Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2745–2748

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Assessment of Substitution in the Second Pharmacophore of Dmt-Tic Analogues

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Received 3 August 2000; accepted 28 September 2000

Abstract—The Dmt-Tic pharmacophore exhibits potent δ-opioid receptor antagonism. Analogues with substitutions in the second pharmacophore with (**1**, **1′**) or without a COOH function (**2**–**9**) were synthesized: several had high δ affinity (**1′**, **2**, **7**, and **9**), but exhibited low to non-selectivity toward μ receptors similar to H-Dmt-Tic-amide and H-Dmt-Tic-ol. Functional bioactivity indicated high δ antagonism (pA_2 7.4–7.9) (**1′**, **2**, and **9**) and modest μ agonism, pEC_{50} (6.1–6.3) (**1′**, **2**, **8**, and **9**), but with E_{max} values analogous to dermorphin. These Dmt-Tic analogues with mixed δ antagonist/μ agonist properties would appear to be better candidates as analgesics than pure μ agonists.

Introduction

The development of the first dipeptide δ antagonist lacking Phe[1] arose from the δ-opioid receptor antagonists of Tyr-Tic-Phe-(Phe) series.[2] Substitution of Dmt for Tyr further produced dipeptides with orders of magnitude higher receptor binding affinity, selectivity and δ antagonism.[3] Additional modification of this unique message domain by *N*-alkylation enhanced in vitro δ antagonism by 20-fold.[4] On the other hand, modification of the COOH function and concomitant elimination of the negative charge by amidation as in H-Dmt-Tic-amide[3] or reduction to an alcohol in H-Dmt-Tic-ol,[3] reduced affinity and selectivity for the δ receptor while concomitantly increasing it for the μ-opioid receptor. However, the C-terminal alcoholic function of H-Dmt-Tic-ol seemed to be better tolerated in terms of δ interaction and enhanced μ receptor binding than the amide function.[3] Other substituents at the C-terminus with higher octanol:water coefficients dramatically increased interaction with the μ site to yield bifunctional ligands with either δ antagonism/μ agonism or δ antagonism/μ antagonism bioactivities.[5] Those data demonstrated that strong hydrophobic or lipophilic groups at the C-terminus of Dmt-Tic peptide antagonists interacted with a putative region in the receptor that determines μ activity.[5]

The requirement for an intact heterocylic ring of Tic for antagonism was delineated through substitutions by benzimidazole-, pyridoindole-, or spriolinden-derivatives,[6] while substitutions at positions 5, 6, and 7 in the Tic pharmacophore of H-Dmt-Tic-OH analogues exhibited altered receptor binding profiles.[7] This communication focused on modification at position 6 or 7 of Tic in the Dmt-Tic pharmacophore with or without a COOH function as analogues of H-Dmt-Tic-amide and H-Dmt-Tic-ol, and documents the effect of electronic perturbation on δ antagonist and μ agonist properties.

Chemistry

Dmt was synthesized according to published methods.[8] Briefly, the peptide antagonists reported in Table 1 (see Fig. 1) were prepared by acylation of H-(*R*,*S*)-(6-F)-Tic-OMe (**1**, **1′**) or substituted tetrahydroisoquinolines **2**–**9** with Boc-Dmt-OH via WSC/HOBt as a standard procedure and removing the Boc group with TFA to give the final compounds.[5] H-(*R*,*S*)-(6-F)-Tic-OH was obtained

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0960-894X/00/$ - see front matter
PII: S0960-894X(00)00569-2

Table 1. Receptor binding affinities of Dmt-Tic dipeptide analogues[a]

Peptide	R	R^1	R^2	$K_i\delta$	$K_i\mu$	$K_i\mu/K_i\delta$
H-Dmt-Tic-amide	$CONH_2$	H	H	1.22±0.09 (6)	276.8±27 (3)	227[b]
H-Dmt-Tic-ol	CH_2OH	H	H	0.44±0.14 (6)	151.4±16.3 (3)	17[b]
1	COOH	F	H	65.3±13 (4)	43.0±6.4 (4)	0.7
1′	COOH	F	H	0.32±0.02 (3)	195.3±26 (4)	610
2	H	H	H	0.97±0.3 (4)	4.60±0.4 (3)	5
3	H	H	NO_2	101.2±18 (5)	33.8±4 (3)	0.33
4	H	H	NH_2	3.94±0.3 (3)	12.5±0.3 (4)	3
5	H	H	OCH_3	3.79±0.3 (4)	18.1±1.1 (3)	5
6	H	H	OH	33.9±9.9 (4)	6.03±1.0 (3)	0.2
7	H	H	Br	1.04±0.2 (4)	18.2±2.1 (3)	18
8	H	H	F	4.09±0.08 (3)	8.98±2.7 (4)	2
9	CH_2OCH_2-Ph	H	H	0.77±0.23 (5)	6.39±1.6 (5)	8

[a]Abbreviations: Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Dmt, 2′,6′-dimethyl-L-tyrosine; WSC, water soluble carbodiimide; HOBt, hydroxybenzotriazole; TFA, trifluoroacetic acid. The ratio $K_i\mu/K_i\delta$ defines the binding selectivity between δ and μ receptor sites. The numbers in parentheses represent independent binding assays.
[b]Data taken from Salvadori et al.[3]

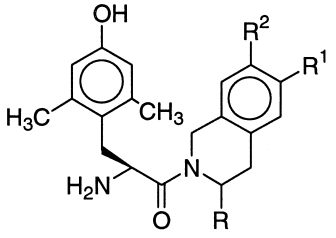

Figure 1. Schematic diagram of the Dmt-Tic pharmacophore with R, R^1, and R^2 substitution sites. See Tables 1 and 2 for details.

from H-(*R*,*S*)-(3-F)-Phe-OH by treatment with formaldehyde and concentrated HCl according to procedures in the literature,[9] esterified at the COOH function with CH_3OH and $SOCl_2$, and used in the acylation step as H-(*R*,*S*)-(6-F)-Tic-OMe. Saponification of the methyl ester followed by removal of the N-protecting group of Boc-Dmt-(*R*,*S*)-(6-F)-Tic-OMe gave the final diastereoisomeric mixture **1** and **1′**. These were separated by preparative reverse-phase HPLC conducted with a Waters Delta Prep 4000 (30×3 cm; 15 mm) column with peptides eluted in a gradient of 0–60% B in 25 min at a flow rate of 40 mL/min. The following mobile phases were used: solvent A (10% acetonitrile in 0.1% TFA, v/v) and solvent B (60% acetonitrile in 0.1% TFA, v/v). Substituted 1,2,3,4-tetrahydroisoquinolines as intermediates in the synthesis of **2**–**9** were prepared according to published methods: $R^2 = NO_2$ (**3**) and NH_2 (**4**);[10] $R^2 = OCH_3$ (**5**), OH (**6**) and F (**8**);[11] R^2 = Br (**7**);[12] and when R = hydroxymethyl (**9**), Tic-ol was esterified with NaH/benzyl bromide as reported.[13] All compounds were purified by preparative HPLC using the same conditions as reported above.

Biological

Receptor binding affinities of the compounds were conducted as previously reported.[3–5,14–16] (Table 1). The determination of antagonist and agonist bioactivity utilized mouse vas deferens and guinea-pig ileum for δ and μ activities, respectively, as reported elsewhere.[14] The measurements for δ antagonism or μ agonism were compared against deltorphin C, a δ agonist[17] and dermorphin, a μ agonist,[18] respectively. For comparison, the pA_2 values of the δ antagonists H-Dmt-Tic-amide and H-Dmt-Tic-ol,[3] and naloxone (μ) were included as controls (Table 2).

Results and Discussion

The competition binding assays for the compounds in Figure 1 are detailed in Table 1. Deletion of the COOH function in compound **2** had a similar δ binding profile to H-Dmt-Tic-amide and H-Dmt-Tic-ol, but with 30- to 60-fold greater interaction for μ receptors. The removal of the COOH function precipitates profound changes at the μ site which results in the loss of δ selectivity as observed in studies with deltorphins.[3,5] Thus, we further explored the effect of substitutions in the second pharmacophore (Tic) of our template with and without a COOH function.

Inclusion of the halogen F at position 6 of Tic (R^1, **1**, **1′**) was tested as a means to increase the hydrophobicity and H bonding capacity inside the ligand-binding domain. The results revealed a different binding pattern than the control dipeptides which depended on the chirality of Tic (Table 1). In fact, the second HPLC peak **1′** showed high δ affinity comparable to the reference compounds, while its diastereoisomer **1** might suggest a possible candidate peptide for future study as a potential μ ligand due to the shift in the μ binding pattern and selectivity. Those data confirmed the hypothesis that while Tyr/Dmt-Tic represents the δ-opioid message domain,[1,3] Tyr/Dmt-D-Tic better defines a μ-opioid message domain.[3,19]

Deletion of the COOH function (R) in combination with the substitution of different chemical functions (**2**–**8**) at position 7 (R^2) of the 1,2,3,4-tetrahydroisoquinoline aromatic nucleus decreased affinity to the δ receptor from approximately 3- (**4**, **5**, and **8**) to 30- (**6**) and 100-fold (**3**), and maintained μ affinity in the low nM range (Table 1). Thus, none of the substitutions indicated receptor preference: NO_2 (**3**) and OH (**5**) were especially not well tolerated in binding to the δ receptor. An aromatic ring (**9**) was added to the C-terminus of H-Dmt-

Table 2. Bioactivity of Dmt-Tic dipeptide analogues[a]

Compound	R	R^1	R^2	MVD (δ)			GPI (μ)		
				Agonist		Antagonist	Agonist		Antagonist
				pEC_{50}	E_{max} (%)	pA_2	pEC_{50}	E_{max} (%)	pA_2
H-Dmt-Tic-amide	$CONH_2$	H	H	i	—	7.2[b]	< 5	—	—
H-Dmt-Tic-ol	CH_2OH	H	H	i	—	7.0[b]	< 5	—	—
1′	COOH	F	H	i	—	7.45	6.09	83±4	ND
2	H	H	H	i	—	7.41	6.31	52±13[†]	ND
8	H	H	F	i	—	6.21	6.2	73±11	ND
9	CH_2OCH_2-Ph	H	H	i	—	7.87	6.28	85±6	ND
Dermorphin				—	—	—	8.8	91±2	—
Deltorphin C				9.48	91±2	—	—	—	—
Naloxone				—	—	—	—	—	8.73

[a]Data are the means±SEM of at least three independent experiments. Definitions: MVD, mouse vas deferens; GPI, guinea-pig ileum; i represents inactivity up to 1 μM; ND, not determined; pEC_{50} is the negative log of the molar concentration of an agonist to produce 50% of the maximum effect; E_{max} (%) is the maximal effect induced by an agonist expressed as percent inhibition of the electrically induced twitches. Antagonism is defined as the pA_2, which is the negative log of the molar concentration of an agonist (deltorphin C)[17] needed to elicit the original submaximal response. These values have been obtained by testing the antagonist at 1 μM and using the equation, $pA_2 = \log[C-1]/[\text{antagonist}]$, where C = concentration.
[b]Data taken from Salvadori et al.[3]

Tic-ol by alkylation with a benzyl group (Ph). Although **9** demonstrated high δ affinity comparable to H-Dmt-Tic-ol, μ affinity rose 23-fold. These data further verify that a free carboxyl group is not required for binding to δ receptors[17] and the aromatic ring of the CH_2OCH_2Ph group (**9**) apparently (and partially) occupies a site normally available to μ agonists,[5] since the compound still exhibited δ antagonism (Table 2).

Pharmacological activity in vitro with MVD and GPI was tested on selected compounds in order to evaluate agonist and antagonist activity at the δ and μ receptors as shown in Table 2. All analogues assayed (**1′**, **2**, **8**, and **9**) exhibited δ antagonism with a pA_2 ranging from 6.2–7.9 that brackets the activity of the standard dipeptide antagonists (H-Dmt-Tic-amide and H-Dmt-Tic-ol). In terms of biological activity on GPI, the compounds are all agonists with a pEC_{50} ranging from 6.1–6.3 with an E_{max} that was comparable to the μ opioid dermorphin (Table 2). Thus, these analogues possess mixed δ antagonist/μ agonist bioactivity and would appear to be better candidates as analgesics with a low propensity to produce tolerance and dependence than pure μ agonists, such as morphine.[20]

Conclusions

We demonstrated that modification of the second pharmacophore in Dmt-Tic yielded analogues with δ affinity and δ antagonism in spite of the lack of COOH function. However, receptor selectivity was dramatically absent in keeping with previous data that indicated the necessity of a negative charge to discriminate between δ and μ receptors. Furthermore, while the electronic alterations to the aromatic nucleus of Tic enhanced μ affinity, δ antagonism remained comparable to the standard peptides, H-Dmt-Tic-amide and H-Dmt-Tic-ol. The addition of a benzyl ether in lieu of the alcohol function in **9** gave similar results to position 6 or 7 substitutions on Tic in Dmt-Tic analogues. The data are especially critical to the future design of Dmt-Tic analogues which exhibit antagonist/agonist activities; those studies are currently in progress.

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