Brief Articles

New 2′,6′-Dimethyl-L-tyrosine (Dmt) Opioid Peptidomimetics Based on the Aba-Gly Scaffold. Development of Unique μ-Opioid Receptor Ligands

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The Aba-Gly scaffold, incorporated into Dmt-Tic ligands (H-Dmt-Tic-Gly-NH-CH_2-Ph, H-Dmt-Tic-Gly-NH-Ph, H-Dmt-Tic-NH-CH_2-Bid), exhibited mixed μ/δ or δ opioid receptor activities with μ agonism. Substitution of Tic by Aba-Gly coupled to −NH−CH_2−Ph (**1**), −NH−Ph (**2**), or −Bid (Bid = 1*H*-benzimidazole-2-yl) (**3**) shifted affinity ($K_i(\mu)$ = 0.46, 1.48, and 19.9 nM, respectively), selectivity, and bioactivity to μ-opioid receptors. These compounds represent templates for a new class of lead opioid agonists that are easily synthesized and suitable for therapeutic pain relief.

Introduction

Modifications of the opioid H-Dmt-Tic[1] pharmacophore revealed that even small modifications changed its pharmacological profile,[2] including enhanced δ-antagonism, δ-agonism, mixed μ-agonism/δ-agonism, and mixed μ-agonism/δ-antagonism. Interestingly, these biological profiles of opioid ligands represent a class of compounds that may have diminished propensity to induce tolerance and dependence in a long-term treatment of pain.[3] One liability of small peptides is cyclization to dioxopiperazine during synthesis and purification,[4,5] which occurs with peptides containing Tic or constrained residues at the C-terminus[5] and other amino acids.[6,7] A reduced peptide bond [ψ(CH_2NH)] between Tic[2] and Phe[3] in TIPP[8] or the N-terminal dimethylation of the Dmt-Tic pharmacophore to yield *N*,*N*-$(CH_3)_2$-Dmt-Tic analogues[9] eliminated cyclization and elevated opioid properties. Aba-Gly,[10] a mimetic of the Phe-Gly or Tic-Gly sequence, prevents dioxopiperazine formation. In dermorphin, a μ-opioid selective agonist, it shifted affinity and selectivity toward δ-opioid receptors.[11] Since opioid ligands with mixed μ-agonist/δ-antagonist, μ-agonist/δ-agonist, or selective δ-agonist activity profile have a diminished propensity to induce tolerance, they may have therapeutic advantages over μ-agonist analgesics for long-term treatment of pain. Aba-Gly analogues C-terminally extended by −NH−CH_2−Ph (**1**) or −NH−Ph (**2**), or transformed in Bid (**3**), might be new antinociceptive therapeutics with potentially fewer side effects.[12]

Chemistry

New compounds (**1**−**3**) were prepared by solution peptide methods (Scheme 1). Boc-Aba-Gly-OH[10] was condensed with benzylamine or aniline via WSC/HOBt. After N-terminal Boc deprotection with TFA, Aba-Gly amides were condensed with Boc-Dmt-OH via WSC/HOBt. Boc deprotection with TFA gave **1** and **2**. Compound **3** was synthesized as follows: mixed carbonic anhydride coupling of Boc-Aba-Gly-OH with *o*-phenylenediamine gave the crude intermediate monoamide, which was converted without purification to the desired benzoimidazole derivative by cyclization and dehydration in acetic acid. After Boc deprotection this derivative was converted into **3** by condensation with Boc-Dmt-OH, as described above. The final products were purified by preparative HPLC.

Receptor Affinity Analysis

δ-Opioid receptor affinities for **1**−**3** were in the range $K_i(\delta)$ = 11−427 nM, considerably weaker than the reference compounds with a loss of affinity ranging from 355- to 13800-fold. The μ-opioid receptor affinity, on the other hand, with $K_i(\mu)$ values in the nanomolar range ($K_i(\mu)$ = 0.46−19.9 nM), is in good accordance with the values of the reference compounds. Selectivity increased and shifted from δ to μ opioid receptors ($K_i(\delta)/K_i(\mu)$ = 18−24) (Table 1). It is interesting to note that the presence of Aba-Gly in the μ-opioid selective dermorphin or in its N-terminal tetrapeptide sequence shifted selectivity to δ-opioid receptors when inserted into the 3- or 4-position in lieu of the Phe-Gly sequence.[11] In contrast, substitution of Tic[2] with Aba-Gly in peptides or pseudopeptides containing the essentially δ-opioid receptor selective Dmt-Tic pharmacophore induces a shift in affinity and selectivity to μ-opioid receptors.

Functional Bioactivity

Ligands **1**−**3** exhibited weak δ-agonism or partial δ-agonist activity (Table 1). In accord with the $K_i(\mu)$ data, the IC_{50} values, based on the function bioactivity in GPI, followed the same trend in activity: benzylamide (51 nM) > anilide (95 nM) > Bid (231 nM). In particular, **1**, which is characterized by its

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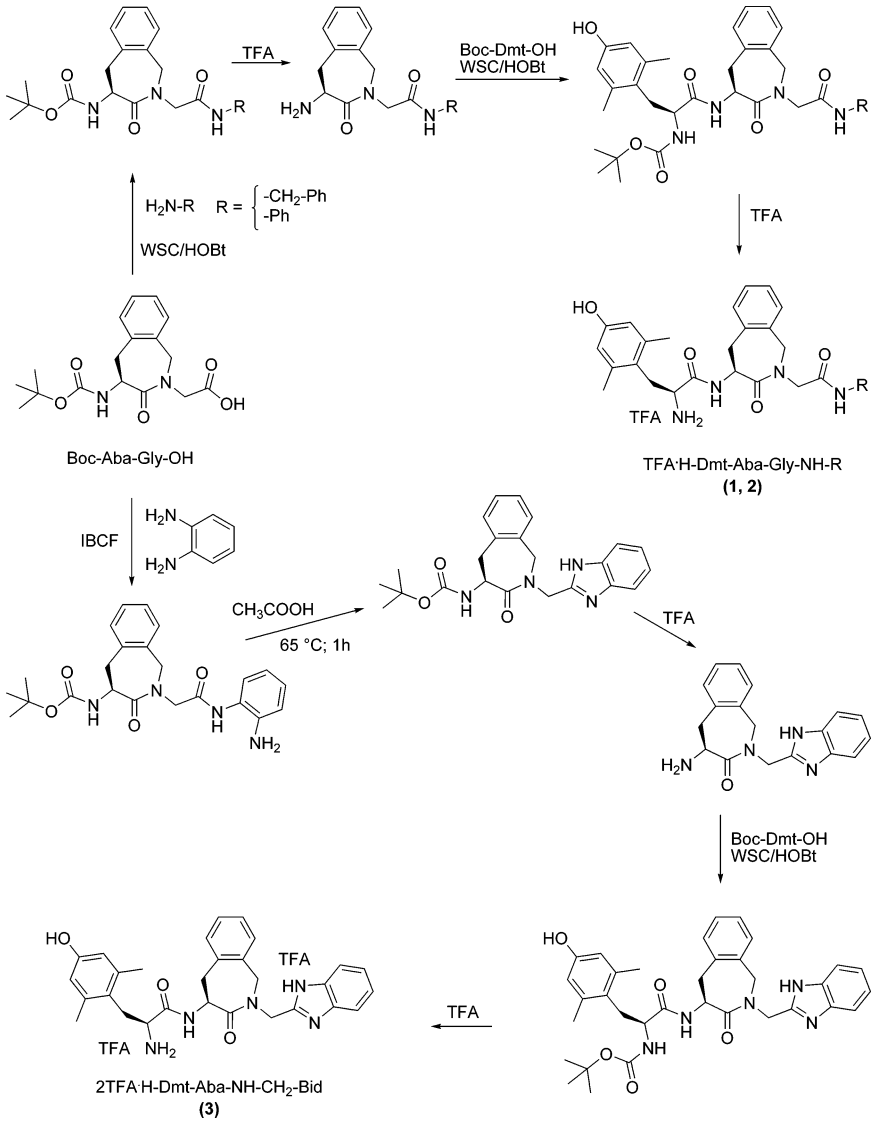
Scheme 1. Synthesis of Compounds **1**−**3**



peptidomimetic structure, has $K_i(\mu)$ and IC_{50} values comparable to the values of μ-selective opioid tetrapeptides endomorphin-1 and -2.[13]

Conclusions

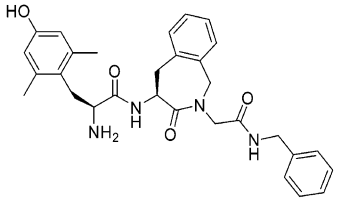
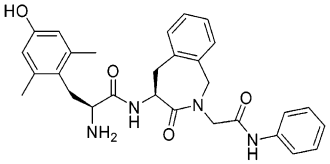
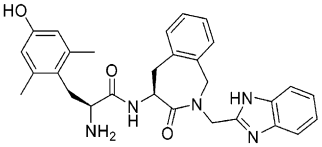
Dioxopiperazine formation, previously observed in Tic[2]-containing peptides,[4,5] was absent using the Aba-Gly scaffold because of its lactam structure and the lack of cis isomerism in the amide bond[14] between Dmt[1] and Tic[2] or Pro.[10,11] The Tic residue allows g(−) and g(+) side chain conformations, whereas only Aba permits g(+) and trans conformation. This important conformational change is derived from the substitution of an iminoacid (Tic) by its surrogate primary amine (Aba-Gly). Furthermore, **1**−**3** could be considered "peptidomimetic" derivatives and potentially be devoid of the drawbacks linked to peptides. Many opioid peptides and pseudopeptides were derived from the substitution of Tic in the Dmt-Tic or Tyr-Tic pharmacophore, some with quite interesting results, but further development was not pursued.[15−17] Furthermore, if we consider substitutions for the tetrahydroisoquinoline nucleus, only a few modifications are permitted without the loss of biological potency. Although substitution of Tic[2] in Dmt-Tic ligands by the Aba-Gly scaffold reduced δ-opioid receptor activity (Table 1), the resultant ligands maintained μ-opioid receptor affinity and functional bioactivity, and this is in good agreement with similar modifications reported by Tourwé et al.[18b] We propose that H-Dmt-Aba-Gly-NH-CH_2-Ph (**1**) has the earmarks of a versatile prototype for a new class of easily synthesizable peptidomimetics.[11,18] Our current ongoing investigations on the incorporation of negatively (Aba-Glu, Aba-Asp) and positively (Aba-Lys, Aba-Arg) charged scaffolds for δ and/or μ opioid ligands further assess the important role of charge in the discrimination of opioid receptor selectivity and bioactivity.

Experimental Section

Boc-Aba-Gly-NH-CH_2-Ph. To a solution of Boc-Aba-Gly-OH[10] (0.1 g, 0.3 mmol) and benzylamine (0.03 mL, 0.3 mmol) in DMF (10 mL) at 0 °C were added HOBt (0.05 g, 0.33 mmol) and WSC (0.06 g, 0.33 mmol). The mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.12 g (92%); R_f(B) = 0.68; HPLC K' = 8.59; mp 78−80 °C; $[\alpha]^{20}_D$ +4.2; MS *m*/*z* $(M + H)^+$ 425.

TFA·H-Aba-Gly-NH-CH_2-Ph. Boc-Aba-Gly-NH-CH_2-Ph (0.12 g, 0.28 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) was added to the solution until the

Table 1. Receptor Binding and Functional Bioactivity

no.	Structure	Receptor affinity[a] (nM) $K_i\delta$	Receptor affinity[a] (nM) $K_i\mu$	Selectivity δ/μ	Selectivity μ/δ	Functional bioactivity (nM) MVD (IC_{50})[b]	Functional bioactivity (nM) MVD (K_e)	Functional bioactivity (nM) GPI (IC_{50})[b]
	H-Dmt-Tic-Gly-NH-CH_2-Ph[c]	*0.031*	*0.16*	*–*	*5.3*	*-*	*0.56*	*2.69*
	H-Dmt-Tic-Gly-NH-Ph[c]	*0.042*	*0.16*	*–*	*3.6*	*3.02*	*-*	*2.57*
	H-Dmt-Tic-NH-CH_2-Bid[c]	*0.035*	*0.50*	*–*	*14*	*0.13*	*-*	*26.9*
	Endomorphin-2		*0.69*					*13.7*
1	 H-Dmt-Aba-Gly-NH-CH_2-Ph	11.0±2.3	0.46±0.07	23.9	-	830±70		51±5
2	 H-Dmt-Aba-Gly-NH-Ph	27.2±5.7	1.48±0.11	18.4	-	Partial Agonist (max 40%) IC_{50} = 650 nM		95±7.5
3	 H-Dmt-Aba-NH-CH_2-Bid	427±38	19.9±0.57	21.5		Partial Agonist (max 40%) IC_{50} = 2,700 nM		231±15

[a] The K_i values (nM) were determined according to Cheng and Prusoff,[19] using published methods.[3,9] The mean ± SEM values with three repetitions are based on independent binding assays conducted in duplicate using five to eight graded doses of peptides with several different synaptosomal preparations. [b] Agonism was expressed as IC_{50} obtained from dose response curves.[20] These values represent the mean ± SE for at least six fresh tissue samples. Deltorphin C and dermorphin were the internal standards for MVD (δ-opioid receptor bioactivity) and GPI (μ-opioid receptor bioactivity) tissue preparations, respectively. [c] Data taken from Balboni et al.[3]

product precipitated: yield 0.12 g (96%); R_f(A) = 0.42; HPLC K' = 5.63; mp 93−95 °C; $[\alpha]^{20}_D$ +5.6; MS *m*/*z* (M + H)$^+$ 324.

Boc-Dmt-Aba-Gly-NH-CH_2-Ph. To a solution of Boc-Dmt-OH (0.05 g, 0.16 mmol) and TFA·H-Aba-Gly-NH-CH_2-Ph (0.07 g, 0.16 mmol) in DMF (10 mL) at 0 °C were added NMM (0.02 mL, 0.16 mmol), HOBt (0.03 g, 0.18 mmol), and WSC (0.04 g, 0.18 mmol). The mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.09 g (88%); R_f(B) = 0.67; HPLC K' = 8.59; mp 105−107 °C; $[\alpha]^{20}_D$ −3.2; MS *m*/*z* (M + H)$^+$ 616.

TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph (1). Boc-Dmt-Aba-Gly-NH-CH_2-Ph (0.09 g, 0.15 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.09 g (96%); R_f(A) = 0.52; HPLC K' = 6.79; mp 120−122 °C; $[\alpha]^{20}_D$ −4.8; MS *m*/*z* (M + H)$^+$ 516; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.48 (m, 7H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 9H).

Boc-Aba-Gly-NH-Ph. This compound was obtained by condensation of Boc-Aba-Gly-OH[10] with aniline via WSC/HOBt as reported for Boc-Aba-Gly-NH-CH_2-Ph: yield 0.1 g (90%); R_f(B) = 0.61; HPLC K' = 8.35; mp 75−77 °C; $[\alpha]^{20}_D$ +4.9; MS *m*/*z* (M + H)$^+$ 410.

TFA·H-Aba-Gly-NH-Ph. Boc-Aba-Gly-NH-Ph was treated with TFA as reported for TFA·H-Aba-Gly-NH-CH_2-Ph: yield 0.09 g (97%); R_f(A) = 0.37; HPLC K' = 5.36; mp 98−100 °C; $[\alpha]^{20}_D$ +6.4; MS *m*/*z* (M + H)$^+$ 310.

Boc-Dmt-Aba-Gly-NH-Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Aba-Gly-NH-Ph via WSC/HOBt as reported for Boc-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.08 g (89%); R_f(B) = 0.59; HPLC K' = 8.21; mp 111−113 °C; $[\alpha]^{20}_D$ −4.8; MS *m*/*z* (M + H)$^+$ 602.

TFA·H-Dmt-Aba-Gly-NH-Ph (2). Boc-Dmt-Aba-Gly-NH−Ph was treated with TFA as reported for TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.07 g (95%); R_f(A) = 0.46; HPLC K' = 6.33; mp 126−128 °C; $[\alpha]^{20}_D$ −5.3; MS *m*/*z* (M + H)$^+$ 502; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.48 (m, 5H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.64 (m, 9H).

Boc-Aba-NH-CH_2-Bid. A solution of Boc-Aba-Gly-OH[10] (0.1 g, 0.3 mmol) and NMM (0.03 mL, 0.3 mmol) in DMF (10 mL) was treated at −20 °C with IBCF (0.04 mL, 0.3 mmol). After 10 min at −20 °C, *o*-phenylendiamine (0.03 g, 0.3 mmol) was added. The mixture was allowed to stir while slowly warming to room temperature (1 h) and was then stirred for 3 h. The solvent was evaporated, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed with 5% $NaHCO_3$ and brine and dried over Na_2SO_4. The solution was filtered, the solvent was evaporated, and the residual solid was dissolved in glacial AcOH (10 mL). The solution was heated at 65 °C for 1 h. After the solvent was evaporated, the residue was crystallized from Et_2O/Pe (1:9, v/v): yield 0.1 g (82%); R_f(B) = 0.51; HPLC K' = 7.23; mp 85−87 °C; $[\alpha]^{20}_D$ +6.4; MS *m*/*z* (M + H)$^+$ 407.

2TFA·H-Aba-NH-CH_2-Bid. Boc-Aba-NH-CH_2-Bid was treated with TFA as reported for TFA·H-Aba-Gly-NH-CH_2-Ph: yield 0.09 g (96%); R_f(A) = 0.28; HPLC K' = 4.40; mp 102−104 °C; $[\alpha]^{20}_D$ +6.9; MS *m*/*z* (M + H)$^+$ 307.

Boc-Dmt-Aba-NH-CH_2-Bid. To a solution of Boc-Dmt-OH (0.12 g, 0.4 mmol) and 2TFA·H-Aba-NH-CH_2-Bid (0.21 g, 0.4 mmol) in DMF (10 mL) at 0 °C were added NMM (0.09 mL, 0.8 mmol), HOBt (0.07 g, 0.44 mmol), and WSC (0.08 g, 0.44 mmol). The mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.21 g (88%); R_f(B) = 0.46; HPLC K' = 6.98; mp 115−117 °C; $[\alpha]^{20}_D$ +4.9; MS *m*/*z* (M + H)$^+$ 599.

2TFA·H-Dmt-Aba-NH-CH_2-Bid (3). Boc-Dmt-Aba-NH-CH_2-Bid was treated with TFA as reported for TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.15 g (90%); R_f(B) = 0.46; HPLC K' = 4.95; mp 134−136 °C; $[\alpha]^{20}_D$ +5.5; MS *m*/*z* $(M + H)^+$ 499; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95 (m, 1H), 4.45−4.48 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.70 (m, 8H).

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Supporting Information Available: Additional experimental details and references, NMR data of intermediates, and elemental analysis data for **1**−**3**. 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 abbreviations inIUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol.* Chem. **1985**, *260*, 14−42), other symbols and abbreviations are as follows: H-Aba-Gly-OH, 2-(4-amino-4,5-dihydro-3-oxo-1*H*-benzo[*c*]azepin-2(3*H*)-yl)acetic acid; Aba-NH-CH_2-Bid, 2-((1*H*-benzo[*d*]imidazol-2-yl)-methyl)-4-amino-1,2,4,5-tetrahydrobenzo[*c*]-azepin-3-one; Ac, acetyl; Boc, *tert*-butyloxycarbonyl; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]-enkephalin; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuteriodimethylsulfoxide; dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2; Dmt, 2′,6′-dimethyl-L-tyrosine; GPI, guinea-pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IBCF, isobutyl chloroformate; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; MVD, mouse vas deferens; NMM, 4-methylmorpholine; Pe, petroleum ether; 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 HCl; Z, benzyloxycarbonyl.

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