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# RING E ANALOGS OF METHYLLYCACONITINE (MLA) AS NOVEL NICOTINIC ANTAGONISTS

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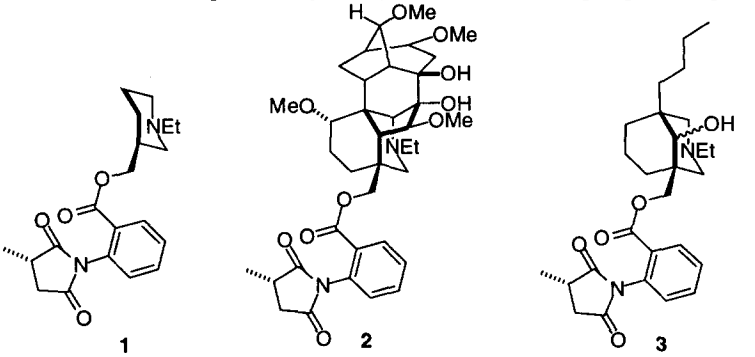
**Abstract:** We have prepared ring E analogs of the diterpenoid alkaloid methyllycaconitine. These compounds have been assayed for nicotinic activity and were found to act as functional antagonists on adrenal nicotinic receptors. 

Neuronal nicotinic acetylcholine receptors (nAChR) are located throughout the central and peripheral nervous systems, which include several different regions of the brain, spinal cord, retina, ganglia, and adrenal medulla. These receptors are composed of multiple subunits that have been divided into two general classes: α subunits and β subunits. Currently, eight neuronal nAChR α subunits (α2, α3, α4, α5, α6, α7, α8, and α9) and three neuronal nAChR β subunits (β2, β3, and β4) have been described.[1] Recent evidence has documented that some neuronal tissues express not only multiple nAChR subunits, but also multiple subtypes of neuronal nAChRs, based on specific subunit composition. The existence of multiple subtypes of neuronal nAChRs has many important physiological implications.[2,3] A specific pattern of sensitivity to cholinergic agonists such as acetylcholine, nicotine, dimethylphenylpiperazinium (DMPP), and cytisine has been demonstrated with various nAChR subtypes.[4] We report here our synthesis of a new class of nicotinic antagonists (**1**) based on the alkaloid methyllycaconitine (MLA, **2**) as well as preliminary biological data on a select group of compounds.




**1** **2** **3**

**Figure 1.** Ring E analog of MLA (**1**), MLA (**2**), and ring A/E analog (**3**)

MLA is extremely interesting as a lead compound for the development of new nAChR antagonists. MLA is the most potent nonpeptide nAChR antagonist currently known and is reported to selectively act at α7 nicotinic receptors.[5] Methyllycaconitine (MLA) is one member of a larger family of diterpene alkaloids.[6] These structurally similar molecules have been isolated from plants of the genera *Aconitum* and *Delphinium*. Both of these families of plants have a long history as a source of poisons and medicinal agents. MLA and a few others are unique in that they are potent and selective ligands for the nAChR.

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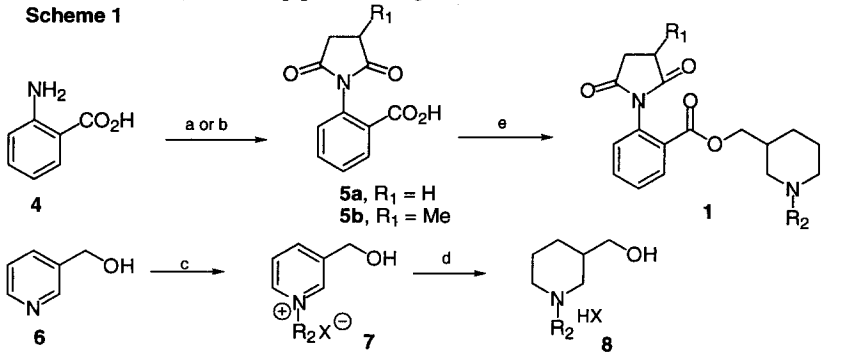
Several structurally less complex analogs of MLA (**3** for example) have been synthesized both as analogs of MLA as well as part of a partial synthesis of MLA.[7] Most of the synthetic efforts have focused on the preparation of the A/E bicyclic ring system of the alkaloid. Only one report of the biological activity of any analogs has been published.[8] In this report the analog **3** was reported to have an $IC_{50}$ of 107 μM. A related A/E/B tricyclic analog had an $IC_{50}$ of 478 nM. Reports on the SAR of MLA indicate that the succinimide moiety is important for optimal activity at the nAChR.[9] Also of importance is the methyl group on the succinimide ring.[10]

As one can appreciate from looking at the structure of MLA, a number of analogs of MLA could conceivably be prepared retaining the essential elements of the structure. One of the simplest analogs that might be prepared are analogs of ring E (a piperidine ring, **1**). For our initial study we have chosen to examine the effect of different groups on the nitrogen of the piperidine ring.

**Scheme 1**




(a) $R_1$ = H, succinic anhydride, toluene, $Et_3N$, reflux, Dean-Stark, 15%. (b) $R_1$ = Me, methylsuccinic anhydride, neat, 145 °C, 0.1 mm Hg, 3 h, 69%. (c) $R_2$-X, $CH_3CN$, reflux, 20 h. **7a**, Et-I, 90%; **7b**, Me-I, 100%; **7c**, *i*Pr-Br, 76%; **7d**, *n*Bu-Br, 100%; **7e**, EtOEt-I, 84%; **7f**, $PhCH_2CH_2$-I, 74%; **7g**, $PhCH_2CH_2CH_2$-I, 100%. (d) $H_2$ (1 atm, balloon), $PtO_2$ (10% w/w), EtOH. **8a**, 95%; **8b**, 100%; **8c**, 92%; **8d**, 94%; **8e**, 84%; **8f**, 95%; **8g**, 100%. (e) TBTU, **8a-g**, $iPr_2NEt$, $CH_3CN$, For yields see Table 1.

We have prepared a series of analogs of MLA by the route shown in Scheme 1. In this initial series of analogs we wished to look at two areas. The major area of investigation is substitution on the nitrogen of the piperidine ring. A second area of investigation was methyl substitution of the succinimide ring. To this end we have prepared two different anthranilate derivatives (**5a** and **5b**) for coupling to the piperidine ring. The synthesis of both **5a** and **5b** followed reported routes. The synthesis of **5a** while straightforward was quite low yielding.[10] The synthesis of **5b** involved fusing methylsuccinic anhydride with anthranilic acid. This procedure gave an excellent yield of the desired imide.[7a,11] All attempts to prepare **5a** by this method were unsuccessful. The synthesis of the piperidine portion of **1** was addressed as follows, starting from the commercially available hydroxymethylpyridine (**6**), a pyridinium salt (**7**) was prepared in excellent (74–100%) yield. The pyridinium salt was then reduced (84–100% yield) to provide the piperidine salt **8**. A variety of palladium and platinum catalysts were examined, only $PtO_2$ gave consistently good yields. Coupling of **8** to the anthranilic acid derivatives **5a** or **5b** produced our target compounds. The use of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as a coupling agent proved to be the most convenient route to the desired esters. The yields for this coupling reaction were quite inconsistent, ranging from 6% to 60%. We were able to obtain sufficient material for our biological evaluations. Our target compounds **1a–1h** were converted to their water soluble hydrochloride salts prior to biological evaluation.

In these studies we have used cultured bovine adrenal chromaffin cells as a neuronal model to study the

functional effects of the MLA analogs. These cells contain several nAChR subunit genes, including the α3, α5, α7, and β4 genes[12] and express multiple nAChR subtypes.[12d, 13] In cultured chromaffin cells, mAb35-nAChRs, which are believed to contain α3, α5 and β4 subunits, are reported to be the principal receptors that mediate adrenal catecholamine secretion.[13] In addition, α7-containing nAChRs are also expressed and recently these receptors have been reported to stimulate adrenal secretion.[14]

**Table 1. Yields and nicotinic antagonist properties of target compounds.**

| Compound | $R_1$ | $R_2$ | Yield | Nicotine-stimulated catecholamine release (% inhibition)[a,b] |
|---|---|---|---|---|
| **1a** | Me | Et | 22% | 33.5% ± 4.3% |
| **1b** | Me | Me | 35% | 30.1% ± 4.4% |
| **1c** | H | nBu | 24% | 38.4% ± 10.1% |
| **1d** | Me | EtOEt | 37% | 45.9% ± 3.7% |
| **1e** | Me | iPr | 18% | 56.5% ± 7.2% |
| **1f** | H | iPr | 35% | 36.4% ± 4.8% |
| **1g** | Me | $Ph(CH_2)_2$ | 60% | 37.0% ± 7.5% |
| **1h** | Me | $Ph(CH_2)_3$ | 15% | 86.3% ± 4.2% |
| **2** (MLA) | - | - | - | 95.2% ± 1.9% |

[a]Cultured adrenal chromaffin cells were isolated and cultured as described previously.[15]

[b]Cells were either not treated (control groups) or treated for 15 min with 50 μM concentrations of the compounds to be tested. The cells were then stimulated for 5 min with 10 μM nicotine in the continuous presence of the compounds. Catecholamine release during this 5 min. stimulation period was determined.[16] Results are expressed as a percentage of the inhibition of control nicotine-stimulated release (% inhibition). Values represent means ± SEM (N = 3-6).

The relative efficacies of the MLA analogs are shown in Table 1. Compound **1a**, which is a direct analog of MLA, produced moderate inhibition of nAChR-stimulated catecholamine release. Shortening (**1b**) or increasing the length of the N-alkyl chain (**1c**) resulted in no significant change in antagonist activity. Placement of an oxygen in the alkyl chain (**1f**) which should inductively decrease the basicity of the nitrogen also produced no significant change in antagonist activity. The preparation of the *N*-iPr analog (**1e**) significantly increased inhibition activity when compared to **1a**. We felt that this compound would be an excellent compound to assay for the importance of the methyl group on the succinimide. Thus, compound **1f** was prepared and showed a marked reduction in potency. This is consistent with the work of Jacyno who reported a marked decrease in activity of MLA that lacked the methyl group on the succinimide.[10] Feeling that larger alkyl groups might produce more potent antagonists, we prepared two further analogs **1g** and **1h**. While **1g** showed no significant improvement in activity, **1h** showed excellent activity being almost as efficacious as MLA at the concentration tested.

It is important to note that all of the compounds tested were racemates and mixtures of diastereomers yet showed significant activity as antagonists. It is also significant that these compounds show activity on adrenal nAChRs in the micromolar range. These compounds have also demonstrated selectivity for nAChR-stimulated secretion; they had no effect on release stimulated by direct depolarization with elevated levels of KCl (data not shown). The preparation of diastereomerically and enantiomerically pure compounds should lead to significantly more potent compounds.

We have discovered a simple analog (**1**) of methyllycaconitine (MLA, **2**) that acts as a micromolar inhibitor at the nAChR. This should be a fertile area from which to find new selective and potent antagonists of subtypes of the nAChR. The goal of our initial work is to quickly outline a SAR (structure–activity relationship) of this new lead compound to assess structural requirements necessary for potency and selectivity. This will provide a rational,

rapid analysis of key features in MLA to arrive at a range of lead compounds for further development, and lead to new pharmacological tools.

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