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Structure activity studies of ring E analogues of methyllycaconitine. Part 2: Synthesis of antagonists to the α3β4* nicotinic acetylcholine receptors through modifications to the ester

Stephen C. Bergmeier,[a,*] Khadiga A. Ismail,[b] Kristjan M. Arason,[a] Susan McKay,[c] Darrell L. Bryant[c] and Dennis B. McKay[c]

[a]*Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701, USA*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt*
[c]*Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*

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Abstract—The development of novel agents for the differentiation of neuronal nicotinic acetylcholine receptors (nAChRs) is important for the treatment of a variety of pathological conditions. We have prepared and evaluated a number of simpler analogues of the norditerpeniod alkaloid methyllycaconitine (MLA) in an effort to understand molecular determinants of nAChR·small molecule interactions. We have previously reported the synthesis and evaluation of a series of ring E analogues of MLA. We report here the optimization of the α3β4* functional activity of this series of compounds through modification of the ester.

The development of agents for the differentiation of specific subtypes of neuronal nicotinic acetylcholine receptors (nAChRs) has significant implications for advancements in the treatment of a variety of pathological conditions.[1–4] Neuronal nAChRs are located throughout the central and peripheral nervous system and have been implicated in several pathologies[2,5,6] including Alzheimer's disease,[7–9] Parkinson's disease,[10–13] and nicotine addiction.[14–17] Currently, several types of neuronal nAChRs, based on subunit composition, have been described, including the heteromeric, α4β2 nAChRs and α3α5β4 nAChRs and the homomeric, α7 nAChRs.[18] The goal of our research is to identify and understand molecular determinants of nAChR·small molecule interactions.

Methyllycaconitine (MLA, **1**) is a diterpene alkaloid isolated from plants of the genera *Aconitum* and *Delphinium*.[19–21] MLA is the most potent nonpeptide nAChR antagonist known, with selectivity for α7 nAChRs,[22–25] and moderate affinity (K_i value, 1.3 μM) for α3β4* nAChRs.[26] MLA inhibits bovine adrenal catecholamine secretion (IC_{50} value, 2.6 μM) mediated through activation of α3β4* nAChRs.[27] The preparation and evaluation of several bicyclic analogues of MLA has been reported.[28–31] We have prepared and evaluated a number of simpler analogues of MLA in an effort to understand the origins of these activities on multiple nAChRs subtypes[27,32,33] (Fig. 1).

A simplification of the MLA core structure provides the piperidine fragment **2**. Further simplification of this fragment provides lead structure **3**. The analogues that we had initially prepared were simplified derivatives of MLA that contained only the E ring and the succinimidoylanthranilate ester (**2**). Based upon these early studies into the structure–activity relationship (SAR) of compound **3**, we determined **3a** ($R = Ph(CH_2)_3$, $R' = CH_3$) to be our lead compound. This compound has low affinity (IC_{50} value, 177 μM) for α7 nAChRs and has no affinity for α3β4* nAChRs; however, **3a** appears to act as a noncompetitive inhibitor (IC_{50} value, 11 μM) of α3β4* nAChRs.[27] All of these analogues varied simply through changes in the substitution on the piperidine nitrogen. One pair of examples, **3b** ($R = i$-Pr, $R' = CH_3$) and **3c** ($R = i$-Pr, $R' = H$) had the same

* Corresponding author. Tel.: +1-740-517-8462; fax: +1-740-593-0148; e-mail: bergmeis@ohio.edu

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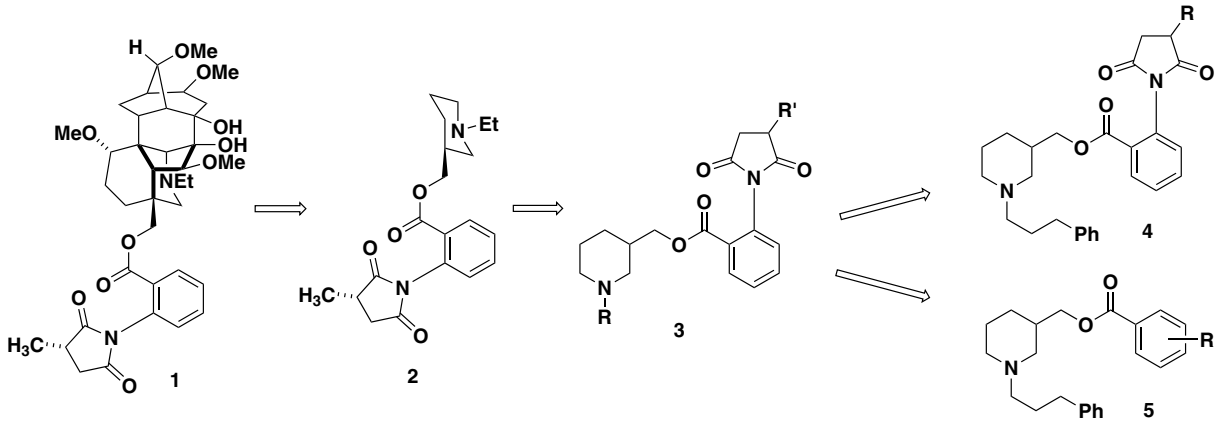
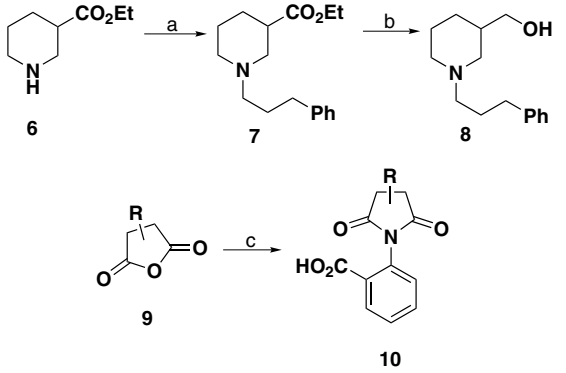


Figure 1. Methyllycaconitine (MLA), **1**, ring E analogue **3** and new analogues **4** and **5**.

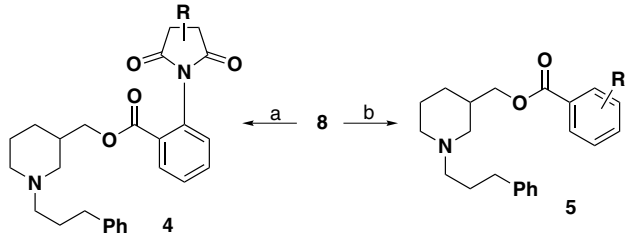
substituent on the nitrogen (R = *i*-Pr) and either the presence or absence of a methyl group on the succinimide ring. The differences in α3 nAChR functional activity between these two compounds (~20 μM) suggested that modification to this part of the molecule could be a fertile area for enhancement of the affinity at the α3β4* nAChR. We have prepared two series of molecules in which this general area of the molecule has been modified. The goals for this work are to optimize the α3β4* functional activity of this series of compounds through modification of the ester. In the first series we have replaced the methyl group on the imide ring with a variety of alkyl chains, **4**. In the second series we have replaced the entire succinimide ring with other substituents, **5**. For both series of analogues we have retained the phenylpropyl chain on the piperidine nitrogen.

The synthesis of the requisite piperidine methanol **8** is shown in Scheme 1. Alkylation of ethyl nipecotate with 1-bromo-3-phenylpropane followed by reduction of the ester with $LiAlH_4$ provides **8** in excellent yield.

For the first series of compounds we needed to prepare a series of substituted succinimido–anthranilic acids. As shown in Scheme 1, this synthesis starts with either commercially available or known succinic anhydrides.[34]



Scheme 1. Reagents and conditions: (a) $Ph(CH_2)_3Br$, K_2CO_3, 85%. (b) $LiAlH_4$, 95%. (c) Anthranilic acid, 145–160 °C, 0.1 mmHg, **10a**, R = 3,3-dimethyl, 34%; **10b**, R = 3,4-dimethyl, 66%; **10c**, R = Et, 63%; **10d**, R = *n*-Bu, 65%; **10e**, R = octyl, 71%; **10f**, R = *i*-Pr, 69%; **10g**, R = cyclopentyl, 65%; **10h**, R = allyl, 63%; **10i**, R = Bn, 70%.



Scheme 2. Reagents and conditions: (a) **10**, DCC, **4a**, R = 3,3-dimethyl, 85%; **4b**, R = 3,4-dimethyl, 83%; **4c**, R = Et, 95%; **4d**, R = *n*-Bu, 97%; **4e**, R = octyl, 76%; **4f**, R = *i*-Pr, 98%; **4g**, R = cyclopentyl, 75%; **4h**, R = allyl, 94%; **4i**, R = Bn, 85%. (b) R–$PhCO_2H$, DCC, **5a**, R = 2-Cl, 92%; **5b**, R = 2-OMe, 80%; **5c**, R = 2-CF_3, 86%; **5d**, R = 2-F, 49%; **5e**, R = 2-acetamide, 45%; **5f**, R = 2-phthalimide, 58%; **5g**, R = 2-phenyl, 80%; **5h**, R = 4-phenyl, 81%.

These succinic anhydrides were coupled to anthranilic acid to provide acid **10**.[35] As shown in Scheme 2, these acids were then coupled to **8** using DCC to provide a series of ring E analogues (**4**). The second series of analogues (**5**) was prepared using a variety of substituted benzoic acids which were coupled to **8** using DCC.

These analogues possess significant inhibitory activity in functional assays involving α3β4* nAChRs (Table 1). However, the analogues showed little or no inhibitory effects on binding to α7, α4β2, or α3β4* nAChRs at concentrations up to 10 μM (data not shown) supporting noncompetitive interactions. Very few competitive antagonists of α3β4* nAChRs exist. Most α3β4* nAChR antagonists are noncompetitive and their potencies are in the micromolar range.[36–38] For example, hexamethoniun and decamethonium have IC_{50} values of ~17 μM; mecamylamine, one of the most potent noncompetitive inhibitors, has an IC_{50} value of 0.1 μM. Tubocurarine, a competitive antagonist, has an IC_{50} value of 2 μM. Our novel analogues have comparable IC_{50} values of 1–11 μM.

Given that the presence or absence of a methyl group at the 3-position of the imide ring is significant, we wished to study the consequences of di-substitution at this position. Compound **4a** shows a small but significant improvement in potency relative to **3a**.[39]

Table 1. Functional effects of analogues on α3β4* nAChR-stimulated adrenal catecholamine secretion

Compound #	R	Catecholamine secretion[a] (IC_{50} value, μM)
MLA	—	2.6 (2.3–3.0)[b]
3a	—	11.4 (10.9–11.9)[b]
4a	3,3-Dimethyl	7.5 (6.8–7.6)
4b	*trans*-3,4-Dimethyl	6.0 (5.5–6.6)
4c	Et	4.5 (4.4–4.5)
4d	*n*-Bu	1.5 (1.5–1.5)
4e	Octyl	1.3 (0.7–2.4)
4f	*i*-Pr	2.5 (2.3–2.6)
4g	Cyclopentyl	1.2 (1.2–1.3)
4h	Allyl	1.7 (1.7–1.7)
4i	Bn	1.3 (1.1–1.4)
5a	2-Cl	3.4 (3.2–3.5)
5b	2-OCH_3	3.2 (3.1–3.4)
5c	2-CF_3	3.2 (3.1–3.2)
5d	2-F	5.2 (4.8–5.6)
5e	2-Acetamide	8.4 (7.8–9.1)
5f	2-Phthalimide	1.3 (1.2–1.6)
5g	2-Phenyl	1.7 (1.6–1.8)
5h	4-Phenyl	3.0 (2.3–3.9)

[a] Secretion studies were performed as previously described.[27] Values represent geometric mean (95% confidence limits) of 5–6 experiments.
[b] Data from Bryant et al.[27]

The 3,4-dimethyl imide analogue (**4b**) was prepared in an effort to address the issue of rotational isomers around the aryl–imide bond. Computational results indicate that the barrier to this rotation is quite low (~1 kcal/mol) thus an analogue with a *trans* disubstituted dimethyl might show enhanced potency as both rotational isomers are present at all times. This compound shows a small, but significant increase in potency relative to **3a**. While the enhancements in potency of **4a** and **4b** relative to **3a** are small (less than twofold), they do suggest that the stereochemistry of substitution on the imide ring is an important consideration in the design of future analogues.

We next chose to examine the effects of increasing chain length on the 3-substituent on the imide ring. The ethyl compound (**4c**) showed significant improvements (twofold) in potency relative to **3a**. Increasing the chain length to an *n*-Bu or octyl (**4d**,**e**) provided significant enhancement (~10-fold) in potency relative to **3a**. Substitution of the ethyl chain (**4c**) with a methyl to provide an isopropyl group (**4f**) again provided significant improvements in potency compared to **3a**. Constraining the methyls of the isopropyl group into a cyclopentyl group (**4g**) provided a significant improvement in potency. Inclusion of a π-bond with an allyl (**4h**) or phenyl (**4i**) also provided significantly improved activity.

We next turned our attention to replacement of the succinimide group altogether. Replacement of the imide ring with very simple heteroatom substituents (**5a**–**d**) provided a small but significant enhancement in potency compared with **3a**. There was little or no difference between electron donating (**5b**) and electron withdrawing (**5a**,**c**,**d**) groups. Replacement of the imide ring (**3a**) with an acetamide (**5e**) provided a very minimal enhancement of activity. However, replacement of the imide ring (**3a**) with a phthalimide ring (**5f**) significantly increased the potency (~10-fold). We next chose to carry out an isosteric replacement of the imide ring (**3a**) with a simple phenyl ring (**5g**). This again provided a significant level of enhancement. Movement of the phenyl ring from the 2-position (**5g**), as in the original molecule, to the 4-position (**5h**) resulted in no significant loss of activity.

In summary the imide ring can be substituted with 3–7 carbon chains and provide enhanced potency at α3β4* nAChRs. Perhaps more intriguing is the total replacement of the imide ring with a phthalimide or phenyl ring to also provide compounds with greatly enhanced potency at α3β4* nAChRs. These studies are beginning to define a novel noncompetitive binding site on neuronal nAChRs.

It is significant that none of these new compounds (**4a**–**i**, **5a**–**h**) showed any affinity for the agonist binding sites of either α7, α4β2, or α3β4* nAChRs. In a comparison of several of these compounds (**4d**,**e**,**g**,**h**,**i**, **5f**, and **5g**) with other known α3β4* nAChR antagonists[37] we find that these ring E analogues with imide substitutions are potent nAChR antagonists. Studies on further modifications to substitution on the aryl ring as well as the piperidine ring are underway and will be reported in due course.

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