triene-4,6-diol (4R) protects acute hippocampal slices against excitotoxicity via a nicotinic mechanism. The data shows that 4R protects against the neurotoxic organophosphates paraoxon (POX) and diisopropylfluorophosphate (DFP) suggesting that cembranoids could be novel antidotes against these neurotoxins. Exposure to organophosphate (OP) insecticides or sublethal doses of OP war nerve toxins cause health impairment. The best-documented detrimental effects involve deficits in behavioral performance and abnormalities in nerve function. Many of the chronic symptoms associated with OP insecticide exposure are indistinguishable from those reported by Gulf War veterans allegedly exposed to OP nerve toxins. Current postexposure medical countermeasures against nerve agents (atropine, oximes, reversible AChE inhibitors and benzodiazepines) are useful in preventing mortality but are not sufficiently effective as far as protecting the CNS against apoptotic neuronal death. We used acute hippocampal slices to study the toxicity of POX and DFP and the protection by 4R. Acute hippocampal slices are a choice preparation to quantitatively measure early neurotoxic and neuroprotective events. This model has been successfully used for more than two decades by others and by us to study the effect of anoxia, oxygen and glucose deprivation, and excitotoxic amino acids. The main parameter measured is the loss of synaptically evoked population spikes (PS), which reflects the sum of axon potentials from a population of neurons and is an early predictor of neuronal apoptosis. Routinely, POX and DFP were superfused for 10 min and washed off for 30 min. Afterwards antidotes were applied for 60 min, and the PS were recorded. Our results show that 50–100 μM POX decreased the PS area by 60-80%; a higher concentration, up to 200 μM POX, did not increase the damage. The effect of POX developed with a half-life of 2 min; the maximum effect was reached by 10 min and remained unchanged for up to 1 hour. Ten μM POX completely inhibited AChE activity in the slice. The classical antidote, 200 μM pralidoxime, applied 30 min after POX provided an almost total remission of the damage caused by POX. One μM atropine, the main antidote against OPs presently used, was not significantly neuroprotective against POX when used alone. As POX, DFP inhibited the activity of AChE; but contrary to POX, DFP caused a concentration dependent loss of PS. 4R, at 2 to 10 μM applied together with 1 μM atropine 30 min after exposure to POX or DFP, protected nearly 100% and after 1 hour 70% of PS area.

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1.15

From acetyl bispidine to an extended bispidine amide framework: Synthesis and structure–affinity relationships for nicotinic acetylcholine receptors (nAChRs)

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Cytisine discovered in the 19th century is an invaluable template in the development of bioactive compounds. Especially its bispidine framework which is fused to a 2-pyridone moiety has been used as a core structure for the synthesis of ligands for numerous biological targets including nAChRs. It is accessible by double Mannich reaction from N-*t*Boc-4-piperidone, formaldehyde and benzylamine and subsequent reduction of the carbonyl group yielding N-benzyl-N′-*t*Boc-bispidine. The N-protected bispidine, especially N-*t*Boc-bispidine after cleavage of the N-benzyl protecting group, served as starting material for the synthesis of diverse bispidine analogs. N-*t*Boc-bispidine itself interacts with nAChRs (e.g. Ki: 45 nM for α4/β2*). The obtained bispidine amides were tested for their affinities for different nAChR subtypes by competition assays with [^{3}H]epibatidine α4/β2*, α3/β4*, muscle type) and [^{3}H]MLA (α7*), respectively, using membrane fractions of native tissues (rat brain, calf/pig adrenals and Torpedo californica electroplax). The simplest analog, acetyl bispidine, displayed high affinity for α4/β2 (Ki: 5.6 nM). Compounds showed a broad affinity spectrum (e.g. Ki values from 1.2 nM to >10,000 nM for α4/β2*), which provided important insight into structure–affinity relationship.

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1.16

Probing the non-competitive binding site within the n-terminal region of α4β2 nicotinic receptors

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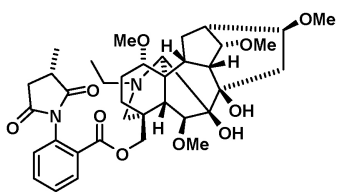
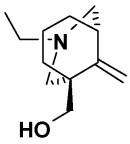
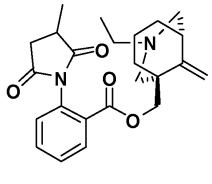
Novel nicotinic acetylcholine receptor (nAChR) antagonists have been derived from methyllycaconitine (MLA). AE Alcohol analogue 1 [(1*S**, 5*S**)-(3-ethyl-9-methylidene-3-azabicyclo[3.3.1]non-1-yl)methanol] is a truncated version and displays non-competitive binding on α4β2, α3β4 and α7 nAChRs. AE Succinimide analogue 2 [(3-ethyl-9-methylene-3-aza-bicyclo[3.3.1]nonan-1-yl)methyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate] contains an anthranilate ester side-chain displaying mixed competitive and non-competitive binding at these receptors.



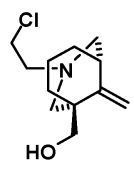
Methyllycaconitine (MLA)



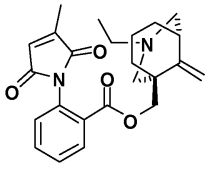
Analogue 1



Analogue 2



Analogue 1 Probe



Analogue 2 Probe

Mutation of the acetylcholine binding protein (AChBP) subunits to mimic the binding site of mammalian nAChRs combined with radioligand binding studies and X-ray crystallography has provided