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Synthesis and Pharmacological Evaluation of Highly Potent [Dmt¹]DALDA Analogs

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Introduction

Structure variation of the highly potent and μ -selective, peripheral analgesic [D-Arg²,Lys⁴] dermorphin-(1–4)-amide (DALDA) [1] led to [Dmt¹]DALDA, with 12-fold higher affinity than DALDA at μ -receptors and potency 180-fold higher in the guinea pig ileum (GPI) assay. Receptor selectivity was maintained as well ($K_i^{\delta}/K_i^{\mu} = 14700$ relative to 11400 for DALDA) [2]. [Dmt¹]DALDA is a potent, systemically active peripheral opioid analgesic [3]. We had access to protected analogs of [Dmt¹]DALDA which were utilized in a fragment condensation approach to the synthesis of gram-amounts of three antioxidants (including [Dmt¹]DALDA) [4]. Encouraged by the recent report on the use of fluorescent analogs of [Dmt¹]DALDA for confocal laser scanning microscopy (CLSM) and flow cytometry studies [5], these synthetic intermediates which may be blood-brain barrier (BBB)-permeant were examined for their bioactivity. Surprisingly, the protected analogs of [Dmt¹]DALDA exhibited higher receptor selectivity compared to the parent peptide. The details of the synthetic approach and biological evaluation of these intermediates, having the masked side chain charges, is described.

Results and Discussion

Synthesis of the three antioxidant peptides Dmt-D-Arg-Phe-Lys-NH₂ (SS-02) (**1**), D-Arg-Dmt-Lys-Phe-NH₂ (SS-31) (**5**), and Phe-D-Arg-Phe-Lys-NH₂ (SS-20) (**6**) involved routinely used side chain protecting groups for amino acid building blocks. Thus, the guanidino group was protected with NO₂ and the ϵ -NH₂ of Lys was protected by Cbz or 2-Cl-Cbz. This afforded the protected analogs Dmt-D-Arg(NO₂)-Phe-Lys-NH₂ (**2**), Dmt-D-Arg(NO₂)-Phe-Lys(Cbz)-NH₂ (**3**) and Dmt-D-Arg(NO₂)-Phe-Lys(2-Cl-Cbz)-NH₂ (**4**), and Boc-D-Arg-Dmt-Lys-Phe-NH₂ (**7**). These peptides were synthesized using Boc/Cbz chemistry and BOP reagent coupling as exemplified by the synthesis of **4**. Starting with the C-terminal Lys residue protected as H-Lys(2-Cl-Cbz)-NH₂, (prepared from the commercially available Boc-Lys(2-Cl-Cbz)-OH in two steps by amidation with NH₄HCO₃ in the presence of DCC/HOBt following a literature procedure [6], followed by exposure to TFA). Selective removal of the 2-Cl-Cbz in the presence of the NO₂ group was accomplished using catalytic transfer hydrogenolysis (CTH) [7]. Opioid activities were determined *in vitro* using the GPI and mouse vas deferens (MVD) assays as previously described [2]. The results are summarized in Table I.

In agreement with the literature, [Dmt¹]DALDA (**1**) has nanomolar potency at μ receptors with 5 fold selectivity over δ receptors. Masking the guanidine side chain of D-Arg with NO₂ (**2**) led to a 2-fold potency enhancement at μ receptors and a similar potency increase at δ receptors. Masking of **2** by protection of the ϵ -NH₂ of Lys with Cbz (**3**) had no effect on potency at the μ receptor and produced only slightly enhanced potency at the δ receptor. Addition of a 2-Cl

substituent to the Cbz protecting group on ϵ -NH₂ of Lys in **3** to give **4** further increased μ agonist potency with δ agonist potency similar to that of **1**. Thus, **4** has significantly higher μ vs. δ selectivity and is 3 fold more potent than **1**. As expected, the other related peptides (**5**, **6** and **7**) were found to be inactive.

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Table IGuinea pig ileum (GPI) and mouse vas deferens (MVD) assay of [Dmt¹]DALDA and related compounds.

<i>No.</i>	<i>Compound</i>	GPI	MVD	MVD/GPI
		IC₅₀[nM]^a	IC₅₀[nM]^a	IC₅₀ ratio
1	Dmt-D-Arg-Phe-Lys-NH ₂ (SS-02)	1.03 ± 0.04	5.38 ± 0.91	5.22
2	Dmt-D-Arg(NO ₂)-Phe-Lys-NH ₂	0.534 ± 0.028	2.92 ± 0.49	5.46
3	Dmt-D-Arg(NO ₂)-Phe-Lys(Cbz)-NH ₂	0.513 ± 0.066	2.19 ± 0.10	2.19
4	Dmt-D-Arg(NO ₂)-Phe-Lys(2-Cl-Cbz)-NH ₂	0.340 ± 0.053	5.79 ± 0.77	17.0
5	D-Arg-Dmt-Lys-Phe-NH ₂ (SS-31)	4900 ± 210	inactive	-
6	Phe-D-Arg-Phe-Lys-NH ₂ (SS-20)	inactive	inactive	-
7	Boc-D-Arg-Dmt-Lys-Phe-NH ₂	inactive	inactive	-

^aMean of 3–6 determinations ± SEM.