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Chemical space screening around Phe^3 in opioid peptides: Modulating μ versus δ agonism by Suzuki-Miyaura cross-couplings

Tom Willemse [a,e,1], Emilie Eiselt [b,1], Karlijn Hollanders [a,e], Wim Schepens [c], Herman W.T. van Vlijmen [c], Nga N. Chung [d], Véronique Blais [b], Brain Holleran [b], Jean-Michel Longpré [b], Peter W. Schiller [d], Bert U.W. Maes [e], Philippe Sarret [b,*], Louis Gendron [b,*], Steven Ballet [a,*]

[a] Research Group of Organic Chemistry, Departments of Bioengineering Sciences and Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[b] Department of Pharmacology-Physiology, Faculty of Medicine and Health Sciences, Institut de Pharmacologie de Sherbrooke, Centre de Recherche du CHUS, Université de Sherbrooke, 3001 12eAvenue Nord, Sherbrooke, Quebec J1H 5N4, Canada
[c] Discovery Sciences, Janssen Research and Development, Turnhoutseweg 30, 2340 Beerse, Belgium
[d] Laboratory of Chemical Biology and Peptide Research, Montreal Clinical Research Institute, 110 avenue des Pins Ouest, Montreal, Quebec H2W 1R7, Canada
[e] Organic Synthesis Division, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

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ABSTRACT

In this study, affinities and activities of derivatized analogues of Dmt-dermorphin[1–4] (i.e. Dmt-D-Ala-Phe-GlyNH_2, Dmt = 2′,6′-dimethyl-(*S*)-tyrosine) for the μ opioid receptor (MOP) and δ opioid receptor (DOP) were evaluated using radioligand binding studies, functional cell-based assays and isolated organ bath experiments. By means of solid-phase or solution-phase Suzuki-Miyaura cross-couplings, various substituted regioisomers of the phenylalanine moiety in position 3 of the sequence were prepared. An 18-membered library of opioid tetrapeptides was generated *via* screening of the chemical space around the Phe^3 side chain. These substitutions modulated bioactivity, receptor subtype selectivity and highly effective ligands with subnanomolar binding affinities, contributed to higher functional activities and potent analgesic actions. In search of selective peptidic ligands, we show here that the Suzuki-Miyaura reaction is a versatile and robust tool which could also be deployed elsewhere.

Alleviation or treatment of pain remains a significant challenge in pain research. Although opioid therapy is the cornerstone of severe and chronic pain management, serious unwanted effects are associated with their (chronic) administration (e.g., physical dependence, tolerance, nausea).[1] The biological effects of these drugs are exerted via binding to the three opioid receptor subtypes (μ-,δ-, and κ-opioid receptors termed MOP, DOP and KOP, respectively), belonging to the superfamily of G protein-coupled receptors.[2,3] One possible approach to overcome the limitations of opioids is the use of ligands with mixed activity profile.[4] It has, for example, previously been shown that dual MOP/DOP agonism or mixed agonism/antagonism were advantageous over highly-selective receptor subtype ligands.[5–11]

The opioid heptapeptidedermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), initially derived from the skin of the South American frog *Phyllomedusasauvagei*, was found to be a potent and selective MOP agonist.[12–14] Compared to morphine, dermorphin was shown to possess higher antinociceptive efficacy with decreased adverse effects.[15] SAR studies indicated the *N*-terminal tetrapeptide to be the minimal sequence required for potent opioid responses. In addition, it had been shown that Dmt[1] replacement of tyrosine afforded ligands with enhanced MOP and DOP bioactivity.[16] Here, we attempted to modulate the phar-macological profiles of the peptidic ligands by modifying the second key aromatic pharmacophore group, the Phe^3 side chain. It was hypothesized that the Dmt[1] residue of the opioid peptide would reach deeply into the orthosteric binding pockets, while the Phe^3 side chain would position itself at the outer boundaries of the binding pocket of the receptors. For DOP, the Phe^3 side chain reaches a potential subpocket created by the side chains of H^{301}, K^{108}, Y^{109}, E^{112} and

Abbreviations: BRET, bioluminescence resonance energy transfer; cAMP, cyclic adenosine monophosphate; Dmt, 2,6-dimethyltyrosine; DOP, δ-opioid receptor; EC_{50}, half maximal effective concentration; EPAC, exchange protein directly activated by cAMP; GPI, guinea pig ileum; IC_{50}, half maximal inhibitory concentration; K_i, inhibitory constant; KOP, κ-ipioid receptor; MOP, μ-opioid receptor; MVD, mouse vas deferens; P.A., partial agonist; TES, triethylsilane.

* Corresponding authors.
E-mail addresses: philippe.sarret@USherbrooke.ca (P. Sarret), louis.gendron@USherbrooke.ca (L. Gendron), steven.ballet@vub.be (S. Ballet).

[1] Equal contribution.

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V^{197}. In case of MOP, the side chain may access a pocket defined by the side chains of Q^{124}, H^{319}, W^{318} and I^{322}, and hence in both cases additional hydrophobic, dipole-dipole and cation-π interactions could for instance be made between ligand and receptor by substituting the second aromatic ring in opioid peptides.

In the present work, we report the synthesis and the *in vitro*/*in vivo* biological evaluation of potent opioid peptide ligands with mixed MOP/DOP activity profile by extension of the Phe^3 aromatic core. Introduction of a 'halogen derivatization handle' allowed structural modification via Suzuki-Miyaura cross-couplings.[17,18]

This robust C—C coupling allows the efficient introduction of substituents on arylated amino acid residues in peptide substrates.[19–23] Previously, this bioorthogonal methodology has been applied for the derivatization of peptides,[24–28] leading to altered biological profile and therapeutic effect[27,29] implementing both solution-phase[21] and solid-phase[30–33] Suzuki-Miyaura reactions. The functional group compatibility of Suzuki-Miyaura reactions with peptidic substrates has recently been reviewed elsewhere.[22,27]

The commercial availability of the different regioisomers of iodo-(*S*)-phenylalanine and straightforward incorporation into opioid tetrapeptides provided, after derivatization, a diverse library including (hetero)aromatic or vinylic substituents (Fig. 1) and imposed profound effects on the biological activity. The dermorphin(1–4) analogues **1**–**9** were prepared via standard N_α-Fmoc-based solid phase peptide synthesis using Rink Amide resin as solid support. The Dmt^1 residue was inserted as Boc-Dmt-OH (2 eq), and with DIC/HOBt (each 2 eq) to avoid acylation on the unprotected phenol moiety. The Suzuki-Miyaura reactions, making use of (hetero)aromatic boronic acids as coupling partners, were performed on the solid support bearing the fully protected halogenated tetrapeptides (Scheme 1) (Fig. 2).

Catalyst screening showed that the $PdCl_2$(dppf) catalyst, which was shown to be compatible to peptide diversification in solution phase,[22] allowed to prepare the cross-coupled products with excellent conversions on support. Hence, the cross-couplings on solid-phase were realized with $PdCl_2$(dppf) (10 mol%) as the precatalyst system in combination with K_2CO_3 (5 eq) and (hetero)aromatic boronic acids (3 eq). Near complete conversions were attained after 30 min in a mixture of THF/H_2O (1/1) at 100 °C, while gently stirring the resin beads in MW vials. After successful cross-coupling, the peptide analogues were cleaved from the resin by treatment with a mixture of TFA/TES/H_2O (95:2.5:2.5 v/v/v). The crude peptides were obtained after evaporation of the cleavage mixture and purified by preparative HPLC to yield the target peptides (>95% purity).

Due to the occurrence of side product formation during cleavage, a different strategy was pursued to access the vinylated analogues. The vinyl group was chosen as a substituent of limited size which additionally offers a possibility for further diversification or peptide cyclization. Here, the peptides were first assembled on solid support and cleaved to obtain the iodinated precursor peptides (Scheme 1). As mentioned above, this (still convergent) strategy was followed due to the limited stability of vinylated products toward highly acidic (95% TFA) conditions. After optimization of the reaction conditions, $PdCl_2$(dppf) (5 mol%) was again found suitable for these transformations in combination with K_2CO_3 (6 eq) as the base and potassium vinyltrifluoroborate (3 eq) as the boron coupling partner. For these couplings trifluoroborates, benchstable analogues of boronic acid, were used.[34,35] In this case, optimal conversion was reached in a 1:1 mixture of H_2O/*i*PrOH at 80 °C for 2 h. The peptides were obtained after purified by preparative HPLC with a yield ranging from 13 to 39% (>95% purity).

The obtained set of peptide analogues (**1**–**9**) was evaluated for biological activity (see Table 1). In addition to MOP and DOP binding and functional signaling bioassay (EPAC cAMP BRET-based

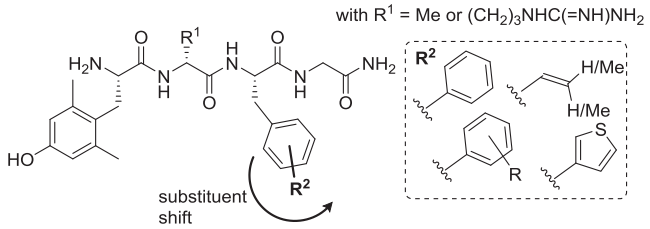
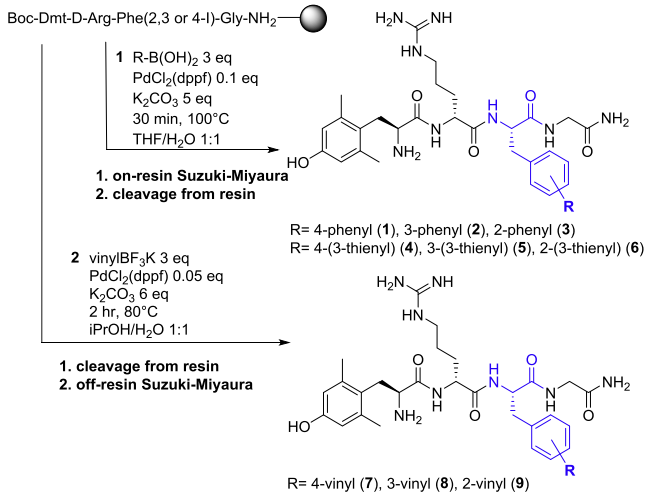


Fig. 1. Phe^3 derivatizations within the opioid tetrapeptides H-Dmt^1-D-Arg/Ala^2-Phe^3-Gly^4-NH_2.



Scheme 1. Preparation of the first peptide set using on-resin and off-resin Suzuki-Miyaura cross-couplings.

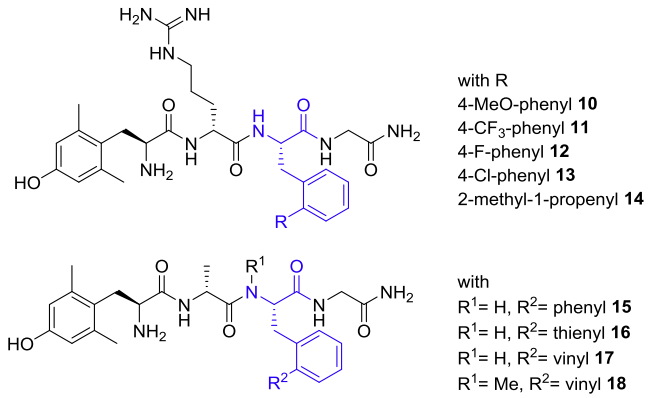


Fig. 2. Structures of the second set [Dmt^1,D-Arg^2]- and [Dmt^1,D-Ala^2]dermorphin (1–4) analogues.

biosensor test), the guinea pig ileum (GPI, functional test representative of MOP agonist activity) and mouse vas deferens (MVD, functional test representative of DOP agonist activity) assays were carried out. From this first data set, it could be concluded that the peptides with highest MOP and DOP agonist activity contained a substituent at the *ortho*-position (**3**, **6**, **9**). Especially the vinyl group (**9**) attracted interest due to its subnanomolar binding affinity for MOP and its potent activity in the functional bioassays. Indeed, compound **9** exhibited respectively 20-fold and 70-fold increase in potency at inhibiting cAMP production and GPI contraction, compared to the reference MOP agonist DAMGO (Tables 1 and 2).

Interestingly, two peptides with *para*-substitution (**1**, **4**) demonstrated *in vitro* μ-opioid antagonism in the functional GPI