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Letter

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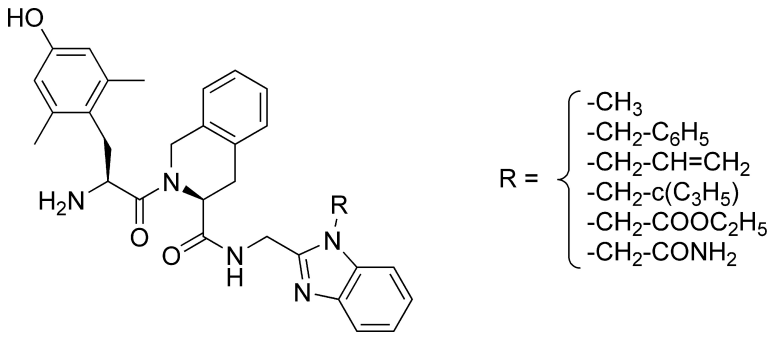

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Conversion of the Potent δ-Opioid Agonist H-Dmt-Tic-NH-CH_2-Bid into δ-Opioid Antagonists by N^1-Benzimidazole Alkylation[1]

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Abstract: N^1-Alkylation of 1*H*-benzimidizole of the δ agonist H-Dmt-Tic-NH-CH_2-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 peptides[2] 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-CH_2-Bid).[9,10] To restore the δ-opioid receptor selectivity, an acidic moiety was introduced by alkylation of N^1-benzimidazole, yielding H-Dmt-Tic-NH-CH_2-Bid(CH_2-COOH),[10] and whose pharmacological behavior highlighted the role of benzimidazole-N^1H 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 N^1-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 methods[9] described in detail in Supporting Information. In brief, mixed carbonic anhydride coupling of *tert*-butyloxycarbonyl-glycine (Boc-Gly-OH) with *o*-phenylendiamine gave intermediate monoamide, which was converted without purification to the desired 1*H*-benzimidazol-2-yl-methyl)-carbamic acid *tert*-butyl ester (Boc-NH-CH_2-Bid) by cyclization and dehydration in acetic acid (AcOH) in scheme. After N-terminal Boc deprotection with TFA, H_2N-CH_2-Bid was condensed with Boc-Tic-OH via WSC/HOBt. Alkylation of N^1-Bid was carried out by treatment of Boc-Tic-NH-CH_2-Bid[9] with K_2CO_3 and iodomethane, benzyl bromide, allyl bromide, cyclopropylmethyl bromide, or ethyl bromoacetate.[10] Boc-Tic-NH-CH_2-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 NH_3 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-CH_2-Bid (a) and H-Dmt-Tic-NH-CH_2-Bid(CH_2-COOH) (b). μ-Opioid receptor affinity was within the same order of magnitude as H-Dmt-Tic-NH-CH_2-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-CH_2-Bid (a), but considerably less selective than H-Dmt-Tic-NH-CH_2-Bid(CH_2-COOH) (b) (Table 1).

Alkylation transformed the δ agonist H-Dmt-Tic-NH-CH_2-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-CH_2-Bid(CH_2-COOH) (b), supporting evidence that the carboxylic function prevents μ-opioid receptor activation.[2a,6] Alkylation of N^1H-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 N^1H-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

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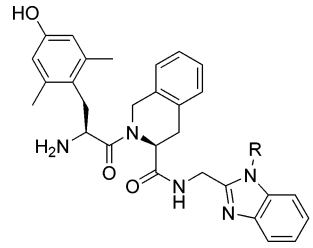
Table 1. Receptor Affinity and Functional Bioactivity of **1**−**6**



R = -CH_3 **1**; -CH_2-C_6H_5 **2**; -CH_2-CH=CH_2 **3**; -CH_2-c(C_3H_5) **4**; -CH_2-$COOC_2H_5$ **5**; -CH_2-$CONH_2$ **6**

	receptor affinity (nM)		functional bioactivity			
			MVD			GPI
compd no.	$K_i(\delta)$	$K_i(\mu)$	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-CH_2-Bid), Balboni et al.[9] [b] [H-Dmt-Tic-NH-CH_2-Bid(CH_2-COOH), Balboni et al.[10] [c] DEL (deltorphin 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 P_2 synaptosomes preincubated to remove endogenous opioids,[6,15] and labeled with 2.1 nM [^{3}H]deltorphin II (45.0 Ci/mmol, Amersham, Buckinghamshire, UK; K_D = 1.4 nM) for δ-opioid receptors, and 3.5 nM [^{3}H]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 deltorphin C (MVD) and dermorphin (GPI) log(concentration)-response curve to the right; pA_2 values were determined using the Schild Plot.[18] Agonism was the inhibition of the electrically evoked twitch; the IC_{50} values (nM) represent the mean ± SE of not less than six tissue samples.

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

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(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*-butyloxycarbonyl; DAMGO, [D-Ala^2,*N*-Me-Phe^4, Gly ol^5] enkephalin; DEL, deltorphin C, (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2); DER, dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2); 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; pA_2, 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′-dimethyl)aminopropyl]-carbodiimide hydrochloride; Z, benzyloxycarbonyl, NMM, 4-methylmorpholine; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight.

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