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Novel μ opioid antagonists derived from the μ opioid agonists endomorphin and [Dmt[1]]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2)

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Abstract

Hybrid analogues of the μ opioid agonists endomorphin and [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2, Dmt = 2',6'-dimethyltyrosine) containing *cis*-4-amino-Pro, *trans*-4-amino-Pro, *cis*-4-aminoethyl-Pro or *cis*-4-guanidinylethyl-Pro in the 2-position of the peptide sequence were synthesized. None of the compounds retained high μ opioid agonist activity and, unexpectedly, substitution of *cis*-4-amino-Pro resulted in a novel class of potent μ opioid antagonists. In particular, the compound H-Dmt-*cis*-4-amino-Pro-Trp-Arg-NH_2 (CZ-1) turned out to be a highly selective μ opioid antagonist with ~ 1 nM μ receptor binding affinity.

Keywords:

[Dmt^1]DALDA, endomorphin, mu opioid receptor antagonists, opioid activity profiles, proline analogues

INTRODUCTION

Selective μ opioid receptor antagonists are important not only as pharmacological tools but also for various clinical applications. They are commonly used as rescue agents to reverse the serious side effects, particularly respiratory depression, induced by μ opioid agonists. Other potential therapeutic indications of μ opioid antagonists include psychosis, Parkinson's disease and obesity (Goodman et al., 2007). Furthermore, peripherally restricted μ opioid antagonists may have therapeutic potential for the treatment of opioid-induced gastrointestinal disorders.

Non-peptide μ opioid antagonists include the irreversibly binding ligands, β-FNA (Takemori et al., 1981) and clocinnamox (Lewis et al., 1988), cyprodime (Schmidhammer et al.,1990), and NAN (Obeng et al., 2018). CTOP And CTAP are peptidic μ opioid antagonists (Hawkins et al., 1989; Abbruscato et al., 1997). The cyclic tetrapeptide c[-D-Arg-Phe-Lys-Dmt-] ("cyclodal") is a selective μ opioid antagonist with subnanomolar μ receptor binding affinity (Weltrowska et al., 2016).

Here we describe the design of hybrid analogues of the endogenous μ opioid agonists endomorphin-1 or -2 (H-Tyr-Pro-Trp(or Phe)-Phe-NH_2) (Zadina et al., 1997) and of the dermorphin-derived μ opioid agonist [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2; Dmt = 2',6'-dimethyltyrosine) (Schiller et al., 2000), which led to the discovery of a novel class of potent μ opioid antagonists. [Dmt^1]DALDA is a potent μ opioid agonist with high μ opioid receptor binding affinity (K_i^{μ} = 0.143 nM) and high μ receptor binding selectivity (selectivity ratio μ/δ/κ = 1:14700:156) (Schiller et al., 2000). In rat and mouse tail-flick assays [Dmt^1]DALDA was a much more potent and longer lasting analgesic than morphine with both intrathecal and subcutaneous administration (Shimoyama et al., 2001; Neilan et al., 2001). Furthermore, [Dmt^1]DALDA turned out to be a much more effective analgesic than morphine in two animal models of neuropathic pain (Shimoyama et al., 2012; Schiller et al., 2015).

The structural motif of [Dmt^1]DALDA are alternating aromatic and basic amino acid residues. In an effort to further explore SAR of this peptide, we designed and synthesized analogues in which the D-Arg residue is replaced by proline derivatives that carry a basic substituent in the 4-position of the

ring structure (Figure 1). Peptide analogues containing *cis*-4-amino-Pro, *trans*-4-amino-Pro, *trans*-4-guanidino-Pro (CZ-series) and analogues containing *cis*-4-aminoethyl-Pro and *cis*-4-guanidinylethyl-Pro (SJ-series) were synthesized. The incorporation of these Pro analogues introduces conformational constraints in the peptide backbone and affects the positioning of their basic substituents in the interaction with the receptor which may have interesting effects on the opioid activity profile. Furthermore, the peptide analogues contained various natural or artificial amino acid residues in the 3-position and a basic residue (Lys or Arg) in the 4-position as present in [Dmt[1]]DALDA (Figure 2):

H-Dmt-*cis*-4-amino-Pro-Trp-Lys-NH_2 (CZ-1, **1**)
H-Dmt-*cis*-4-amino-Pro-Tmp-Lys-NH_2 (CZ-2, **2)**
H-Dmt-*cis*-4-amino-Pro-Phe-Arg-NH_2 (CZ-3, **3**)
H-Dmt-*cis*-4-amino-Pro-Tmp-Arg-NH_2 (CZ-4, **4**)
H-Dmt-*trans*-4-amino-Pro-Phe-Arg-NH_2 (CZ-5, **5**)
H-Dmt-*trans*-4-guanidino-Pro-Phe-Lys-NH_2 (CZ-6, **6**)
H-Dmt-*cis*-4-aminoethyl-Pro-Trp-Arg-NH_2 (SJ-1, **7**)
H-Dmt-*cis*-4-aminoethyl-Pro-Tmp-Arg-NH_2 (SJ-2, **8**)
H-Dmt-*cis*-4-aminoethyl-Pro-1-Nal-Arg-NH_2 (SJ-3, **9**)
H-Dmt-*cis*-4-guanidinoethyl-Pro-Trp-Lys-NH_2 (SJ-4, **10**)
H-Dmt-*cis*-4-guanidinoethyl-Pro-Tmp-Lys-NH_2 (SJ-5, **11**)
H-Dmt-*cis*-4-guanidinoethyl-Pro-1-Nal-Lys-NH_2 (SJ-6, **12**)

Because the peptides contain a modified Pro residue in the 2-positon, they can also be considered as endomorphin-[Dmt[1]]DALDA hybrid peptides. .

The in vitro opioid activity profiles of the compounds were determined in opioid receptor binding assays and the functional guinea pig ileum (GPI) and mouse vas deferens (MVD) assays. Surprisingly, this analogue design led to the discovery of a new class of μ opioid antagonists.

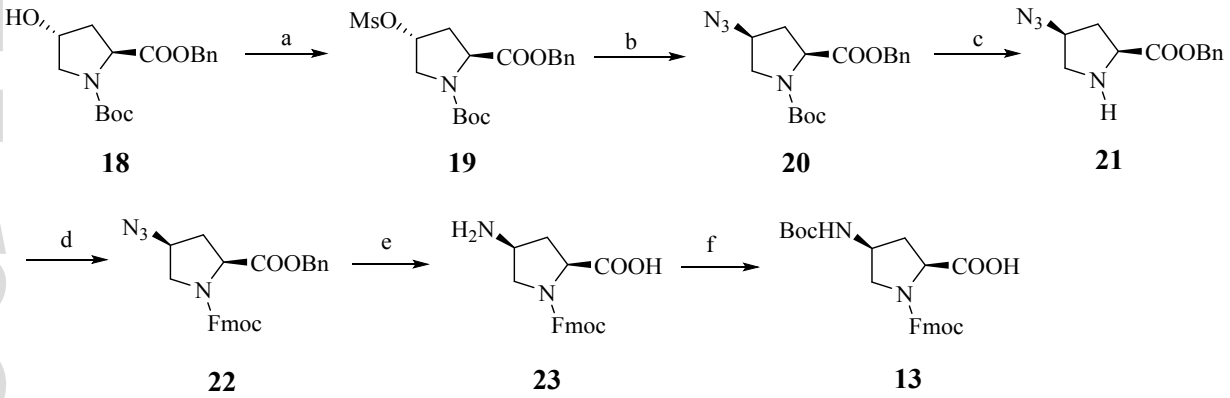
2 | METHODS AND MATERIALS

2.1 | Chemistry

2.1.1 | Synthesis of proline analogues

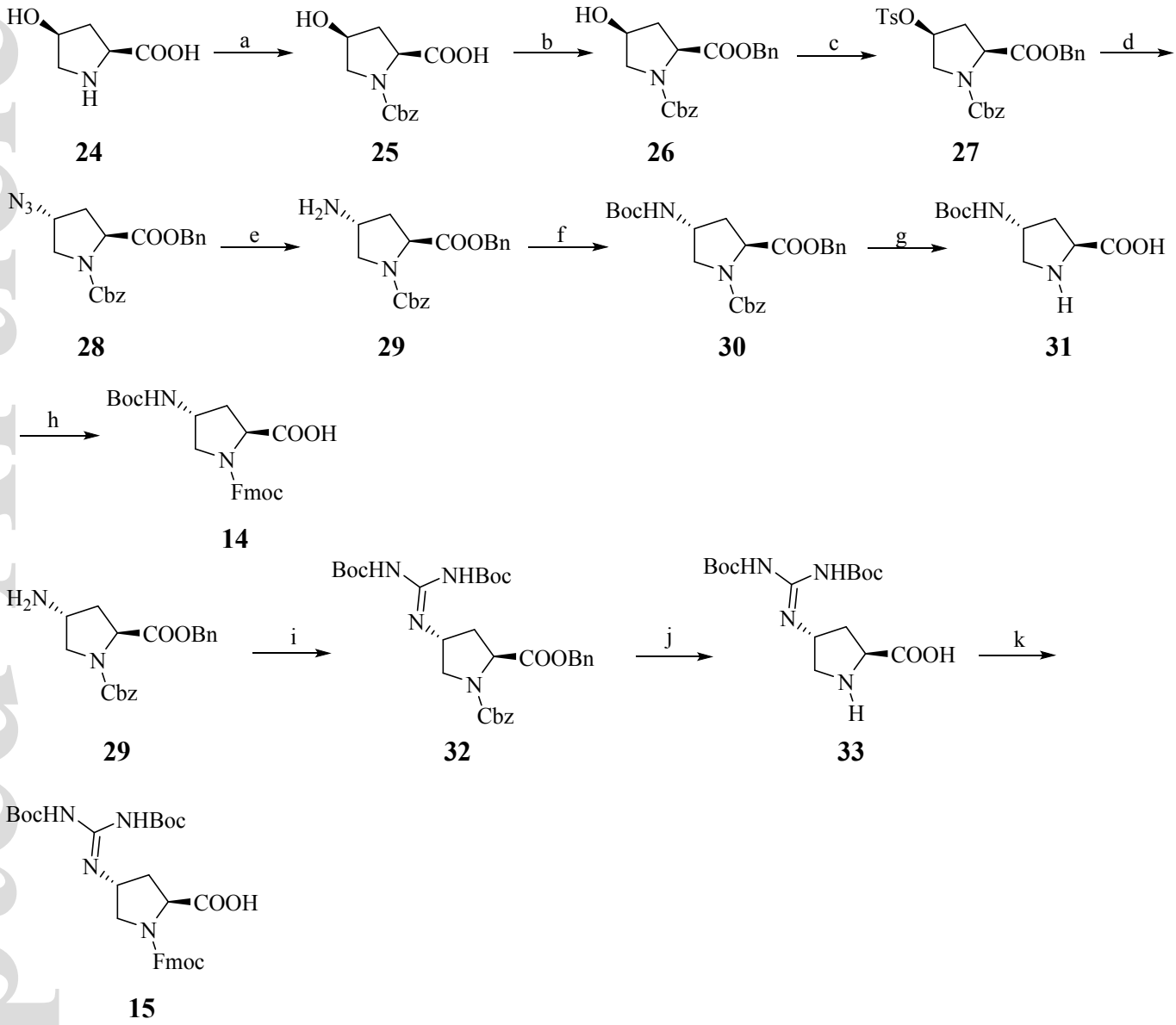
The five orthogonally protected Pro analogues were synthesized from commercially available *trans*-4-hydroxy-L-proline. They are *cis*-4-BocNH-N$^{\alpha}$-Fmoc-Pro-OH (**13**), *trans*-4-BocNH-N$^{\alpha}$-Fmoc-Pro-OH (**14**), *trans*-4-(diBoc-guanidino)-N$^{\alpha}$-Fmoc-Pro-OH (**15**), *cis*-4-(2-BocNH-ethyl)-N$^{\alpha}$-Fmoc-Pro-OH (**16**) and *cis*-4-(2-diBoc-guanidlinoethyl)-N$^{\alpha}$-Fmoc-Pro-OH (**17**).

The synthesis of *cis*-4-BocNH-N$^{\alpha}$-Fmoc-Pro-OH (**13**) was performed following a published method (Tamaki et al., 2001) with some modifications (Scheme 1). Briefly, the 4-hydroxyl group of Boc-*trans*-Hyp-OBzl (**18**) was activated with mesyl chloride to give **19**. Nucleophilic substitution of the sulfonate **19** with NaN_3 yielded in the *cis*-4-azide **20** with the chiral center at the 4 position inverted. Boc deprotection with TFA, and Fmoc re-protection gave **22**. The azide group was then reduced by Pd-catalyzed hydrogenation to yield **23** and Boc protection gave the orthogonally protected compound **13**.



Scheme 1. Synthesis of *cis*-4-BocNH-N^{a}-Fmoc Pro-OH (**13**).

Reagents and conditions (a) MsCl, pyridine, 0 °C to rt, overnight, 69%; (b) NaN_3, DMF, 65 °C, overnight, 57%; (c) TFA, DCM, 2 h, 60%; (d) Fmoc-Osu, 6% $NaHCO_3$/1,4-dioxane, rt, overnight, 78%; (e) Pd/C, H_2 (8 atm), rt, 3 d, 30%; (f) $(Boc)_2O$, TEA, H_2O/1,4-dioxane, 0 °C to rt, overnight, 50%.



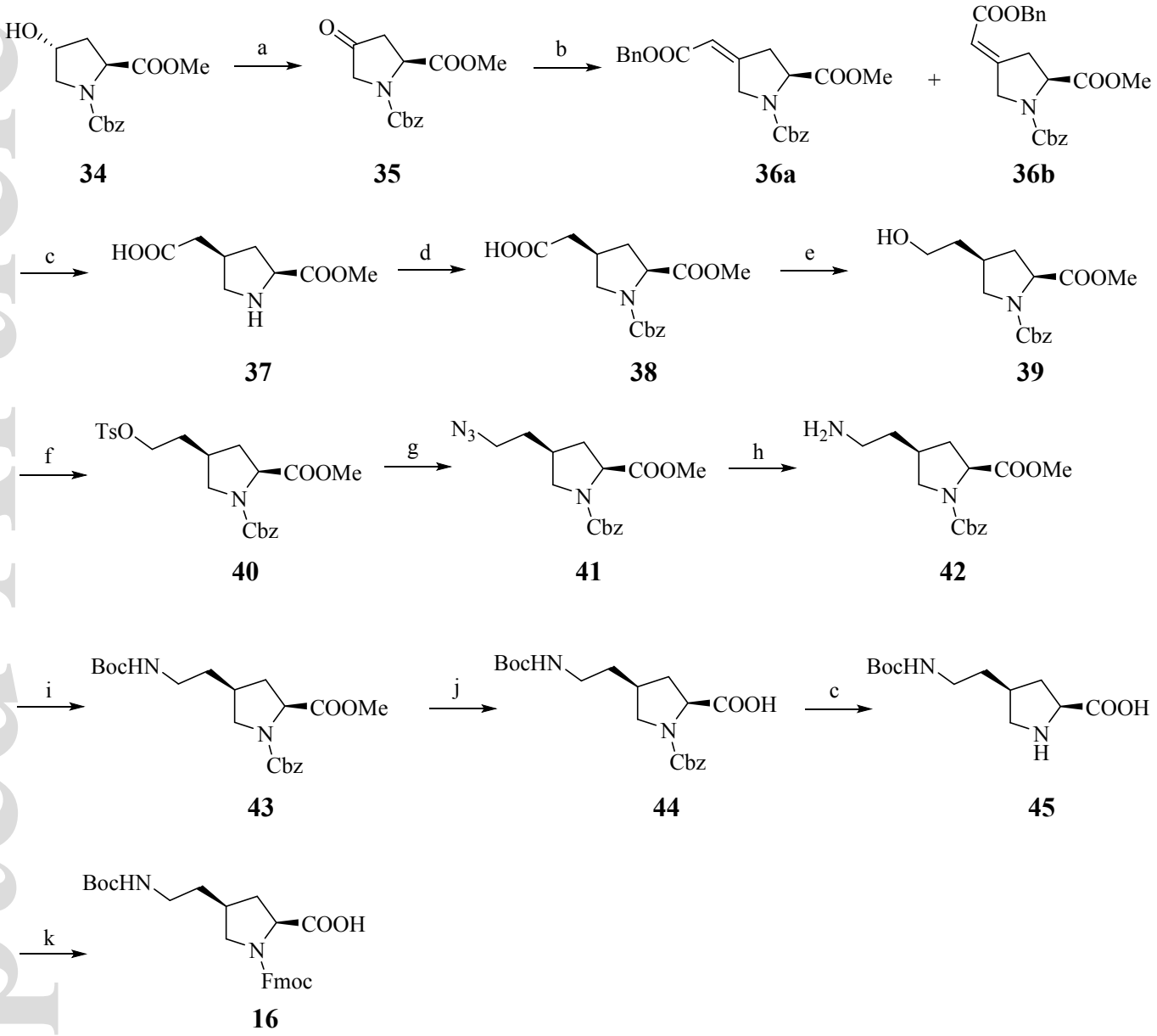
Scheme 2. Synthesis of *trans*-4-BocNH-N^a-Fmoc-Pro-OH (**14**) and *trans*-4-(diBoc-gunidino)-N^a-Fmoc-Pro-OH (**15**).

Reagents and conditions: (a) Cbz-Cl, THF/$NaHCO_3$, rt, overnight, 84%; (b) BnBr, TEA, THF, 0 °C to rt, overnight, 82%; (c) TsCl, TEA, DMAP, DCM, 0 °C to rt, overnight, 58%; (d) NaN_3, DMF, 65 °C, overnight, 91%; (e) PPh_3, H_2O, THF, reflux, overnight; (f) $(Boc)_2O$, TEA, H_2O/1,4-dioxane, 0 °C to rt, overnight, 78%(e and f 2 steps); (g) Pd/C, H_2, rt, overnight; (h) Fmoc-Osu, 6% $NaHCO_3$/1,4-dioxane, rt, overnight, 24%(g and h 2 steps); (i) N,N'-Di-Boc-N"-trifluoromethane sulfonyl-guanidine, DIPEA,

dry DCM, 0 °C to rt, overnight, 55%; (j) Pd/C, H_2, rt, overnight; (k) Fmoc-Osu, 6% $NaHCO_3$/1,4-dioxane, rt, overnight, 51% (j and k 2 steps).

For the synthesis of *trans*-4-BocNH-N$^{\alpha}$-Fmoc-Pro-OH (**14**) (Scheme 2), *cis*-4-hydroxyproline (**24**) was reacted with Cbz-Cl to give N$^{\alpha}$-Cbz-Hyp-OH (**25**), its carboxyl group was then protected as the benzyl ester (**26**) and the 4-position hydroxyl group was activated by tosylation (**27**). SN_2 displacement in **27** with NaN_3 gave **28**. The azide group of **28** was then reduced by PPh_3 in water and THF. The amino group of the resulting **29** was protected with Boc-group to give **30**. Simultaneous removal of the Cbz- and Bn- groups of **30** by hydrogenation gave **31**, the α-NH group of which was protected with Fmoc to give compound **14**. Compound **29** was reacted with the guanidinilating reagent N,N'-di-Boc-N''-trifluoromethanesulfonyl-guanidine (Tamaki et al., 2001) to give **32**. After removal of the Cbz-group and re-protection with Fmoc, *trans*-4-(diBoc-guanidino-N$^{\alpha}$-Fmoc-Pro -OH (**15)** was obtained.

The synthesis of *cis*-4-(2-BocNH-ethyl)-N$^{\alpha}$-Fmoc-Pro-OH (**16**) is depicted in Scheme 3. Commercially available *trans*-N$^{\alpha}$-Cbz-hydroxyproline (**34**) was oxidized to **35** by using a combination of TCCA and TEMPO. Key intermediate **36** was obtained via Horner-Wadsworth- Emmons reaction that is capable of carbon chain elongation (McCarthy, & Naylor, 2013) The reaction resulted in a pair of isomers **36a** and **36b** which could be separated by silica gel column chromatography. Hydrogenation of **36b** proceeded in a diastereoselective manner to afford **37**. As determined by NMR, an 80% diastereomeric excess was observed. The *cis* configuration of **37** was confirmed by NOESY measurements, where the correlation between 2-C*H* and 4-C*H* of the pyrrolidine moiety was observed (Grygorenko et al., 2009). The α-amino group of **37** was then re-protected with Cbz (**38**). The carboxyl group of **38** was selectively reduced to alcohol by IBCF/$NaBH_4$ to give **39**, the hydroxyl group of which was then activated by reaction with TsCl to give **40**. Substitution with NaN_3 yielded azide **41** which was reduced by PPh_3 to give amine **42**. Protecting the amino group with Boc and converting the Cbz- to the Fmoc-group by the usual methods yielded the targeted Pro analogue **16**.

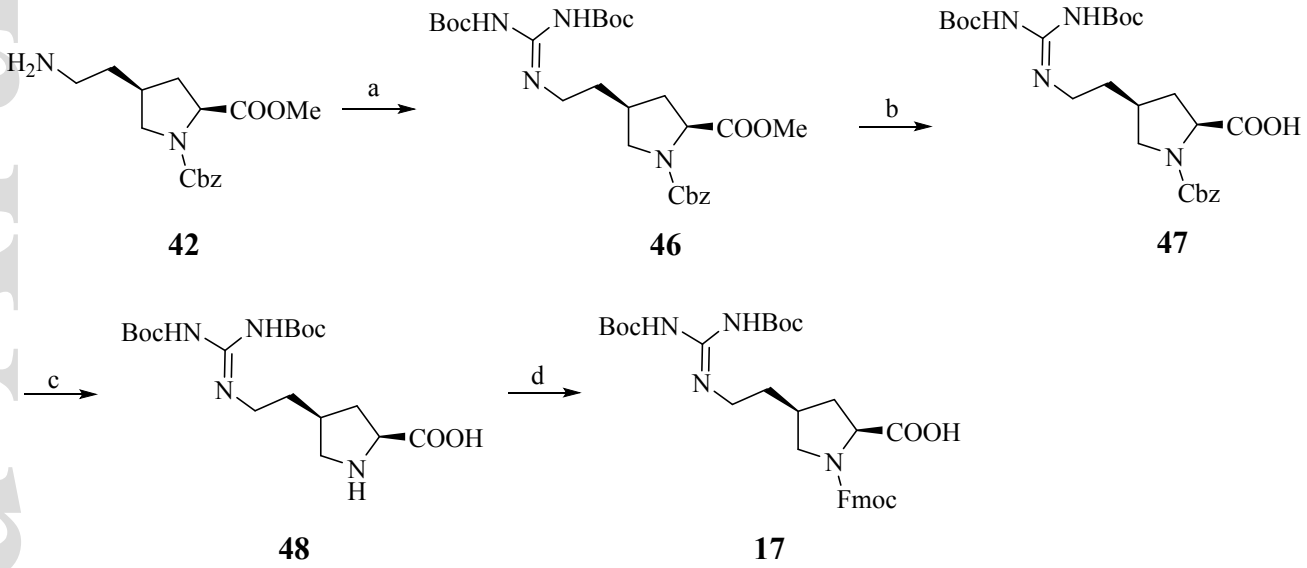


Scheme 3. Synthesis of *cis*-4-(2-BocNH-Et)-N^a-Fmoc-Pro-OH (**16**).

Reagents and conditions: (a) TCCA, DCM, 0 °C,15 min, then TEMPO 1 h, 75%.; (b) benzyl 2-(diethoxyphosphoryl)acetate, 60% NaH, dry THF, -20 °C, 30 min, then **35**, 30 min, 84%.; (c) Pd/C, H_2 (4 atm), MeOH, overnight; (d) Cbz-Cl, THF/$NaHCO_3$, rt, overnight, 86% (c and d 2 steps); (e) NMM, IBCF, THF, 0 °C,10 min, then $NaBH_4$, 0 °C, 20 min, 90%.; (f) TsCl, TEA, DMAP, DCM, 0 °C to rt, overnight, 80%; (g) NaN_3, DMF, 65 °C, overnight; (h) PPh_3, H_2O, THF, reflux, overnight, 86% (g and h

2 steps); (i) $(Boc)_2O$, TEA, H_2O/1,4-dioxane, 0 °C to rt, overnight, 95%; (j) LiOH, THF/H_2O, rt, overnight, 80%; (k) Fmoc-OSu, $NaHCO_3$, acetone/water, rt, overnight, 45% (c and k 2 steps).

Starting from intermediate **42**, *cis*-4-(2-diBoc-guanidinoethyl)-Nα-Fmoc-Pro-OH (**17**) was synthesized by guanidination, followed by deprotection and reprotection strategy (Scheme 4).



42 **46** **47**

48 **17**

Scheme 4. Synthesis of *cis*-4-(2-diBoc-guanidinoethyl)-N[a]-Fmoc-Pro-OH (**17**)

Reagents and conditions: (a) N,N'-Di-Boc-N"-trifluoromethanesulfonyl-guanidine, DIPEA, dry DCM, 0 °C to rt, overnight, 63%; (b) LiOH, THF/H_2O, rt, overnight, 85%; (c) Pd/C, H_2 (4 atm), MeOH, overnight; (d) Fmoc-OSu, $NaHCO_3$, acetone/water, rt, overnight, 64%(c and d 2 steps).

2.1.2 | Peptide synthesis

Peptide synthesis was performed by the manual solid phase technique on a Rink Amide resin. Crude peptides were purified by semi-preparative RP-HPLC. Each peptide was at least 98% pure, as assessed by analytical RP-HPLC. Molecular weights were confirmed by MS (Table 1).

2.2 | *In vitro* pharmacological characterization

2.2.1 | Opioid receptor binding assays

Opioid receptor binding studies were performed as described in detail elsewhere (Schiller et al., 2000). Binding affinities for μ and δ receptors were determined by displacing, respectively, [^{3}H]DAMGO (Multiple Peptide Systems, San Diego, CA) and [^{3}H]DSLET (Multiple Peptide Systems) from rat brain membrane binding sites, and κ opioid receptor binding affinities were measured by displacement of tritiated (5α,7α,8β-(–)-*N*-methyl-*N*-[7-(1-pyrrolidinyl-1-oxaspiro[4.5]-dec-8-yl]benzeneacetamide ([^{3}H]U69,593) (Lahti et al., 1985) (Amersham) from guinea pig brain membrane binding sites. Incubations were performed for 2 h at 0° C with [^{3}H]DAMGO, [^{3}H]DSLET and [^{3}H]U69,593 at respective concentrations of 0.72, 0.78 and 0.80 nM. IC_{50} values were determined from log dose-displacement curves and K_i values were calculated from the obtained IC_{50} values by means of the equation of Cheng and Prusoff (1973) using values of 1.3, 2.6 and 2.9 nM for the dissociation constants of [^{3}H]DAMGO, [^{3}H]DSLET and [^{3}H]U69,593, respectively.

2.2.2 | Functional guinea pig (GPI) and mouse vas deferens (MVD) assays

The GPI (Paton, 1957) and MVD (Henderson et al., 1972) functional assays were carried out as reported in detail elsewhere (Schiller et al., 1978; DiMaio et al., 1982). A dose-response curve was determined with [Leu^5]enkephalin as standard for each ileum and vas preparation, and IC_{50} values of the compounds being tested were normalized according to a published procedure (Waterfield et al., 1979). K_e values for antagonists were determined from the ratios of IC_{50} values obtained with an agonist in the presence and absence of a fixed antagonist concentration (Kosterlitz et al., 1968). μ antagonist K_e values were determined in the GPI assay against the μ agonist TAPP (H-Tyr-D-Ala-Phe-Phe-NH_2) (Schiller et al.,1989). κ antagonist K_e values of compounds were also determined in the GPI assay against the κ agonist U50,488. δ antagonist K_e values were determined in the MVD assay against the δ agonist DPDPE.

3 | Results and Discussion

[Dmt[1]]DALDA and endomorphin, that both can be considered as parent peptides of the CZ- and SJ-peptides, are potent and selective μ opioid agonists. Surprisingly, none of the peptides described here showed the expected high μ agonist potency in the GPI assay (Table 3). Most of them displayed μ antagonist activity (compounds CZ-1, CZ-2, CZ-3, CZ-4, SJ-1, SJ-2 and SJ-3) with varying μ receptor binding affinities (Table 2) and represent *a new class of selective μ opioid antagonists.* Three of the peptides (CZ-5, SJ-4 and SJ-6) turned out to be selective μ opioid partial agonists and one of them was a moderately potent μ opioid full agonist (CZ-6).

The most potent μ antagonists contain *cis*-4-amino-Pro in the 2-position of the peptide sequence (CZ-1 CZ-4), whereas a *trans*-4-amino-Pro[2]-containing analogue (CZ-5) was identified a weak μ partial agonist. Substitution of *trans*-4-guanidino-Pro in the 2-position produced the only compound with modest μ opioid agonist activity (CZ-6). Peptides containing a Pro analogue with the amino- or guanidino group attached to the 4-position via an ethyl linker (SJ-series) in general showed weak μ antagonist activity or modest μ partial agonist activity. All compounds displayed weak δ receptor binding affinity with weak δ partial agonist activity or weak δ antagonist activity, as determined in the MVD assay. All of them showed relatively weak κ receptor binding affinity and for some of them weak κ antagonist activity could be determined in the GPI assay (this was not possible with some of the compounds because of very weak κ antagonist activity and was indicated as N.D. in Table 3).

Most notable was compound CZ-1 which is a potent μ opioid antagonist, showing high μ opioid receptor binding affinity (K_i^{μ} = 1.62 nM) and high μ receptor selectivity in the opioid receptor binding assays, and high μ antagonist activity in the functional GPI assay (K_e^{μ} = 22.6 nM). In comparison with the widely used μ opioid antagonist CTOP, CZ-1 showed about equal μ receptor binding affinity and 2-fold higher μ opioid antagonist activity in the GPI assay (Table 3). In the opioid receptor binding assays it displayed higher μ versus δ receptor selectivity than CTOP, but lower μ versus κ receptor selectivity (Table 2). Compound CZ-5 turned out to be a μ partial agonist with high μ receptor binding affinity and high μ receptor binding selectivity.

4 | Conclusions

Unexpectedly, substitution of *cis*- 4-amino-Pro in the 2-position of the peptide sequence of peptides structurally related to [Dmt[1]]DALDA and endomorphin resulted in a novel class of potent μ opioid antagonists. In particular, the compound CZ-1 is of interest as a pharmacological tool because

of its high μ opioid antagonist activity and high μ opioid receptor binding selectivity. A limitation of using the opioid antagonist naloxone to reverse respiratory arrest caused by opioid overdose is its relatively short duration of action (Collins et al., 2018; Baumann et al., 2018). Depending on the results of future in vivo studies, CZ-1 or further analogues of the new μ opioid antagonist class described here may also turn out to be useful to counter opioid-induced respiratory depression.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

FIGURE LEGENDS

Figure 1. Proline analogues containing a basic residue in the 4-position.

Figure 2. Structures of endomorphin-[Dmt^{1}]DALDA hybrid peptides containing Pro analogues in the 2-position.

Table 1. Analytical parameters [Dmt[1]]DALDA analogues

compd	peptide	Formula	TOF-MS $(M\text{-}3TFA+H)^+$ Obsd.	HPLC[a] (t_R min)
1	H-Dmt-*cis*-4-amino-Pro-Trp-Lys-NH_2	$C_{39}H_{49}F_9N_8O_{11}$	635.4	12.61
2	H-Dmt-*cis*-4-amino-Pro-Tmp-Lys-NH_2	$C_{40}H_{54}F_9N_7O_{11}$	638.4	13.5
3	H-Dmt-*cis*-4-amino-Pro-Phe-Arg-NH_2	$C_{37}H_{48}F_9N_9O_{11}$	624.4	11.94
4	H-Dmt-*cis*-4-amino-Pro-Tmp-Arg-NH_2	$C_{40}H_{54}F_9N_9O_{11}$	666.4	13.59
5	H-Dmt-*trans*-4-amino-Pro-Phe-Arg-NH_2	$C_{37}H_{48}F_9N9O_{11}$	624.4	12.03
6	H-Dmt-*trans*-4-guanidino-Pro-Phe-Lys-NH_2	$C_{38}H_{50}F_9N_9O_{11}$	638.4	11.85
7	H-Dmt-*cis*-4-aminoethyl-Pro-Trp-Arg-NH_2	$C_{41}H_{53}F_9N_{10}O_{11}$	691.7	12.43
8	H-Dmt-*cis*-4-aminoethyl-Pro-Tmp-Arg-NH_2	$C_{42}H_{58}F_9N_9O_{11}$	694.7	13.69
9	H-Dmt-*cis*-4-aminoethyl-Pro-1-Nal-Arg-NH_2	$C_{43}H_{54}F_9N_9O_{11}$	702.7	13.2
10	H-Dmt-*cis*-4-guanidinoethyl-Pro-Trp-Lys-NH_2	$C_{42}H_{55}F_9N_{10}O_{11}$	705.5	12.34
11	H-Dmt-*cis*-4-guanidinoethyl-Pro-Tmp-Lys-NH_2	$C_{43}H_{60}F_9N_9O_{11}$	708.5	13.55
12	H-Dmt-*cis*-4-guanidinoethyl-Pro-1-Nal-Lys-NH_2	$C_{44}H_{56}F_9N_9O_{11}$	716.5	13.2

[a] Performed on a Sunfire™ Prep C18 column (4.6×150 mm) with a linear gradient of 95-10% solvent A (0.05% TFA in H_2O) over 30 min at a flow rate of 1.2 ml/min.

Table 2. Opioid receptor binding affinities of [Dmt[1]]DALDA analogues[a]

Compound		Binding affinities			Selectivity ratio
		K_i^{μ} [nM]	K_i^{δ} [nM]	K_i^{κ} [nM]	μ/δ/κ
CZ-1	**1**	1.62 ± 0.23	1520 ± 160	108 ± 19	1/938/67
CZ-2	**2**	9.73 ± 0.57	210 ± 4	152 ± 1	1/22/16
CZ-3	**3**	9.72 ± 1.37	2430 ± 80	64.2 ± 4.2	1/250/7
CZ-4	**4**	2.38 ± 0.49	39.0 ± 0.5	85.7 ± 25.3	1/16/36
CZ-5	**5**	1.55 ± 0.15	8230 ± 960	163 ± 6	1/5310/105
CZ-6	**6**	96.6 ± 15.4	7590 ± 570	457 ± 72	1/79/5
SJ-1	**7**	300 ± 14	1160 ± 140	607 ± 132	1/4/2
SJ-2	**8**	84.0 ± 5.2	389 ± 20	3820 ± 280	1/5/45
SJ-3	**9**	99.8 ± 15.2	5750 ± 1970	1670 ± 100	1/58/17
SJ-4	**10**	14.1 ± 1.7	1410 ± 270	444 ± 129	1/100/31
SJ-5	**11**	1.91 ± 0.16	679 ± 210	149 ± 21	1/355/78
SJ-6	**12**	45.3 ± 6.7	727 ± 27	240 ± 24	1/16/5
CTOP		1.30 ± 0.23	258 ± 59	14900 ± 500	1/198/11500

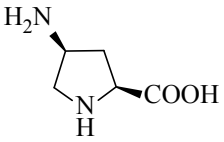
[a] Mean of 3-4 determinations ± SEM.

Table 3. GPI and MVD assay of [Dmt[1]]DALDA analogues[a]

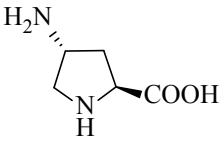
Compound		GPI			MVD	
		IC_{50} (nM)	K_e^{μ} (nM)[b]	K_e^{κ} (nM)[c]	IC_{50} (nM)	K_e^{δ} (nM)[d]
CZ-1	**1**		22.6 ± 4.3	1160 ± 160		6380 ± 160
CZ-2	**2**		33.5 ± 6.3	3660 ± 180		1660 ± 250
CZ-3	**3**		211 ± 19	1700 ± 90	3060 ± 370 (IC_{20})	
CZ-4	**4**		16.8 ± 3.0	970 ± 260		463 ± 29
CZ-5	**5**	1420 ± 70 (IC_{30})		N.D.	3160 ± 840 (IC_{20})	
CZ-6	**6**	1220 ± 210		N.D.	4370 ± 270 (IC_{25})	
SJ-1	**7**		4840 ± 130	N.D.	PA[e]	
SJ-2	**8**		1440 ± 240	N.D.		4410 ± 600
SJ-3	**9**		1520 ± 80	N.D.	PA	
SJ-4	**10**	1380 ± 170 (IC_{30})		N.D.	450 ±70 (IC_{20})	
SJ-5	**11**		2310 ± 440	1490 ± 10	PA	
SJ-6	**12**	1820 ± 110 (IC_{20})		N.D.	348 ± 23 (IC_{30})	
CTOP			40.9 ± 2.7	6940 ± 580	PA	

[a] Mean of 3-4 determinations ± SEM. [b] Determined against TAPP.

[c] Determined against U50,488. [d] Determined against DPDPE. [e] PA = partial agonist.



cis-4-amino-Pro-OH



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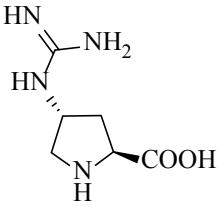

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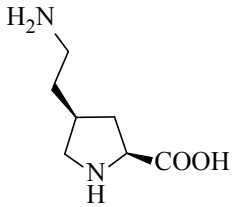
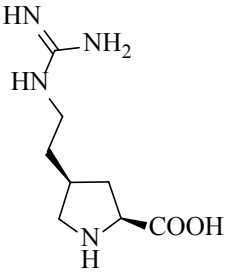


cis-4-(2-aminoethyl)-N-Fmoc-Pro-OH



cis-4-(2-gunidinoethyl)-N-Fmoc-Pro-OH