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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 non peptide 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 behaviour highlighted the role of benzimidazole-$N^1$H 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-/delta;-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 the Scheme. After N-terminal Boc deprotection with TFA, $H_2$N-$CH_2$-Bid was condensed with Boc-Tic-OH via WSC/HOBt. Alkylation of $N^1$-Bid was carried out

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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 NaOH 1N 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) had subnanomolar affinity for δ-opioid receptors ($K_i$δ 0.12–0.36 nM); alkylation decreased affinity by approximately an 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 non-selective, 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).

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 $N^1$H-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, ethylester, amide) modify δ-opioid receptor activation which suggests the importance of $N^1$H-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 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 labelled 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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-$d_6$, hexadeuterodimethyl 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-methyl morpholine; MALDI-TOF, matrix assisted laser desorption ionization time of flight.

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**Table**

**Receptor affinity and functional bioactivity of 1–6**

See text for summary and pertinent references. Parent compounds: [a](H-Dmt-Tic-NH-$CH_2$-Bid), Balboni et al.[9] and [b][H-Dmt-Tic-NH-$CH_2$-Bid($CH_2$-COOH), Balboni et al.[10]. Standard compounds: [c]DEL (deltorphin C) Lazarus et al.[19] and [d]DER (dermorphin) Melchiorri and Negri.[20] –, No activity. The number of independent repetitions (*n*) is listed for the radioreceptor assays conducted in duplicate; bioassays represent means ± SE for at least 6 different tissue samples.

| Cmpd no. | Receptor affinity (nM) | | | Functional bioactivity | | |
|---|---|---|---|---|---|---|
| | | | | MVD | | GPI |
| | $K_i(\delta)$ | $K_i(\mu)$ | 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 | - | 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 |

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