Notes

Nudicauline and Elatine as Potent Norditerpenoid Ligands at Rat Neuronal α-Bungarotoxin Binding Sites: Importance of the 2-(Methylsuccinimido)benzoyl Moiety for Neuronal Nicotinic Acetylcholine Receptor Binding

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Methyllycaconitine (MLA, **1**) is a novel, potent probe for mammalian and insect nicotinic acetylcholine receptors (nAChR) and displays remarkable selectivity toward neuronal [^{125}I]-α-bungarotoxin (αBgTX) binding sites that correspond to α7-type nAChR in mammalian brain. We have shown that, among a number of selected norditerpenoid alkaloids, elatine (**2**) and nudicauline (**3**) are equipotent with, or better than, MLA (**1**) in binding to brain [^{125}I]-αBgTX binding sites, with IC_{50} values of 6.1, 1.7, and 7.6 nM, respectively. The 2-((*S*)-methylsuccinimido)benzoyl moiety of these ligands is crucial for high-affinity binding, whereas structural modifications to the norditerpenoid core of the ligand can be tolerated without loss of activity or selectivity. In addition to MLA (**1**), elatine (**2**), and nudicauline (**3**), we have examined lycoctonine (**4**), inuline (**6**), lappaconitine (**7**), *N*-desacetyllappaconitine (**8**), delsoline (**10**), delcorine (**11**), deltaline (**12**), condelphine (**13**), and karacoline (**14**). This study therefore extends the range of norditerpenoids, other than MLA, which can be used to probe this important class of nAChR. All 12 alkaloids were assessed for activity at [^{3}H]nicotine binding sites which are considered to represent α4β2 nAChR. Furthermore, the ^{1}H and ^{13}C NMR spectroscopic data of MLA and elatine have been critically compared.

Introduction

Methyllycaconitine (MLA, **1**), isolated and purified from various species of *Delphinium* and *Consolida*, is a hexacyclic norditerpenoid alkaloid which is esterified at the C18 oxygen atom with the 2-((*S*)-methylsuccinimido)benzoyl moiety.[1,2] MLA (**1**) is the most potent, nonproteinaceous competitive antagonist at neuronal vertebrate and invertebrate [^{125}I]-α-bungarotoxin (αBgTX) binding sites,[3,4] which, in mammalian brain, correspond to nicotinic acetylcholine receptors (nAChR) containing the α7 subunit.[4,5] Unlike αBgTX, MLA (**1**) displays a 1000-fold preference for the neuronal αBgTX-sensitive nAChR subtypes relative to muscle nAChR[3,6] and blocks α7-like nAChR in hippocampal neurons and *Xenopus* oocytes with picomolar affinity.[5,7]

nAChR are pentameric ligand-gated cation channels. Whereas the well-characterized nAChR from vertebrate muscle and *Torpedo* electroplaques are comprised of two α and one each of β, γ (or ε), and δ subunits, a plethora of distinct but homologous subunits have been identified in mammalian and avian nervous systems.[8,9] Thus, there is potential for enormous heterogeneity of neuronal nAChR, and the identification and characterization of these distinct nAChR would be greatly facilitated by a range of selective probes. Historically, the alkaloids nicotine and muscarine were used to distinguish nicotinic and muscarinic acetylcholine responses.[10] More recently, the snake polypeptide toxin αBgTX has been crucial in the characterization of muscle nAChR[11] and certain neuronal nAChR that correlate with functional receptors reconstituted from α7, α8, and α9 subunits expressed in *Xenopus* oocytes.[7−9,12]

Several hundred lycoctonine-type norditerpenoid alkaloids have now been isolated from *Delphinium* and *Consolida*,[13−15] and some of these may have similar potential to MLA (**1**) in terms of potency and selectivity.[16,17] A few of these natural products (<5%) possess the 2-((*S*)-methylsuccinimido)benzoyl moiety of MLA (**1**). Elatine (**2**) and nudicauline (**3**) (Figure 1) are two such ligands which differ from MLA (**1**) within the norditerpenoid core but contain this anthranilate ester moiety. These probes are candidates for exploring the differences in the agonist/competitive antagonist recognition site between various members of the nAChR family and are lead compounds for the design and synthesis of novel high-affinity ligands for neuronal nAChR subtypes. Benn and Jacyno have reviewed the chemical and biological perspectives of these norditerpenoid alkaloids.[16] Indeed, Jacyno and colleagues have recently published that lycaconitine (similar to MLA (**1**), but lacking the angular methyl group on the succinimide) is 5-fold less active than MLA.[17]

We have recently established[18] that hydrolysis of the C18 ester bond in MLA (**1**) to yield lycoctonine (**4**)

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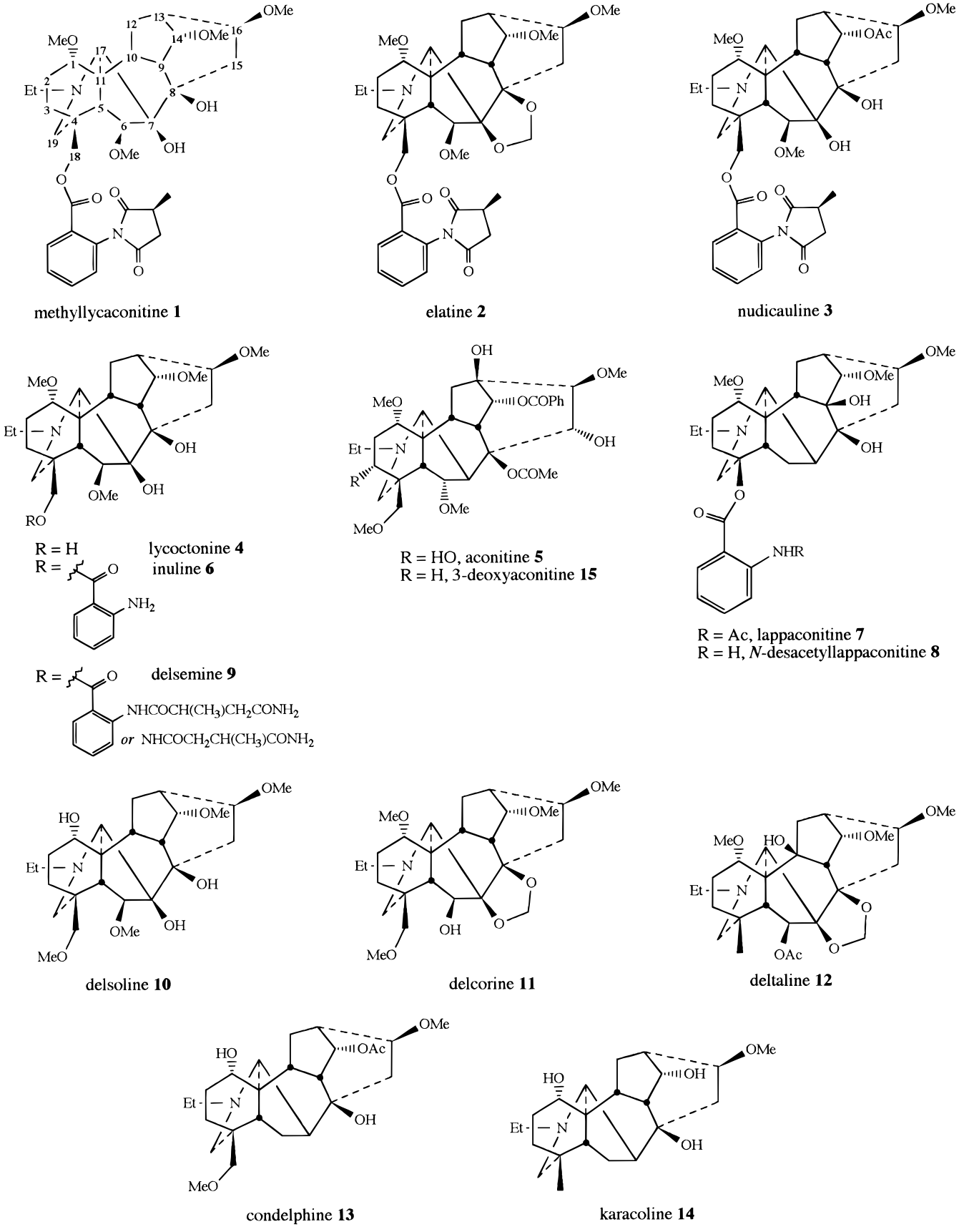


Figure 1. Structures of norditerpenoid alkaloids.

greatly diminishes nicotinic potency. In contrast, addition of the 2-((*S*)-methylsuccinimido)benzoyl moiety to the norditerpenoid alkaloid aconitine (**5**) converts it from a sodium channel activator with only weak affinity for rat brain [^{125}I]-αBgTX binding sites into a nicotinic ligand with potency comparable to that of MLA (**1**).[18] We now demonstrate the importance of this moiety for nicotinic potency, and we report that a number of natural and semisynthetic alkaloids which contain this ester group are potent neuronal nAChR probes, as a result of our biological analysis of 12 norditerpenoid alkaloids at α7 and α4β2 nAChR.

Chemistry

In order to obtain alkaloids to test the structure−activity relationships among these substituted norditerpenoids quickly, we used readily available MLA (**1**) as the template for functional group transformations. This alkaloid was isolated and purified from Garden Hybrid *Delphinium* according to literature procedures.[1,2] Saponification of ester **1**, with sodium hydroxide, afforded neopentyl-like alcohol **4** which could be readily purified from even trace amounts of MLA (**1**).[19] The high affinity (nanomolar) of this nAChR antagonist means that essentially all traces of this starting material must be removed prior to assaying the resulting semisynthetic ligands.[19] Lycoctonine (**4**) was prepared free from detectable MLA (**1**) and then converted into inuline (**6**) by treatment with isatoic anhydride catalyzed by 4-(*N*,*N*-dimethylamino)pyridine. Inuline (**6**) was also synthesized by the controlled hydrolysis of succinimide **1**, but we found that inuline (**6**) and any small quantities of unreacted MLA (**1**) were difficult to separate by silica gel column chromatography, making the anthranoylation of lycoctonine (**4**) a more expedient route to the free aniline **6** than the partial hydrolysis of MLA (**1**). Elatine (**2**) was prepared by the reaction of MLA (**1**) with a large excess of formaldehyde diethylacetal (as the solvent), in order to introduce the C7/C8 methylenedioxy bridge.[20,21] We found this procedure to be more practical than azeotropic distillation (Dean−Stark) from benzene−formalin.[21]

NMR Data

The ^{1}H NMR data for lycoctonine (**4**) are in good agreement with the published data in the recent comprehensive review of the ^{1}H NMR spectra of norditerpenoid alkaloids by Hanuman and Katz.[22] For lycoctonine (**4**), the C8 hydroxyl functionality was assigned at δ 4.15 by comparison with the ^{1}H NMR spectrum of MLA (**1**). The C18 hydroxyl functionality was assigned within δ 3.58−3.62 following the conversion of lycoctonine (**4**) into inuline (**6**). The C7 hydroxyl functionality was not observed for **4** (in $CDCl_3$). In the 400 MHz ^{1}H NMR spectrum of semisynthetic inuline (**6**) (from lycoctonine), the C18 methylene protons resonated as two doublets at δ 4.12 and 4.13 (J_{AB}), and the signal at δ 1.66 was assigned to the H7 hydroxyl proton. These spectral data are in good agreement with those obtained with inuline (**6**) obtained by the partial hydrolysis of MLA (**1**). The ^{1}H NMR spectrum of elatine (**2**) displayed the characteristic methylenedioxy functional group resonance at δ 5.07 and broadened succinimido resonances in the ^{13}C NMR spectrum similar to those observed for MLA (**1**).

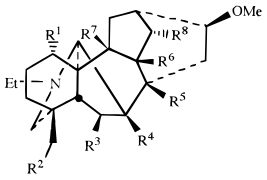
The ^{13}C NMR data for elatine (**2**) are all in good agreement with those previously assigned by Pelletier and co-workers, and the assignments presented here are based upon those published data.[21] There is considerable similarity between the structures of the norditerpenoid alkaloids MLA (**1**) and elatine (**2**), the only difference being the introduction of a C7/C8 methylenedioxy bridge in **2** compared to the two tertiary alcohols in **1** (see Figure 1). Interestingly, the C9 and C10 carbon resonances appear at significantly different frequencies to those found in MLA (**1**). Hence, our results indicate that C9 and C10 resonate at δ 43.22 and 46.07, respectively, in MLA (**1**), compared to elatine (**2**) where C9 and C10 have been assigned in the reverse order at 48.42 and 39.86 ppm, respectively.[21] Furthermore, we have assigned the elatine resonances at 36.99 and 35.39/35.19 ppm as the C2″ and C3″ succinimido carbons, respectively, based on Pelletier's assignments of 37.0 (C2″ succinimide) and 35.3 (C3″ succinimide) ppm for elatine. However, in our DEPT and correlation spectroscopic studies with MLA, the C3″ methylene succinimido carbon resonates at 37.00 ppm, while the C2″ methine succinimido signal is unambiguously assigned to the split peak at 35.45 and 35.21 ppm. The inconsistency in these literature assignments for elatine is due to incorrect numbering of the methylsuccinimido ring rather than an error in the interpretation of the ^{13}C NMR spectrum. The C5″ succinimido methyl carbon resonance in MLA also appears as either a broadened signal or a split peak (*vide infra*) at approximately 16.4 ppm, and the same phenomenon is also observed in elatine. If the signal at 36.99 ppm, in our spectrum, can be attributed to the succinimido methylene carbon, then it is possible that the split peaks observed for the methine and methyl carbons are a result of racemization at the chiral center under the experimental conditions employed. However, this is unlikely as this split peak phenomenon is also observed with MLA which has been unambiguously synthesized from inuline and homochiral (*S*)-methylsuccinic anhydride where racemization of the carbon bearing the methyl group is unlikely to occur.[23] In the published ^{13}C NMR spectral data for elatine (**2**), the signals at 28.4 and 27.9 ppm have been assigned to C2 and C12, respectively.[21] In the spectral data obtained with our samples of elatine, these signals resonated at 27.80 and 26.36 ppm. However, in the ^{13}C NMR spectra of MLA (**1**), C12 resonates at lower field (28.70 ppm) compared to C2 (26.07 ppm) unambiguously identified by DEPT and ^{13}C/^{1}H heteronuclear correlation spectroscopy. Following comparison with our MLA ^{13}C NMR data, it is possible that the published assignments for C2/C12, C9/C10, and the succinimido methine/methylene in elatine (**2**) may have to be revised (interchanged).

Results and Discussion

The binding constants (IC_{50}) of 12 lycoctonine- and aconitine-type norditerpenoid alkaloids for inhibition of [^{125}I]-αBgTX binding to rat brain membranes are given in Table 1; inhibition curves for six of these compounds, representing the range of potencies observed, are illustrated in Figure 2. Three of these compounds, MLA (**1**), elatine (**2**), and nudicauline (**3**), are potent inhibitors of binding with IC_{50} values of 7.6, 6.1, and 1.7 nM, respectively, showing that nudicauline (**3**) is even more potent than MLA (**1**). These high-affinity alkaloids are distinguished by the presence of the 2-((*S*)-methylsuccinimido)benzoyl moiety, but they differ in substituents around the norditerpenoid core. In comparison, the greatly reduced potency of lycoctonine (**4**) emphasizes the importance of the 2-((*S*)-methylsuccinimido)benzoyl moiety for activity.

We have previously postulated[6] that the ester within MLA (**1**) provides a carbonyl oxygen that, together with the tertiary nitrogen atom, conforms to the Beers and Reich pharmacophore[24] for nicotinic binding. Thus, the ester carbonyl oxygen in MLA (**1**) could donate a lone pair of electrons to form a hydrogen bond at the ligand binding site. However, inuline (**6**) is the anthranoyl ester of lycoctonine (**4**); therefore inuline incorporates the carbonyl ester functional group of MLA (**1**), yet it is

Table 1. Affinity of a Series of Norditerpenoid Alkaloids at Rat Neuronal α7-Type nAChR



compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50} (M)
methyllycaconitine (**1**)	MeO	2-(methylsuccinimido)benzoate	MeO	OH	OH	H	H	OMe	$7.6 \pm 3.4 \times 10^{-9}$
nudicauline (**3**)	MeO	2-(methylsuccinimido)benzoate	MeO	OH	OH	H	H	OAc	$1.7 \pm 0.7 \times 10^{-9}$
lycoctonine (**4**)	MeO	HO	MeO	OH	OH	H	H	OMe	$1.0 \pm 0.1 \times 10^{-5}$
inuline (**6**)	MeO	2-aminobenzoate	MeO	OH	OH	H	H	OMe	$1.6 \pm 0.6 \times 10^{-6}$
lappaconitine (**7**)	MeO	(see Figure 1)	H	H	OH	OH	H	OMe	$9.6 \pm 5.5 \times 10^{-5}$
N-desacetyllappaconitine (**8**)	MeO	(see Figure 1)	H	H	OH	OH	H	OMe	$6.8 \pm 0.9 \times 10^{-6}$
delsoline (**10**)	HO	MeO	MeO	OH	OH	H	H	OMe	$1.9 \pm 0.5 \times 10^{-5}$
elatine (**2**)	MeO	2-(methylsuccinimido)benzoate	MeO	OCH_2O		H	H	OMe	$6.1 \pm 1.5 \times 10^{-9}$
delcorine (**11**)	MeO	MeO	HO	OCH_2O		H	H	OMe	$5.3 \pm 1.4 \times 10^{-5}$
deltaline (**12**)	MeO	H	AcO	OCH_2O		H	HO	OMe	$1.1 \pm 0.4 \times 10^{-4}$
condelphine (**13**)	HO	MeO	H	H	OH	H	H	OAc	$1.6 \pm 0.3 \times 10^{-6}$
karacoline (**14**)	HO	H	H	H	OH	H	H	OH	$1.8 \pm 0.5 \times 10^{-6}$

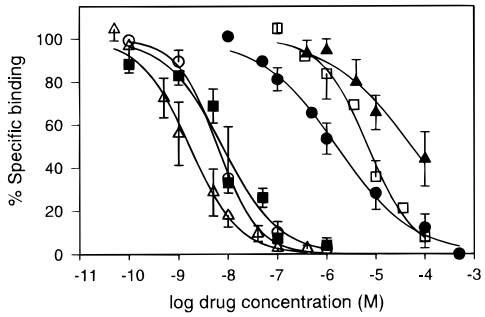


Figure 2. Binding curves for inhibition of [^{125}I]-αBgTX binding to rat brain membranes by representative norditerpenoids. Brain membranes were incubated with [^{125}I]-αBgTX in the presence and absence of excess unlabeled αBgTX (to define nonspecific binding) or serial dilutions of norditerpenoids, to determine their ability to compete for specific ligand binding sites: (△) nudicauline (**3**), (■) MLA (**1**), (○) elatine (**2**), (●) condelphine (**13**), (□) *N*-desacetyllappaconitine (**8**), (▲) delcorine (**11**). Values are the means from at least three independent assays, with SEM indicated by the vertical bars. Data points have been fitted to the nonlinear Hill equation to generate the curves shown; IC_{50} values derived from the curves are given in Table 1.

at least 2 orders of magnitude less active than MLA at inhibiting [^{125}I]-αBgTX binding. This result indicates that the anthranoyl ester alone is insufficient for optimum potency, and we propose that the 2-(*S*)-methylsuccinimido moiety of MLA is significant for its activity. Recent toxicity studies (LD_{50} values) in mice have revealed that the removal of this moiety from MLA (**1**) reduces the LD_{50} by more than 2 orders of magnitude.[25] This work highlighted the importance of an amide or succinimide functionality at the anthranilic acid aniline functional group to enhance mammalian toxicity and concluded that anthranoyl lycoctonine (inuline, **6**) was a pivotal toxic alkaloid in the transition between low and high toxicity.[25]

Lappaconitine (**7**) and *N*-desacetyllappaconitine (**8**) are both norditerpenoid alkaloids which possess an anthranoyl moiety directly esterified, via a tertiary alcohol, to the A ring of the norditerpenoid core structure (at C4). MLA (**1**) and other potent norditerpenoid alkaloids studied in this series differ at this point by having an extra carbon atom (C18) between the A ring and the alkyl oxygen of the anthranoyl ester bond. The distance between the tertiary nitrogen atom and the ester carbonyl oxygen atom of MLA (**1**) is crucial in the Beers and Reich pharmacophore,[24] and this distance will be similar in lappaconitine (**7**) and *N*-desacetyllappaconitine (**8**) but different from that found in MLA (**1**). Both **7** and **8** are weak inhibitors of [^{125}I]-αBgTX binding (IC_{50} = 96 and 6.8 μM, respectively). This may reflect alterations in the distance geometry (*vide supra*), the absence of the 2-(*S*)-methylsuccinimido ring, or other structural changes (see Figure 1) within these ligands.

Delsemine (**9**) is similar in structure to MLA (**1**) except that the 2-(*S*)-methylsuccinimido ring has been opened with ammonia to produce the corresponding bis-amides. Ammonia can react with either carbonyl of the 2-(*S*)-methylsuccinimido ring to give bis-amides with the methyl group either α or β to the primary amide.[26] Delsemine (**9**), as a mixture of these regioisomers, has been reported to be over 2 orders of magnitude less active (IC_{50} = 0.36 μM) than MLA (**1**) at rat brain [^{125}I]-αBgTX binding sites,[27] a result which provides further evidence that this succinimido ring is significant for high potency.

The methylsuccinimido ring, which is found in MLA (**1**) and other related potent ligands, may help to maintain the appropriate distance geometry between the tertiary nitrogen atom of the norditerpenoid and the carbonyl oxygen of the ester bond.[24,28] ^{1}H and ^{13}C NMR spectroscopic studies with MLA (**1**), elatine (**2**), and related small molecule analogues containing the 2-(methylsuccinimido)benzoyl moiety (on a neopentyl alcohol) display broad lines for the methyl resonances, indicating that the 5-membered heterocycle may undergo slow rotation on the NMR time scale.[23] Inuline (**6**) (anthranoyllycoctonine) satisfies the pharmacophore requirements of the electronegative oxygen atom and the tertiary nitrogen atom,[24,28] but it is possible that the anthranoyl aromatic ring is no longer in the correct orientation for high-affinity binding. *N*-Acylated analogues of inuline (**6**), e.g., delsemine (**9**), should possess similar electron bond distributions to those found in MLA (**1**), but the observed potency of this alkaloid **9** (*vide supra*) is significantly reduced when compared to that of MLA (**1**). Furthermore, the modest activity of inuline (**6**) (IC_{50} = 1.6 μM, Table 1) cannot be attributed even to trace impurities of MLA (**1**), as it was synthesized from a sample of lycoctonine (**4**) itself prepared

and efficiently purified from MLA (**1**). The same source of lycoctonine (**4**) had little activity ($IC_{50} = 10\ \mu M$, Table 1).

From our detailed examination of the relative potencies of these alkaloids, the importance of substituents on the norditerpenoid core can also be appraised. In particular, elatine (**2**) was equipotent (Table 1) with MLA (**1**), despite the replacement of C7 and C8 hydroxyl groups with a methylenedioxy bridge (see Figure 1). We therefore conclude that the C7 and C8 hydroxyl groups are not essential for the donation of a hydrogen atom to form a hydrogen bond at the receptor binding site. They are the only two (tertiary) hydroxyl groups present in MLA (**1**), although they cannot be discounted as the source of lone pairs of electrons to form a hydrogen bond. Nudicauline (**3**), in which the C14 *O*-methyl ether of MLA (**1**) has been replaced with an acetate functional group, was marginally more potent than MLA at [^{125}I]-αBgTX binding sites (Table 1, Figure 2). Kukel and Jennings have recently shown that within a series of five 2-(methylsuccinimido)benzoyl norditerpenoid alkaloids tested at rat neuronal [^{125}I]-αBgTX binding sites, all were within 1 order of magnitude in binding affinity when compared to MLA (**1**).[27] Hence, glaudelsine, which has the lowest binding affinity in their series and differs from MLA (**1**) in having a *β*-hydroxy functional group at C6 (i.e., not a *β*-methoxy functional group), inhibited [^{125}I]-αBgTX binding in rat brain membranes with an IC_{50} of 16 nM compared to MLA (**1**) with an IC_{50} of 1.7 nM. Unfortunately, the key chemical structures of these norditerpenoid alkaloids are incorrect in their paper and also in the corresponding erratum, where active compounds are shown with incorrect stereochemistry.[27]

Other norditerpenoid alkaloids tested in our study, none of which contains the 2-((*S*)-methylsuccinimido)-benzoyl functionality, were only weakly active at rat neuronal [^{125}I]-αBgTX binding sites as shown in Table 1. Hence, we propose that structural modifications to selective functional groups within the norditerpenoid alkaloid can be tolerated without loss of significant activity or selectivity compared to altering the 2-((*S*)-methylsuccinimido)benzoyl moiety. The C18 *O*-methyl ethers delsoline (**10**) and delcorine (**11**) were of low activity. The least potent compound in our study was deltaline (**12**) (Table 1) with an IC_{50} of 110 μM. Deltaline lacks an oxygen atom at C18 (Figure 1), and it is also the only compound examined which contains an acetate group at C6 on the *β*-face. Deltaline (**12**) contains an additional tertiary alcohol at C10 (R^7 = HO, Table 1). However, in other respects, e.g., the presence of the C7/C8 methylenedioxy bridge, deltaline is similar in its norditerpenoid core substitution pattern to elatine (**2**), one of the most potent ligands in our study. Delcorine (**11**) also contains this methylenedioxy bridge, yet it is 4 orders of magnitude less active than elatine (**2**) (see Table 1). This difference in activity is analogous to that observed between lycoctonine (**4**) and MLA (**1**).

The results presented here for lycoctonine-type alkaloids are also in agreement with our studies with aconitine-type norditerpenoids which we have synthesized substituted with anthranoyl and 2-((*S*)-methylsuccinimido)benzoyl moieties respectively.[18] Thus, condelphine (**13**) and karacoline (**14**), which are substituted with hydrogen at C7 (R^4 in Table 1) and are therefore typical *Aconitum* alkaloids, displayed low activity. Furthermore, when semisynthetic 3-deoxyaconitine (**15**) was 18-*O*-demethylated and regioselectively esterified at the unmasked C18 neopentyl alcohol with the 2-((*S*)-methylsuccinimido)benzoyl moiety, the binding affinity at rat [^{125}I]-αBgTX binding sites was equal to that recorded for MLA (**1**).[18] This is despite the fact that 3-deoxyaconitine (**15**) is significantly different from lycoctonine (**4**) within the norditerpenoid core, e.g., the aconitine-type alkaloid **15** contains 6α-methoxy, 7*β*-hydrido, 8*β*-acetate, 13*β*-hydroxy, 14α-benzoate, and 15α-hydroxy substitutents (see Figure 1) compared to a lycoctonine-type alkaloid. Furthermore, aconitine (**5**) and MLA (**1**) display markedly different pharmacology. However, without the 2-(*S*)-methylsuccinimido group and with the anthranoyl ester functional group intact (cf. inuline, **6**), a decrease in the binding affinity of 2 orders of magnitude was observed.[18] This is analogous to the difference in activity we have observed between MLA (**1**) (which contains the succinimido group) and inuline (**6**).

The α3- and α4-containing nAChR are several orders of magnitude less sensitive to MLA than α7-containing nAChR.[29] All compounds were assessed for activity at [^{3}H]nicotine binding sites which are considered to represent nAChR comprised of α4 and *β*2 subunits.[30] Only those compounds with nanomolar affinities at [^{125}I]-αBgTX binding sites showed any appreciable activity at [^{3}H]nicotine binding sites. IC_{50} values for MLA (**1**), nudicauline (**3**), and elatine (**2**) were 44, 9.4, and 8.3 μM, respectively. Thus, these norditerpenoids are at least 1000-fold more active at α7-containing nAChR. A preference for [^{125}I]-αBgTX binding sites is retained by all the other compounds which displayed micromolar affinities for this (α7) nAChR but were unable to displace [^{3}H]nicotine binding even at the highest concentration tested (100 μM).

Conclusions

These results demonstrate unequivocally the importance of the methylsuccinimido ring for potency of lycoctonine-type norditerpenoid alkaloids at mammalian neuronal nAChR labeled with [^{125}I]-αBgTX. There is now considerable scope for the synthesis of alternative substituted succinimide or maleimide analogues of MLA (**1**) which can be used to explore the domain surrounding this region of the ligand at the receptor (agonist) binding site. These results also demonstrate that hydrogen atom donation from C7 and/or C8 hydroxyl functional groups, in forming a putative hydrogen bond, is not an absolute requirement for the binding of MLA (**1**) to α7 nAChR. There appears to be a tolerance of certain functional group alterations/deletions within the MLA norditerpenoid core which do not affect binding affinity. These results have implications for the design and synthesis of less structurally complex, subtype-selective nAChR ligands.[31,32]

Experimental Section

NMR spectra were recorded using Jeol GX 270 (operating at 270 MHz for ^{1}H) or Jeol EX 400 (operating at 400 MHz for ^{1}H or 100 MHz for ^{13}C) spectrometers. Chemical shift values are on the δ scale (parts per million) and referenced to either tetramethylsilane, δ 0.00 in $CDCl_3$ for ^{1}H NMR, or deuteriated chloroform, at δ 77.00 for ^{13}C NMR. NMR data are reported sequentially as integral, multiplicity, assignment, and coupling constant (*J*). The ^{1}H and ^{13}C NMR assignments for all norditerpenoid alkaloids were based on detailed comparisons with ^{1}H/^{1}H homonuclear, ^{1}H/^{13}C heteronuclear, and ^{13}C DEPT

spectroscopic studies of MLA and elatine and are in agreement with recently reported ^{1}H and ^{13}C NMR spectroscopic data for these alkaloids.[21,22] Thin layer chromatography (TLC) was performed using aluminum-backed TLC plates coated with Kieselgel 60 F_{254} purchased from Merck. Silica gel 60 (35−75 μm) was purchased from Prolabo. Solvent A is dichloromethane−methanol−concentrated aqueous ammonia, 100:10:1, v/v/v. High-resolution mass spectra (FAB, mNBA matrix) were recorded with a VG Analytical Autospec mass spectrometer (by the EPSRC MS service at Swansea), and HPLC was performed with a Jasco PU-980 pump equipped with a Jasco UV-975 detector. Melting points were determined using a Kofler hot-stage apparatus (Cambridge Instruments) and are uncorrected.

MLA was isolated and purified from Garden Hybrid *Delphinium* as previously described and was >99.9% pure by HPLC (using UV detection at 270 nm, with lappaconitine as the internal standard).[1,2,19] Furthermore, our isolated MLA cochromatographs with and displays identical biological activity with an authentic sample of MLA, kindly provided for us by Prof. M. Benn (University of Calgary, Canada). Unless otherwise stated, all solvents were purchased from Fisons. Acetonitrile, chloroform, dichloromethane, and methanol were all HPLC grade. Absolute ethanol was purchased from Haymans, and anhydrous DMF and DMSO were purchased from Aldrich. Isatoic anhydride was recrystallized (absolute ethanol) to give a brown powder, mp 210−213 °C dec (lit.[33] mp 240 °C dec, lit.[34] mp 243 °C dec).

Lycoctonine (4). MLA (870 mg, 1.27 mmol) was dissolved in absolute ethanol (20 mL) and aqueous potassium hydroxide solution (2 M, 2 mL). The reaction mixture was stirred at 20 °C for 13 h, after which time TLC (solvent A, detection by dipping in ninhydrin dissolved in *n*-butyl alcohol followed by heating) showed that MLA had been hydrolyzed to a more polar alkaloid. The pH of the ethanolic solution was adjusted to 7 (paper) with aqueous hydrochloric acid solution (1 M) before concentration *in vacuo* and lyophilization. The residue was then suspended in dichloromethane, inorganic salts were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was then purified by flash chromatography (solvent A) to give pure lycoctonine **4** (458 mg, 77%), homogeneous by TLC and >99.9% free from MLA by HPLC:[19] ^{1}H NMR ($CDCl_3$) δ 4.15 (1H, s, H8 (OH)), 3.85 (1H, s, H6), 3.62−3.58 (2H, m, H14, H18 (OH)), 3.45 (3H, s, OMe), 3.44 (1H, d, $H18_a$, 14.3 Hz), 3.41 (3H, s, OMe), 3.37 (1H, d, $H18_b$, 14.3 Hz), 3.33 (3H, s, OMe), 3.25 (3H, s, OMe), 3.21 (1H, t, H16, 7.6 Hz), 3.06 (1H, t, H9, 5.2 Hz), 3.00−2.72 (4H, m, H1, H17, NCH_2), 2.66−2.50 (2H, m, $H19_a$, $H15_a$), 2.41 (1H, dd, $H12_a$, 13.0, 2.2 Hz), 2.33 (1H, dd, H13, 6.0, 4.8 Hz), 2.27 (1H, d, $H19_b$, 12.0 Hz), 2.20−2.00 (2H, m, H2), 1.96−1.76 (2H, m, H10, $H12_b$), 1.72−1.45 (4H, m, H5, $H15_b$, H3), 1.04 (3H, t, NCH_2CH_3, 7.1 Hz) (for lycoctonine, H7 OH was not observed,[22] in $CDCl_3$); ^{13}C NMR ($CDCl_3$) δ 90.40 (C6), 88.21 (C7), 84.15 and 83.78 (C1, C14), 82.50 (C16), 77.37 (C8), 67.38 (C18), 64.74 (C17), 57.73, 57.62, 56.09, and 55.62 (C1, C6, C14, C16, 4 × OMe), 52.58 (C19), 50.99 (NCH_2), 49.45 (C5), 48.70 (C11), 45.92 (C10), 43.09 (C9), 38.37 (C4), 37.82 (C13), 33.42 (C15), 31.44 (C3), 28.57 (C12), 25.94 (C2), 13.99 (NCH_2CH_3); HRMS (FAB, mNBA matrix) lycoctonine ($C_{25}H_{41}NO_7$) found 468.2956 (MH^+ calcd 468.2961).

Inuline (Anthranoyllycoctonine) (6). Lycoctonine (54 mg, 0.11 mmol) was dissolved in anhydrous DMF (1 mL), and to this were added isatoic anhydride (38 mg, 0.23 mmol) and 4-(*N*,*N*-dimethylamino)pyridine as catalyst (6 mg, 0.4 equiv). The reaction mixture was then heated to 70 °C and stirred at this temperature for 17 h. TLC (solvent A) indicated that starting material was still present, so a further aliquot of isatoic anhydride (10 mg, 61 μmol) was added. After another 7 h, only a trace of starting material remained (TLC analysis, solvent A), so the reaction mixture was cooled to 20 °C and the DMF was removed *in vacuo* using a Kugelrohr distillation apparatus. Dichloromethane (5 mL) was added to the residue, and the resultant brown precipitate was filtered off. The residual brown oil (54 mg) was purified by flash chromatography on silica gel, eluting first with 2% methanol in dichloromethane followed by gradually increasing methanol concentrations to 5% in dichloromethane. Homogeneous fractions were concentrated to afford pure inuline (**6**) (5 mg, 7%), while a further quantity of impure inuline (**6**) was also recovered (15 mg): ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, dd, aromatic H6, 8.5, 1.5 Hz), 7.29 (1H, td, aromatic H4, 7.0, 1.8 Hz), 6.68−6.64 (2H, m, aromatic H3, H5), 5.78 (2H, br s, NH_2), 4.13 (1H, d, $H18_a$, 11.6 Hz (J_{AB})), 4.12 (1H, d, $H18_b$, 11.6 Hz (J_{AB})), 4.01 (1H, s, H8 (OH)), 3.91 (1H, s, H6), 3.61 (1H, t, H14, 4.6 Hz), 3.42 (3H, s, OMe), 3.37 (3H, s, OMe), 3.35 (3H, s, OMe), 3.26 (3H, s, OMe), 3.23 (1H, dd, H16, 8.8, 7.0 Hz), 3.08 (1H, dd, H9, 6.7, 4.6 Hz), 3.00 (1H, dd, H1, 10.1, 7.3 Hz), 2.98−2.88 (2H, m, H17, H_a of NCH_2), 2.86−2.78 (1H, m, H_b of NCH_2), 2.73 (1H, d, $H19_a$, 11.6 Hz), 2.61 (1H, dd, H15, 15.3, 8.8 Hz), 2.50−2.42 (2H, m, $H12_a$, $H19_b$), 2.34 (1H, dd, H13, 7.0, 4.6 Hz), 2.25−2.12 (1H, m, H2), 2.12−2.04 (1H, m, H2), 2.00−1.94 (2H, m, H10), 1.89−1.80 (1H, m, $H12_b$), 1.80−1.72 (2H, m, H5, $H3_a$), 1.67 (1H, dd, H15, 15.3, 7.0 Hz), 1.66 (1H, s, H7 (OH)), 1.63−1.53 (1H, m, $H3_b$), 1.07 (3H, t, NCH_2CH_3, 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 167.72 (CO, ester), 150.73 (aromatic C2), 134.23 and 130.63 (aromatic C4, C6), 116.77 and 116.15 (aromatic C3, C5), 110.20 (aromatic C1), 90.82 (C6), 88.42 (C7), 83.96 and 83.86 (C1, C14), 82.53 (C16), 77.45 (C8), 68.53 (C18), 64.45 (C17), 57.90, 57.72, 56.18, and 55.69 (C1, C6, C14, C16, 4 × OMe), 52.40 (C19), 50.92 (N-CH_2), 50.27 (C5), 48.99 (C11), 46.04 (C10), 43.17 (C9), 38.13 (C13), 37.49 (C4), 33.52 (C15), 32.16 (C3), 28.64 (C12), 26.08 (C2), 14.09 (NCH_2CH_3).

Elatine (2). Purified MLA (**1**) (270 mg, 0.39 mmol) was suspended in formaldehyde diethylacetal (20 mL) containing *p*-toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol). The resulting white suspension was then heated to 80 °C whereupon it cleared. A gummy precipitate appeared at this stage, so DMSO (3 mL) was added to aid solubilization. The reaction mixture was heated at 80 °C for 44 h and then cooled to 25 °C, and benzene (8 mL) was added. The orange-colored solution was heated for a further 2 h, with azeotropic distillation using a Dean−Stark trap, before being cooled to 25 °C. The solvents were then removed *in vacuo* to furnish an orange oil which was dissolved in dichloromethane (20 mL) and then washed with saturated aqueous sodium hydrogen carbonate solution (15 mL). The dichloromethane solution was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give an orange oil (301 mg). Purification by flash chromatography (silica gel, 5% methanol−dichloromethane) gave pure MLA, 83 mg, and a mixture of MLA and elatine (**2**), 106 mg. This mixture was purified further by HPLC using the following conditions: column, C8-IK5 Inertpak (Capital HPLC, 25 cm × 10 mm i.d.); eluent, 64% aqueous ammonium acetate (0.05 M buffered to pH 5.0 with glacial acetic acid), 36% acetonitrile; flow rate, 4 mL/min; detection at 270 nm. Combined collected fractions were adjusted to pH 8 with a few drops of saturated aqueous sodium hydrogen carbonate solution before removing the acetonitrile *in vacuo*. The remaining aqueous component was then lyophilized (16 h) to afford a white powder. This was suspended in distilled water (25 mL) and washed with dichloromethane (4 × 20 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford the title compound (28 mg, 10%), homogeneous by TLC and free of (<0.1%) detectable MLA by HPLC:[19] ^{1}H NMR ($CDCl_3$) *inter alia* δ 8.05 (1H, d, aromatic H6, 7.3 Hz), 7.67 (1H, td, aromatic H4, 7.7, 1.6 Hz), 7.53 (1H, td, aromatic H5, 7.9, 1.3 Hz), 7.27 (1H, dd, aromatic H3, 7.7, 1.1 Hz), 5.07 (2H, s, OCH_2O), 4.13−4.00 (2H, m, H18), 3.43 (3H, s, OMe), 3.35 (3H, s, OMe), 3.33 (3H, s, OMe), 3.26 (3H, s, OMe), 3.30−3.22 (1H, m, H16), 2.80−2.90 (1H, m, NCH_{2a}), 2.76 (1H, d, $H19_a$, 11.6 Hz), 2.73−2.63 (1H, m, NCH_{2b}), 2.60 (1H, dd, $H12_a$, 13.9, 3.8 Hz), 2.44 (1H, dd, $H15_a$, 14.8, 8.8 Hz), 2.40−2.30 (2H, m, $H19_b$, H13), 1.86 (1H, dd, $H15_b$, 14.8, 7.7 Hz), 1.75−1.68 (1H, m, $H12_b$), 1.06 (3H, t, NCH_2CH_3 7.1) (succinimido proton resonances at δ 3.05 (CH and CH_2), 2.55 (CH_2), and 1.50 (CH_3) were assigned using ^{1}H/^{1}H correlation spectroscopy); ^{13}C NMR ($CDCl_3$) δ 179.87 (CO N), 175.92 (CO N), 164.09 (CO O), 133.55 (C4 aromatic), 132.96 (C2 aromatic), 131.21 (C6 aromatic), 129.94 (C3 aromatic), 129.38 (C5 aromatic), 127.11 (C1 aromatic), 93.53 (OCH_2O), 92.14 (C7), 89.27 (C6), 83.36 and 83.33 (C1, C8), 81.55 (C16)*, 81.24 (C14)*, 69.76 (C18), 64.14 (C17), 58.93 (OMe), 57.80 (OMe), 56.15 (OMe), 55.20 (OMe), 53.41 (C5), 52.79 (C19), 50.48 (NCH_2CH_3), 49.95 (C11), 48.42 (C9)*, 39.86 (C10)*, 38.59 (C13), 37.15 (C4), 36.99 (C2″ succinimide)*,

35.39 and 35.19 (C3″ succinimide)*, 34.83 (C15), 31.70 (C3), 27.80 (C2)*, 26.36 (C12)*, 16.56 and 16.28 (C5″ succinimide), 13.91 (NCH_2CH_3) (* denotes that these assignments may have to be interchanged). The HPLC sample (>99.9% pure) displayed appropriate HRMS data: 695.3603 (MH^+, 100), also 694.3476 (7); calcd $C_{38}H_{51}O_{10}N_2$ requires 695.3544 and $C_{38}H_{50}O_{10}N_2$ requires 694.3465.

All synthesized alkaloids gave satisfactory ^1H and ^{13}C NMR spectral data consistent with the proposed structures.[13,22] All other alkaloids were from Latoxan (Rosans, France).

Nicotinic Binding Assays. Competition binding assays for [^{125}I]-αBgTX or [^{3}H]nicotine binding sites in rat brain membranes were carried out as previously described.[35] In brief, rat brain P2 membranes (1−2 mg of protein/mL) were incubated with serial dilutions of competing ligand and 1 nM [^{125}I]-αBgTX or 10 nM [^{3}H]nicotine for 3 h at 37 °C or for 1 h at 20 °C, respectively. Bound radioligand was separated by centrifugation in the case of [^{125}I]-αBgTX or filtration in the case of [^{3}H]nicotine. Total and nonspecific binding were determined in the absence and presence, respectively, of 1 μM αBgTX or 10^{-4} M nicotine. Potential ligands (as their free bases, except for nudicauline perchlorate and lappaconitine hydrobromide) were dissolved in ethanol to a stock concentration of 10^{-3} M and stored at 4 °C. Concentrations that inhibit radioligand binding by 50% (IC_{50} value) were determined by fitting the data to the Hill equation.

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