ($K_i = 0.95$ nM); at the α3β4- and α7-subtype receptors, the binding affinity of cytisine were 840 nM and 4200 nM respectively. However, at 10 µM concentration, the agonist potency of cytisine at α4β2-subtype receptor was only 56% relative to nicotine, inferring that cytisine is a partial α4β2-nAChR agonist. Coe and co-workers reported that the agonist potency of nicotine was reduced by 30% upon co-administration with cytisine, indicating that cytisine partially antagonizes the agonist effect of nicotine. Taken together, these findings established that cytisine is a selective, low-efficiency partial α4β2-nAChR agonist. The effect of cytisine on dopamine release in rat striatum has also been studied. Dopaminergic toxicity caused by oxidopamine (6 µg; a selective neurotoxin that destroys dopaminergic neurons in the brain) was significantly attenuated when the rats were pre-administered with cytisine (2 mg/kg), whilst the amount of dopamine expression in substantia nigra was increased from 30% to 60%. The neuroprotective properties displayed by cytisine may be beneficial in neurodegenerative disorders.

2.9. N-Hydroxybenzylanabasine

(*Angian chinense* (Lour.) is a Chinese deciduous shrub commonly used in traditional Chinese medicine. Powdered *A. chinense* roots were found to contain (2S)-N-hydroxybenzylanabasine (Fig. 9), an N-substituted analogue of anabasine. Subsequent biological activity investigations indicated that (2S)-N-hydroxybenzylanabasine displayed moderate neuritis inhibitory properties against microglial nitric oxide inflammation ($IC_{50} = 6.7$ µM) when compared with curcumin (positive control; $IC_{50} = 3.1$ µM).

2.8. Metanicotine

Metanicotine in humans is currently available. Clinical trials of metanicotide were advanced into phase II as an enema formulation in 2003; a phase II placebo-controlled trial with 200 ulcerative colitis patients was carried out but unsatisfactory primary efficacy resulted in the discontinuation of the trial in 2005. No published reports on the efficacy or pharmacokinetics of metanicotide in humans is currently available.

2.7. Cytisine

Cytisine acts as a competitive antagonist in the presence of nicotine, attenuating nicotine’s effect at α4β2-nAChR by shielding nicotine-induced dopaminergic activation and therefore limiting the rewarding effect from tobacco consumption; in the absence of nicotine during smoking cessation, cytisine behaves as a partial agonist and increases dopamine levels in the brain, dampening nicotine withdrawal symptoms.

![Fig. 9. (2S)-N-hydroxybenzylanabasine.](image)

![Fig. 10. Anabasamine.](image)

![Fig. 11. Cytisine and Varenicline.](image)
Cytisine served as the lead compound for the development of a more efficacious α4β2-nAChR partial agonist for smoking cessation, which led to the development of Varenicline (Fig. 11) by Pfizer, approved by the FDA in 2006 for the treatment of nicotine addiction and smoking cessation.\textsuperscript{84,85,98,100} Varenicline displays a selective binding affinity (Kᵢ = 0.06 nM) and potent partial agonist activity (EC\textsubscript{50} = 3.1 μM) at the α4β2- nAChR in rats; dopamine release in rat brain was reduced by 45% upon co-administration of nicotine and varenicline.\textsuperscript{86} Full scale clinical trials indicated that varenicline possesses a similar partial agonist profile at the α4β2-nAChR as cytisine in humans.\textsuperscript{84,85,98,100} Nausea and insomnia are the two most common side-effects in the first month of treatment, occurring in ~30% and ~26% of patients respectively, but these mostly subside upon extended administration and/or dose titration.\textsuperscript{100} Varenicline displays a greater efficacy than bupropion (an atypical antidepressant and nicotinic receptor antagonist) and NRTs in sustaining abstinence from smoking.\textsuperscript{84,100} It is worth noting that early postlaunch surveillance and meta-analysis advised potential neuropsychiatric and cardiovascular conditions associated with varenicline use, but subsequent clinical studies revealed that the probability of these adverse events is low in patients that do not have a pre-existing psychiatric condition, such as depression, anxiety or schizophrenia.\textsuperscript{100}

3-Hydroxy-11-norcytisine (Fig. 12) is a 5-membered ring skeletal congener of cytisine isolated from the Leguminosae family.\textsuperscript{102,103} It was extracted from the seeds of \textit{L. anagyroides} in 1989 by Hayman and Gray,\textsuperscript{102} but has received little attention in the pharmacology literature despite its obvious structural and potential biosynthetic relationship with cytisine.\textsuperscript{103} Almost two decades later, Yohannes and Bhatti examined the biological activities of 3-hydroxy-11-norcytisine at rat α4β2- and α7-nAChRs.\textsuperscript{104} The binding affinity (Kᵢ) of 3-hydroxy-11-norcytisine was ~35000- and ~153-fold less effective than cytisine at α4β2- (14 μM vs 0.4 μM) and α7-nAChRs (260 μM vs 1.7 μM), respectively.\textsuperscript{103} The subtle structural differences in these natural products clearly results in large discrepancies in binding at the nicotinic receptors.\textsuperscript{103}

The \textit{Ormosia} genus of the \textit{Leguminosae} contains pyridine alkaloids structurally related to cytisine.\textsuperscript{104} The roots of \textit{O. hosiei} contain hostieines A and B, whilst the stems contain hostieines C and D (Fig. 13).\textsuperscript{104} These cytisine-type alkaloids comprise a structurally unprecedented 2-azabicyclo-[3.2.1]-octane ring system. The binding affinity and agonist potency of hostieines A–D at the α4β2- nAChR were examined using a [3H]-cytisine displacement assay.\textsuperscript{105} Hostiene A was found to have the most potent binding affinity at α4β2- nAChR, with nanomolar potency ~5-fold stronger than nicotine.\textsuperscript{104}

2.12. Huperzines

Abundant throughout China, \textit{Huperzia serrata} is a plant renowned for its diverse therapeutic applications in traditional Chinese medicine, including the treatment of contusions, swellings, pain and schizophrenia.\textsuperscript{105-109} In a photochemical study examining the biochemical significance of \textit{H. serrata}, the aqueous extract of dried \textit{H. serrata} was found to contain huperzine A (Fig. 14), an alkaloid comprising an unusual bicyclo[3.3.1] ring system fused with an ethyldiene group and a 2-pyridone moiety.\textsuperscript{110} Much effort has focused on the extraction of huperzine A from other plants due to the limited quantity available from \textit{H. serrata}.\textsuperscript{108-109} Huperzine A has also been isolated from \textit{Lycopodiaceae} and \textit{Selaginellaceae} families, but also in poor yield; \textit{Phlegmariurus carinatus} and \textit{P. mingechensis} of the \textit{Huperziaceae} family have been reported to produce the highest yields of huperzine A.\textsuperscript{111,112} Several detailed reviews on the therapeutic potential of huperzine A are available.\textsuperscript{105-109}

The cholinesterases (ChE) are a family of enzymes present in the CNS that break down choline-based esters.\textsuperscript{113}\textsuperscript{114} Two types of ChE have been characterized: acetylcholinesterase (AChE) and butryrylcholinesterase (BuChE).\textsuperscript{114} AChE specifically hydrolyzes ACh into choline and acetic acid to avoid over-stimulation in post-synaptic nerves; BuChE (also known as pseudocholinesterase) is a nonspecific ChE that breaks down different choline-based esters.\textsuperscript{113}\textsuperscript{114} ACh is a neurotransmitter found predominantly in the human brain and has an important role in arousal, attention, memory and motivation.\textsuperscript{114} AD patients have lower ACh levels due to age related degeneration of their cholinergic system and/or brain injuries.\textsuperscript{113} The cholinergic hypothesis of AD suggests a strategy to treat neurodegeneration is to restore ACh deficiency.\textsuperscript{114} Therefore, AChE inhibitors are served as cognition enhancing agents to treat patients with mild to moderate AD, including tacrine, donepezil, physostigmine and rivastigmine.\textsuperscript{113,114}

The inhibitory activity of huperzine A has been examined against both AChE and BuChE in \textit{vitro}.\textsuperscript{115} The inhibitory activity on AChE induced by huperzine A (IC\textsubscript{50} = 0.082 μM) was slightly more potent than tacrine (a nonselective ChE inhibitor; IC\textsubscript{50} = 0.093 μM), but ~8-fold weaker than the drug donepezil (a selective AChE inhibitor; IC\textsubscript{50} = 0.010 μM).\textsuperscript{115} The inhibitory activity on BuChE induced by huperzine A (IC\textsubscript{50} = 74.43 μM) was ~15 and ~1000-fold less potent than donepezil (IC\textsubscript{50} = 5.01 μM) and tacrine (IC\textsubscript{50} = 0.074 μM) respectively.\textsuperscript{115} These findings indicated that huperzine A displays high selectivity for AChE over BuChE.\textsuperscript{105-109,115} Oral administration of huperzine A to rats led to significant inhibition of AChE (16% inhibition at 1 μmol/kg), ~15 and 140-fold more potent than donepezil (9% inhibition at 8 μmol/kg) and tacrine (7% inhibition at 60 μmol/kg), respectively.\textsuperscript{115} However, upon intracerebroventricular (ICV) injection, the anti-AChE activity of huperzine A (21% inhibition at 0.066 μmol/kg) was ~3-fold less potent than donepezil (35% inhibition at 0.038 μmol/kg) but ~2-fold stronger than tacrine (11% inhibition at 0.068 μmol/kg), a pattern similar to the \textit{in vitro} results.\textsuperscript{115} The different administration routes clearly affect the bioavailability of huperzine A, with the ICV route facilitating access to the brain.\textsuperscript{106-107,115} In a subsequent \textit{in vivo} experiment, the level of ACh in the whole rat brain upon huperzine A administration was also measured.\textsuperscript{115} Huperzine A displayed the most prolonged increase in ACh level when compared with donepezil and tacrine, lasting for at least 6 h after administration.\textsuperscript{115} Moreover, the activity of choline acetyltransferase and the level of choline did not change, indicating that the increase of ACh level was not a result of an increase in ACh synthesis.\textsuperscript{108,109,115} A clear inverse relationship was observed between AChE activity and ACh level, further confirming that the increase was mediated through the AChE inhibition induced by huperzine A.\textsuperscript{111} Given that the lack of ACh in the brain is a common symptom in AD patients, the high binding specificity to AChE of huperzine A suggests therapeutic potential.\textsuperscript{106-109}

The effect of huperzine A on glutamate-induced neuron toxicity was investigated by Ved and co-workers.\textsuperscript{116} Neurons derived from rat embryonic forebrain were treated for 45 min with either 100 μM glutamate, 100 nM huperzine A or a mixture of 100 nM huperzine A and 100 μM glutamate. Neuronal cell death caused by glutamate-induced toxicity was found to be ~55% upon treatment with glutamate alone, which was ~50% greater than the group treated with huperzine A alone. Treatment
with both huperzine A and glutamate resulted in a ~30% neuron death, suggesting that huperzine A had partially suppressed the glutamate-induced toxicity in neurons. In the same study, the effect of huperzine A on calcium mobilization was also investigated. Neurons derived from embryonic forebrain were exposed to 10 µM of either glutamate or Bay-K8644 (a potent calcium channel agonist), followed by huperzine A (100 nM) and the level of evation was measured. Upon huperzine A treatment, the glutamate-induced calcium mobilization was reduced from 811 to 668 nM, but a minimal effect was observed in the neurons that were exposed to Bay-K8644 (calcium elevation: 505 nM vs 521 nM). These results inferred that huperzine A acts on glutamate receptors to exert neuroprotective properties on glutamate-induced toxicity.

Studies examining the effect of huperzine A on the NMDA receptor, an ionotropic glutamate receptor that controls synaptic plasticity and memory function, have also been performed. Huperzine A (100 µM) did not inhibit the binding of [3H]-glutamate (an agonist that binds to the NMDA agonist site), [3H]-selfotel (an antagonist that binds to the NMDA regulatory site) or [3H]-ifenprodil (an antagonist that binds to the NMDA polyamine regulatory site), however, both [3H]-dizocilpine and [3H]-thienylcyclohexylpiperidine (non-competitive antagonists that bind to the NMDA ion channel) were displaced by huperzine A at Kᵢ = 5.6 and 9.5 µM respectively, indicating that huperzine A interacts with NMDA receptor by binding to the ion channel via a non-competitive inhibition mechanism. The NMDA-induced neuronal toxicity was prevented as the survival rate of neurons that were exposed to Bay-K8644 (calcium elevation: 505 nM vs 521 nM). These results revealed that huperzine A acts on glutamate receptors to exert neuroprotective properties on glutamate-induced toxicity.

Fig. 13. Hosieines A-D with their binding affinities (Kᵢ) and inhibitory concentration (IC₅₀) at the human α4β2-nAChR.

Fig. 14. Huperzine A.

Fig. 15. Nicotine and Cytisine.

Huperzine A is less toxic and induces less adverse effects than classic AChE inhibitors that are currently used to treat AD (e.g. donepezil and tacrine). Memory tests that are used to measure the progression of AD are applied in clinical trials to assess the performance of a treatment; clinical trials performed in China used these memory tests (e.g. mini-mental state evaluation, memory quotient and AD assessment scale-cognitive section) to evaluate the efficacy of huperzine A in the treatment of patients suffering from age related memory dysfunction or dementia. Huperzine A reportedly led to significant cognitive enhancement in these patients and as a result, the China Food and Drug Administration (CFDA) approved huperzine A (0.2 mg; twice daily) to treat the cognitive symptoms of AD. However, the lack of randomization in these clinical trials and evidence-based literature on the safety and efficacy have prevented medical approval outside China. Interestingly, huperzine A was not protected under patent upon its initial release and has been sold as a dietary supplement in the United States. Several natural products containing the huperzine A scaffold have been isolated from various plant sources; those with reported biological activities relevant to the subject of this review are shown in Table 1.

2.13. Multijuguinones

*Senna multijuga* is a popular ornamental plant in many Brazilian regions because of its brightly yellow coloured flowers. This species belongs to the *Senna* genus which is renowned for their diverse...
Table 1  
Source and biological activity of huperzine derivatives.

| Natural product       | Source | Activity                          | Refs                  |
|-----------------------|--------|-----------------------------------|-----------------------|
| H. serrata            | AChE inhibitor (0.082 µM)                  | 105-120               |
|                       | BuChE inhibitor (74.43 µM)                  |                       |
|                       | NMDA ion channel antagonist               |                       |
| H. serrata            | AChE inhibitor (2.57 µM)                  | 106,107,110,121       |
|                       | BuChE inhibitor (169 µM)                  |                       |
| L. casuarinoides      | AChE inhibitor (0.6 µM)                   | 121,122               |
| H. carinata           | AChE inhibitor (2.63 µM)                  | 124                   |
| L. carinatum          | AChE inhibitor (4.6 µM)                   | 125                   |
| L. carinatum          | AChE inhibitor (7.0 µM)                   | 125                   |
| L. casuarinoides      | AChE inhibitor (87.3 µM)                  | 121                   |

(continued on next page)
The seeds of *S. multijuga* have been used in Brazilian traditional medicine to treat ophthalmic and skin infections, whilst the leaves are used as a sedative during rituals by indigenous South American tribes. In a phytochemical study, two unusual 2-methyl-3-hydroxy-6-alkyl pyridine alkaloids, 7\'-multi-

juguinone 1 and 12\'-hydroxy-7\'-multijuguinone 2 were isolated from the leaves of *S. multijuga*. In a subsequent study, five structurally related pyridine alkaloids were also isolated from *S. multijuga*; 7\'-multijuguinol 3, 12\'-hydroxy-7\'-multijuguinol 4, 8\'-multijuguinol 5, 12\'-hydroxy-8\'-multijuguinol 6 and methyl-multijuguinate 7 (Fig. 15).

The AChE inhibitory activity of 1–7 were investigated in both isolation reports by standard bioautography (TLC assay) and microplate tests. Using physostigmine (a reversible AChE inhibitor) as the positive control, the preliminary bioautography studies revealed that all the pyridine alkaloids 1–7 shown in Fig. 15 were ~3- to 120-fold less active than physostigmine. A microplate test confirmed the bioactivity of the natural products as the assay data indicated weak anti-AChE activity (13%–52% inhibition at 350 mM) when compared with physostigmine (87% inhibition at 350 mM). The data suggests that the constitution of the alkyl chain at C6 is vital and the presence of the C7\'-OH appears to be important for higher levels of inhibition.

### 2.14. Euphorbialoids and derivatives

*Euphorbia* is a genus of flowering plants in the *Euphorbiaceae* family renowned for their use in traditional Chinese medicine, such as the treatment of skin diseases, gonorrhoea, migraine and intestinal parasites. In a phytochemical study on *E. prolifera*, ten myrsinol diterpenes named euphorbialoids A–J and two previously reported congeners 1 and 2 (Fig. 16) were isolated by Guo and co-workers in 2012. These natural products are myrsinol diterpenes comprising a 5–7-6 ring system bound to nicotinoyloxy group at different positions on the

| Natural product | Source | Activity | Refs |
|----------------|--------|---------|------|
| 16-hydroxyhuperzine B | *L. casuarinoides* | AChE inhibitor (20.9 µM) | 126 |
| Casuarinine C (11R) | *L. casuarinoides* | AChE inhibitor (12.1 µM) | 126 |
| Casuaranine I (7R,11S,12S,13R) | *L. casuarinoides* | Neuroprotective against H2O2-induced neuron damage in human SH-SY5Y cells (10 µM) | 126 |
| Casuaranine H (15R) | *L. casuarinoides* | AChE inhibitor (1.9 µM) | 121,127 |
| N-demethylhuperzine | *L. casuarinoides* | AChE inhibitor (23.9 µM) | 121,128 |
| Lycoparin C | | | |

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myrsinane skeleton.\textsuperscript{133}

Nitric oxide (NO) is a membrane-permeable gas that acts as a neuromodulator at the synaptic junctions.\textsuperscript{135} High levels of NO will result in oxidative stress and hence neuronal inflammation in the CNS, a condition thought to play a role in neurodegenerative diseases (e.g. AD and Parkinson’s disease).\textsuperscript{135} The neuroprotective properties of euphorbialoids A–J and analogues 1–2 were investigated by evaluating their inhibitory activity on lipopolysaccharide (LPS)-induced NO production in murine microglial BV-2 cells.\textsuperscript{133} Euphorbialoids A–J and their unnamed congeners 1 and 2 inhibited the production of NO to varying degrees in microglial BV-2 cells when compared with 2-methyl-2-thiopseudourea sulfate (positive control; 13.2 \( \mu \)M),\textsuperscript{133} suggesting that these alkaloids represent therapeutic leads for the treatment of neurodegenerative diseases.

2.15. Cerpegin

Ceropegia of the Apocynaceae family is a genus of plants that are native to Africa, Southern Asia and Australia.\textsuperscript{136} The phytoconstituents of Ceropegia species have been routinely used in Ayurvedic medicine to treat gastric disorders, dysentery, hepatic disease, urinary tract diseases and diarrhoea.\textsuperscript{136} In one phytochemical study, cerpegin (Fig. 17), a pyridine alkaloid consisting a 2-pyridone fused with a 2-furanone ring was isolated from the fleshy stem of \textit{C. juncea}.\textsuperscript{137} Cerpegin has been shown to possess many diverse biological and pharmacological activities, such as analgesic, tranquilizing, anti-inflammatory, anti-ulcer and anti-cancer properties.\textsuperscript{136,138} Relevant to the subject of this review, cerpegin was found to exhibit dose-related analgesic properties against acetic acid-induced writhing in mice.\textsuperscript{136,138} No automatic or behavioural changes were observed up to a dose of 20 mg/kg; however, excitation, respiratory paralysis and later convulsions was produced by cerpegin with dosing >400 mg/kg.\textsuperscript{138} Tranquilizers act on the CNS to moderate brain activities and relieve hyperactive nerves to treat patients with anxiety, sleeping disorders and psychoses (e.g. schizophrenia).\textsuperscript{136} Cerpegin displays tranquilizing properties through an unknown mechanism, but it has been suggested that this furanopyridone alkaloid competitively antagonizes both dopamine (D\(_2\)) and serotonin (5-HT) receptors.\textsuperscript{136,138}

2.16. Cantleyine

Many pharmacologically active compounds have been detected from Brazilian Strychnos, a genus of flowering plants belonging to the Loganiaceae family.\textsuperscript{139} In a methodology study attempting to modify the isolation procedure of biologically active alkaloids from Strychnos species, a monoterpene tri-substituted cyclopentapyridine alkaloid named cantleyine (Fig. 18) was isolated from the aqueous extract of \textit{S. trinervis} roots.\textsuperscript{139} The effect of cantleyine against CaCl\(_2\) on voltage dependent calcium channels was investigated.\textsuperscript{139} It was reported that when the concentration of cantleyine was increased from 120 to 490 \( \mu \)M, CaCl\(_2\) induced maximal smooth muscle contraction decreased from 90% to 45% in guinea pig ileum. These results suggested that cantleyine induces a reversible but nonselective spasmolytic action on the vascular and visceral smooth muscles due to the inhibition of Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels, similar to that exhibited by common calcium channel inhibitors such as verapamil and nifedipine.\textsuperscript{139}

2.17. Haplophyllidine

The furanopyridine alkaloid haplophyllidine (Fig. 19) was isolated from the seeds of \textit{Haplophyllum perforatum} by Shakirov and co-workers.\textsuperscript{140,141} Subsequent studies showed that this alkaloid is also present in the stems and leaves of \textit{H. perforatum}.\textsuperscript{140} Haplophyllidine is a potent CNS depressant and synergizes the effects...
of narcotic/hypnotic drugs in mice, rats and rabbits. Simultaneous subcutaneous injection (s.c.) of luminal (<72.5 mg/kg) and haplophyllidine (<130 mg/kg) produced strong neurological deficits; upon co-administration with hexanal (40 mg/kg) or chloral hydrate (230 mg/kg), haplophyllidine (2–40 mg/kg) prolonged sleep duration by 50% and 70% respectively. Haplophyllidine (100 mg/kg) produced complete mortality protection against strychnine (1.44 mg/kg), whilst at 250 mg/kg reduced death by 80% against caffeine (210 mg/kg). These studies suggested that haplophyllidine exhibits pronounced sedative and antianaleptic properties.

2.18. Gentianine

Gentianine (Fig. 20), an alkaloid comprising a 3-vinylpyridine fused with pyranone, was isolated from *Gentiana kirilowii* and its chemical structure was established upon total synthesis. Gentianine has also been extracted from many other Gentiana and Swertia species of the Gentianaceae family, such as *E. littoralis* and *S. chirata*. 
3.1. Epibatidine

Gentianine significantly potentiated the sleeping time induced by hexobarbital (100 mg/kg; i.p.) by 80%; amphetamine-induced (10 mg/kg; s.c.) stereotypic behaviours (including continuous sniffing, biting and compulsive gnawing) were blocked; gentianine completely inhibited lysergide-induced symptoms such as piloerection and tremors (10 mg/kg; i.p.) toxicity was reduced by 88% and amphetamine-induced hypnosis, amphetamine toxicity and lysergide-induced symptoms in rats. 3. In a study examining the chemical constituents of the skin secretions, the methanolic extract of 750 mg/kg, the alkaloid is thought to possess only a moderate to low potency, whereas 276 mg/kg was examined in both the Straub-tail response (generally used as a high-dose syndrome or alkaloid contamination. Moreover, the anti-nociceptive activity of epibatidine was almost unaffected when the nonselective opioid antagonist naloxone was pre-administered to rats, proving that epibatidine does not exert its analgesic properties through the opioid receptors. However, subsequent studies inferred that synthetic epibatidine did not generate a Straub tail response in rats, suggesting previous results were linked to high-dose syndrome or alkaloid contamination. Regardless, epibatidine clearly displayed a non-opioid mode of action, and more biological investigations were subsequently performed to re-evaluate the biological properties of this compound. In 1993, Qian and co-workers reported that epibatidine is a potent nAChR agonist. A group of mice was divided in half and administered with either (–)-nicotine (5 mg/kg; positive control) or (+)-epibatidine (20 µg/kg). Both groups had a rapid anti-nociceptive response; the control group reached maximum response at 2 min and lasted for 10 min, whilst the epibatidine group took 5 min to reach a maximum response that lasted for 20 min. In the same study, if mice were pre-administered the nAChR antagonist mecamylamine (1 mg/kg) and then treated with (+)-epibatidine, the anti-nociceptive dose was 289.2 µg/kg, ~22-fold greater than the group that did not receive mecamylamine (13.6 µg/kg). Providing further evidence that epibatidine is exerting its effects via the nAChRs. Based on these results, a radioligand binding assay was performed to investigate the binding affinity of epibatidine at nAChRs and a range of other neurotransmitter receptors. The study showed that the IC50 value of [3H] (+)-epibatidine required to displace [3H]-cytisine (a potent α4β2-nAChR ligand) was 70 pM, ~100-fold greater than [3H]-nicotine (IC50 = 7.8 nM). However, at 10 µM, epibatidine failed to displace any specific ligands at a range of other neuronal receptors (i.e. GABA, benzodiazepine, dopaminergic, serotonergic, adrenergic, glutamate/aspartate, neuropeptide, brain, cholecystokinin and calcium gene-related peptide). These detailed studies provide very strong evidence that epibatidine exerts its anti-nociceptive effects through nAChR agonism. There are seventeen nAChR subtypes in vertebrates and sixteen in humans, with three different receptor sites abundant in the mammalian brain (α4β2- and α3β4- and α7-nAChR). A series of experiments were conducted to investigate the binding selectivity of epibatidine at these different nAChR subtypes. Using (–)-nicotine as the control, Gopakrishnan and co-workers re-evaluated the binding affinity (Kd) and the agonist potency (ED50) of (+)-epibatidine at four different nAChR subtypes (α4β2-, α3β4-, α7- and α1β1γ- subunits) to establish receptor binding specificity (Table 2). The α1β1γ-nAChR was also selected as part of the investigation as it is a commonly expressed nAChR at neuromuscular junctions.
Table 3

| M1-mACHR binding profile of (+)-epibatidine and (-)-epibatidine vs carbachol and atropine. |
|-----------------------------------------------|----------|---------|-------|-----------|
| Kapp(NMS) (µM) | Kapp(oxo-M) (µM) | NMS/oxo-M ratio |
| (+)-Epibatidine | 6.9 | 1.4 | 4.9 |
| (-)-Epibatidine | 16 | 1.4 | 11.4 |
| Carbachol | 22 | 0.0049 | 4490 |
| Atropine | 0.0010 | 0.00048 | 2.1 |

These studies showed that while (+)-epibatidine and (-)-nicotine are both α4β2-nAChR agonists, fundamental differences were apparent. The binding affinity and the agonist potency of nicotine are higher at the α4β2-nAChR than the other receptor subtypes, whilst epibatidine displays strong binding affinity and agonist activity at all four nAChRs, with some slight specificity at the α4β2 and α3β4 subtypes.154,155 Overall, epibatidine is a much stronger nAChR agonist than nicotine. In a separate study, Rupniak and co-workers reported that both (+)- and (-)-epibatidine have very similar binding affinity at the α4β2-nAChR subtype (K_i = 0.04 vs 0.06 nM) and near identical anti-nociceptive properties (IC_{50} = 0.10 vs 0.24 nM) in rats.154 These interesting results indicated that the absolute stereochemistry of epibatidine has a negligible effect on binding with the α4β2-nAChR, nor its pharmacological profile.154 The same group also discovered that epibatidine binds to the muscarinic acetylcholine receptor (mACHR) M1 subtype at high doses.148,154 There are five mACHR subtypes (M1-M5) in humans, but only the M1 subtype has been found in the brain.148 Rupniak and co-workers investigated the binding affinity of epibatidine at M1-mACHR and nAChRs using a radioligand binding assay.154 Epibatidine displaced [³H]-pirenzepine (a selective M1-mACHR ligand) and [³H]-cytisine in the cerebral cortex of rats (70% and 98% inhibition at 10 μM, respectively).154 The Relative Affinity Ratio was also calculated for both (+)- and (-)-epibatidine to predict their antagonist/agonist efficacy at the M1-mACHR.157 The ability of (+)-epibatidine to displace the mACHR antagonist [³H]-N-methylscopolamine (NMS) and the mACHR agonist [³H]-oxotremorine-M (oxo-M) was measured as the Apparent Affinity Constant (K_{app}) using the radioligand binding assay; K_{app}(NMS) was then divided by the K_{app}(oxo-M) to provide a measurement of antagonist/agonist efficacy.154 The experiment was also repeated for (-)-epibatidine and the results were compared to those of carbachol (a potent nonselective mAChR agonist) and atropine (a potent nonselective mAChR antagonist). These results are summarised in Table 3.

The Relative Affinity Ratios of both (+)- and (-)-epibatidine are significantly lower than that of the mACHR agonist carbachol, indicating that both epibatidine enantiomers are not agonists at the M1-mACHR.149,154 The affinity profile of (+)-epibatidine suggested that it has a similar affinity as the classic mAChR antagonist atropine (Ratio = 4.2 vs 2.1), whereas (-)-epibatidine resembles a partial mAChR agonist.154,155 Given that (+)- and (-)-epibatidine is a potent nACHR agonist and a moderate M1-mACHR antagonist, this natural product was initially considered a promising lead for the non-opioid treatment of pain.146,149 However, no in vivo experiments have been performed in non-rodents due to its low therapeutic index in rats (LD_{50} < 125 nmol/kg; intravenously).149 The toxicity of epibatidine is caused by its potent, non-selective binding at the nAChRs.146,147 Because these nicotinic receptors are widely distributed within the human body (e.g. brain, heart and smooth muscle) and are involved in many neurological and physiological conditions (e.g. schizophrenia, Parkinson’s disease, AD, muscular paralysis, hypertension and seizures), epibatidine binding would lead to many off-target effects at important districts.148-150 As a result, epibatidine itself is no longer investigated for therapeutic development, but its unique scaffold provides a platform for the development of safer therapeutic agents through medicinal chemistry studies.148,149,154

3.2. Phantasmidine

Phantasmidine (Fig. 22) is an epibatidine congener isolated from Anthony’s poison arrow frog (E. anthonyi) by Fitch and co-workers.159 Due to the small quantity obtained (20 µg), the chemical structure was tentatively inferred from the limited spectroscopic data (MS, IR and NMR) and analogy to epibatidine.159 The absolute configuration of phantasmidine was later established through total synthesis and it was revealed that the compound exists as a 4:1 scemilic mixture of (2aR,4aS,9aS) and (2aS,4aR,9aR) enantiomers.160

Initial investigations by Fitch and co-workers showed that phantasmidine displayed a specific agonist activity at nAChRs expressing the β4-subunits (data not shown), suggesting that the compound might possess a different nAChR subtype specificity to epibatidine.159 The group later conducted a more detailed pharmacological investigation on phantasmidine to elucidate its binding selectivity and agonist activity at the nAChRs.160 The binding affinities and agonist potency of (2aR, 4aS, 9aS)-phantasmidine, (2aS, 4aR, 9aR)-phantasmidine, racemic samples of phantasmidine and epibatidine (positive control) to nAChRs (α4β2-, α3β4- and α7-subtypes) are shown in Table 4.

The results revealed that the binding affinity and the agonist activity of (±)-phantasmidine at nAChRs were ~2- to 50-fold weaker than (+)-epibatidine, whilst the binding affinity and the agonist activity of the (2aR,4aS,9aS)-enantiomer were ~2- to 45-fold greater than the (2aS,4aR,9aR)-enantiomer.160 Interestingly, these data indicate that phantasmidine is selective for the α4β2-subtype, contradicting the initial results reported by Fitch and co-workers.159 Toxicity investigations showed that the LD_{50} values of (±)-phantasmidine and the (2aR,4aS,9aS)-enantiomer were 270 and 72 µg/kg respectively, at least 10- and 3-fold less toxic than (+)-epibatidine (LD_{50} < 26 µg/kg), whilst the (2aS,4aR,9aR)-isomer was much less toxic, producing similar effects at LD_{50} > 10 mg/kg.160 It is clear that the stereochemistry of phantasmidine plays an important role in nAChR binding and toxicity.160 Similar to epibatidine, phantasmidine itself is also no longer considered as a potential therapeutic candidate due to its low therapeutic index, however, its agonist selectivity at the neuronal α4β2-nAChR makes it a useful pharmacologic tool for the investigation of specific nAChR subtypes.159,160

3.3. Noranabasamine

Noranabasamine (Fig. 23) is a des-N-methyl analogue of...
anabasamine that was isolated from the skins of Columbian poison dart frog *Phyllobates terribilis* [163]. Due to its structural similarity with nicotine and anabasine (*Nicotiana* alkaloids), it has been suggested that noranabasamine might highly also possess agonist activity at nAChRs [162]. However, no biological evaluation on noranabasamine is currently available.

### 4. Fungal and bacterial-derived

#### 4.1. 4-Hydroxy-2-pyridones

Entomogenous deuteromycetes are a taxonomically diverse group of imperfect fungi known to produce biologically active secondary metabolites due to their complex association with insect hosts. In a study investigating the CNS-related secondary metabolites of entomogenous fungi, militarinone A (Fig. 24) was isolated from the mycelial extract of *Paecilomyces militaris* strain RCEF0095 [164]. Militarinone A is a 1,4-dihydroxy-2-pyridone alkaloid comprising a cis-1,4-dihydroxycyclohexane moiety and a polyene side chain. In subsequent studies, two structurally related 4-hydroxy-2-pyridone alkaloids, (+)-N-deoxymilitarinone A [165] and farinosone A [166] (Fig. 24), were isolated from *P. farinosus* strains RCEF0097 and RCEF0101 respectively. The neurotrophic properties of militarinone A, (+)-N-deoxymilitarinone A and farinosone A were investigated by examining their potential to stimulate neuronal differentiation in PC-12 cell lines [164–166]. The cell viability data showed that all three compounds exhibited potent neuritogenic activities when compared with an endogenous glycoprotein, the nerve growth factor (positive control; induces 80% neurite outgrowth at 50 ng/mL). Militarinone A produced 80%, 70% and 30% neurite outgrowth at 33, 10 and 3.3 µM respectively; (+)-N-deoxymilitarinone A displayed a weaker neurotrophic activity than militarinone A, inducing a neurite outgrowth of 51% and 12% at 100 and 33 µM respectively, which inferred that the hydroxy group at N1 is essential for neuronal differentiation and survival; farinosone A exhibited 70% and 40% neurite outgrowth at 50 and 20 µM respectively. These findings showed that militarinone A, (+)-N-deoxymilitarinone A and farinosone A exhibit pronounced neuronal proliferation and are interesting therapeutic candidates for the prevention of neuronal decline [164–166].

Biological investigations on the secondary metabolites extracted

### Table 4

|             | α4β2 | α3β4 | α7  |
|-------------|------|------|-----|
| Ki          | EC50 | Ki   | EC50 |
| (2aR,4aS,9aS)-Phantasmidine | 0.27 | 200  | 9.1  | 570  | 4.4 |
| (2aS,4aR,9aS)-Phantasmidine | 12   | 56,000 | 390  | 27,000 | 130 |
| (±)-Phantasmidine     | 0.35 | 200  | 11   | 750  | 5.4 |
| (±)-Epibatidine       | 0.033| 25   | 0.22 | 41   | 2.7 |

![Fig. 23. Noranabasamine.](image)

![Fig. 24. 4-Hydroxy-2-pyridone alkaloids with CNS activity.](image)
Acromelic and acromelobic acids

Acromelic acids A and B (Fig. 25) are neuroexcitatory amino acids first isolated from Clitocybe acromelalga by Konno, Shirahama and Matsumoto in 1983.\(^{170}\) \(C.\) acromelalga is a toxic Japanese mushroom that elicits symptoms similar to acromelalgia and erythromelalgia upon ingestion.\(^{170-173}\) The fresh fruiting bodies of \(C.\) acromelalga contain acromelic acids A and B;\(^{174}\) stereospecific total synthesis of acromelic acid A affirmed the proposed structures and assigned the absolute configuration of both natural products in the process.\(^{172}\) Acromelic acids A and B are classified as kainoids, analogues of kainic acid (Fig. 25), a classic \(\alpha\)-amino acid (\(\alpha\)-AA) agonist at glutamate receptors and is used as a neurodegenerative agent in neuroscience research to mimic glutamate excitotoxicity in neurodegenerative models (e.g. AD and epilepsy).\(^{174}\) Therefore, the neuroexcitatory properties of acromelic acids A and B were investigated.\(^{171,172,176,177}\) Electrophysiological tests were performed to examine the neuroexcitatory potential of acromelic acid A at the crayfish neuromuscular junction and mice spinal cord.\(^{173,175}\) These studies showed that acromelic acid A is a powerful glutamate receptor agonist that exhibits significant depolarizing action in central neurons and ionotropic neuroexcitatory activity in both muscle fibre and brain, with a potency ~100-fold greater than kainic acid.\(^{171-174}\) Similar results were obtained on the frog spinal cord (data not shown).\(^{175}\) In 1990, Nozoe and co-workers reported the isolation of acromelic acid C (Fig. 25) from \(C.\) acromelalga.\(^{177}\) The chemical structure and absolute configuration of acromelic acid C was established by detailed NMR spectroscopy and by analogy to acromelic acids A and B.\(^{177}\) The neurotoxicity of acromelic acid C was investigated via intraperitoneal injection into a mouse; the median lethal dose was 10 mg/kg, slightly higher than acromelic acids A and B (7 and 8 mg/kg respectively).\(^{177}\)

Two neuroexcitatory amino acids, acromelic acid and an acromelic acid analogue 1 (Fig. 25) were also isolated from \(C.\) acromelalga by Shirahama and Yamano in 1992 and 1993, respectively;\(^{178,179}\) the chemical structures and absolute configurations were established by detailed NMR spectroscopy and total synthesis in the process. Both compounds exhibit weak glutamate depolarizing activity on the spinal cord of infant mice (data not shown).\(^{178,179}\)

4.3. Fusaric acid

Fusarium is a genus of filamentous fungi that produce diverse biologically active secondary metabolites, including the mycotoxin fusaric acid (Fig. 26).\(^{180-185}\) Fusaric acid (5-butylpicolinic acid) was first isolated from \(F.\) heterosporum in 1934 by Yabuta\(^{180}\) and was found to exhibit weak antimicrobial properties.\(^{180-181}\) More than three decades later, fusaric acid was found to be a potent inhibitor of dopamine \(\beta\)-hydroxylase (DBH),\(^{182}\) an enzyme that catalyses the conversion of dopamine to norepinephrine.\(^{184}\) In vitro studies suggested that fusaric acid is a very potent inhibitor of DBH, ~10-fold more active than picolinic acid.\(^{184,185}\) The percentage inhibition of DBH induced by fusaric acid at concentration of 0.005, 0.05, 0.5 and 1 \(\mu\)M was 20%, 58%, 89% and 92%, respectively; whilst picolinic acid at 0.5 and 5 \(\mu\)M inhibited DBH by 55% and 83% respectively, inferring that the butyl side chain at C5 increases the DBH inhibitory effects.\(^{184}\) Inhibition of DBH by fusaric acid was found to be competitive and completely reversible, indicating that this compound affects the enzyme-substrate complex.\(^{184}\) The binding specificity of fusaric acid was studied by examining its inhibitory activity on other

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**Fig. 25.** Acromelic acids A–C, acromelobic acids and kainic acid.

**Fig. 26.** Fusaric acid and Phenopicolinic acid.
oxidoreductases.\textsuperscript{184} At 10 µM, fusaric acid did not inhibit MAO, tyrosine hydroxylase or aldehyde dehydrogenase, indicating that the compound has a high degree of binding specificity for DBH.\textsuperscript{184} A study examining the dopamine and norepinephrine levels in rat brains after a single dose administration of fusaric acid (100 mg/kg; intraperitoneal injection) have been performed.\textsuperscript{182,184} The norepinephrine expression in rat brains decreased significantly from 0.22 to 0.10 µg/g three hours after fusaric acid administration, whilst the level of dopamine remained constant (from 0.42 to 0.40 µg/g). The significant decrease in the level of norepinephrine without a corresponding decrease in dopamine expression in rat brains implies that fusaric acid inhibits DBH and thus reduces the production of norepinephrine.\textsuperscript{185} These investigations showed that fusaric acid is a potent DBH inhibitor both in vivo and in vitro,\textsuperscript{182,184} which led to clinical trials of fusaric acid in patients with mania and depression being conducted in 1974.\textsuperscript{185} The levels of 3-methoxy-4-hydroxyphenylglycol (the major metabolite of norepinephrine) in the cerebrospinal fluid was reduced by ~25% when patients were administered fusaric acid compared to placebo, whilst the mean concentration of homovanillic acid (the major metabolite of dopamine) almost doubled, indicating an accumulation of dopamine in the brain.\textsuperscript{185} However, adverse behavioural changes were observed in patients with stage III mania and/or other severe pre-existing psychotic features; a few depression being conducted in 1974.\textsuperscript{185} The levels of 3-methoxy-4-hydroxyphenylglycol (the major metabolite of norepinephrine) in the cerebrospinal fluid was reduced by ~25% when patients were administered fusaric acid compared to placebo, whilst the mean concentration of homovanillic acid (the major metabolite of dopamine) almost doubled, indicating an accumulation of dopamine in the brain.\textsuperscript{185} However, adverse behavioural changes were observed in patients with stage III mania and/or other severe pre-existing psychotic features; a few patients with mild hypomanic symptoms showed no change or slight improvements, suggesting that the effects of fusaric acid relate to the pre-existing clinical state of the patients.\textsuperscript{182,185} The results from these clinical trials indicated that a reduction in norepinephrine by DBH inhibition did not improve manic symptoms and therefore fusaric acid was not approved for therapeutic use.\textsuperscript{185} Subsequent in vivo investigations have demonstrated other neurochemical effects of fusaric acid in the brain.\textsuperscript{182,186} In addition to inhibiting the biosynthesis of norepinephrine, fusaric acid was also found to alter the levels of melatonin, serotonin, tyrosine, tryptophan and luteinizing hormone.\textsuperscript{186} However, inconsistent results (data not shown) were obtained from these different studies which inferred that the neurochemical effects of this mycotoxin vary with species (i.e. rodents, rabbits and swine).\textsuperscript{186} Although fusaric acid has been shown to cause behavioural changes in test subjects, it is primarily used as a research tool as its mode of action in the brain is still not fully understood.\textsuperscript{182,186}

Another potent DBH inhibitor named phenopicolinic acid (Fig. 26) was isolated from \textit{Paecilomyces} sp. strain AF2562 by Nakamura and co-workers in 1975.\textsuperscript{187} The DBH inhibitory activity of phenopicolinic acid was reported to be nearly double that of fusaric acid (IC$_{50}$ = 0.039 µM).\textsuperscript{185} In vivo experiments demonstrated that phenopicolinic acid (50 mg/kg; oral administration) reduced the blood pressure of hypertensive rats by 21%, 16%, and 23% in 1, 3 and 5 h respectively; the LD$_{50}$ (50 mg/kg; oral administration) of either coprismycin A or B for 24 h. The assay revealed 100% cell viability, suggesting that coprismycins A and B are not toxic to dopaminergic neurons.\textsuperscript{182} In a subsequent experiment, SH-SY5Y cells were pre-treated with different concentrations of either coprismycin A or B, pre-treated with different concentrations of either coprismycin A or B, and the cell viability was measured. The cell viability was ~80% when cells were treated with 1.0, 2.5 or 5.0 µM respectively, suggesting that the MPP$^+\text{ exposure was improved from 63.0% to 69.7%, 74.2% and 80.3% upon treatment of coprismycin A at concentration of 1.0, 2.5 or 5.0 µM respectively, suggesting that the MPP$^+$-induced neurotoxicity was suppressed.\textsuperscript{191} Coprismycin B exerted similar neuroprotective properties, producing cell viability of 76.4% and 88.4% at 1 and 2.5 µM respectively; however, at 5 µM the cell viability dropped to 69.8%, indicating neurotoxicity of coprismycin B at higher concentrations.\textsuperscript{191} Nonetheless, these findings have provided strong evidence to show that both coprismycins A and B exhibit pronounced neuroprotective properties that warrant further investigation.

Two 2,2'-bipyridyl alkaloids, SF2738 D\textsuperscript{1} and SF2738 F\textsuperscript{2} were discovered in 1994 from a culture of \textit{Streptomyces} sp. in Japan (Yokohama, Kanagawa).\textsuperscript{192} Some years later, these natural products were also isolated from the bacterial strain SNA015 of \textit{Streptomyces} sp. found in \textit{C. tripartitus}.\textsuperscript{193} The structural similarity of SF2738 D and SF2738 F with coprismycins A and B led to an investigation of their neuroprotective properties.\textsuperscript{197} Upon treating SF2738 D and SF2738 F to SH-SY5Y cells, there was no loss in cell viability. Moreover, the cell viability of SH-SY5Y cells increased (data not shown) when they were treated with SF2738 D and SF2738 F prior to exposure to MPP$^+$, suggesting they have a similar neuroprotective effect to the coprismycins and are themselves also promising therapeutic leads for treating pronounced neuroprotective properties against neuronal stimulation caused by glutamic acid and quisqualic acid and as a result comprises a promising lead neuroprotective agent.\textsuperscript{199}

4.5. 3-Thiopyridines

Dung beetles roll faecal matter into balls for ease of transport and subsequent storage as a food source. These dung balls contain unique microorganisms that have been studied as a source of structurally unique secondary metabolites with biological active properties.\textsuperscript{191} In one such study, Oh and co-workers isolated coprismycins A and B (Fig. 28) from a SNA015 strain of a \textit{Streptomyces} species found in the dung balls of indigenous Korean dung beetle \textit{Copris tripartitus}.\textsuperscript{191} Coprismycins A and B are densely substituted 2-aryl-3-thiopyridine alkaloids that differ in their aldoxime geometry.

Coprismycins A and B are structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a metabolic precursor to the neurotoxin 1-methyl-4-phenylpyridinium (MPP$^+$).\textsuperscript{191} MPP$^+$ is known to induce neuropathological changes by killing dopamine-producing neurons in the pars compacta of the substantia nigra. Neurodegenerative diseases (e.g. Parkinson’s disease) are often associated with low levels of functional dopaminergic neurons in the brain. Due to their structural similarity to MPTP, the coprismycins were evaluated against human-derived SH-SY5Y cells that express dopaminergic neuron markers.\textsuperscript{191} SH-SY5Y cells were treated with three concentrations (1.0, 2.5 and 5.0 µM) of either coprismycin A or B for 24 h. The assay revealed 100% cell viability, suggesting that coprismycins A and B are not toxic to dopaminergic neurons.\textsuperscript{191} In a subsequent experiment, SH-SY5Y cells were pre-treated with different concentrations of either coprismycin A or B, and the cell viability was measured. The cell viability was ~63.0% to 69.7%, 74.2% and 80.3% upon pre-treatment of coprismycin A at concentration of 1.0, 2.5 or 5.0 µM respectively, suggesting that the MPP$^+$-induced neurotoxicity was suppressed.\textsuperscript{197} Coprismycin B exerted similar neuroprotective properties, producing cell viability of 76.4% and 88.4% at 1 and 2.5 µM respectively; however, at 5 µM the cell viability dropped to 69.8%, indicating neurotoxicity of coprismycin B at higher concentrations.\textsuperscript{191} Nonetheless, these findings have provided strong evidence to show that both coprismycins A and B exhibit pronounced neuroprotective properties that warrant further investigation.

![Fig. 27. Aspernigrin B.](image-url)
neurodegenerative disorders.\textsuperscript{191}

5. Marine-derived

5.1. Anabaseine

A potent neurotoxin was initially discovered from the marine worm *Rhynchocoea* by Bacq\textsuperscript{192} in 1936 and was found to exhibit significant toxicity when injected into crabs, causing convulsions, flaccid paralysis and eventually death.\textsuperscript{193} The structure of this compound remained unknown for many years as attempts at crystallization using standard alkaloidal precipitants were unsuccessful. Three and a half decades later, Kem and co-workers isolated the same compound from the hoplonemerteine *Paranemertes peregrine* and elucidated its chemical structure by both total synthesis and comparison with previously published literature data.\textsuperscript{194} Named anabaseine (Fig. 29), this nemertine alkaloid is a double bond isomer of anatabine, possessing a tetrahydropyridyl ring with an internal imine double conjugated with the pyridine moiety.\textsuperscript{46,194,195} Subsequent studies reported that anabaseine is the primary compound found in the poison glands of *Messor* and *Aphenaenogaster* ants;\textsuperscript{46,194,195} anabaseine has not yet been detected in plants.

Anabaseine has been shown to stimulate the release of ACh and norepinephrine from rat brains upon injection;\textsuperscript{195} several synthetic anabaseine-related analogues also displayed significant cognitive enhancement and avoidance behaviours.\textsuperscript{46,195} As a result, the pharmacological properties of anabaseine were examined by Kem and co-workers on rat α4β2- and α7-receptors, the two predominant nAChRs in mammalian CNS.\textsuperscript{195} The binding affinity (K\textsubscript{i}) and the agonist potency (EC\textsubscript{50}) of anabaseine (K\textsubscript{i} = 0.032 μM; EC\textsubscript{50} = 4.2 μM) at the α4β2-nAChR were ~8-fold less active and ~3-fold more potent than nicotine (K\textsubscript{i} = 0.0041 μM; EC\textsubscript{50} = 14 μM) respectively;\textsuperscript{195} at the α7-nAChR, anabaseine (K\textsubscript{i} = 0.058 μM; EC\textsubscript{50} = 6.7 μM) was ~7-fold more potent than nicotine (K\textsubscript{i} = 0.40 μM; EC\textsubscript{50} = 47 μM).\textsuperscript{195} These findings indicated that anabaseine exhibits different binding selectivity and agonist potency at the nAChRs, inferring the significance of structural conformation with nicotinic receptor recognition sites. Further studies are required to fully understand the impact of the subtle structural differences between this neurotoxin and the tobacco alkaloids.\textsuperscript{195}

5.2. Isoanatabine

Isoanatabine (Fig. 30) was isolated from the hoplonemerteine *Amphiporus angulus* by Kem and co-workers in 2009.\textsuperscript{196} This alkaloid is an anatabine isomer possessing a carbon–carbon double bond in the 3,4-position of the piperidine scaffold.\textsuperscript{46,196} The naturally occurring enantiomeric form of isoanatabine has not yet been reported. Due to the structural similarity with nicotine, the pharmacological properties of both (−)- and (+)-isoanatabine were examined by Kem and co-workers at rat and human α4β2-nAChRs.\textsuperscript{196} The binding affinity of (−)-isoanatabine (K\textsubscript{i} = 108 nM) at rat α4β2-nAChR was slightly stronger than the (−)-enantiomer (K\textsubscript{i} = 136 nM); however, at the human α4β2-nAChR, (−)-isoanatabine was less efficacious (EC\textsubscript{50} = 1.01 μM; I\textsubscript{max} = 78.7%\textsuperscript{196}) than the (−)-enantiomer (EC\textsubscript{50} = 0.31 μM; I\textsubscript{max} = 102%).\textsuperscript{196} These findings indicate that isoanatabine is a more potent ACh stimulant at the α4β2-receptors relative to anabasine and anatabine, suggesting the presence and the position of the double bond improves nicotinic receptor binding.\textsuperscript{196}

5.3. 2,3’-Bipyridyl and nemertelline

2,3’-Bipyridyl and nemertelline (Fig. 31) are neurotoxins isolated from the marine hoplonemerteine worm *A. angulus* by Kem and co-workers in 1976.\textsuperscript{197} Pharmacological investigations have demonstrated that the paralytic properties of 2,3’-bipyridyl (LD\textsubscript{50} = 94 μg) were stronger than nemertelline (LD\textsubscript{50} > 240 μg) in crustaceans; both natural products exhibit comparable activity when tested against barnacle larvae (IC\textsubscript{50} = 4.1 and 3.2 μM, respectively).\textsuperscript{197}

5.4. Platisidines A–C

Platisidines A–C (Fig. 32) were isolated from an Okinawan marine sponge in the genus of *Plakortis* (sp. SS-11) by Koboyashi and co-workers.\textsuperscript{198} These nicotinic acid derivatives comprise an *N*-methylated pyridinium-β-carboxylate with a hexadecanoyl side chain. Platisidines A–C exhibited weak inhibitory activities against AChE (IC\textsubscript{50} = 2.8, 2.6 and 2.1 mM respectively) when compared with galantamine (positive control; IC\textsubscript{50} = 6.4 μM).\textsuperscript{198}
5.5. Cyclostellettamines A–F

mAChRs play important roles in various physiological functions in the human body, including memory and learning. Six structurally unprecedented macrocyclic bis-1,3-disubstituted pyridiniums, cyclostellettamines A–F (Fig. 33), were isolated from a Japanese marine sponge *Stelletta maxima*. Cyclostellettamines A–F inhibited the binding of [3H]-methylquinuclidinyl benzilate (a selective mAChR antagonist) to mAChR subtypes M1, M2 and M3. As mAChRs are known to be correlated with CNS-related diseases, these data suggest that the cyclostellettamines A–F are structurally unique leads that warrant further investigation.

5.6. Agelongine and daminin

Agelas, a genus of marine sponges commonly found on the Caribbean and Indo-Pacific coral reefs, is a rich source of pharmacologically active bromo-alkaloids. In a study examining the biologically active secondary metabolites of *A. longissimi*, the methanolic extract of the sponge was partitioned and purified to give agelongine (Fig. 34), an alkaloid comprising a pyridinium-β-carboxylate moiety bonded to a 4-bromopyrrole-2-carboxylic unit through an aliphatic chain. In a subsequent study, the structurally related pyridinium alkaloid daminin (Fig. 34) was isolated from the sponge *Axinella damicornis*.

Agelongine exhibited a competitive, reversible antagonism at serotoninergic receptors subfamily 1 (5-HT1) in vitro. The agonist properties of 5-hydroxytryptamine at 5-HT1 receptors were inhibited when agelongine was introduced (IC50 = 80 µM). Moreover, agelongine (100 µM) did not affect the concentration–response of histamine, ACh and prostaglandin E2, suggesting that agelongine is selective for 5-HT1 receptors; however, the specific 5-HT1 receptor subtype used in these preliminary pharmacological tests was not stated. For example, 5-HT1A, the most common 5-HT receptor subtype and highly saturated in the hippocampus, is involved in the emotional mechanism; the classic anxiolytic agent buspirone is an agonist at this site. In contrast, both the 5-HT1B and 5-HT1D receptors are widely distributed in the basal ganglia and act as autoreceptors to decrease the transmission of the neurotransmitter glutamate in the neuronal terminals. The triptans are 5-HT1B/1D receptor agonists used to treat migraine attacks in adults with or without aura. Further investigations are required to fully understand how agelongine binds across the serotonergic system and therefore determine its therapeutic potential as a neuropsychiatric lead compound.

Calcium ions regulate many critically important functions in the CNS, including the release of neurotransmitters and intracellular signal transductions. The neuroprotective properties of daminin were determined by measuring its effect on the changes of [Ca2+]i levels in...
neuronal cells, using glutamic acid and NMDA (neuroexcitatory agonists) as positive controls. Upon treatment with either glutamic acid (200 µM) or NMDA (200 µM) alone, the [Ca2+]i level in neuronal cells was increased by 305% and 235% respectively. However, if the neuronal cells were pre-treated with 0.5, 1.0 or 3.0 µg/mL of daminin for 5 min, the increase in [Ca2+]i level induced by glutamic acid dropped to 58.1%, 65.4% and 25.1% respectively; daminin (1.0 µg/mL) also suppressed the increase in neuronal [Ca2+]i level induced by NMDA to 63.5%. These findings indicated that daminin displayed pronounced neuroprotective properties.

6. Multiple sources

6.1. Trigonelline

Trigonella foenum-graecum L. (fenugreek) of the Leguminosae family is a herbal plant that has been used for centuries to treat a wide range of ailments including diabetes, fever, memory loss, epilepsy and migraine. The vitamin B6 derivative trigonelline (Fig. 35) is a N-methylnicotinic acid that has been isolated from the seeds of fenugreek, a legume crop used as a spice and medicines in East Asia and Northern Africa. Trigonelline occurs in raw coffee beans and is converted into nicotinic acid upon roasting at ~230 °C. Trigonelline is a water-soluble secondary metabolite formed from nicotinate (Fig. 35) and is responsible for the bitterness in coffee. Many studies on the biological activities of trigonelline in animals and a variety of cell systems are available, those with psychopharmacological properties relevant to the subject of this review are included herein.

Extension of dendrites and axons in neurons can compensate neural loss and repair damaged neuronal network in people with dementia. Komatsu and co-workers demonstrated that trigonelline extracted from coffee beans exhibits functional neurite outgrowth activity by inducing axonal extension in human neuroblastoma SK-N-SH cells. Trigonelline (30 µM) significantly increased the percentage of human SK-N-SH cells with neurites >50 µm by 15% after three days of treatment. In a subsequent study, the same group also revealed that upon oral administration of trigonelline (500 mg/kg; q.d.; 15 days), male ddY mice pre-treated with Aβ (5 nmol) were able to complete more successful crossings over a previous platform position in the water maze test, indicating an improvement in memory retention. Trigonelline also displays other CNS-related properties, including protection against cerebral ischemia by decreasing neuronal spike frequency from single action potential to multiple firing (0.1 mM), stimulation of dopamine release (136% at 4.977 µM), competitive inhibition of GABA_A receptors (K_i = 13 nM), and weak inhibition of AChE (IC_50 = 233 µM). Taken together, these findings indicate that trigonelline exhibits pronounced neuroprotective properties that warrant further investigation.

7. Conclusions

In summary, pyridine alkaloids possess a diverse array of properties in the CNS that validate this class of natural products as a source of potential therapeutic leads for CNS disorders. Many pyridine alkaloids structurally related to nicotine are themselves nAChR agonists, including cytisine and epibatidine, with the former providing the basis for the development of Varenicline for smoking cessation. As a result of their efficacious stimulation of neurotransmitters at the nAChRs, several nicotine derivatives have become pharmaceutical leads for dementia. For instance, cotinine improves the ED50 of cholinergic medications when taken adjunctively; the metanicholine core structure provided a safer scaffold for the development of new AD therapeutic agents with a good safety profile. The structural diversity of the acetylcholinesterase inhibitors (AChEIs) is striking, which includes the huperzines, anabasine, the multijuguinones and the platisidines. AChEIs block ACh hydrolysis and hence comprise potential new leads for the symptomatic treatment of dementia. Huperzine A has been approved as an AD drug in China and is sold as a dietary supplement in the USA. An array of pyridine alkaloids are promising leads for the prevention and the treatment of neurodegenerative disorders as they possess neurotrophic and neuroprotective properties, such as militarione A, arthpyrone C, (25)-N-hydroxybenzylanabasine, casuarine H, paeclimide, coprismycin A/B, trigonelline, daminin, euphorbiaioids, aspermgin B and cantleyine. This class may be useful for the treatment of mood disorders as many bind receptors and enzymes linked to neurotransmitter levels, including serotonin (agoneligne, cerpegin), dopamine (fusaric acid, phenipolocinic acid, cerpegin) and glutamate (acromelic and acromelobic acids). The cyclostellettamines are nAChR antagonists that inhibit the activation of neuronal potentials in the nervous system and are therefore potential therapeutic leads for various CNS-related disorders, including Parkinson’s and AD. Many of the pyridine alkaloids described herein have pronounced effects in the CNS, but their exact mode of action is not well understood. For example, haplophyllidine possesses sedative and anti-analeptic properties, while gentianine is a CNS stimulant with a promising antipsychotic profile. We hope that this review has provided thought-provoking insight into the therapeutic utility of pyridine alkaloids for the treatment for CNS disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1 Goyal K, Koul V, Singh Y, Anand A. Targeted drug delivery to central nervous system (CNS) for the treatment of neurodegenerative disorders: trends and advances. Con Nerv Syst Agents Med Chem. 2014;1(4):41–58.
2 Jain KK. Drug delivery to the central nervous system. Springer; 2010.
3 Kumar V. Potential medicinal plants for CNS disorders: an overview. Phytother Res. 2006;20(12):1023–1035.
4 Lane TE, Carson M, Bergmann C, Wyss-Coray T. Central nervous system diseases and inflammation. Springer; 2007.
5 Lee DC, Song H, Colamarino SA, Ming G, Gage FH. Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu Rev Pharmacol Toxicol. 2004;44:399–421.
6 Misra A, Ganeh S, Shahihalla A, Shah SP. Drug delivery to the central nervous system: A review. J Pharm Sci. 2003;92(2):252–273.
7 Mansoor N, Nordin N, Mohamed F, Ling KII, Rosli R, Hassan Z. crossing the blood-brain barrier: A review on drug delivery strategies for treatment of the central nervous system diseases. Curr Drug Deliv. 2019;16(8):698–711.
8 Pangalos MN, Schencher LE, Hurko O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. Nat Rev Drug Discov. 2007;6(7):521–532.
9 Di Nunzio JC, Williams III RO. CNS Disorders—Current Treatment Options and the Prospects for Advanced Therapies. Drug Dev Ind Pharm. 2008;34(11):1141–1167.
10 Nichols E, Szeoke CE, Vollnet SE, et al. Global, regional, and national burden of Alzheimer’s disease and other dementias, 1990–2016: A systematic analysis for the global burden of disease study 2016. Lancet Neurol. 2019;18(1):88–106.
11 Fazel S, Khosla V, Doll H, Geddes J. The prevalence of mental disorders among the homeless in western countries: systematic review and meta-regression analysis. PLoS Med. 2008;5(12):e225.
The structures of huperzine A and B, two new alkaloids from the roots of <i>Platycodon grandiflorus</i> and <i>Huperzia serrata</i>. 

Liu J-S, Zhu Y-L, Yu C-M, et al. J Nat Prod. 2013;76(6):1058-1063.

Pharmacological profile of huperzine A, a novel cholinesterase inhibitor, on the central cholinergic system of the Rat. 

J Neurosci Res. 1986;16(3):223-231.

Tang X, De Sarno P, Sugaya K, Giacobini E. Effect of huperzine A, a new cholinesterase inhibitor, on Alzheimer’s disease: An assessment on chemistry, pharmacology, and clinical studies. 

CNS Drugs. 1994;14(2):146-161.

Tang X, De Sarno P, Sugaya K, Giacobini E. Effect of huperzine A, a new cholinesterase inhibitor, on Alzheimer’s disease: An assessment on chemistry, pharmacology, and clinical studies. 

CNS Drugs. 1994;14(2):146-161.

Veit HS, Koenig ML, Dave JR, Doctor BP. Huperzine A, a potential therapeutic agent for dementia, reduces neuronal cell death caused by glutamate. 

NeuroReport. 1997;8(9):1463-1467.

Gordon RK, Nigmat SV, Weitz JA, Dave JR, Doctor BP, Veit HS. The NMDA receptor ion channel: A site for binding of huperzine A. 

J Appl Toxicol. 2001;21(5):547-551.

Wang T, Tang XC. Reversal of scopolamine-induced deficits in radial maze performance by (−) huperzine A and its relationship with E2020 and tacrine. 

Eur J Pharmacol. 1998;349(2-3):137-142.

Gao Y, Tang X, Guan L, Kuang P. Huperzine A reverses scopolamine- and muscimol-induced memory deficits in chick. 

Acta Pharm Sin. 2008;20(12):1169-1173.

Zhao Y, Tang XC, Cai J, Jing Y, et al. Huperzine A on working memory in reserpine- or yohimbine-treated monkeys. 

Eur J Pharmacol. 2001;433(2-3):151-156.

Zhang D-B, Chen J-J, Song Q-Y, Zhang L. Lycodine-type alkaloids from <i>lycopodium cauvaria</i> and their acetylcholinesterase inhibitory activity. 

J Nat Prod. 2011;74(9):1389-1394.

Jia-Sen L, Mei-Fen H. The alkaloids huperzines C and D and HUPERZINE FROM <i>Huperzia serrata</i>. 

Pharm Biol. 2007;45(3):276-285.

Lamy FP. The role of cholinesterase inhibitors in Alzheimer’s disease. 

CNS Drugs. 1994;14(2):146-161.
