Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Bioorg. Med. Chem. 28 (2020) 115820

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Pyridine alkaloids with activity in the central nervous system

Simon X. Lin [a], Maurice A. Curtis [b,c], Jonathan Sperry [a,*]

[a] School of Chemical Sciences, University of Auckland, Auckland, New Zealand
[b] Centre for Brain Research, University of Auckland, Auckland, New Zealand
[c] Department of Anatomy and Medical Imaging, University of Auckland, Auckland, New Zealand

ARTICLE INFO

Keywords:
Natural products
Pyridine
Alkaloids
CNS

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 benzo-fused (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

* Corresponding author.
E-mail address: j.sperry@auckland.ac.nz (J. Sperry).

https://doi.org/10.1016/j.bmc.2020.115820
Received 27 August 2020; Received in revised form 27 September 2020; Accepted 5 October 2020
Available online 16 October 2020

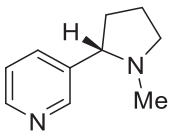


Fig. 1. Nicotine.

(Fig. 1), was isolated from dried *N. tabacum* leaves by Posselt and Reimann.[21] The chemical structure of nicotine was later established by Pictet and Crépieux through total synthesis in 1895.[22] Nicotine is also present (albeit in lower amounts) in other genera of the *Solanaceae* family, such as potatoes, tomatoes and green peppers.[19,20] Today, *N. tabacum* is cultivated in over 120 countries worldwide, where it is used to make cigarettes and as the source of nicotine for replacement therapy (NRT).[20] Many reviews on the biological activities of nicotine in animals and a variety of cell systems are available.[17–20]

Upon smoking tobacco, nicotine is carried to the lungs, where it quickly enters the bloodstream and into the brain.[19] The effects of nicotine include heightened arousal, reduced stress and anxiety, energy increase and enhanced pain thresholds.[17–20] Nicotine has also been shown to improve learning, problem-solving ability, reaction time, selective attention and vigilance in those performing repetitive tasks.[19,20] These positive reinforcing effects induced from acute nicotine administration are critical factors in tobacco addiction.[17–20] 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.[17–20] 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.[17–20] 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.[20] 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.[17–20] In particular, the release of dopamine in the mesolimbic system leads to the rewarding effects associated with nicotine use.[19,20] Picciotto and Zoli have demonstrated that knocking out the α4β2-subunit gene in rats eliminated the effects of nicotine and the release of dopamine.[20,23] In related studies, the α3β4-nAChR is implicated in the cardiovascular effects of nicotine[20,24] and the α7-nAChR is involved in learning, memory and sensory gating.[20,25]

Systematic nicotine administration increases the levels of opioid peptides in the nucleus accumbens, leading to analgesic effects.[20,26] *In vivo* experiments have demonstrated that the reinforcing effects of nicotine in mice were eliminated upon co-administration with naltrexone (a non-selective opioid receptor antagonist); mice with their μ-opioid receptor gene removed exhibited reduced analgesic effects upon nicotine administration, suggesting endogenous opioids are involved in the rewarding properties of nicotine.[26] In more recent studies, it was proposed that nicotine also binds to *N*-methyl-*D*-aspartate (NMDA) receptors, causing a release of dopamine in the nucleus accumbens.[20,27] Upon acute nicotine administration in rats, the levels of glutamate were increased in the ventral tegmental area due to the activation of nAChRs located pre-synaptically on the glutamatergic nerve terminals.[27] The increase of glutamate induces firing of dopaminergic neurons and thus elevates dopamine levels.[20,27] However, when dizocilpine (a non-competitive NMDA receptor antagonist) was co-administered, the effects of nicotine were attenuated, inferring that the nicotine-NMDA receptor interaction is indeed important for the addictive properties of nicotine.[27]

Tobacco addiction is a huge global health concern, causing >7 million smoking-related deaths worldwide in 2017.[28] The World Health Organization (WHO) has reported that >1.3 billion people smoke tobacco daily, and the cost for the treatment of nicotine related health conditions in the USA is ~US$155 billion per annum.[20] Pure nicotine is used in NRT as a smoking cessation agent to help abate tobacco withdrawal.[17–20,29] Nicorette® was approved as an NRT by the US Food and Drug Administration (FDA) in 1984 as a patch, gum, tablet and spray that enables controlled levels of nicotine to be administered during smoking cessation.[20,29] There is some evidence that nicotine consumption reduces the risk of developing neurodegenerative diseases (e.g. Parkinson's disease)[30] and mood disorders (e.g. anxiety and depression).[31] Recently, preliminary investigations have reported lower rates of SARS-CoV-2 (COVID-19) infection among smokers.[32–34]

Several natural products structurally related to nicotine have also been isolated from a variety of sources; many reviews on their biological activities are available, thus only key points relevant to the subject of this account are included herein.

2.2. Nornicotine

Nornicotine (Fig. 2) is a minor peripheral metabolite of nicotine in various mammal species (e.g. humans, monkeys and rodents).[35,36] This pyridine alkaloid possesses a demethylated pyrrolidine ring and was extracted from *N. glutinosa* by Ehrenstein in 1931.[37] 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).[38–40] Kisaki and Tamaki established that both *S*-(−)- and *R*-(+)-nornicotine are present in *N. tabacum* L.[41]

Nornicotine displays significant agonist properties at nAChR subtypes in the CNS.[35,42] Dwoskin and co-workers have demonstrated that nornicotine evokes the release of dopamine by stimulating nAChRs from the dopaminergic presynaptic terminals in a concentration dependent manner.[42] Both mecamylamine and dihydro-β-erythroidine (nonselective nAChR antagonists) inhibited the [^{3}H]-overflow effect of nornicotine (<100 μM) on rat striatal slices with preloaded [^{3}H]-dopamine.[42] However, the inhibitory activities of both nAChR antagonists were not observed when the nornicotine concentration was increased (>100 μM), suggesting a nAChR-mediated mechanism was involved.[42] The effect of (−)-nornicotine 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.[42] Subsequently, Papke and co-workers demonstrated that nornicotine is more potent at the rat α7-nAChR ($EC_{50} = 17.4$ μmol/L) than at the α4β2- ($EC_{50} = 375$ μmol/L) and α3β4- ($EC_{50} = 614$ μmol/L) subtype receptors.[35] These findings imply that nornicotine contributes to the neuropharmacological effects of tobacco smoking via a nicotinic receptor stimulation.[35,42]

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;[36,43,44] its chemical structure was established by Smith in the same year through total synthesis.[45] 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)

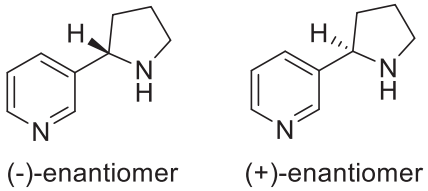


Fig. 2. (−)- and (+)-Nornicotine.

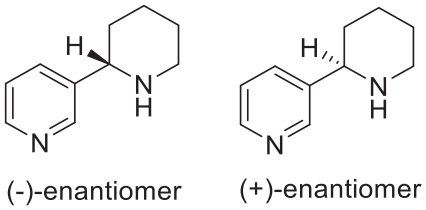


Fig. 3. (−)- and (+)-Anabasine.

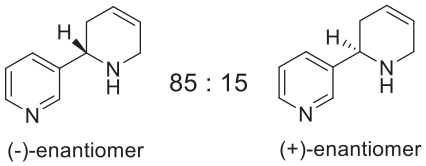


Fig. 4. (−)- and (+)-Anatabine.

leaves;[38,46] small traces of anabasine have also been detected in hoplonemertines, *Messor* and *Aphaenogaster* ants.[43,47]

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.[46] The binding affinities (K_i) and the agonist potencies (EC_{50}) of the two anabasine enantiomers at rat α4β2- and α7-nAChRs were examined.[46] Using nicotine (K_i = 0.0056 μM; EC_{50} = 19 μM) as a comparison, *in vitro* experiments have demonstrated that (−)-anabasine is a more potent agonist at the α7-nAChR (K_i = 0.39 μM; EC_{50} = 18 μM) than at the α4β2-receptor (K_i = 1.1 μM; EC_{50} > 30 μM); however, (+)-anabasine binds more selectively at the α4β2-nAChR (K_i = 0.91 μM) than at the α7-receptor (K_i = 3.7 μM).[46] Subsequent toxicity analysis showed that (+)-anabasine (LD_{50} = 11 mg/kg) is more toxic than (−)-anabasine (LD_{50} = 16 mg/kg), implying that the stereochemistry affects both pharmacological activities and lethality.[46]

2.4. Anatabine

Anatabine (Fig. 4) is the second most abundant alkaloid (~4%) present in *Nicotiana* species.[36,48] 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.[49] Anatabine is a pyridine-dihydropyridine structurally related to anabasine.[48] Subsequent studies reported that anatabine exists as an 85:15 scalemic mixture of (−)- and (+)-enantiomers in *Nicotiana* species.[50]

The binding affinities (K_i) of (−)- and (+)-anatabine were evaluated at rat α4β2-nAChR by displacement of [^{3}H]-cytisine radioligand binding using anabasine and nicotine as comparisons.[48] Kem and co-workers demonstrated that (+)-anatabine (K_i = 119 nM) exhibited a higher binding affinity than (−)-anatabine (K_i = 249 nM), ~8- and 4-fold more potent than anabasine (K_i = 910 nM) respectively;[48] the nicotine K_i value was found to be ~2 nM.[48] In the same study, the agonist potency (EC_{50}) and ACh stimulation efficacies (I_{max}) of both anatabine enantiomers at human α4β2- and α7-nAChRs were also examined.[48] At the α4β2-subtype receptor, (−)-anatabine (EC_{50} = 2.65 μM; I_{max} = 43.2%) was ~2-fold more efficacious than (+)-anatabine (EC_{50} = 0.74 μM, I_{max} = 25.0%); much higher efficacies were observed at the α7-receptors for both (−)-anatabine (EC_{50} = 69.7 μM; I_{max} = 113%) and (+)-anatabine (EC_{50} = 51.8 μM; I_{max} = 105%).[48] These findings suggest that anatabine is a selective and potent ligand at α7-nAChRs.[48] Recent publications have reported that anatabine also lowers the production of β-amyloid in human brain cells[48,51] and enhances memory and attention dysfunction in rats,[48,52] suggesting that anatabine may represent a potential therapeutic candidate for dementia disorders such as Alzheimer's disease (AD).[48,51,52]

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;[53] its chemical structure was affirmed through total synthesis in the same study.[53] Although structurally similar to nicotine and anatabine, 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;[54] 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).[54] 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.[55] The agonist potency (EC_{50}) and the maximal receptor response (I_{max}) of *N*-methylanatabine (EC_{50} = 6.2 μM; I_{max} = 26%) at the human α4β2-receptor were ~8- and 6-fold less active than nicotine (EC_{50} = 0.8 μM; I_{max} = 159%) respectively.[55]

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).[56–59] Between 70% and 80% of the nicotine consumed by humans is oxidized to cotinine by cytochrome P450 2A6 (CYP2A6) and cytoplasmic aldehyde oxidase.[57–59] The chemical structure of cotinine was initially proposed by Pinner[60] in 1893 and later confirmed by Frankenburg and Vaitekunas[61] in 1957 through oxidative degradation. Subsequent phytochemical investigations revealed that cotinine is also present in *Nicotiana* plants (e.g. *N. tabacum*)[61] and *Duboisia hopwoodii*.[62] Several reviews and studies on the pharmacological properties of cotinine are available.[56,58,59,63,64]

Cotinine crosses the blood brain barrier and acts as a low affinity nAChR agonist at brain receptors.[56,65] Vainio and co-workers demonstrated that cotinine displays weak binding affinity (K_i) and agonist potency (EC_{50}) at rat brain nAChRs labelled with [^{3}H]-epibatidine when compared with nicotine.[65] Cotinine (K_i = 3.0 μM; EC_{50} = 21 μM) was ~270-fold less active than nicotine (K_i = 11 nM; EC_{50} = 77 nM) at competing for nAChRs binding sites with [^{3}H]-epibatidine (250 pM) from rat frontal cortex;[65] a similar pattern was observed in rat hippocampus cells (data not shown).[65] These findings indicated that cotinine exhibits weak binding potency to nAChRs in the CNS and mediates its various pharmacological effects upon voluntary nicotine administration.[65]

In a subsequent study, the specific receptor subtype that cotinine primarily acts on was examined by Terry Jr and co-workers.[63] More than seventy neurotransmitter receptors, transporters and enzymes (including dopamine $D_{1\text{-}4}$, adrenergic α1-2, $GABA_{A\text{-}B}$, glutamate, histamine $H_{1\text{-}3}$, muscarinic acetylcholine receptor $M_{1\text{-}5}$, opioid, acetylcholine$_{1\text{-}7}$, 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.[63] 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.[56,63] Subsequent behavioural studies also showed that cotinine (1.0–10 mg/kg) increased the

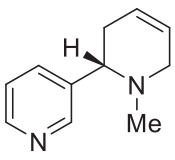


Fig. 5. *N*-methylanatabine.

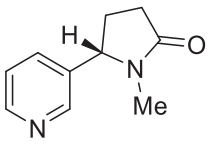


Fig. 6. Cotinine.

exploration time in rats when it was co-administered (intraperitoneal injection) with donepezil (0.5 mg/kg).[63] 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.[56,58,63–65] 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).[58] 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),[66,67] thus implicating cotinine as a potential therapeutic agent for attention deficit hyperactivity disorder (ADHD) and neuropsychiatric disorders (e.g. anxiety, depression and psychosis).[58,66,67] It was also shown that cotinine is efficacious in treating dementia-related memory impairments;[68] 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.[68] These findings indicate that the neuroprotectivity and the absence of toxicity exhibited by cotinine is due to its agonist property at the α7-nAChRs.[56,58,63–68] 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;[69] the levels of serotonin and noradrenaline in rat brains increased when cotinine (2 mg/kg) was given in repeated doses,[70] suggesting potential use as an antidepressant agent through activation of α7-nAChRs.[58] Taken together, cotinine displays a safer therapeutic profile than nicotine due to its much longer half-life and a lower risk of abuse.[56,58,59,64,66–68] Cotinine induces positive changes in synaptic plasticity which warrant further investigations.

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;[71] structural elucidation was affirmed by total synthesis and this 3,5-dinicotine alkaloid was consequently named DINIC (Fig. 7) due to the presence of the two *N*-methylpyrrolidine rings.[71] The pharmacological activities of DINIC was investigated by evaluating its ability to displace the binding of [^{3}H]-nicotine and [^{3}H]-methyllycaconitine at rat α4β2- and α7-nAChRs respectively.[71] DINIC displayed potent binding affinity (K_i = 1.18 μM) and inhibited the effect of [^{3}H]-nicotine at the α4β2-nAChR in the [^{3}H]-dopamine release assay (<64% inhibition at 100 nM);[71] no inhibition on [^{3}H]-methyllycaconitine binding at the α7-nAChR was observed, indicating that DINIC is selective for the α4β2-receptor.[71]

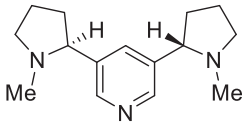


Fig. 7. DINIC.

2.8. Metanicotine

Metanicotine (Fig. 8), also known as Rivanicline, TC-2403 and RJR-2403, is a nicotine alkaloid examined as a potential therapeutic candidate for the treatment of neurodegenerative disorders, including AD.[72,73] The chemical structure of metanicotine was first assigned by Pinner in 1895 through the degradation of nicotine.[74] In 1953, Wahl reported the natural occurrence of metanicotine as the biological degradation of nicotine upon its isolation from fermented tobacco.[75] Subsequent phytochemical studies revealed that metanicotine is also present in *Solanaceae* plants (e.g. *N. tabacum* and *D. hopwoodii*)[62,76] and tobacco smoke.[77]

The pharmacological properties of metanicotine in the CNS were characterized by Bencherif and co-workers in 1996.[72,78] The *in vitro* receptor binding studies using [^{3}H]-nicotine radioligand displacement showed a high binding affinity exhibited by metanicotine at the α4β2-nAChRs in rat brains (K_i = 26 nM);[72,78] at other receptor sites (i.e. angiotensin II, cholecystokinin$_{A-B}$, endothelin ET_{A-B}, muscarinic acetylcholine receptor M_{1-3}, histamine H_3, bradykinin B_2, leukotriene B_4, neurokinin NK, phencyclidine, neuropeptide Y_2, thromboxane A_2, dopamine D_{3-4}, NMDA, 5-HT_{1A-3} and sodium channel 2), metanicotine was a poor competitive inhibitor and failed to displace [^{3}H]-nicotine ligand (IC_{50} > 10 μM).[72,78] The potency (EC_{50}) and efficacy (E_{max}) of metanicotine in evoking [$^{86}Rb^+$] release from rat thalamic synaptosomes (EC_{50} = 732 nM; E_{max} = 79%) were slightly less active than nicotine (EC_{50} = 591 nM; E_{max} = 87%) when compared with the full agonist tetramethylammonium at 300 μM.[72,78] The ability of metanicotine to induce dopamine release from rat striatal synaptosomes (EC_{50} = 1.2 μM; E_{max} = 81%) is equally efficacious and potent as ABT-418 (EC_{50} = 1.1 μM; E_{max} = 91%), a neuroprotective anxiolytic agent used in the treatment of AD and ADHD; but ~10-fold less potent than nicotine (EC_{50} = 100 nM; E_{max} = 113%).[72,78]

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.[72,78] The agonist properties of metanicotine were evaluated in *Xenopus oocytes* expressing the rat α4 and β2 subunits using nicotine and ACh for comparison. At concentrations of 10 and 100 μM, metanicotine produced a higher peak activation (492%) than both nicotine (333%) and ACh (242%), suggesting that metanicotine is a highly potent agonist at the α4β2-nAChR.[72,78] The physiological and behavioural profiles of metanicotine administration were investigated using the passive avoidance test, water-navigation performance and radial arm maze performance in rats.[72,78] 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 mecamylamine-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.[72,78] Acute toxicity studies indicated that metanicotine is much less toxic than nicotine after single or repeated doses in rats and dogs (LD_{50} = 1.8 mol/kg).[72,78] 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.[72,78] 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.

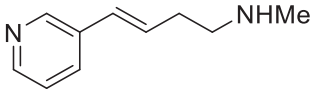


Fig. 8. Metanicotine.

(USA).[79,80] However, the development was discontinued in the pre-clinical phase in 2001 due to undesired adverse effects.[79] Subsequently, metanicotine (as TC-2403) was examined for ulcerative colitis (a chronic inflammatory bowel disease) due to its ability to inhibit the production of Interleukin-8.[80] Clinical trials of metanicotine 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.[79,80] No published reports on the efficacy or pharmacokinetics of metanicotine in humans is currently available.[80]

2.9. N-Hydroxybenzylanabasine

Alangium chinense (Lour.) is a Chinese deciduous shrub commonly used in traditional Chinese medicine.[81] Powdered *A. chinense* roots were found to contain (2*S*)-*N*-hydroxybenzylanabasine (Fig. 9), an *N*-substituted analogue of anabasine. Subsequent biological activity investigations indicated that (2*S*)-*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).[81]

2.10. Anabasamine

An alkaloid named anabasamine (Fig. 10), possessing a 2,3′-bipyridyl scaffold bonded to a piperidine ring, was isolated from the seeds of *Anabasis aphylla* (Central Asian shrub) by Mukhamedzhanov and co-workers in 1967.[82] In a subsequent phytochemical study investigating the inhibition of cholinesterases, anabasamine was found to exhibit weak but selective anti-acetylcholinesterase properties.[83] The binding affinity (K_i) of anabasamine at human blood erythrocyte acetylcholinesterase was ~8.6-fold more effective than at horse blood serum butyrylcholinesterase (51 μM *vs* 440 μM).[83]

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.[84–89] 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.[84–89] In several phytochemical studies examining the biological active secondary metabolite of *L. anagyroides*,[90,91] the aqueous extract of the seeds was found to contain cytisine (Fig. 11), a quinolizidone alkaloid fused to a bispidine ring with absolute configuration later assigned as 1*R*,5*S* through stereoselective total synthesis.[92] 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%).[93–95] Pure cytisine has been used as a respiratory analeptic, diuretic and an insecticide in Europe.[84–89] Several detailed reviews on the biological properties of cytisine and its therapeutic applications are available.[84–89]

The binding affinity (K_D) and the maximum number of binding sites

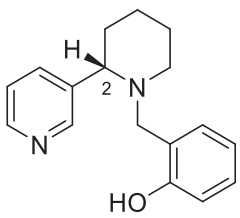


Fig. 9. (2*S*)-*N*-hydroxybenzylanabasine.

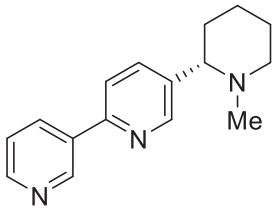


Fig. 10. Anabasamine.

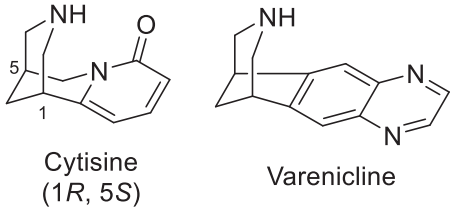


Fig. 11. Cytisine and Varenicline.

(B_{max}) of cytisine at nAChRs have been examined in whole rat brains.[96] Cytisine has a significantly higher binding affinity (K_D = 0.145 nM) when compared with nicotine (K_D = 0.89 nM); with a similar binding density between the two compounds (B_{max} = 99.1 and 114.5 fmol/mg, respectively).[96] Cytisine displays a million-fold binding specificity for nAChRs (K_i = 0.16 nM) over muscarinic acetylcholine receptors (K_i > 400 μM).[84,96] Cytisine binds with high density in the thalamus of both rat and human brain, where the α4β2-subtype is the predominant nAChRs.[96] 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);[96,97] at the α3β4- and α7-subtype receptors, the binding affinity of cytisine were 840 nM and 4200 nM respectively.[97,98] 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.[98] Coe and co-workers[98] 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-efficacy partial α4β2-nAChR agonist.[84–89] The effect of cytisine on dopamine release in rat striatum has also been studied.[99] 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%.[99] The neuroprotective properties displayed by cytisine may be beneficial in neurodegenerative disorders.[84–89,99,100] In a related study, Picciotto and co-workers observed antidepressant-like properties of cytisine in mouse models.[101] Upon cytisine treatment (1.5 mg/kg), mice showed similar acute antidepressant-like responses in the tail suspension and the forced swim tests when compared with mecamylamine (a nonselective nAChR antagonist that has shown antidepressant effects), inferring that the antidepressant-like efficacy of cytisine is a result of its partial agonist property in which it competitively inhibits ACh signalling through α4β2-nAChRs.[84,101]

Given that cytisine is a selective α4β2-nAChR partial agonist that induces dopamine release and displays antidepressant-like effects, it became a therapeutic candidate as a smoking cessation agent.[84–89,100] 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

that include irritability, depression, insomnia and fatigue.[85–89,100] Unsystematic clinical trials during the 1960 s and 1970 s in Central and Eastern Europe suggested that cytisine maintains smoking abstinence superior to placebo[84–86,88] and as a result, cytisine was marketed in pill form (Tabex®) in 1964 to treat smoking dependence in Bulgaria, East Germany, Poland and Russia.[85–89] However, cytisine displays low efficacy due to limited crossing of the blood–brain barrier;[84–89,100] it was also reported to cause adverse effects, including nausea, vomiting and sleep disorders.[85–87,89] Therefore, the use of cytisine as a smoking cessation aid in humans is not approved by the FDA or the European Union (EU), but is still available in some European countries.[84–89,100] 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.[84–89,98,100] Varenicline displays a selective binding affinity (K_i = 0.06 nM) and potent partial agonist activity (EC_{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.[98] Full scale clinical trials indicated that varenicline possesses a similar partial agonist profile at the α4β2-nAChR as cytisine in humans.[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.[100] Varenicline displays a greater efficacy than bupropion (an atypical antidepressant and nicotinic receptor antagonist) and NRTs in sustaining abstinence from smoking.[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.[100]

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

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

2.12. Huperzines

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

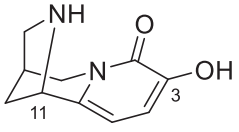


Fig. 12. 3-Hydroxy-11-norcytisine.

The cholinesterases (ChE) are a family of enzymes present in the CNS that break down choline-based esters.[113,114] Two types of ChE have been characterized; acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).[113] 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.[113,114] ACh is a neurotransmitter found predominantly in the human brain and has an important role in arousal, attention, memory and motivation.[114] AD patients have lower ACh levels due to age related degeneration of their cholinergic system and/or brain injuries.[113] The cholinergic hypothesis of AD suggests a strategy to treat neurodegeneration is to restore ACh deficiency.[114] Therefore, AChE inhibitors are served as cognition enhancing agents to treat patients with mild to moderate AD, including tacrine, donepezil, physostigmine and rivastigmine.[113,114]

The inhibitory activity of huperzine A has been examined against both AChE and BuChE *in vitro*.[115] The inhibitory activity on AChE induced by huperzine A (IC_{50} = 0.082 μM) was slightly more potent than tacrine (a nonselective ChE inhibitor; IC_{50} = 0.093 μM), but ~8-fold weaker than the drug donepezil (a selective AChE inhibitor; IC_{50} = 0.010 μM).[115] The inhibitory activity on BuChE induced by huperzine A (IC_{50} = 74.43 μM) was ~15 and ~1000-fold less potent than donepezil (IC_{50} = 5.01 μM) and tacrine (IC_{50} = 0.074 μM) respectively.[115] These findings indicated that huperzine A displays high selectivity for AChE over BuChE.[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.[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 *in vitro* results.[115] The different administration routes clearly affect the bioavailability of huperzine A, with the ICV route facilitating access to the brain.[105–107,115] In a subsequent *in vivo* experiment, the level of ACh in the whole rat brain upon huperzine A administration was also measured.[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.[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.[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.[115] 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.[105–109]

The effect of huperzine A on glutamate-induced neuron toxicity was investigated by Ved and co-workers.[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

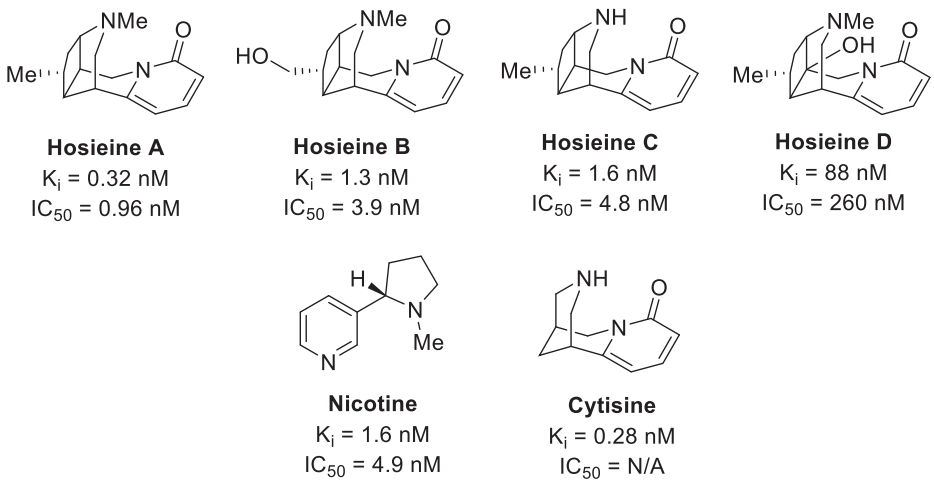


Fig. 13. Hosieines A–D with their binding affinities (K_i) and inhibitory concentration (IC_{50}) at the human α4β2-nAChR.

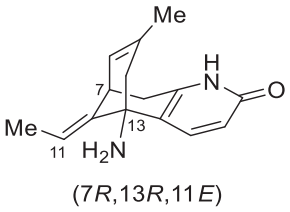


Fig. 14. Huperzine A.

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.[105–109,116] In the same study, the effect of huperzine A on calcium mobilization was also investigated.[116] 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).[116] These results inferred that huperzine A acts on glutamate receptors to exert neuroprotective properties on glutamate-induced toxicity.[105–109,116]

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.[117] Huperzine A (100 μM) did not inhibit the binding of [^{3}H]-glutamate (an agonist that binds to the NMDA agonist site), [^{3}H]-selfotel (an antagonist that binds to the NMDA agonist site), [^{3}H]-dichlorokynurenic acid (an antagonist that binds to the NMDA glycine regulatory site) or [^{3}H]-ifenprodil (an antagonist that binds to the NMDA polyamine regulatory site);[117] however, both [^{3}H]-dizocilpine and [^{3}H]-thienylcyclohexylpiperidine (non-competitive antagonists that bind to the NMDA ion channel) were displaced by huperzine A at $K_i = 5.6$ 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.[105–109,117] The NMDA-induced neuronal toxicity was prevented as the survival rate of cell culture increased from 35% to 85% upon pre-treatment with huperzine A.[117] Thus, huperzine A is a potent non-competitive NMDA ion channel antagonist and exerts neuroprotective properties by blocking NMDA-induced toxicity in neuronal cells.[105–109,117]

The effect of huperzine A on cognitive enhancement has been examined in several animal models (e.g. rats, chicks and monkeys).[105–109,118] Tang and co-workers demonstrated that both working and reference memory deficits induced by scopolamine (a mAChR antagonist commonly used to induce cognitive deficits) were significantly improved upon huperzine A administration.[106,108,118] The improvements were more pronounced on working memory than on reference memory.[118] Upon oral administration to aged rats, huperzine A was ~3.5 and 9-fold more potent at ameliorating memory impairments than donepezil and tacrine respectively, in agreement with the results reported by Tang and co-workers.[115] In a subsequent study, the cognitive enhancement induced by huperzine A was investigated on memory deficits induced by both scopolamine and GABA in chicks.[119] The results revealed that huperzine A was able to reverse the memory disruptions caused by scopolamine and GABA, suggesting huperzine A improved memory formation processes through both AChE inhibition and GABA receptor antagonism.[108,119] Cai and co-workers also established that huperzine A (0.01 mg/kg; intramuscularly) significantly improved the spatial working memory impairments induced by catecholamine depleting agent reserpine (0.1 mg/kg; intramuscular) in monkeys.[120] The results revealed an inverted U-shaped dose–response pattern, inferring that huperzine A putatively improves the working memory deficits through an adrenergic mechanism.[108,120]

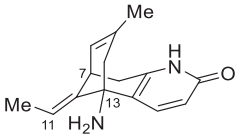
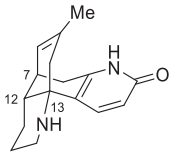
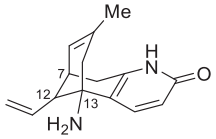
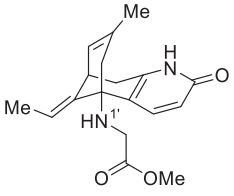
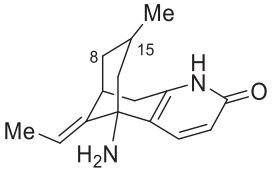
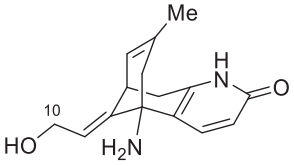
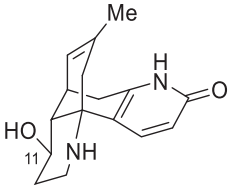
The LD_{50} of huperzine A lies between 2 and 4 mg/kg in female rats and >4 mg/kg in male rats.[105–109] Huperzine A is less toxic and induces less adverse effects than classic AChE inhibitors that are currently used to treat AD (e.g. donepezil and tacrine).[106,108] Memory tests that are used to measure the progression of AD are applied in clinical trials to assess the performance of a treatment;[106,108] 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.[105–109] 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.[106,108] However, the lack of randomization in these clinical trials and evidence-based literature on the safety and efficacy have prevented medical approval outside China.[105–109] Interestingly, huperzine A was not protected under patent upon its initial release and has been sold as a dietary supplement in the United States.[108]

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

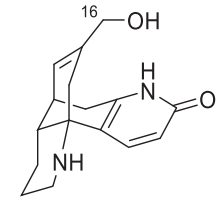
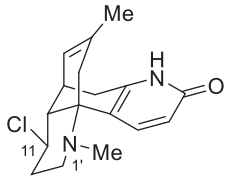
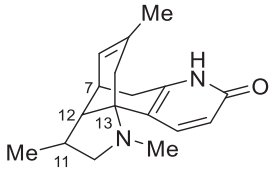
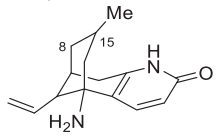
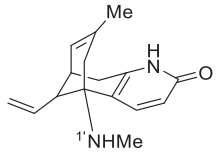
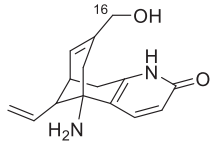
Senna multijuga is a popular ornamental plant in many Brazilian regions because of its brightly yellow coloured flowers.[129] This species belongs to the *Senna* genus which is renowned for their diverse

Table 1
Source and biological activity of huperzine derivatives.

Natural product	Source	Activity	Refs
 Huperzine A (7*R*,13*R*,11*E*)	*H. serrata*	AChE inhibitor (0.082 μM) BuChE inhibitor (74.43 μM) NMDA ion channel antagonist	105–120
 Huperzine B (7*S*,12*R*,13*R*)	*H. serrata*	AChE inhibitor (2.57 μM) BuChE inhibitor (169 μM)	106,107,110,121
 Huperzine C (7*S*,12*S*,13*R*)	*L. casuarinoides*	AChE inhibitor (0.6 μM)	121,122
 Huperserine E	*H. serrata*	AChE inhibitor (6.71 μM)	123
 8,15-dihydrohuperzine A (15*R*)	*H. carinata*	AChE inhibitor (2.63 μM)	124
 Carinatumin A	*L. carinatum*	AChE inhibitor (4.6 μM)	125
 Carinatumin B (11*R*)	*L. carinatum*	AChE inhibitor (7.0 μM)	125
	L. casuarinoides	AChE inhibitor (87.3 μM)	121

(*continued on next page*)

Table 1 (*continued*)

Natural product	Source	Activity	Refs
 16-hydroxyhuperzine B	*L. casuarinoides*	AChE inhibitor (20.9 μM)	126
 Casuarinine C (11*R*)	*L. casuarinoides*	AChE inhibitor (12.1 μM)	126
 Casuarinine I (7*R*,11*S*,12*S*,13*R*)	*L. casuarinoides*	Neuroprotective against H_2O_2-induced neuron damage in human SH-SY5Y cells (10 μM)	126
 Casuarinine H (15*R*)	*L. casuarinoides*	AChE inhibitor (1.9 μM)	121,127
 N-demethylhuperzinine	*L. casuarinoides*	AChE inhibitor (23.9 μM)	121,128
 Lycoparin C			

biological and pharmaceutical properties.[129,130] 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.[130] In a phytochemical study, two unusual 2-methyl-3-hydroxy-6-alkyl pyridine alkaloids, 7′-multijuguinone **1** and 12′-hydroxy-7′-multijuguinone **2** were isolated from the leaves of *S. multijuga*.[129] In a subsequent study,[130] 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.[129,130] 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.[129,130] 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).[129,130] 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.[129,130]

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.[131,132] In a phytochemical study on *E. prolifera*,[133] ten myrsinol diterpenes named euphorbialoids A–J and two previously reported congeners **1** and **2**[134] (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

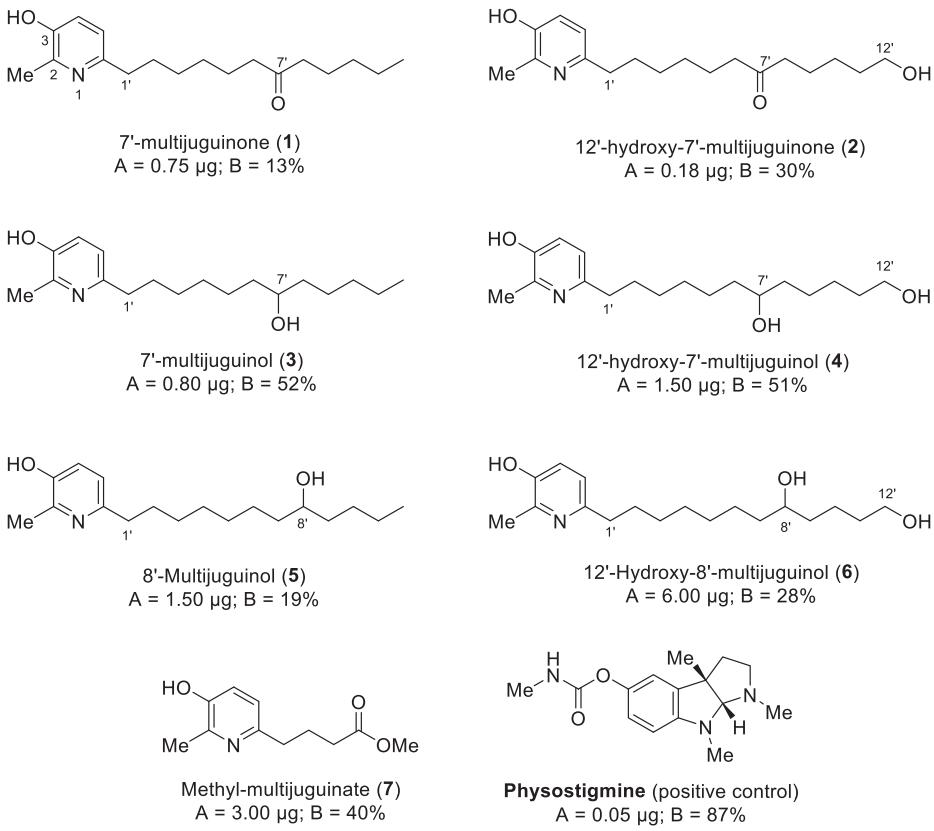


Fig. 15. Multijuguinones and their anti-AChE activity (A = Minimum amount for inhibition of AChE; B = AChE inhibition at 350 mM). Physostigmine is included for comparison.

myrsinane skeleton.[133]

Nitric oxide (NO) is a membrane-permeable gas that acts as a neuromodulator at the synaptic junctions.[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).[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.[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 μM),[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.[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.[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 *C. juncea*.[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.[136] Relevant to the subject of this review, cerpegin was found to exhibit dose-related analgesic properties against acetic acid-induced writhing in mice.[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.[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).[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.[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.[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 *S. trinervis* roots.[139] The effect of cantleyine against $CaCl_2$ on voltage dependent calcium channels was investigated.[139] It was reported that when the concentration of cantleyine was increased from 120 to 490 μM, $CaCl_2$-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.[139]

2.17. Haplophyllidine

The furopyridine alkaloid haplophyllidine (Fig. 19) was isolated from the seeds of *Haplophyllum perforatum* by Shakirov and coworkers.[140,141] Subsequent studies showed that this alkaloid is also present in the stems and leaves of *H. perforatum*.[140]

Haplophyllidine is a potent CNS depressant and synergizes the effects

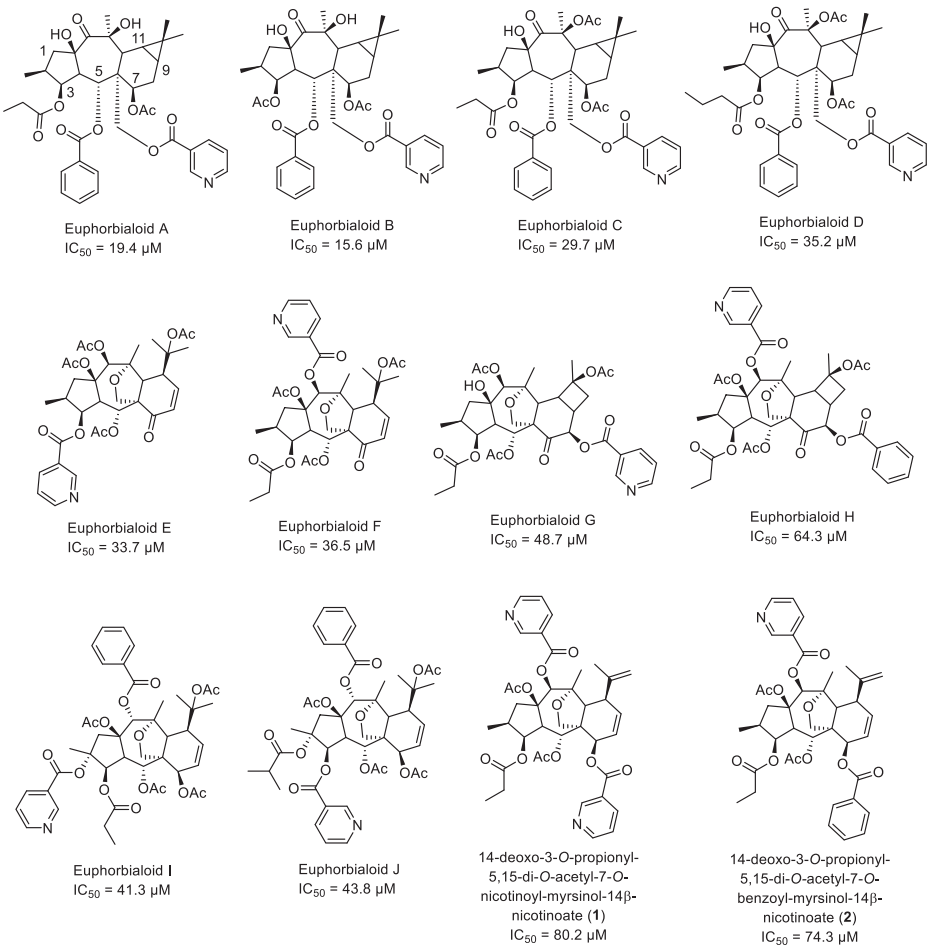


Fig. 16. Euphorbialoids with their inhibitory activity (IC_{50}) on LPS-induced NO production in BV-2 cells.

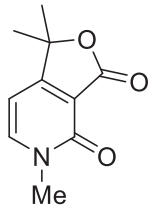


Fig. 17. Cerpegin.

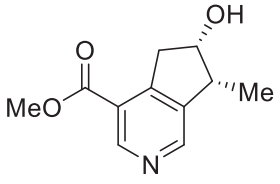


Fig. 18. Cantleyine.

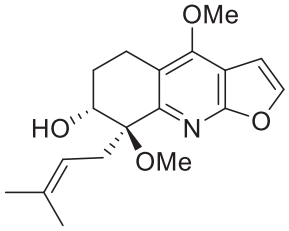


Fig. 19. Haplophyllidine.

of narcotic/hypnotic drugs in mice, rats and rabbits.[140,142] 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.[142] In a subsequent study, the antagonism properties of haplophyllidine against analeptic agents, including corazol, camphor, strychnine and caffeine were studied in mice.[143] At 25 mg/kg, haplophyllidine inhibited 50% of corazol-induced convulsions and completely eliminated the convulsion effects at 75 mg/kg; co-administration of camphor (525 mg/kg) and haplophyllidine (125–150 mg/kg) lowered the convulsion effects by 50% and lethality by 100%; 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).[143] These studies suggested that haplophyllidine exhibits pronounced sedative and anti-analeptic properties.[140,142,143]

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.[144,145] Gentianine has also been extracted from many other *Gentiana* and *Swertia* species of the *Gentianaceae* family, such as *E. litteorale* and *S. chirata*.[145]

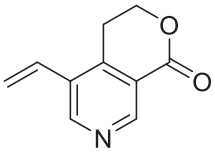


Fig. 20. Gentianine.

Initial biological observations of gentianine reported that the alkaloid acts as a CNS stimulant in low doses, but becomes paralytic in higher amounts.[146,147] The antipsychotic profile of gentianine was demonstrated by Bhattacharya and co-workers in 1974.[146] Gentianine (10–20 mg/kg; i.p.) diminished spontaneous motility and produced sedation/ptosis in rats; when dosage was increased (50–100 mg/kg; i.p.), hind-limb paralysis and catalepsy was observed, inferring that gentianine exhibits an antipsychotic activity.[146] Subsequent pharmacological studies evaluated the effects of gentianine on hexobarbital-induced hypnosis, amphetamine toxicity and lysergide-induced symptoms in rats.[146] Gentianine significantly potentiated the sleeping time induced by hexobarbital (100 mg/kg; i.p.) by 80%; amphetamine (20 mg/kg; i.p.) toxicity was reduced by 88% and 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 in mice.[146] The effects of gentianine on mice in a rotarod performance test, conditioned avoidance response and induced aggressive behaviour were examined.[146] Gentianine inhibited the ability of trained mice to remain on a rotating rod by 80%; it selectively blocked the avoidance response to the conditioned stimulus (buzzer) without affecting the escape response to the unconditioned stimulus (electric shock); aggressive behaviours induced by foot-shocks were inhibited by 60%.[146]

The effects of gentianine on morphine analgesia, the anticonvulsant action of diphenylhydantoin and pentylenetetrazol-induced convulsions have also been examined in rats.[146] Upon gentianine administration, the analgesic activity of morphine (2 mg/kg; i.p.) was increased by 150%; the anticonvulsant activity of diphenylhydantoin (2.5 mg/kg; i.p.) was potentiated by 60%; pentylenetetrazol (70 mg/kg; s.c.)-induced convulsions were inhibited by 70%.[146] The LD_{50} of gentianine was found to be 276 mg/kg; the alkaloid is thought to possess only a moderate to low order of toxicity due to the lack of obvious toxicity present upon prolonged i.p. administration (20 mg/kg; q.d.; 21 days) in rats.[146] Gentianine exhibits a broad range of interesting neuropharmacological properties that warrant further investigation with modern biological testing.[146,147]

3. Amphibian-derived

3.1. Epibatidine

Native to Central and South America, the poison dart frog *Epipedobates anthonyi* is renowned for its brightly coloured body and high toxicity.[148–150] Indigenous South American Indians apply the skin secretions to poison the tips of blow-dart weaponry for hunting and self-defence against large predators.[149] In a study examining the chemical constituents of the skin secretions, the methanolic extract of 750 *E. anthonyi* frog skins was concentrated and dried *in vacuo* to give a crude alkaloid fraction that upon purification gave (+)-epibatidine (Fig. 21), a structurally unprecedented alkaloid comprising a 2-chloropyridine bonded to an *exo*-7-aza-bicycloheptane.[150] The absolute configuration of (+)-epibatidine was assigned to be 1*R*,2*R*,4*S* through stereoselective total synthesis.[151,152] A detailed review on the bioactivity of epibatidine and its therapeutic potential has recently been published,[149] thus only key points will be included herein.

Amphibians produce biologically active alkaloids that have aided the development of lead compounds for the treatment of various

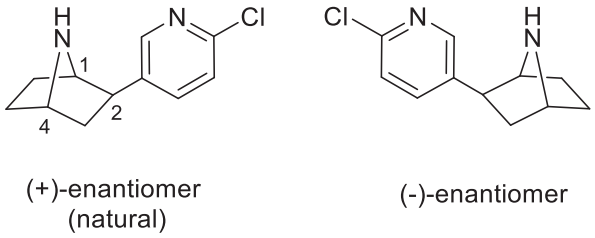


Fig. 21. (+)- and (−)-Epibatidine.

pathologies.[149] Therefore, upon the isolation of (+)-epibatidine, efforts to decipher its biological mechanism were forthcoming. (+)-Epibatidine was examined in both the Straub-tail response (generally used as a positive indicator of morphine-like mechanism) and heat nociception activity.[150] In these experiments, (+)-epibatidine was reported to be a potent anti-nociceptive, ~200 times more effective than morphine (Straub-tail response ED_{50}: 0.020 *vs* 10 mg/kg; hot plate analgesia ED_{50}: 0.005 *vs* 1 mg/kg, respectively).[150] Epibatidine was shown to have a non-opioid mode of action due to the binding at opioid receptors being ~9000-fold less potent than morphine (IC_{50} = 8800 nM *vs* 1 nM, respectively). 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.[148–150] 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.[153–155] 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.[149] In 1993, Qian and co-workers reported that epibatidine is a potent nAChR agonist.[153] A group of mice was divided in half and administered with either (−)-nicotine (5 mg/kg; positive control) or (+)-epibatidine (20 μg/kg).[153] 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.[153] 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),[153] 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 IC_{50} value of [^{3}H]-(+)-epibatidine required to displace [^{3}H]-cytisine (a potent α4β2-nAChR ligand) was 70 pM, ~100-fold greater than [^{3}H]-nicotine (IC_{50} = 7.8 nM).[153] However, at 10 μM, epibatidine failed to displace any specific ligands at a range of other neuronal receptors (i.e. $GABA_A$, benzodiazepine, dopaminergic, serotonergic, adrenergic, glutamate/aspartate, neurokinin, bradykinin, cholecystokinin and calcium gene-related peptide).[153] These detailed studies provide very strong evidence that epibatidine exerts its anti-nociceptive effects through nAChR agonism.[148,149,153]

There are seventeen nAChR subtypes in vertebrates and sixteen in humans, with three different receptor sites abundant in the mammalian brain (α4β2-, α3β4- and α7-nAChR).[148,149] A series of experiments were conducted to investigate the binding selectivity of epibatidine at these different nAChR subtypes.[155] Using (−)-nicotine as the control, Gopalakrishnan and co-workers re-evaluated the binding affinity (K_i) and the agonist potency (ED_{50}) of (+)-epibatidine at four different nAChR subtypes (α4β2-, α3β4-, α7- and α1β1δγ-subunits) to establish receptor binding specificity (Table 2).[155] The α1β1δγ-nAChR was also selected as part of the investigation as it is a commonly expressed nAChR at neuromuscular junctions.

Table 2
Binding affinity and agonist potency of (+)-epibatidine and (−)-nicotine at different nAChR subtypes (nM).

	α4β2		α3β4		α7		α1β1δγ	
	K_i	ED_{50}	K_i	ED_{50}	K_i	ED_{50}	K_i	ED_{50}
(+)-Epibatidine	0.05	17	0.23	19	2.2	1100	2.5	1600
(−)-Nicotine	1.0	4000	5.0	14,000	2000	40,000	10,000	250,000

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 at the muscarinic acetylcholine receptor (mAChR) M_1-subtype at high doses.[148,154] There are five mAChR subtypes (M_1–M_5) in humans, but only the M_1-subtype has been found in the brain.[156] Rupniak and co-workers investigated the binding affinity of epibatidine at M_1-mAChR and nAChRs using a radioligand binding assay.[154] Epibatidine displaced [^{3}H]-pirenzepine (a selective M_1-mAChR ligand) and [^{3}H]-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 M_1-mAChR.[157] The ability of (+)-epibatidine to displace the mAChR antagonist [^{3}H]-*N*-methylscopolamine (NMS) and the mAChR agonist [^{3}H]-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 M_1-mAChR.[149,157] 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 antagonist.[154,157] Given that (+)- and (−)-epibatidine is a potent nAChR agonist and a moderate M_1-mAChR antagonist, this natural product was initially considered a promising lead for the non-opioid treatment of pain.[148,149] 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).[158] The toxicity of epibatidine is caused by its potent, non-selective binding at the nAChRs.[148,149] 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]

Table 3
M_1-mAChR binding profile of (+)- and (−)-epibatidine *vs* carbachol and atropine.

	K_{app}(NMS) (μM)	K_{app}(oxo-M) (μM)	NMS/oxo-M ratio
(+)-Epibatidine	6.9	1.6	4.3
(−)-Epibatidine	16	1.4	11.4
Carbachol	22	0.0049	4490
Atropine	0.0010	0.00048	2.1

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 scalemic mixture of (2a*R*,4a*S*,9a*S*) and (2a*S*,4a*R*,9a*R*) enantiomers.[160,161]

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 (2a*R*, 4a*S*, 9a*S*)-phantasmidine, (2a*S*, 4a*R*, 9a*R*)-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 (2a*R*,4a*S*,9a*S*)-enantiomer were ~2- to 45-fold greater than the (2a*S*,4a*R*,9a*R*)-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 (2a*R*,4a*S*,9a*S*)-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 (2a*S*,4a*R*,9a*R*)-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

(2a*R*,4a*S*,9a*S*) (2a*S*,4a*R*,9a*R*)

Fig. 22. Phantasmidine.

Table 4
Binding affinity (K_i) and agonist potency (EC_{50}) at different nAChRs (nM).

	α4β2		α3β4		α7
	K_i	EC_{50}	K_i	EC_{50}	K_i
(2a*R*,4a*S*,9a*S*)-Phantasmidine	0.27	200	9.1	570	4.4
(2a*S*,4a*R*,9a*R*)-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

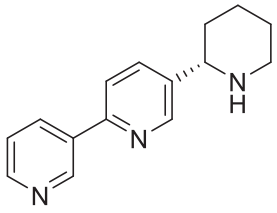


Fig. 23. Noranabasamine.

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.[164,165] 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;[164] (+)-*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;[165] farinosone A exhibited 70% and 40% neurite outgrowth at 50 and 20 μM respectively.[166] 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

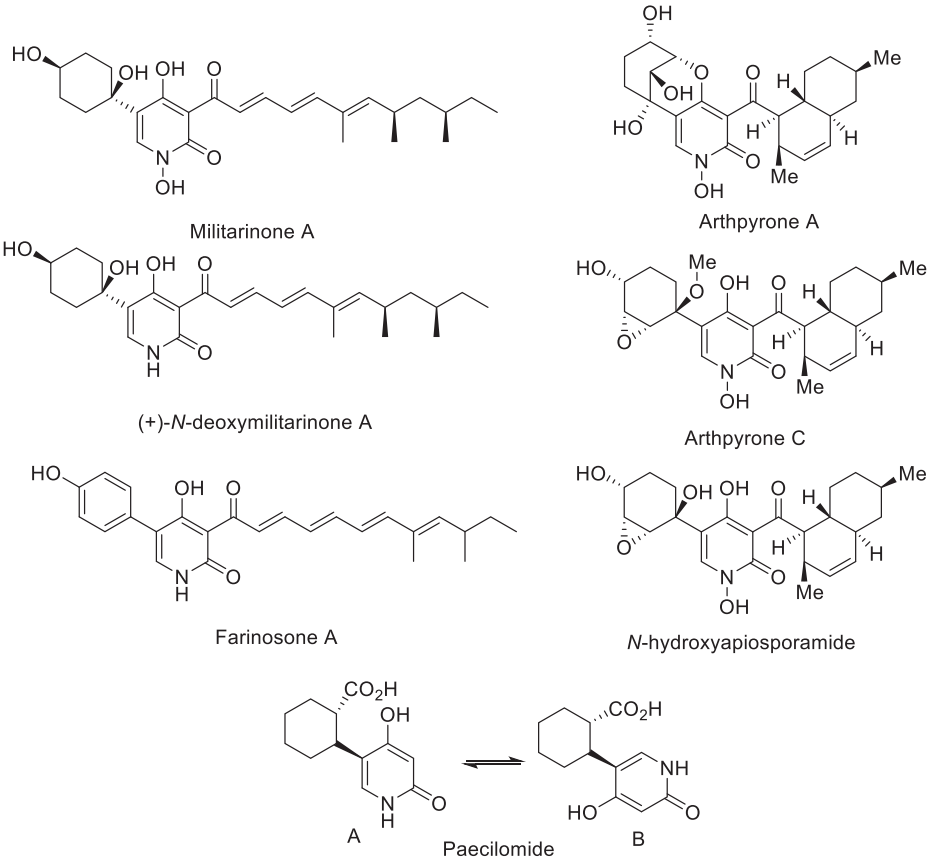


Fig. 24. 4-Hydroxy-2-pyridone alkaloids with CNS activity.

from sponge-derived fungi led to the discovery of arthpyrone A and C (Fig. 24) from *Arthrinium arundinis* strain ZSDS1-F3 derived from *Phakellia fusca*.[167] These alkaloids comprise a 1,4-dihydroxy-2-pyridone bonded to a cyclohexanol moiety and a decalin ring system. One previously discovered 1,4-dihydroxy-2-pyridone alkaloid, *N*-hydroxyapiosporamide (Fig. 24), was also isolated.[168] Arthpyrone C displayed pronounced anti-AChE activity (IC_{50} = 0.81 μM) when compared with classic AChE inhibitor tacrine (positive control; IC_{50} = 0.48 μM), whilst arthpyrone A and *N*-hydroxyapiosporamide both exhibited moderate AChE inhibition (IC_{50} = 47 and 39 μM, respectively).[167] These results suggest that arthpyrone C is an interesting lead compound for the development of treatments for neurodegenerative conditions.

The 4-hydroxy-2-pyridone alkaloid paecilomide (Fig. 24) was isolated from *P. lilacinus* by Takahashi and co-workers in 2013.[169] Two possible rotational forms, paecilomide A and B, were observed in the NOESY contour map between both H6 and H5′ as well as between H7 and H4′, due to the possibility of free rotation of the C7-C4′ sigma bond.[169] Paecilomide displayed potent AChE inhibitory activity (57.5% inhibition at 25 μL) when compared with physostigmine (98% inhibition at 10 mg/mL), suggesting therapeutic potential.[169]

4.2. 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* contains acromelic acids A and B;[170] stereoselective 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 natural product found in *Digenea simplex* that is related to the excitatory neurotransmitter glutamate.[170,172,174] Kainic acid acts as a potent 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.[171,173,175,176] Electrophysiological tests were performed to examine the neurological potency 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).[176] 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, acromelobic acid and an acromelobic 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. heterosporium* in 1934 by Yabuta[181] and was found to exhibit weak antimicrobial properties.[180–183] More than three decades later, fusaric acid was found to be a potent inhibitor of dopamine β-hydroxylase (DBH),[183] 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 μM was 20%, 58%, 89% and 92%, respectively; whilst picolinic acid at 0.5 and 5 μM 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 uncompetitive 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

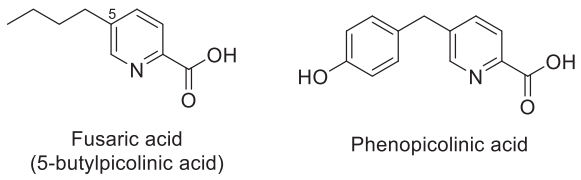


Fig. 26. Fusaric acid and Phenopicolinic acid.

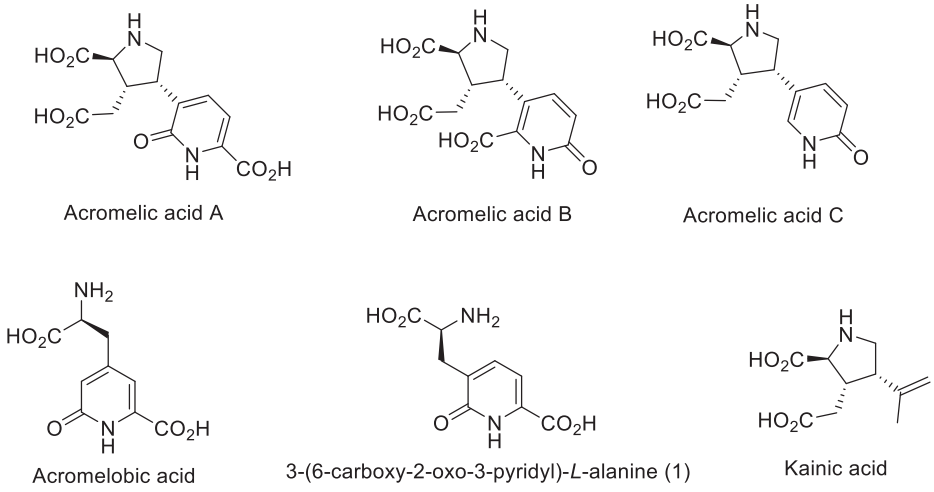


Fig. 25. Acromelic acids A–C, acromelobic acids and kainic acid.

oxidoreductases.[184] At 10 μM, fusaric acid did not inhibit MAO, tyrosine hydroxylase or aldehyde hydrogenase, indicating that the compound has a high degree of binding specificity for DBH.[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.[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.[184] These investigations showed that fusaric acid is a potent DBH inhibitor both *in vivo* and *in vitro*,[182,184] which led to clinical trials of fusaric acid in patients with mania and depression being conducted in 1974.[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.[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.[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.[185] Subsequent *in vivo* investigations have demonstrated other neurochemical effects of fusaric acid in the brain.[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.[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).[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.[182,186]

Another potent DBH inhibitor named phenopicolinic acid (Fig. 26) was isolated from *Paecilomyces* sp. strain AF2562 by Nakamura and co-workers in 1975.[187] The DBH inhibitory activity of phenopicolinic acid was reported to be nearly double that of fusaric acid (IC_{50} = 0.039 *vs* 0.05 μM).[187] *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} of phenopicolinic acid was ~350 mg/kg upon intraperitoneal injection.[187]

4.4. Aspernigrin B

Filter feeding marine species, such as sponges, host microorganisms that produce biologically active secondary metabolites with therapeutic potential.[188,189] The aqueous extract of an *Aspergillus niger* strain isolated on the sponge *Axinella damicornis* was found to contain aspernigrin B (Fig. 27), a structurally unprecedented 4-pyridone alkaloid.[189,190]

The neuroprotective properties of aspernigrin B were determined by measuring its effect on the changes of intracellular calcium ($[Ca^{2+}]_i$) levels in neuronal cells, using the neuroexcitants glutamic acid and quisqualic acid as positive controls.[189] Upon treatment with either glutamic acid (200 μM) or quisqualic acid (317 μM) alone, the $[Ca^{2+}]_i$ level in neuronal cells was increased by 376 and 275 nM respectively.[189] However, if the neuronal cells were pre-treated with 1 or 5 μg/mL of aspernigrin B for five minutes, the increase in $[Ca^{2+}]_i$ level induced by glutamic acid was suppressed to 280 and 169 nM respectively; aspernigrin B (1 μg/mL) reduced the increase in neuronal $[Ca^{2+}]_i$ level induced by quisqualic acid to 182 nM.[189] Aspernigrin B displayed pronounced neuroprotective properties against neuronal stimulation caused by glutamic acid and quisqualic acid and as a result comprises a promising lead neuroprotective agent.[189]

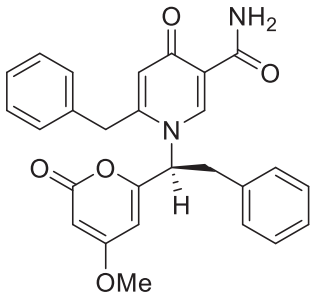


Fig. 27. Aspernigrin B.

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.[191] In one such study, Oh and co-workers isolated coprismycins A and B (Fig. 28) from a SNA015 strain of a *Streptomyces* species found in the dung balls of indigenous Korean dung beetle *Copris triparititus*.[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^+).[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.[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.[191] In a subsequent experiment, SH-SY5Y cells were pre-treated with different concentrations of either coprismycin A or B, followed by MPP^+ (800 μM).[191] The cell viability after MPP^+ exposure was improved from 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.[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.[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 **1** and SF2738 F **2** (Fig. 28) were discovered in 1994 from a culture of *Streptomyces* sp. in Japan (Yokohama, Kanagawa).[192] Some years later, these natural products were also isolated from the bacterial strain SNA015 of *Streptomyces* sp. found in *C. triparititus*.[191] The structural similarity of SF2738 D and SF2738 F with coprismycins A and B led to an investigation of their neuroprotective properties.[191] 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

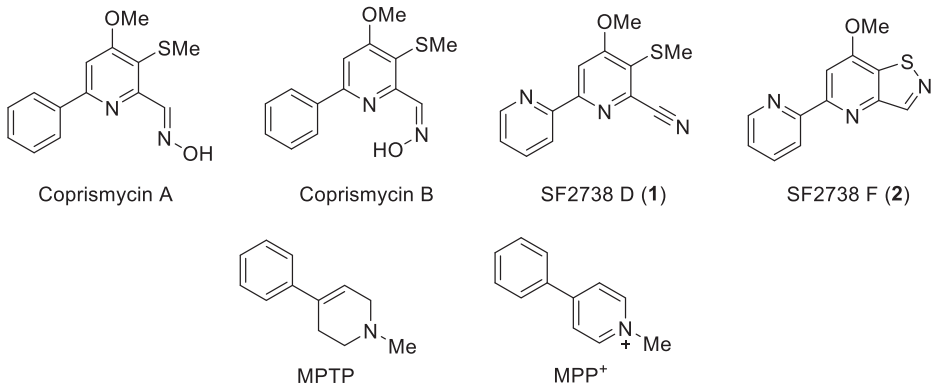


Fig. 28. 3-Thiopyridine alkaloids with neuroprotective properties. MPTP and MPP^+ included for structural comparison.

neurodegenerative disorders.[191]

5. Marine-derived

5.1. Anabaseine

A potent neurotoxin was initially discovered from the marine worm *Rhynchocoela* by Bacq[193] in 1936 and was found to exhibit significant toxicity when injected into crabs, causing convulsions, flaccid paralysis and eventually death.[194] 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 hoplonemertine *Paranemertes peregrine* and elucidated its chemical structure by both total synthesis and comparison with previously published literature data.[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.[46,194,195] Subsequent studies reported that anabaseine is the primary compound found in the poison glands of *Messor* and *Aphenaenogaster* ants;[46,195] anabaseine has not yet been detected in plants.[46,195]

Anabaseine has been shown to stimulate the release of ACh and norepinephrine from rat brains upon injection;[195] several synthetic anabaseine-related analogues also displayed significant cognitive enhancement and avoidance behaviours.[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.[195] The binding affinity (K_i) and the agonist potency (EC_{50}) of anabaseine ($K_i = 0.032$ μM; $EC_{50} = 4.2$ μM) at the α4β2-nAChR were ~8-fold less active and ~3-fold more potent than nicotine ($K_i = 0.0041$ μM; $EC_{50} = 14$ μM) respectively;[195] at the α7-nAChR, anabaseine ($K_i = 0.058$ μM; $EC_{50} = 6.7$ μM) was ~7-fold more potent than nicotine ($K_i = 0.40$ μM; $EC_{50} = 47$ μM).[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.[195]

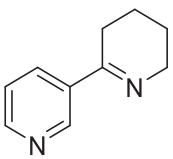


Fig. 29. Anabaseine.

5.2. Isoanatabine

Isoanatabine (Fig. 30) was isolated from the hoplonemertine *Amphiporus anagulatus* by Kem and co-workers in 2009.[196] This alkaloid is an anatabine isomer possessing a carbon–carbon double bond in the 3,4-position of the piperidine scaffold.[48,196] 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.[196] The binding affinity of (−)-isoanatabine ($K_i = 108$ nM) at rat α4β2-nAChR was slightly stronger than the (+)-enantiomer ($K_i = 136$ nM); however, at the human α4β2-nAChR, (−)-isoanatabine was less efficacious ($EC_{50} = 1.01$ μM; $I_{max} = 78.7\%$) than the (+)-enantiomer ($EC_{50} = 0.31$ μM; $I_{max} = 102\%$).[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.[196]

5.3. 2,3′-Bipyridyl and nemertelline

2,3′-Bipyridyl and nemertelline (Fig. 31) are neurotoxins isolated from the marine hoplonemertine worm *A. angulatus* by Kem and co-workers in 1976.[197] Pharmacological investigations have demonstrated that the paralytic properties of 2,3′-bipyridyl ($LD_{50} = 94$ μg) were stronger than nemertelline ($LD_{50} > 240$ μg) in crustaceans; both natural products exhibit comparable activity when tested against barnacle larvae ($IC_{50} = 4.1$ and 3.2 μM, respectively).[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.[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 ($IC_{50} = 2.8$, 2.6 and 2.1 mM respectively) when compared with galantamine (positive control; $IC_{50} = 6.4$ μM).[198]

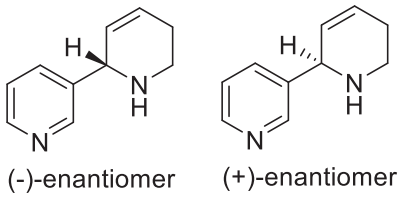


Fig. 30. Isoanatabine.

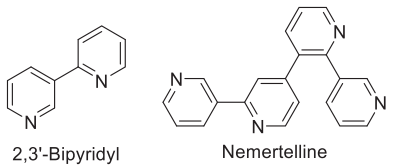


Fig. 31. 2,3′-Bipyridyl and Nemertelline.

5.5. Cyclostellettamines A–F

mAChRs play important roles in various physiological functions in the human body, including memory and learning.[156] Six structurally unprecedented macrocyclic bis-1,3-disubstituted pyridiniums, cyclostellettamines A–F (Fig. 33), were isolated from a Japanese marine sponge *Stelletta maxima*.[199] Cyclostellettamines A–F inhibited the binding of [^{3}H]-methylquinuclidinyl benzilate (a selective mAChR antagonist) to mAChR subtypes M_1, M_2 and M_3.[199] As mAChRs are known to be correlated with CNS-related diseases,[156] these data suggest that the cyclostellettamines A–F are structurally unique leads that warrant further investigation.[199]

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.[200,201] 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.[200] In a subsequent study, the structurally related pyridinium alkaloid daminin (Fig. 34) was isolated from the sponge *Axinella damicornis*.[201]

Agelongine exhibited a competitive, reversible antagonism at serotonergic receptors subfamily 1 (5-HT_1) *in vitro*.[200] The agonist properties of 5-hydroxytryptamine at 5-HT_1 receptors were inhibited when agelongine was introduced (IC_{50} = 80 μM).[200] Moreover, agelongine (100 μM) did not affect the concentration–response of histamine, ACh and prostaglandin E_2, suggesting that agelongine is selective for 5-HT_1 receptors; however, the specific 5-HT_1 receptor subtype used in these preliminary pharmacological tests was not stated.[200] 5-HT_1 receptor subtypes, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} have different distribution and function in the human body.[202] For example, 5-HT_{1A}, the most common 5-HT_1 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.[202] In contrast, both the 5-HT_{1B} and 5-HT_{1D} receptors are widely distributed in the basal ganglia and act as autoreceptors to decrease the transmission of the neurotransmitter glutamate in the neuronal terminals.[202] The triptans are 5-$HT_{1B/1D}$ receptor agonists used to treat migraine attacks in adults with or without aura.[202] 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.[203] The neuroprotective properties of daminin were determined by measuring its effect on the changes of $[Ca^{2+}]_i$ levels in

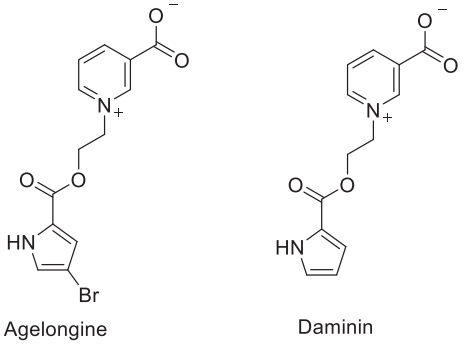


Fig. 34. Agelongine and Daminin.

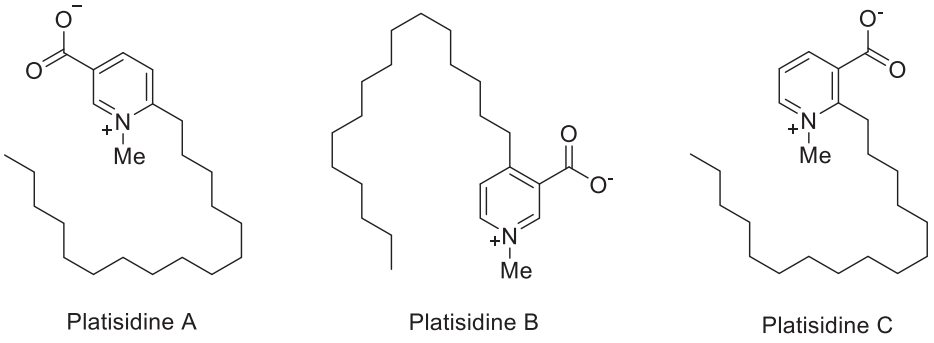
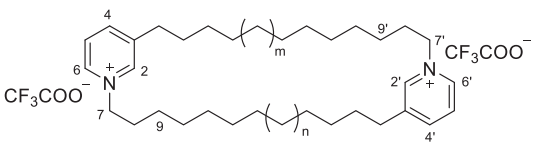


Fig. 32. Platisidines A–C.



A: m = 1, n = 1
IC_{50}: M_1 = 0.068, M_2 = 0.026, M_3 = 0.071
B: m = 1, n = 2
IC_{50}: M_1 = 0.081, M_2 = 0.031, M_3 = 0.109
C: m = 2, n = 2
IC_{50}: M_1 = 0.121, M_2 = 0.054, M_3 = 0.144
D: m = 1, n = 3
IC_{50}: M_1 = 0.174, M_2 = 0.059, M_3 = 0.211
E: m = 2, n = 3
IC_{50}: M_1 = 0.212, M_2 = 0.133, M_3 = 0.257
F: m = 3, n = 3
IC_{50}: M_1 = 0.364, M_2 = 0.150, M_3 = 0.474

Fig. 33. Cyclostellettamines A–F and their inhibition activity (IC_{50}, mg/mL) against $M_{1\text{-}3}$-mAChRs.

neuronal cells, using glutamic acid and NMDA (neuroexcitatory agonists) as positive controls.[201] Upon treatment with either glutamic acid (200 μM) or NMDA (200 μM) alone, the $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ level induced by NMDA to 63.5%.[201] 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.[204] The vitamin B_6 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.[204,205] Subsequent studies revealed that trigonelline is also widely distributed in plants within the dicotyledonae subclass, as well as in various animal species, such as arthropods, marine poriferans and mammals.[204] Trigonelline occurs in raw coffee beans and is converted into nicotinic acid upon roasting at ~230 °C.[204,206] Trigonelline is a water-soluble secondary metabolite formed from nicotinate (Fig. 35) and is responsible for the bitterness in coffee.[204] Many studies on the biological activities of trigonelline in animals and a variety of cell systems are available,[204] 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.[207,208] 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.[207] Trigonelline (30 μM) significantly increased the percentage of human SK-*N*-SH cells with neuritis >50 μm by 15% after three days of treatment.[207] 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.[207] 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),[209] stimulation of dopamine release (136% at 4.977 μM),[210] competitive inhibition of $GABA_A$ receptors (K_i = 13 nM),[211] and weak inhibition of AChE (IC_{50} = 233 μM).[212] 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 ED_{50} of cholinergic medications when taken adjunctively; the metanicotine 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

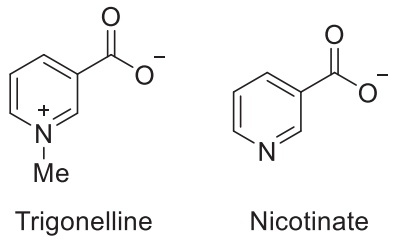


Fig. 35. Trigonelline and nicotinate.

inhibitors (AChEIs) is striking, which includes the huperzines, anabasamine, 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 neuritogenic and neuroprotective properties, such as militarinone A, arthpyrone C, (2*S*)-*N*-hydroxybenzylanabasine, casuarinine H, paecilomide, coprismycin A/B, trigonelline, daminin, euphorbialoids, aspernigrin 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 (agelongine, cerpegin), dopamine (fusaric acid, phenopicolinic acid, cerpegin) and glutamate (acromelic and acromelobic acids). The cyclostellettamines are mAChR 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. *Cen Nerv Syst Agents Med Chem*. 2014;14(1):43–59.
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 Lie 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, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: A review. *J Pharm Sci*. 2003;6(2):252–273.
7 Mansor NI, Nordin N, Mohamed F, Ling KH, 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, Schechter 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 DiNunzio 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, Szoeke CE, Vollset 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.

12 Xu J, Wang J, Wimo A, Qiu C. The economic burden of mental disorders in china, 2005–2013: implications for health policy. *BMC Psychiatry*. 2016;16(1):1–9.
13 Ritchie H. Roser, M. Mental Health. Our World in Data; 2018. http://www.ourworldindata.org/mental-health/; Accessed 21 September 2020.
14 Bharate SS, Mignani S, Vishwakarma RA. Why are the majority of active compounds in the CNS domain natural products? A critical analysis. *J Med Chem*. 2018;61(23):10345–10374.
15 Vitaku E, Smith DT, Njardarson JT. Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among Us FDA approved pharmaceuticals: miniperspective. *J Med Chem*. 2014;57(24):10257–10274.
16 Pennington LD, Moustakas DT. The necessary nitrogen atom: A versatile high-impact design element for multiparameter optimization. *J Med Chem*. 2017;60(9):3552–3579.
17 Benowitz NL. Nicotine addiction. *Prim Care*. 1999;26(3):611–631.
18 Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol*. 2009;49:57–71.
19 Yildiz D. Nicotine, its metabolism and an overview of its biological effects. *Toxicon*. 2004;43(6):619–632.
20 Koob G, Arends M, Le Moal M. *Drugs, addiction, and the brain* Chapter 7-Nicotine. Academic Press; 2014:221–259.
21 Posselt W, Reimann L. Chemical investigation of tobacco and the preparation of the characteristic active principle of this plant. *Magazin f Pharmazie*. 1828;24:138–161.
22 Pictet A, Crépieux P. Ueber phenyl-und pyridylpyrrole und die constitution des nicotins. *Ber Dtsch Chem Ges*. 1895;28(2):1904–1912.
23 Picciotto MR, Zoli M, Rimondini R, et al. Acetylcholine receptors containing the B2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998;391(6663):173–177.
24 Aberger K, Chitravanshi V, Sapru H. Cardiovascular responses to microinjections of nicotine into the caudal ventrolateral medulla of the rat. *Brain Res*. 2001;892(1):138–146.
25 Hajos M, Hurst R, Hoffmann W, et al. The selective Α7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-Azabicyclo [2.2. 2] Oct-3-Yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. *J Pharmacol Exp Ther*. 2005;312(3):1213–1222.
26 Kishioka S, Kiguchi N, Kobayashi Y, Saika F. Nicotine effects and the endogenous opioid system. *J Pharmacol Sci*. 2014;125(2):117–124.
27 Jain R, Mukherjee K, Balhara YPS. The role of NMDA receptor antagonists in nicotine tolerance, sensitization, and physical dependence: A preclinical review. *Yonsei Med J*. 2008;49(2):175–188.
28 World Health Organization; Bloomberg Philanthropies. WHO Report on the Global Tobacco Epidemic, 2017: Monitoring Tobacco Use and Prevention Policies; 2017. http://www.who.int/tobacco/global_report/2017/en/; Accessed 21 September 2020.
29 Stead LF, Perera R, Bullen C, et al. Nicotine replacement therapy for smoking cessation. *Cochrane Database Syst Rev*. 2012;11:CD000146.
30 Soto-Otero R, Méndez-Álvarez E, Hermida-Ameijeiras Á, López-Real AM, Labandeira-García JL. Effects of (−)-Nicotine and (−)-cotinine on 6-hydroxydopamine-induced oxidative stress and neurotoxicity: relevance for Parkinson's disease. *Biochem Pharmacol*. 2002;64(1):125–135.
31 Salas R, Pieri F, Fung B, Dani JA, De Biasi M. Altered anxiety-related responses in mutant mice lacking the B4 subunit of the nicotinic receptor. *J Neurosci*. 2003;23(15):6255–6263.
32 Tindle HA, Newhouse PA, Freiberg MS. Beyond smoking cessation: investigating medicinal nicotine to prevent and treat COVID-19. *Nicotine Tob Res*. 2020;22(9):1669–1670.
33 Farsalinos K, Barbouni A, Niaura R. Systematic review of the prevalence of current smoking among hospitalized COVID-19 patients in China: could nicotine Be a therapeutic option? *Intern Emerg Med*. 2020;15(5):845–852.
34 Farsalinos K, Niaura R, Le Houezec J, et al. Nicotine and SARS-CoV-2: COVID-19 May Be a disease of the nicotinic cholinergic system. *Toxicol Rep*. 2020;7:658–663.
35 Papke RL, Dwoskin LP, Crooks PA. The pharmacological activity of nicotine and nornicotine on nachrs subtypes: relevance to nicotine dependence and drug discovery. *J Neurochem*. 2007;101(1):160–167.
36 Bush LP, Fannin FF, Chelvarajan RL, Burton HR. *Nicotine and related alkaloids* Chapter 1-Biosynthesis And Metabolism Of Nicotine And Related Alkaloids. Springer; 1993:1–30.
37 Ehrenstein M. Zur kenntnis der alkaloide des tabaks. *Arch Pharm*. 1931;269(40–45):627–659.
38 Saitoh F, Noma M, Kawashima N. The alkaloid contents of sixty nicotiana species. *Phytochemistry*. 1985;24(3):477–480.
39 Jacob III P, Yu L, Liang G, Shulgin AT, Benowitz NL. Gas chromatographic—mass spectrometric method for determination of anabasine, anatabine and other tobacco alkaloids in urine of smokers and smokeless tobacco users. *J Chromatogr*. 1993;619(1):49–61.
40 Armstrong DW, Wang X, Lee J, Liu Y. Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco. *Chirality*. 1999;11(1):82–84.
41 Kisaki T, Tamaki E. Phytochemical studies on the tobacco alkaloids. I. Optical rotatory power of nornicotine. *Arch Biochem Biophys*. 1961;92(2):351–355.
42 Teng L, Crooks PA, Buxton ST, Dwoskin LP. Nicotinic-Receptor Mediation of S (-) nornicotine-evoked [3H] overflow from rat striatal slices preloaded with [3H] dopamine. *J Pharmacol Exp Ther*. 1997;283(2):778–787.
43 Ujváry I. *Hayes' handbook of pesticide toxicology* Chapter 3-Pest Control Agents from Natural Products. Elsevier; 2010:119–229.
44 Orechoff A, Menschikoff G. Über die alkaloide von anabasis aphylla L. (I. Mitteil.). *Berichte der deutschen chemischen. Gesellschaft (A and B Series)*. 1931;64(2):266–274.
45 Smith C. Neonicotine recently found as an alkaloid in anabasis Aphylla L (Scientific Note). *J Econ Ent*. 1931;24:1108.
46 Lee ST, Wildeboer K, Panter KE, et al. Relative toxicities and neuromuscular nicotinic receptor agonistic potencies of anabasine enantiomers and anabaseine. *Neurotoxicol Teratol*. 2006;28(2):220–228.
47 Leclercq S, Charles S, Daloze D, Braekman J-C, Aron S, Pasteels JM. Absolute configuration of anabasine from messor and aphaenogaster ants. *J Chem Ecol*. 2001;27(5):945–952.
48 Xing H, Keshwah S, Rouchaud A, Kem WR. A pharmacological comparison of two isomeric nicotinic receptor agonists: the marine toxin isoanatabine and the tobacco alkaloid anatabine. *Mar Drugs*. 2020;18(2):106–117.
49 Späth E, Kesztler F. L-Anatabin, ein neues tabakalkaloid (XI. Mitteil. Über Tabak-Basen). *Berichte der deutschen chemischen. Gesellschaft (A and B Series)*. 1937;70(2):239–243.
50 Ji H, Wu Y, Fannin F, Bush L. Determination of tobacco alkaloid enantiomers using reversed phase UPLC/MS/MS. *Heliyon*. 2019;5(5):e01719.
51 Paris D, Beaulieu-Abdelahad D, Bachmeier C, et al. Anatabine lowers alzheimer's aβ production in vitro and in vivo. *Eur J Pharmacol*. 2011;670(2–3):384–391.
52 Levin ED, Hao I, Burke DA, Cauley M, Hall BJ, Rezvani AH. Effects of tobacco smoke constituents, anabasine and anatabine, on memory and attention in female rats. *J Psychopharmacol*. 2014;28(10):915–922.
53 Späth E, Kesztler F. Über Neue Basen Des Tabaks (XIII. Mitteil. Über Tabak-Alkaloide). *Berichte der deutschen chemischen. Gesellschaft (A and B Series)*. 1937;70(12):2450–2454.
54 Castagnoli K, Steyn SJ, Magnin G, et al. Studies on the interactions of tobacco leaf and tobacco smoke constituents and monoamine oxidase. *Neurotox Res*. 2002;4(2):151–160.
55 Alijevic O, McHugh D, Rufener L, Mazurov A, Hoeng J, Peitsch M. An electrophysiological characterization of naturally occurring tobacco alkaloids and their action on human Α4β2 and Α7 nicotinic acetylcholine receptors. *Phytochemistry*. 2020;170:112187.
56 Fox AM, Moonschi FH, Richards CI. The nicotine metabolite, cotinine, alters the assembly and trafficking of a subset of nicotinic acetylcholine receptors. *J Biol Chem*. 2015;290(40):24403–24412.
57 Balhara YP, Sarkar S. *Neuropathology of drug addictions and substance misuse* Chapter 34-Cotinine Urinalysis for Tobacco Use. Elsevier; 2016:363–372.
58 Grizzell JA, Echeverria V. New insights into the mechanisms of action of cotinine and its distinctive effects from nicotine. *Neurochem Res*. 2015;40(10):2032–2046.
59 Echeverria Moran V. Cotinine: beyond that expected, more than a biomarker of tobacco consumption. *Front Pharmacol*. 2012;3(173):1–9.
60 Pinner A. Ueber Nicotin. Die constitution des alkaloids. *Berichte der deutschen chemischen Gesellschaft*. 1893;26(1):292–305.
61 Frankenburg W, Vaitekunas A. The chemistry of tobacco fermentation. I. Conversion of the Alkaloids. D. Identification of cotinine in fermented leaves. *J Am Chem Soc*. 1957;79(1):149–151.
62 Luanratana O, Griffin WJ. Alkaloids of duboisia hopwoodii. *Phytochemistry*. 1982;21(2):449–451.
63 Terry AV, Callahan PM, Bertrand D. R-(+) and S-(−) isomers of cotinine augment cholinergic responses in vitro and in vivo. *J Pharmacol Exp Ther*. 2015;352(2):405–418.
64 Terry Jr AV, Hernandez CM, Hohnadel EJ, Bouchard KP, Buccafusco JJ. Cotinine, a neuroactive metabolite of nicotine: potential for treating disorders of impaired cognition. *CNS Drug Rev*. 2005;11(3):229–252.
65 Vainio PJ, Tuominen RK. Cotinine binding to nicotinic acetylcholine receptors in bovine chromaffin cell and rat brain membranes. *Nicotine Tob Res*. 2001;3(2):177–182.
66 Terry Jr AV, Buccafusco JJ, Schade RF, et al. The Nicotine metabolite, cotinine, attenuates glutamate (NMDA) Antagonist-related effects on the performance of the five choice serial reaction time task (5C-SRTT) in rats. *Biochem Pharmacol*. 2012;83(7):941–951.
67 Grizzell JA, Iarkov A, Holmes R, Mori T, Echeverria V. Cotinine reduces depressive-like behavior, working memory deficits, and synaptic loss associated with chronic stress in mice. *Behav Brain Res*. 2014;268:55–65.
68 Gao J, Adam B-L, Terry Jr AV. Evaluation of nicotine and cotinine analogs as potential neuroprotective agents for alzheimer's disease. *Bioorg Med Chem Lett*. 2014;24(6):1472–1478.
69 Dwoskin LP, Teng L, Buxton ST, Crooks PA. (S)-(−)-Cotinine, the major brain metabolite of nicotine, stimulates nicotinic receptors to evoke [3H] dopamine release from rat striatal slices in a calcium-dependent manner. *J Pharmacol Exp Ther*. 1999;288(3):905–911.
70 Fuxe K, Everitt BJ, Hökfelt T. On the action of nicotine and cotinine on central 5-hydroxytryptamine neurons. *Pharmacol Biochem Behav*. 1979;10(5):671–677.
71 Wei X, Sumithran SP, Deaciuc AG, et al. Identification and synthesis of novel alkaloids from the root system of nicotiana tabacum: affinity for neuronal nicotinic acetylcholine receptors. *Life Sci*. 2005;78(5):495–505.
72 Bencherif M, Byrd G, Caldwell W, Hayes J, Lippiello P. Pharmacological characterization of RJR-2403: A nicotinic agonist with potential therapeutic benefit in the treatment of Alzheimer's disease. *CNS Drug Rev*. 1997;3(4):325–345.
73 Wilson K, Chang R, Bowman E, McKennis H. Nicotine-like actions of Cis-metanicotine and trans-metanicotine. *J Pharmacol Exp Ther*. 1976;196(3):685–696.
74 Pinner A. Ueber Nicotin. *Arch Pharm*. 1895;233(8):572–612.
75 Wahl R. Über Das Vorkommen von Metanikotin in Natürlich Nikotinfreien Tabaksorten. *Tabak-Forsch Sonderheft*. 1953;36.
76 Parups E. Effect of gibberellic acid applications to leaves of nicotiana on nornicotine, anabasine, metanicotine, oxynicotine, and nicotinic acid content. *Tobacco*. 1960;151(7):19–22.

77 Schmeltz I, Stedman R, Chamberlain W, Burdick D. Composition studies on Tobacco. XX.—Bases of cigarette smoke. *J Sci Food Agric*. 1964;15(11):774–781.
78 Lippiello P, Benchérif M, Caldwell W, et al. Metanicotine: A nicotinic agonist with central nervous system selectivity—in vitro and in vivo characterization. *Drug Dev Res*. 1996;38(3–4):169–176.
79 Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: from basic science to therapeutics. *Pharmacol Ther*. 2013;137(1):22–54.
80 Meyer MD. *xPharm: The comprehensive pharmacology reference*. Trans-Metanicotine. Elsevier; 2007:1–4.
81 Zhang Y, Liu Y-B, Li Y, et al. Sesquiterpenes and alkaloids from the roots of alangium chinense. *J Nat Prod*. 2013;76(6):1058–1063.
82 Mukhamedzhanov S, Aslanov KA, Sadykov A, Leont'ev V, Kiryukhin V. Structure of Anabasamine. *Chem Nat Compd*. 1968;4(3):136–138.
83 Tilyabaev Z, Abduvakhabov A. Alkaloids of anabasis aphylla and their cholinergic activities. *Chem Nat Compd*. 1998;34(3):295–297.
84 Rouden J, Lasne M-C, Blanchet J, Baudoux J. (−)-Cytisine and derivatives: synthesis, reactivity, and applications. *Chem Rev*. 2014;114(1):712–778.
85 Paduszyńska A, Banach M, Rysz J, Dąbrowa M, Gąsiorek P, Bielecka-Dąbrowa A. Cytisine-from the past to the future. *Curr Pharm Des*. 2018;24(37):4413–4423.
86 Tzankova V, Danchev N. Cytisine—from ethomedical use to the development as a natural alternative for smoking cessation. *Biotechnol Biotech Eq*. 2007;21(2):151–160.
87 Pérez EG, Méndez-Gálvez C, Cassels BK. Cytisine: A natural product lead for the development of drugs acting at nicotinic acetylcholine receptors. *Nat Prod Rep*. 2012;29(5):555–567.
88 Tutka P, Zatoński W. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. *Pharmacol Rep*. 2006;58(6):777.
89 Tutka P, Vinnikov D, Courtney RJ, Benowitz NL. Cytisine for nicotine addiction treatment: A review of pharmacology, therapeutics and an update of clinical trial evidence for smoking cessation. *Addiction*. 2019;114(11):1951–1969.
90 Husemann A, Marmé W. Vorläufige mittheilung über cytisin und laburnin, zwei neue pflanzenbasen in cytisus laburnum. *Zeitschr f Chemie*. 1865;1:161.
91 Partheil A. Zur Frage Der Identität von Cytisin Und Ulexin. *Arch Pharm*. 1894;232(6):486–488.
92 Okuda S, Tsuda K, Kataoka H. Absolute configurations of (-)-cytisine and of related alkaloids. *Chem Ind-London*. 1961;43, 1751–1751.
93 El-Shazly A, Sarg T, Ateya A, Abdel Aziz E, Witte L, Wink M. Quinolizidine alkaloids from argyrolobium uniflorum. *Pharmazie*. 1996;51:768–772.
94 Greinwald R, Ross JH, Witte L, Czygan F-C. Alkaloids of templetonia incana. *Biochem Syst Ecol*. 1996;24(5):423–426.
95 Veen G, Günther S, Greinwald R, et al. Quinolizidine alkaloids in petteria ramentacea. *Phytochemistry*. 1992;31(10):3487–3490.
96 Anderson DJ, Arneric SP. Nicotinic receptor binding of [3H] cytisine,[3H] nicotine and [3H] methylcarbamylcholine in rat brain. *Eur J Pharmacol*. 1994;253(3):261–267.
97 Hall M, Zerbe L, Leonard S, Freedman R. Characterization of [3H] cytisine binding to human brain membrane preparations. *Brain Res*. 1993;600(1):127–133.
98 Coe JW, Brooks PR, Vetelino MG, et al. Varenicline: An A4β2 Nicotinic receptor partial agonist for smoking cessation. *J Med Chem*. 2005;48(10):3474–3477.
99 Abin-Carriquiry JA, Costa G, Urbanavicius J, et al. In vivo modulation of dopaminergic nigrostriatal pathways by cytisine derivatives: implications for Parkinson's disease. *Eur J Pharmacol*. 2008;589(1–3):80–84.
100 Jordan CJ, Xi Z-X. Discovery and development of varenicline for smoking cessation. *Expert Opin Drug Discov*. 2018;13(7):671–683.
101 Mineur YS, Somenzi O, Picciotto MR. Cytisine, a partial agonist of high-affinity nicotinic acetylcholine receptors, has antidepressant-like properties in male C57BL/6J mice. *Neuropharmacology*. 2007;52(5):1256–1262.
102 Hayman AR, Gray DO. Hydroxynorcytisine, a quinolizidone alkaloid from laburnum anagyroides. *Phytochemistry*. 1989;28(2):673–675.
103 Yohannes D, Hansen CP, Akireddy SR, et al. First total synthesis of (±)-3-hydroxy-11-norcytisine: structure confirmation and biological characterization. *Org Lett*. 2008;10(23):5353–5356.
104 Pouny I, Batut M, Vendier L, et al. Cytisine-like alkaloids from ormosia hosiei Hemsl. and EH Wilson. *Phytochemistry*. 2014;107:97–101.
105 Yang H, Ma Y, Wang X, Zhu D. Huperzine A: A mini-review of biological characteristics, natural sources, synthetic origins, and future prospects. *Russ J Org Chem*. 2020;56(1):148–157.
106 Ha GT, Wong RK, Zhang Y. Huperzine A as potential treatment of Alzheimer's disease: An assessment on chemistry, pharmacology, and clinical studies. *Chem Biodivers*. 2011;8(7):1189–1204.
107 Bai D. Development of huperzine A and B for treatment of Alzheimer's disease. *Pure Appl Chem*. 2007;79(4):469–479.
108 Zhu D-Y, Tan C-H, Li Y-M. *Medicinal chemistry of bioactive natural products* Chapter 4-The Overview of Studies on Huperzine A: A Natural Drug for the Treatment of Alzheimer's Disease. Wiley; 2006:143–182.
109 Tang XC, Han YF. Pharmacological profile of huperzine A, a novel acetylcholinesterase inhibitor from chinese herb. *CNS Drug Rev*. 1999;5(3):281–300.
110 Liu J-S, Zhu Y-L, Yu C-M, et al. The structures of huperzine A and B, two new alkaloids exhibiting marked anticholinesterase activity. *Can J Chem*. 1986;64(4):837–839.
111 Huang J, He C. Population structure and genetic diversity of huperzia serrata (Huperziaceae) based on amplified fragment length polymorphism (AFLP) markers. *Biochem Syst Ecol*. 2010;38(6):1137–1147.
112 Lim W-H, Goodger JQ, Field AR, Holtum JA, Woodrow IE. Huperzine alkaloids from australasian and southeast asian huperzia. *Pharm Biol*. 2010;48(9):1073–1078.
113 Cummings JL. Cholinesterase inhibitors: A new class of psychotropic compounds. *Am J Psychiatry*. 2000;157(1):4–15.
114 Lamy PP. The role of cholinesterase inhibitors in Alzheimer's disease. *CNS Drugs*. 1994;1(2):146–165.
115 Tang X, De Sarno P, Sugaya K, Giacobini E. Effect of huperzine A, a new cholinesterase inhibitor, on the central cholinergic system of the Rat. *J Neurosci Res*. 1989;24(2):276–285.
116 Ved 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(4):963–967.
117 Gordon RK, Nigam SV, Weitz JA, Dave JR, Doctor BP, Ved HS. The NMDA receptor ion channel: A site for binding of huperzine A. *J Appl Toxicol*. 2001;21(S1):S47–S51.
118 Wang T, Tang XC. Reversal of scopolamine-induced deficits in radial maze performance by (−)-Huperzine A: comparison with E2020 and tacrine. *Eur J Pharmacol*. 1998;349(2–3):137–142.
119 Gao Y, Tang X, Guan L, Kuang P. Huperzine A reverses scopolamine-and muscimol-induced memory deficits in chick. *Acta Pharmacol Sin*. 2000;21(12):1169–1173.
120 Ou LY, Tang XC, Cai JX. Effect of Huperzine A on working memory in reserpine-or yohimbine-treated monkeys. *Eur J Pharmacol*. 2001;433(2–3):151–156.
121 Zhang D-B, Chen J-J, Song Q-Y, Zhang L, Gao K. Lycodine-type alkaloids from lycopodiastrum casuarinoides and their acetylcholinesterase inhibitory activity. *Molecules*. 2014;19(7):9999–10010.
122 Jia-Sen L, Mei-Fen H. The alkaloids huperzines C and D and HUPERZININE FROM LYCOPODIASTRUM CASUARinoides. *Phytochemistry*. 1994;37(6):1759–1761.
123 Jiang W-W, Liu F, Gao X, et al. Huperserines A-E, lycopodium alkaloids from huperzia serrata. *Fitoterapia*. 2014;99:72–77.
124 Thorroad S, Worawittayanont P, Khunnawutmanotham N, et al. Three new lycopodium alkaloids from huperzia carinata and huperzia squarrosa. *Tetrahedron*. 2014;70(43):8017–8022.
125 Choo CY, Hirasawa Y, Karimata C, et al. Carinatumins A-C, New alkaloids from lycopodium carinatum inhibiting acetylcholinesterase. *Bioorgan Med Chem*. 2007;15(4):1703–1707.
126 Tang Y, Fu Y, Xiong J, et al. Casuarinines A-J, lycodine-type alkaloids from lycopodiastrum casuarinoides. *J Nat Prod*. 2013;76(8):1475–1484.
127 Shen Y-C, Chen C-H. Alkaloids from lycopodium casuarinoides. *J Nat Prod*. 1994;57(6):824–826.
128 Hirasawa Y, Kato E, Kobayashi J, et al. Lycoparins A-C, new alkaloids from lycopodium casuarinoides inhibiting acetylcholinesterase. *Bioorgan Med Chem*. 2008;16(11):6167–6171.
129 Serrano MA, Pivatto M, Francisco W, et al. Acetylcholinesterase inhibitory pyridine alkaloids of the leaves of senna multijuga. *J Nat Prod*. 2010;73(3):482–484.
130 Francisco W, Pivatto M, Danuello A, et al. Pyridine alkaloids from senna multijuga as acetylcholinesterase inhibitors. *J Nat Prod*. 2012;75(3):408–413.
131 Shi Q-W, Su X-H, Kiyota H. Chemical and pharmacological research of the plants in genus euphorbia. *Chem Rev*. 2008;108(10):4295–4327.
132 Zhang W, Chen D, Hou A. New myrsinol diterpenes from euphorbia prolifera. *Chinese J Chem*. 2004;22(1):103–108.
133 Xu J, Jin D, Guo Y, et al. New myrsinol diterpenes from euphorbia prolifera and their inhibitory activities on LPS-Induced No production. *Bioorg Med Chem Lett*. 2012;22(11):3612–3618.
134 Jeske F, Jakupovic J, Berendsohn W. Diterpenes from euphorbia seguieriana. *Phytochemistry*. 1995;40(6):1743–1750.
135 Cho N, Moon EH, Kim HW, Hong J, Beutler JA, Sung SH. Inhibition of nitric oxide production in BV2 microglial cells by triterpenes from tetrapanax papyriferus. *Molecules*. 2016;21(4):459.
136 Basak S. Cerpegin alkaloid and its analogues: chemical synthesis and pharmacological profiles. *J Indian Chem Soc*. 2018;95(10):1175–1190.
137 Adibatti N, Thirugnanasambantham P, Kulothungan C, et al. A pyridine alkaloid from ceropegia juncea. *Phytochemistry*. 1991;30(7):2449–2450.
138 Sukumar E, Gopal RH, Rao RB, Viswanathan S, Thirugnanasambantham P. Pharmacological actions of cerpegin, a novel pyridine alkaloid from ceropegia juncea. *Fitoterapia (Milano)*. 1995;66(5):403–406.
139 Da Silva T, Da Silva B, Mukherjee R. The monoterpene alkaloid cantleyine from strychnos trinervis root and its spasmolytic properties. *Phytomedicine*. 1999;6(3):169–176.
140 Openshaw H Chapter 6-Quinoline Alkaloids, Other than Those of Cinchona *The alkaloids: chemistry and physiology*. 1967;vol. 9:223–267.
141 Avazmukhamedov L, Shakirov T, Tel'nov V. The technology of the isolation of the alkaloids dubinidine and haplophylidine. *Chem Nat Compd*. 1966;2(2), 114–114.
142 Magrupova M, Kamilov I, Polievtsev N. Synergy of the alkaloid haplophyllidin with sleep-inducing and narcotic drugs. *Farmakol Alkaloidov, Akad Nauk Uz USSR, Inst Khim Rast Veshchestv*. 1962;1:160–168.
143 Magrapova M, Kamilov I, Polievtsev N. Antagonism between Haplophyllidin and Analeptics. *Farmakol Alkaloidov, Akad Nauk Uz SSR, Inst khim Rast Veshch*. 1962;1:169.
144 Govindachari T, Nagarajan K, Rajappa S. 110. Structure of gentianine. *J Chem Soc (Resumed)*. 1957:551–556.
145 Govindachari T, Nagarajan K, Rajappa S. 526. Synthesis of gentianine. *J Chem Soc (Resumed)*. 1957:2725–2726.
146 Bhattacharya S. Chemical constituents of gentianaceae XI: antipsychotic activity of gentianine. *J Pharm Sci*. 1974;63(8):1341–1342.
147 Seigler DS. *Plant secondary metabolism*. Alkaloids of Terpenoid Origin (excepting indole alkaloids and ergot alkaloids). Springer; 1998:668–669.
148 Traynor J. Epibatidine and Pain. *Br J Anaesth*. 1998;81(1):69–76.
149 Salehi B, Sestito S, Rapposelli S, et al. Epibatidine: A promising natural alkaloid in health. *Biomolecules*. 2019;9(1):6.

150 Spande TF, Garraffo HM, Edwards MW, Yeh HJ, Pannell L, Daly JW. Epibatidine: A novel (chloropyridyl) azabicycloheptane with potent analgesic activity from an ecuadoran poison frog. *J Am Chem Soc*. 1992;114(9):3475–3478.
151 Fletcher SR, Baker R, Chambers MS, Hobbs SC, Mitchell PJ. The synthesis of (+)- and (–)-epibatidine. *J Chem Soc Chem Comm*. 1993;15:1216–1218.
152 Watt A, Verrier H, O'Connor D. Resolution of synthetic (+)-and (-)-epibatidine by chiral high performance liquid chromatography and identification of the natural enantiomer. *J Liq chromatogr*. 1994;17(6):1257–1264.
153 Qian C, Li T, Shen T, et al. Epibatidine is a nicotinic analgesic. *Eur J Pharmacol*. 1993;250(3):R13–R14.
154 Rupniak N, Patel S, Marwood R, et al. Antinociceptive and toxic effects of (+)-epibatidine oxalate attributable to nicotinic agonist activity. *Br J Pharmacol*. 1994;113(4):1487–1493.
155 Gopalakrishnan M, Monteggia LM, Anderson DJ, et al. Stable expression, pharmacologic properties and regulation of the human neuronal nicotinic acetylcholine alpha 4 beta 2 receptor. *J Pharmacol Exp Ther*. 1996;276(1):289–297.
156 Jiang S, Li Y, Zhang C, et al. M1 Muscarinic acetylcholine receptor in Alzheimer's disease. *Neurosci Bull*. 2014;30(2):295–307.
157 Freedman S, Harley E, Iversen L. Relative affinities of drugs acting at cholinoceptors in displacing agonist and antagonist radioligands: The NMS/Oxo-M ratio as an index of efficacy at cortical muscarinic receptors. *Br J Pharmacol*. 1988;93(2):437.
158 Wiener SW, Nelson LS. *Ciottone's disaster medicine*. Cholinergic Agent Attack (Nicotine, Epibatidine, and Anatoxin-a). Elsevier; 2016:688–691.
159 Fitch RW, Spande TF, Garraffo HM, Yeh HJ, Daly JW. Phantasmidine: An Epibatidine congener from the ecuadorian poison frog epipedobates anthonyi. *J Nat Prod*. 2010;73(3):331–337.
160 Fitch RW, Snider BB, Zhou Q, et al. Absolute configuration and pharmacology of the poison frog alkaloid phantasmidine. *J Nat Prod*. 2018;81(4):1029–1035.
161 Zhou Q, Snider BB. Synthesis of phantasmidine. *Org Lett*. 2011;13(3):526–529.
162 Miao L, DiMaggio SC, Shu H, Trudell ML. Enantioselective syntheses of both enantiomers of noranabasamine. *Org Lett*. 2009;11(7):1579–1582.
163 Tokuyama T, Daly J. Steroidal Alkaloids (Batrachotoxins And 4β-Hydroxybatrachotoxins), "Indole Alkaloids" (calycanthine and chimonanthine) and a piperidinyldipyridin. *Tetrahedron*. 1983;39(1):41–47.
164 Schmidt K, Günther W, Stoyanova S, Schubert B, Li Z, Hamburger M. Militarinone A, a neurotrophic pyridone alkaloid from paecilomyces m ilitaris. *Org Lett*. 2002;4(2):197–199.
165 Cheng Y, Schneider B, Riese U, Schubert B, Li Z, Hamburger M. (+)-N-Deoxymilitarinone A, a neuritogenic pyridone alkaloid from the insect pathogenic fungus paecilomyces f arinosus. *J Nat Prod*. 2006;69(3):436–438.
166 Cheng Y, Schneider B, Riese U, Schubert B, Li Z, Hamburger M. Farinosones A– C, neurotrophic alkaloidal metabolites from the entomogenous deuteromycete paecilomyces f arinosus. *J Nat Prod*. 2004;67(11):1854–1858.
167 Wang J, Wei X, Qin X, et al. Arthpyrones A-C, pyridone alkaloids from a sponge-derived fungus arthrinium arundinis ZSDS1-F3. *Org Lett*. 2015;17(3):656–659.
168 Lee JC, Coval SJ, Clardy J. A cholesteryl ester transfer protein inhibitor from an insect-associated fungus. *J Antibiot (Tokyo)*. 1996;49(7):693–696.
169 Teles APC, Takahashi JA. Paecilomide, a new acetylcholinesterase inhibitor from paecilomyces lilacinus. *Microbiol Res*. 2013;168(4):204–210.
170 Konno K, Shirahama H, Matsumoto T. Isolation and structure of aromelic acid A and B. new kainoids of clitocybe acromelalga. *Tetrahedron Lett*. 1983;24(9):939–942.
171 Konno K, Hashimoto K, Ohfune Y, Shirahama H, Matsumoto T. Acromelic acids A and B. Potent neuroexcitatory amino acids isolated from clitocybe acromelalga. *J Am Chem Soc*. 1988;110(14):4807–4815.
172 Konno K, Hashimoto K, Ohfune Y, Shirahama H, Matsumoto T. Synthesis of acromelic acid A, a toxic principle of clitocybe acromelalga. *Tetrahedron Lett*. 1986;27(5):607–610.
173 Shinozaki H, Ishida M, Okamoto T. Acromelic Acid, a novel excitatory amino acid from a poisonous mushroom: effects on the crayfish neuromuscular junction. *Brain Res*. 1986;399(2):395–398.
174 Zheng X-Y, Zhang H-L, Luo Q, Zhu J. Kainic acid-induced neurodegenerative model: potentials and limitations. *J BioMed Biotechnol*. 2011;2011.
175 Ishida M, Shinozaki H. Acromelic acid is a much more potent excitant than kainic acid or domoic acid in the isolated rat spinal cord. *Brain Res*. 1988;474(2):386–389.
176 Maruyama M, Takeda K. Effects of acromelic acid A on the binding of [3H] glutamic acid and [3H] kainic acid to synaptic membranes and on the depolarization at the frog spinal cord. *Brain Res*. 1989;504(2):328–331.
177 Fushiya S, Sato S, Kanazawa T, Kusano G, Nozoe S. Acromelic acid C. A new toxic constituent of clitocybe acromelalga: an efficient isolation of acromelic acids. *Tetrahedron Lett*. 1990;31(27):3901–3904.
178 Yamano K, Hashimoto K, Shirahama H. Novel neuroexcitatory amino acid from clitocybe acromelalga. *Heterocycles (Sendai)*. 1992;34(3):445–448.
179 Yamano K, Shirahama H. New amino acids from clitocybe acromelalga. possible intermediates in the biogenesis of mushroom toxins, acromelic acids. *Tetrahedron*. 1993;49(12):2427–2436.
180 Bacon C, Porter J, Norred W, Leslie J. Production of fusaric acid by fusarium species. *Appl Environ Microbiol*. 1996;62(11):4039–4043.
181 Yabuta T. Biochemistry of fusarium heterosporium in rice plant, part 1: newly found product of fusaric acid. *J Agr Chem Soc Japan*. 1934;10:1059–1068.
182 Wang H, Ng T. Pharmacological activities of fusaric acid (5-butylpicolinic acid). *Life Sci*. 1999;65(9):849–856.
183 Hidaka H, Nagatsu T, Takeya K, et al. Fusaric acid, a Hypotensive agent produced by Fungi. *J Antibiot*. 1969;22(5):228–230.
184 Toshiharu N, Hiroyoshi H, Hiroshi K, et al. Inhibition of dopamine β-hydroxylase by fusaric acid (5-butylpicolinic acid) in vitro and in vivo. *Biochem Pharmacol*. 1970;19(1):35–44.
185 Goodwin FK, Sack RL. Behavioral effects of a new dopamine-β-hydroxylase inhibitor (fusaric acid) in man. *J Psychiatr Res*. 1974;11:211–220.
186 Dhani S, Ghazi T, Nagiah S, Baijnath S, Singh SD, Chuturgoon AA. Fusaric acid alters akt and ampk signalling in C57bl/6 mice brain tissue. *Food Chem Toxicol*. 2020:111252.
187 Nakamura T, Yasuda H, Obayashi A, et al. Phenopicolinic acid, a new microbial product inhibiting dopamine β-hydroxylase. *J Antibiot*. 1975;28(6):477–478.
188 Blunt J, Copp B, Keyzers R, Munro M, Prinsep M. Marine natural products. *Nat Prod Rep*. 2017;34(3):235–294.
189 Hiort J, Maksimenka K, Reichert M, et al. New natural products from the sponge-derived fungus aspergillus niger. *J Nat Prod*. 2004;67(9):1532–1543.
190 Hiort J, Maksimenka K, Reichert M, et al. New natural products from the sponge-derived fungus aspergillus niger. *J Nat Prod*. 2005;68(12), 1821–1821.
191 Kim S-H, Ko H, Bang H-S, et al. Coprismycins A and B, neuroprotective phenylpyridines from the dung beetle-associated bacterium. Streptomyces Sp. *Bioorg Med Chem Lett*. 2011;21(19):5715–5718.
192 Gomi S, Amano S, Sato E, Miyadoh S, Kodama Y. Novel antibiotics SF2738A, B and C, and their analogs produced by Streptomyces Sp. *J Antibiot (Tokyo)*. 1994;47(12):1385–1394.
193 Bacq Z. Les poisons des nemertiens. *Bull Cl Sci Acad R Belg*. 1936;22:1072–1079.
194 Kem WR, Abbott BC, Coates RM. Isolation and structure of a hoplonemertine toxin. *Toxicon*. 1971;9(1):15–22.
195 Kem WR, Mahnir VM, Papke RL, Lingle CJ. Anabaseine is a potent agonist on muscle and neuronalalpha-bungarotoxin-sensitive nicotinic receptors. *J Pharmacol Exp Ther*. 1997;283(3):979–992.
196 Xing H, Rouchaud A, Keshwah S, Kem W. Isoanatabine, a naturally occurring Α4β2 nicotinic receptor agonist. *Biochem Pharmacol*. 2009;7(78):906–907.
197 Kem W, Scott KN, Duncan J. Hoplonemertine worms—a new source of pyridine neurotoxins. *Experientia*. 1976;32(6):684–686.
198 Kubota T, Ishiguro Y, Yamamoto S. Platisidines A-C, N-methylpyridinium alkaloids from an okinawan marine sponge of plakortis species. *Heterocycles*. 2010;80(2):1407–1412.
199 Fusetani N, Asai N, Matsunaga S, Honda K, Yasumuro K. Cyclostellettamines A-F, pyridine alkaloids which inhibit binding of methyl quinuclidinyl benzilate (QNB) to muscarinic acetylcholine receptors, from the marine sponge, Stelletta Maxima. *Tetrahedron Lett*. 1994;35(23):3967–3970.
200 Cafieri F, Fattorusso E, Mangoni A, Taglialatela-Scafati O, Carnuccio R. A Novel bromopyrrole alkaloid from the sponge agelas longissima with antiserotonergic activity. *Bioorg Med Chem Lett*. 1995;5(8):799–804.
201 Aiello A, D'Esposito M, Fattorusso E, et al. Daminin, a bioactive pyrrole alkaloid from the mediterranean sponge axinella damicornis. *Tetrahedron*. 2005;61(30):7266–7270.
202 Hoyer D, Clarke DE, Fozard JR, et al. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev*. 1994;46(2):157–203.
203 DeCoster MA. Calcium dynamics in the central nervous system. *Adv Neuroimmunol*. 1995;5(3):233–239.
204 Zhou J, Chan L, Zhou S. Trigonelline: A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Curr Med Chem*. 2012;19(21):3523–3531.
205 Jayaweera D. *Medicinal plant*. Part III Flacourtiaceae-Lythraceae. The National Science Council Of Sri Lanka; 1981:255–256.
206 Allred KF, Yackley KM, Vanamala J, Allred CD. trigonelline is a novel phytoestrogen in coffee beans. *J Nutr*. 2009;139(10):1833–1838.
207 Tohda C, Nakamura N, Komatsu K, Hattori M. Trigonelline-induced neurite outgrowth in human neuroblastoma SK-N-SH cells. *Biol Pharm Bull*. 1999;22(7):679–682.
208 Tohda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. *Neurosignals*. 2005;14(1–2):34–45.
209 Temraz TA, Houssen WE, Jaspars M, et al. A Pyridinium derivative from red sea soft corals inhibited voltage-activated potassium conductances and increased excitability of rat cultured sensory neurones. *BMC Pharmacol*. 2006;6(1):10.
210 Walker J, Rohm B, Lang R, Pariza M, Hofmann T, Somoza V. Identification of coffee components that stimulate dopamine release from pheochromocytoma cells (PC-12). *Food Chem Toxicol*. 2012;50(2):390–398.
211 Hossain SJ, Aoshima H, Koda H, Kiso Y. Effects of coffee components on the response of GABAA receptors expressed in xenopus oocytes. *J Agric Food Chem*. 2003;51(26):7568–7575.
212 Satheeshkumar N, Mukherjee PK, Bhadra S, Saha B. Acetylcholinesterase enzyme inhibitory potential of standardized extract of trigonella foenum graecum L and its constituents. *Phytomedicine*. 2010;17(3–4):292–295.