

Conversion of the Potent δ -Opioid Agonist H-Dmt-Tic-NH-CH₂-Bid into δ -Opioid Antagonists by N¹-Benzimidazole Alkylation¹

Gianfranco Balboni,^{||} Remo Guerrini,[†] Severo Salvadori,[†] Lucia Negri,[‡] Elisa Giannini,[‡] Sharon D. Bryant,[§] Yunden Jinsmaa,[§] and Lawrence H. Lazarus^{§,*}

Department of Toxicology, University of Cagliari, I-09124, Cagliari, Italy, Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy, Department of Human Physiology and Pharmacology "Vittorio Erspamer," University La Sapienza, I-00185 Rome, Italy, and Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Received August 8, 2005

Abstract: N¹-Alkylation of 1*H*-benzimidazole of the δ agonist H-Dmt-Tic-NH-CH₂-Bid with hydrophobic, aromatic, olefinic, acid, ethyl ester, or amide (**1–6**) became δ antagonists (pA_2 = 8.52–10.14). δ - and μ -Opioid receptor affinities were high ($K_i\delta$ = 0.12–0.36 nM and $K_i\mu$ = 0.44–1.42 nM). Only δ antagonism (pA_2 = 8.52–10.14) was observed; μ agonism (IC_{50} = 30–450 nM) was not correlated with changes in alkylating agent or δ antagonism, and some compounds yielded mixed δ antagonism/ μ agonism.

Numerous opioid peptides² and nonpeptide opiates^{3–5} interact with opioid receptors. H-Dmt-Tic-OH,⁶ which evolved from H-Tyr-Tic-OH,⁷ as a simplified form of TIP-(P),⁸ represents the minimal sequence that selectively interacts with δ -opioid receptors as a potent δ -antagonist. The dipeptide was transformed into a potent δ agonist by replacing the carboxylic function with an alkyl amide terminated with 1*H*-benzimidazole (H-Dmt-Tic-NH-CH₂-Bid).^{9,10} To restore the δ -opioid receptor selectivity, an acidic moiety was introduced by alkylation of N¹-benzimidazole, yielding H-Dmt-Tic-NH-CH₂-Bid(CH₂-COOH),¹⁰ and whose pharmacological behavior highlighted the role of benzimidazole-N¹H in δ -receptor interaction and activation. Similarly, the presence of a nitrogen was required in C-terminally modified endomorphin-2 with naphthyl or isoquinolyl groups resulting in mixed μ/δ agonists.¹¹ To investigate the role of the N¹-benzimidazole on δ and μ bioactivity, alkylation with various groups was initiated. All compounds reverted to potent δ antagonists, and in several cases, μ agonism increased.

Pseudopeptides were prepared stepwise by solution peptide synthetic methods⁹ described in detail in Supporting Information. In brief, mixed carbonic anhydride coupling of *tert*-butyloxycarbonyl-glycine (Boc-Gly-OH) with *o*-phenyldiamine gave intermediate monoamide, which was converted without purification to the desired

1*H*-benzimidazol-2-yl-methyl)-carbamic acid *tert*-butyl ester (Boc-NH-CH₂-Bid) by cyclization and dehydration in acetic acid (AcOH) in scheme. After N-terminal Boc deprotection with TFA, H₂N-CH₂-Bid was condensed with Boc-Tic-OH via WSC/HOBt. Alkylation of N¹-Bid was carried out by treatment of Boc-Tic-NH-CH₂-Bid⁹ with K₂CO₃ and iodomethane, benzyl bromide, allyl bromide, cyclopropylmethyl bromide, or ethyl bromoacetate.¹⁰ Boc-Tic-NH-CH₂-Bid(R) (R = alkyl groups) was deprotected with TFA and condensed with Boc-Dmt-OH via WSC/HOBt. Compound **6** was obtained from Boc-protected **5** after hydrolysis with 1 N NaOH and reaction with NH₃ via mixed anhydrides. Final compounds **1–6** were obtained after TFA treatment and purified by preparative HPLC.

Compounds **1–6** (Table 1) had subnanomolar affinity for δ -opioid receptors ($K_i\delta$ = 0.12–0.36 nM); alkylation decreased affinity by approximately 1 order of magnitude relative to the reference compounds H-Dmt-Tic-NH-CH₂-Bid (a) and H-Dmt-Tic-NH-CH₂-Bid(CH₂-COOH) (b). μ -Opioid receptor affinity was within the same order of magnitude as H-Dmt-Tic-NH-CH₂-Bid, and the lack of a carboxylic function caused a significant increase in μ -opioid receptor affinity.^{6,15,18} Thus, the analogues remained essentially neutral and nonselective, except **5** which was comparable to H-Dmt-Tic-NH-CH₂-Bid (a), but considerably less selective than H-Dmt-Tic-NH-CH₂-Bid(CH₂-COOH) (b) (Table 1).

Alkylation transformed the δ agonist H-Dmt-Tic-NH-CH₂-Bid (IC_{50} = 0.035 nM, MVD) (a) into δ antagonists **1–6** without effect on μ -opioid receptors (GPI). The analogues demonstrated high δ antagonism (pA_2 = 8.52 to 10.14) without μ antagonism; a 15-fold difference in μ -opioid agonism occurred among **1–6**. Although the alkylating agent per se does not appear important, methyl **1** improved δ antagonism slightly more than the bulky substituents (**2–4**), particularly the aromatic benzyl group (**2**). Interestingly, a single methyl converted naltrindole, an opiate δ antagonist, into a μ agonist.¹² Modification of the carboxylic function into an ester (**5**) or amide (**6**) did not change δ antagonism, suggesting these functional groups are weakly implicated in δ -receptor interactions. Compounds **1–6** had improved μ -opioid receptor affinity and agonism compared to H-Dmt-Tic-NH-CH₂-Bid(CH₂-COOH) (b), supporting evidence that the carboxylic function prevents μ -opioid receptor activation.^{2a,6} Alkylation of N¹H-benzimidazole did not modify the pharmacological activity toward μ -opioid receptors, indicating that this nitrogen is not implicated in μ -opioid receptor activation. Thus, **1–6** had a pattern of pharmacological activities as mixed μ agonists/ δ antagonists.

In summary, the alkyl groups (hydrophobic, aromatic, olefinic, acid, ethyl ester, amide) modify δ -opioid receptor activation which suggests the importance of N¹H-benzimidazole in these events. The allyl and cyclopropylmethyl (**3**, **4**) substituents induce antagonism when present at the amino function of alkaloid opiates.¹³ The δ -antagonism/ μ -agonism profile of **1–6** is similar to the bioactivity of opioids that elicit analgesia and display a

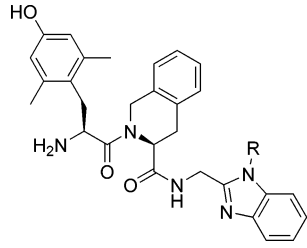
* To whom correspondence should be addressed. Tel.: +1-919-541-3238; Fax: +1-919-541-0696. E-mail: lazarus@niehs.nih.gov.

^{||} University of Cagliari.

[†] University of Ferrara.

[‡] University La Sapienza.

[§] National Institute of Environmental Health Sciences.

Table 1. Receptor Affinity and Functional Bioactivity of 1–6


compd no.	receptor affinity (nM)		functional bioactivity			
	$K_i(\delta)$	$K_i(\mu)$	MVD			GPI
			μ/δ	IC ₅₀ (nM)	pA ₂	IC ₅₀ (nM)
a	0.035 ± 0.006 (3)	0.50 ± 0.054 (3)	14	0.035 ± 0.003	—	40.7 ± 5
b	0.021 ± 0.0025 (4)	6.92 ± 0.25 (4)	330	— ^e	9.57	3193 ± 402
1	0.16 ± 0.03 (3)	0.83 ± 0.07 (5)	5	—	10.14	450 ± 51
2	0.20 ± 0.06 (4)	1.02 ± 0.19 (4)	5	—	8.52	245 ± 35
3	0.13 ± 0.02 (4)	0.44 ± 0.04 (3)	3	—	9.34	72 ± 6
4	0.36 ± 0.05 (4)	0.52 ± 0.08 (4)	1	—	9.47	64 ± 5
5	0.12 ± 0.02 (3)	1.42 ± 0.08 (3)	12	—	9.77	30 ± 4
6	0.16 ± 0.03 (4)	0.49 ± 0.02 (3)	3	—	9.26	77 ± 5
DEL ^c	0.24 ± 0.06 (6)	272 ± 50 (11)	1133	0.17 ± 0.02	—	1300 ± 150
DER ^d	178.6 ± 18 (15)	1.22 ± 0.13 (22)	0.0068	15.2 ± 2	—	1.9 ± 0.3

^a (H-Dmt-Tic-NH-CH₂-Bid), Balboni et al.⁹ ^b [H-Dmt-Tic-NH-CH₂-Bid(CH₂-COOH), Balboni et al.¹⁰ ^c DEL (deltorphan C) Lazarus et al.¹⁹ ^d DER (dermorphin) Melchiorri and Negri.²⁰ ^e —, No activity. The number of independent repetitions (*n*) is listed for the radioreceptor assays conducted in duplicate; bioassays represent means ± SE for at least six different tissue samples.

lower degree of tolerance as seen with analgesics of the μ -selective opiates.¹⁴

Binding assays were conducted as described elsewhere using rat brain P₂ synaptosomes preincubated to remove endogenous opioids,^{6,15} and labeled with 2.1 nM [³H]deltorphan II (45.0 Ci/mmol, Amersham, Buckinghamshire, UK; *K_D* = 1.4 nM) for δ -opioid receptors, and 3.5 nM [³H]DAMGO (50.0 Ci/mmol, Amersham, Buckinghamshire, UK; *K_D* = 1.5 nM) for μ -opioid receptors; the affinity constants (*K_i*) were calculated.¹⁷

In vitro activity utilized guinea-pig ileum (μ) and mouse vas deferens (δ) in competitive bioassays.⁶ Antagonism was the shift of deltorphan C (MVD) and dermorphin (GPI) log(concentration)-response curve to the right; pA₂ values were determined using the Schild Plot.¹⁸ Agonism was the inhibition of the electrically evoked twitch; the IC₅₀ values (nM) represent the mean ± SE of not less than six tissue samples.

Acknowledgment. This research was supported in part in part by the University of Ferrara and in part by the Intramural Research Program of the NIH, and NIEHS. The authors appreciate the professional services of the library staff of the NIEHS.

Supporting Information Available: Additional experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Abbreviations. In addition to the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14–42), this paper uses the following additional symbols and abbreviations: Bid, 1*H*-benzimidazol-2-yl; Boc, *tert*-butoxycarbonyl; DAMGO, [D-Ala², N-Me-Phe⁴, Gly ol⁵] enkephalin; DEL, deltorphan C, (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH₂); DER, dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH₂); DMF, N,N-dimethylformamide; DMSO-*d*₆, hexadeuteriodimethyl sulfoxide; Dmt, 2',6'-dimethyl-L-tyrosine; GPI, guinea-pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MVD, mouse vas deferens; pA₂, negative log of the molar concentration required to double the agonist concentration to achieve the original response; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP(P), H-Tyr-Tic-Phe-(Phe)-OH; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[3'-dimethylaminopropyl]-carbodiimide hydrochloride; Z, benzyloxycarbonyl; NMM, 4-methylmorpholine; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight.
- (2) (a) Bryant, S. D.; Jinsmaa, Y.; Salvadori, S.; Okada, Y.; Lazarus, L. H. Dmt and opioid peptides: a potent alliance. *Biopolymers/Peptide Sci.* **2003**, *71*, 86–102. (b) Li, T.; Fujita, Y.; Tsuda, Y.; Miyazaki, A.; Ambo, A.; Sasaki, Y.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H.; Okada, Y. *J. Med. Chem.* **2005**, *48*, 586–592. (c) Van den Eynde, I.; Laus, G.; Schiller, P. W.; Kosson, P.; Chung, N. N.; Lipkowski, A. W.; Tourwé, D. *J. Med. Chem.* **2005**, *48*, 3644–3648.
- (3) Farouz-Grant, F.; Portoghesi, P. S. Pyrrolomorphinans as δ opioid receptor antagonists. The role of steric hindrance in conferring selectivity. *J. Med. Chem.* **1997**, *40*, 1977–1981.
- (4) Nagase, H.; Kawai, K.; Hayakawa, J.; Wakita, H.; Mizusuna, A.; Matsuura, H.; Tajima, C.; Takezawa, Y.; Endoh, T. Rational drug design and synthesis of a highly selective nonpeptide δ -opioid antagonist, (4aS*,12aR*)-4a-(3-hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12a-ocatahydropyridol[3,4-*b*]acridine (TAN-67). *Chem. Pharm. Bull.* **1998**, *46*, 1695–1702.
- (5) Calderon, S. N.; Rothman, R. B.; Porreca, F.; Flippen-Anderson, J. L.; McNutt, R. W.; Xu, H.; Smith, L. E.; Bilsky, E. J.; Davis, P.; Rice, K. C. Probes for narcotic receptor mediated phenomena. 19. Synthesis of (+)-4-[(α R)- α -(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC 80): a highly selective, nonpeptide δ opioid receptor agonist. *J. Med. Chem.* **1994**, *37*, 2125–2128.
- (6) (a) Salvadori, S.; Attila, M.; Balboni, G.; Bianchi, C.; Bryant, S. D.; Crescenzi, O.; Guerrini, R.; Picone, D.; Tancredi, T.; Temussi, P. A.; Lazarus, L. H. δ Opioidmimetic antagonists: prototypes for designing a new generation of ultrasensitive opioid peptides. *Mol. Med.* **1995**, *1*, 678–689. (b) Salvadori, S.; Guerrini, R.; Balboni, G.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. Further studies on the Dmt-Tic pharmacophore: hydrophobic substituents at the C-terminus endow δ antagonists to manifest μ agonism or μ antagonism. *J. Med. Chem.* **1999**, *42*, 5010–5019.
- (7) Temussi, P. A.; Salvadori, S.; Amodeo, P.; Bianchi, C.; Guerrini, R.; Tomatis, R.; Lazarus, L. H.; Tancredi, T. Selective opioid peptides. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 933–939.
- (8) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Wilkes, B. C.; Marsden, B. J.; Lemieux, C.; Chung, N. N. Differential stereochemical requirements of μ vs δ opioid receptors for ligand binding and signal transduction: development of a class of potent and highly δ -selective peptide antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11871–11875.

- (9) Balboni, G.; Guerrini, R.; Salvadori, S.; Bianchi, C.; Rizzi, D.; Bryant, S. D.; Lazarus, L. H. Evaluation of the Dmt-Tic pharmacophore: conversion of a potent δ -opioid receptor antagonist into a potent δ -agonist and ligands with mixed properties. *J. Med. Chem.* **2002**, *45*, 713–720.
- (10) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. Potent δ -opioid agonists containing the Dmt-Tic pharmacophore. *J. Med. Chem.* **2002**, *45*, 5556–5563.
- (11) Fujita, Y.; Tsuda, Y.; Li, T.; Motoyama, T.; Takahashi, M.; Shimizu, Y.; Yokoi, T.; Sasaki, Y.; Ambo, A.; Kita, A.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H.; Okada, Y. Development of potent bifunctional endomorphin-2 analogues with mixed μ -/ δ -opioid agonist and δ -opioid antagonist properties. *J. Med. Chem.* **2004**, *47*, 3591–3599.
- (12) Coop, A.; Jacobson, A. E.; Aceto, M. D.; Harris, L. S.; Traynor, J. R.; Woods, J. H.; Rice, K. C. *N*-Cyclohexylethyl-*N*-noroxymorphindole: a μ -opioid preferring analogue of naltrindole. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2449–2451.
- (13) Portoghese, P. S. From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. *J. Med. Chem.* **2001**, *44*, 2259–2269.
- (14) Abdelhamid, E.; Sultana, M.; Portoghese, P.; Takemori, A. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 299–303.
- (15) (a) Lazarus, L. H.; Salvadori, S.; Santagada, V.; Tomatis, R.; Wilson, W. E. Function of negative charge in the “address domain” of deltorphins. *J. Med. Chem.* **1991**, *34*, 1350–1355.
- (16) Lazarus, L. H.; Guglietta, A.; Wilson, W. E.; Irons, B. J.; de Castiglione, R. Dimeric dermorphin analogues as μ -receptor probes on rat brain membranes. Correlation between central μ -receptor potency and suppression of gastric acid secretion. *J. Biol. Chem.* **1989**, *264*, 354–362.
- (17) Cheng, Y.-C.; Prusoff, W. H. Relationships between the inhibition constant (K_i) and the concentration of inhibition which cause 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
- (18) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Bryant, S. D.; Jinsmaa, Y.; Lazarus, L. H. Direct Influence of C-terminally substituted amino acids in the Dmt-Tic pharmacophore on δ -opioid receptor selectivity and antagonism. *J. Med. Chem.* **2004**, *47*, 4066–4071.
- (19) Lazarus, L. H.; Bryant, S. D.; Cooper, P. S.; Salvadori, S. What peptides these deltorphins be. *Prog. Neurobiol.* **1999**, *57*, 377–420.
- (20) Melchiorri, P.; Negri, L. The dermorphin peptide family. *Gen. Pharmacol.* **1996**, *27*, 1099–1107.

JM058259L