# NOP-Targeted Peptide Ligands

Delia Preti, Girolamo Caló, and Remo Guerrini

## Contents

1 Introduction ..... 18
2 Structure–Activity Relationship Studies on N/OFQ-Related Peptides ..... 20
2.1 Linear N/OFQ Derivatives ..... 20
2.2 Cyclic N/OFQ Derivatives ..... 23
3 Structure–Activity Relationship Studies on N/OFQ-Unrelated Peptides ..... 24
4 Bivalent NOP Peptide Ligands ..... 27
5 Tetrabranched NOP Peptide Ligands ..... 29
6 Concluding Remarks ..... 30
References ..... 31

**Abstract**

The nociceptin/orphanin FQ (N/OFQ)-N/OFQ peptide (NOP) receptor system is widely distributed at both the peripheral and central level where it modulates important biological functions with increasing therapeutic implications. This chapter wants to provide a comprehensive and updated overview focused on the available structure–activity relationship studies on NOP receptor peptide ligands developed through different rational approaches. Punctual modifications and cyclizations of the N/OFQ sequence have been properly combined furnishing potent NOP selective ligands with different pharmacological activities (full and partial agonists, pure antagonists) and enhanced metabolic stability in vivo. The screening of peptide libraries provided a second family of NOP ligands that have been successfully optimized. Moreover, recent findings suggest the possibility to

D. Preti · R. Guerrini (✉)
Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy
e-mail: grm@unife.it

G. Caló
Department of Medical Sciences, Section of Pharmacology, University of Ferrara and National Institute of Neurosciences, Ferrara, Italy



M.-C. Ko, G. Caló (eds.), *The Nociceptin/Orphanin FQ Peptide Receptor*,
Handbook of Experimental Pharmacology 254, https://doi.org/10.1007/164_2018_198

apply different multimerization strategies for the realization of multi-target NOP/opioid receptor ligands or tetrabranched N/OFQ derivatives with extraordinarily prolonged duration of action in vivo. The diverse approaches led to the identification of important pharmacological tools along with drug candidates currently in clinical development such as Rec 0438 (aka UFP-112) for the treatment of overactive bladder and SER 100 (aka ZP120) for the clinical management of systolic hypertension.

**Keywords**
Nociceptin/orphanin FQ · NOP · Peptide ligands · SAR studies

## 1 Introduction

Nociceptin/orphanin FQ (N/OFQ; FGGFTGARKSARKLANQ, see Fig. 1) shows significant similarities in the primary sequence with other endogenous peptides of the opioid family of which dynorphin A can be considered its closer analogue (Calo' and Guerrini 2013). Nevertheless, due to its unique structure, N/OFQ is unable to interact with classical opioid receptors (MOP, DOP, and KOP), as well as opioid peptides are unable to bind the N/OFQ peptide (NOP) receptor. Typically, the first four N-terminal residues of the heptadecapeptide sequence of N/OFQ (FGGF) represent the "message" domain responsible for NOP activation, while the "address" fragment is composed of the last C-terminal residues (7–17) and promotes NOP binding affinity and receptor selectivity (Calo' et al. 2013; Mustazza et al. 2018). The central dipeptide $Thr^5Gly^6$ constitutes a hinge region between message and address sequences. The message tetrapeptide of N/OFQ is clearly superimposable to the canonical YGGF N-terminus of mammalial opioid peptides. Thus, the simple $Tyr^1$/$Phe^1$ replacement is fundamental for N/OFQ selectivity being able to preclude NOP/opioid receptors cross activation (Calo' et al. 2013). A series of crystallographic studies performed with NOP (Thompson et al. 2012) and classical opioid receptors (Wu et al. 2012; Manglik et al. 2012; Granier et al. 2012) in complex with specific antagonists suggested that the $Phe^1$ phenyl ring of N/OFQ would face a hydrophobic region of the receptor binding pocket while the phenolic function of $Tyr^1$ in opioid peptides would be involved in a hydrogen bond network with the conserved His residue present in position 52 of TM VI of opioid receptors.

Truncation studies on N/OFQ indicated the exclusive possibility to shorten the C-terminal portion up to the identification of N/OFQ(1–13) as the minimal active sequence (Calo' et al. 1996; Dooley and Houghten 1996). Moreover, C-terminal amidation proved to reduce the susceptibility to carboxypeptidases of both N/OFQ and N/OFQ(1–13) (Guerrini et al. 1997). It has to be remarked that, according to NMR studies, the C-terminal "address" portion of N/OFQ would assume a typical alpha helix conformation in physiological conditions (Orsini et al. 2005; Tancredi et al. 2005). This region is characterized by the presence of two couples of Arg-Lys dipeptide units at 8–9 and 12–13 positions, which are important for the affinity/selectivity of the peptide because of the capability to promote the α-helix bioactive

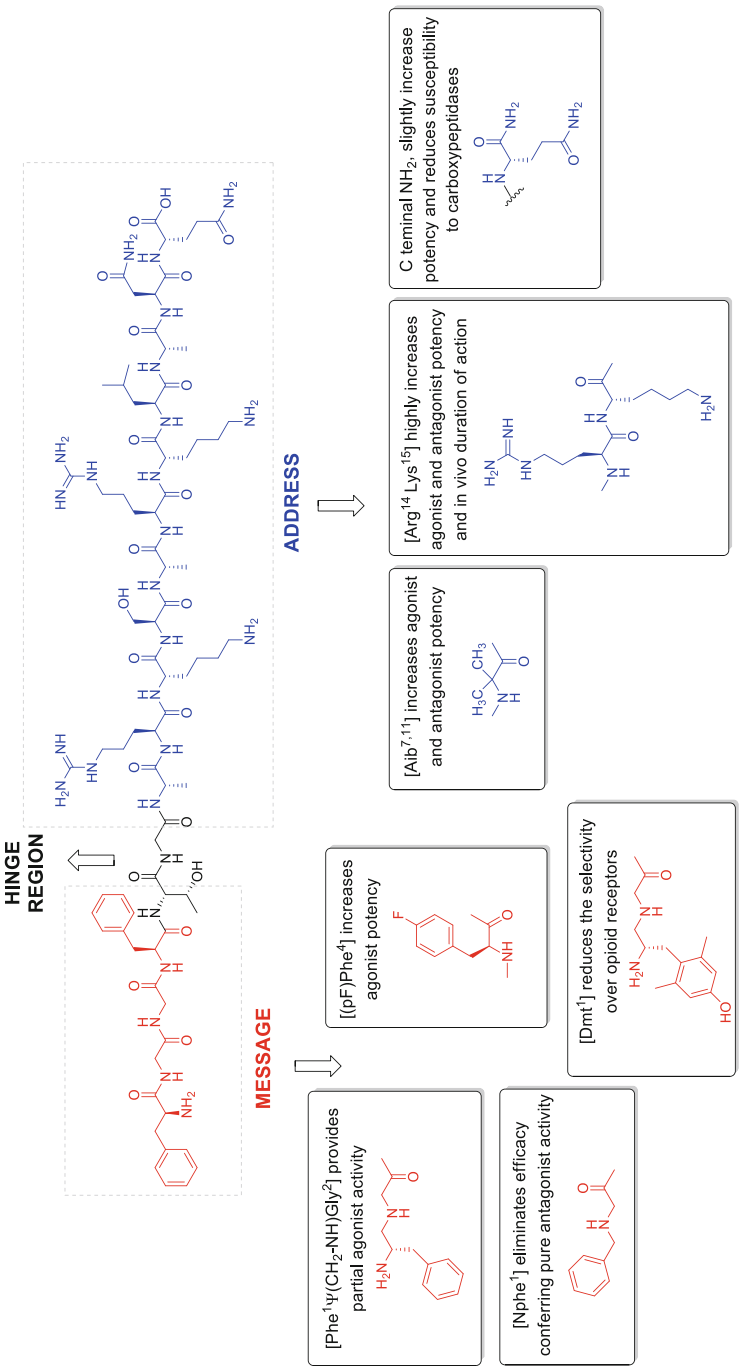



**Fig. 1** Structure–activity relationship studies on the native sequence of N/OFQ

motif as well as to interact with negative residues in the second extracellular loop of the NOP receptor (Daga and Zaveri 2012).

Advances in the pharmacology and medicinal chemistry of the N/OFQ-NOP system have been recently reviewed by different authors (Toll et al. 2016; Mustazza et al. 2018; Zaveri 2016). This chapter wants to provide a comprehensive and updated overview focused on SAR studies on NOP receptor peptide ligands that have been thus far developed following different rational approaches.

## 2 Structure–Activity Relationship Studies on N/OFQ-Related Peptides

### 2.1 Linear N/OFQ Derivatives

The key structural modifications applied to N/OFQ sequence for generating useful NOP peptide ligands have been summarized in Fig. 1. $Phe^1$ is one of the most essential residues for NOP binding/activation/selectivity (Calo' et al. 2013). The saturation of the $Phe^1$ benzyl moiety ($Cha^1$) and the replacement of $Phe^1$ with a $Leu^1$ are highly tolerated while $Ala^1$ derivatives are essentially inactive. In addition, the inversion of the configuration of $Phe^1$ (D-$Phe^1$) abolished the activity. This suggests that a bulky and lipophilic side chain at the first residue is required while aromaticity is not mandatory for receptor binding. The N-terminal residue seems to be of key relevance for ligand efficacy if considering that the reduction of the peptide bond between $Phe^1$ and $Gly^2$ [$Phe^1\Psi(CH_2\text{-}NH)Gly^2$] or the shift of the benzyl side chain to the N-terminal nitrogen ($Nphe^1$) led to partial agonism (i.e., [F/G]N/OFQ(1–13)$NH_2$) or antagonism (i.e., [$Nphe^1$]N/OFQ(1–13)-$NH_2$), respectively (see Table 1) (Calo' et al. 2013; Toll et al. 2016). Of note, a significant loss of selectivity over opioid receptors was observed when $Phe^1$ has been replaced with $Tyr^1$ that was even more evident with the introduction of a 2,6-dimethyltyrosine (Dmt) residue at the same position (Molinari et al. 2013). This led to the identification of [$Dmt^1$]N/OFQ (1–13)-$NH_2$ and [$Dmt^1$]N/OFQ-$NH_2$ as mixed NOP/opioid agonists with an interesting potential as innovative spinal analgesics. In contrast, the replacement of $Phe^1$ with an amino phosphonate moiety in N/OFQ(1–13)$NH_2$ resulted in low potency though NOP selective agonists (Todorov et al. 2012).

Unlike other opioid ligands, N/OFQ is particularly sensitive to substitutions at the $Gly^2$-$Gly^3$ dipeptide spacer. X-ray analysis (Thompson et al. 2012) and docking studies (Daga and Zaveri 2012) confirmed the importance of both the composition and length of the $Gly^2$-$Gly^3$ unit that imposes the right distance between $Phe^1$ and $Phe^4$ and confers high conformation flexibility. This allows the N-terminal nitrogen atom of the message tetrapeptide to establish an ionic interaction with the $Asp^{130}$ of the NOP receptor (Toll et al. 2016). Different substitutions have been also performed at the $Phe^4$ residue that is critical for NOP binding/activation (Guerrini et al. 2001). Noteworthy, the potency of the endogenous peptide was significantly enhanced with the introduction of electron withdrawing moieties (especially a fluorine atom) at the para-position of the $Phe^4$ phenyl ring (Guerrini et al. 2001; McDonald et al. 2002).

**Table 1** In vitro biological activity of N/OFQ-related peptide ligands

| | Human NOP | | | Mouse NOP | |
|---|---|---|---|---|---|
| | | Functional potency | | Functional potency | |
| | Binding affinity | [$^{35}$S] GTPγS | $Ca^{2+}$ | mVD | Reference |
| *NOP agonists* | p$K_i$ | $pEC_{50}$ | $pEC_{50}$ | $pEC_{50}$ | |
| N/OFQ | 9.91 | 8.75 | 9.54 | 7.47 | McDonald et al. (2003), Camarda et al. (2009), and Toll et al. (2016) |
| N/OFQ(1–13)-$NH_2$ | 10.24 | 9.28 | 9.30 | 7.40 | McDonald et al. (2003), Camarda et al. (2009), and Toll et al. (2016) |
| [Dmt$^1$]N/OFQ(1–13)-$NH_2$ | 10.59 | 9.46 | 8.94 | ND | Molinari et al. (2013) and Cerlesi et al. (2017) |
| [(pF)Phe$^4$]N/OFQ (1–13)-$NH_2$ | 9.40 | 9.55 | nd | 8.19 | Guerrini et al. (2001) and McDonald et al. (2002) |
| [Arg$^{14}$Lys$^{15}$]N/OFQ | 9.49 | 9.85 | 9.56 | 8.93 | Okada et al. (2000), Rizzi et al. (2002a), and Camarda et al. (2009) |
| UFP-112 | 10.55 | 10.55 | 9.05 | 9.24 | Arduin et al. (2007) and Camarda et al. (2009) |
| *NOP partial agonists* | p$K_i$ | $pEC_{50}$ | $pEC_{50}$ | $pEC_{50}$ | |
| [F/G]N/OFQ(1–13)-$NH_2$ | 9.27 | 8.05 | 8.03 | Slight transient effect | Wright et al. (2003) and Camarda et al. (2009) |
| UFP-113 | 10.26 | 9.73 | 7.97 | Variable effects | Arduin et al. (2007), Camarda et al. (2009) |
| *NOP antagonists* | p$K_i$ | $pA_2$ | $pA_2$ | $pA_2$ | |
| [Nphe$^1$]N/OFQ(1–13)-$NH_2$ | 8.39 | 7.33 | 6.29 | 6.04 | McDonald et al. (2003), Camarda et al. (2009), and Calo' et al. (2000) |
| UFP-101 | 10.24 | 8.85 | 7.66 | 7.29 | McDonald et al. (2003), Camarda et al. (2009), and Calo' et al. (2002) |

[$^{35}$S]GTPγS: [$^{35}$S]GTPγS binding in membranes from CHO cells expressing the human NOP; $Ca^{2+}$: calcium mobilization in CHO cells coexpressing the human NOP and the $G\alpha_{qi5}$ chimeric protein; mVD electrically stimulated mouse vas deferens; $pEC_{50}$: agonist potency; $pA_2/pK_B$: antagonist potency; UFP-112: [(pF)Phe$^4$Aib$^7$Arg$^{14}$Lys$^{15}$]N/OFQ-$NH_2$; [F/G]N/OFQ(1–13)-$NH_2$: [Phe$^1\Psi$($CH_2$-NH)Gly$^2$]N/OFQ(1–13)-$NH_2$; UFP-113: [Phe$^1\Psi$($CH_2$-NH)Gly$^2$(pF)Phe$^4$Aib$^7$Arg$^{14}$Lys$^{15}$]N/OFQ-$NH_2$; UFP-101: [Nphe$^1$Arg$^{14}$Lys$^{15}$]N/OFQ-$NH_2$

A series of N/OFQ(1–13)-$NH_2$ analogues in which the $Thr^5$ residue was substituted with both natural and nonnatural amino acids has been recently investigated (Guerrini et al. 2015). These analogues behaved as NOP full agonists with highly variable potency thus suggesting that $Thr^5$ would contribute to the binding to the receptor more than to its activation. Yet, neither the size of $X^5$ side chain nor its lipo/hydrophilic nature and hydrogen bond capability seemed of significant relevance for receptor binding. In particular, the simple removal of the side chain hydroxyl function of $Thr^5$ ([$Abu^5$]N/OFQ(1–13)-$NH_2$) determined a substantial preservation of NOP activity.

The effect of various modifications of the C-terminal motif of N/OFQ and N/OFQ (1–13)-$NH_2$ has been also investigated (Calo' et al. 2013). Notably, a highly potent NOP agonist was obtained when a third Arg-Lys couple was introduced at the 14–15 positions of the native peptide sequence (Okada et al. 2000). [$Arg^{14}Lys^{15}$]N/OFQ displayed indeed higher binding affinity (threefold) and potency (17-fold) compared to N/OFQ. Similar results have been achieved with the introduction at the same positions of different combinations of positively charged residues such as Lys-Arg, Lys-Lys, and Arg-Arg (Okada et al. 2008). On the contrary, when a single positive residue was introduced in either 14 or 15 position, a moderate enhancement of binding and biological activity was observed. In addition, with the aim to investigate the importance of the C-terminal secondary structure, both alpha helix inducers (Aib, alpha-aminoisobutyric acid) and breakers (Pro) have been alternatively introduced at key positions of the address domain (Zhang et al. 2012; Tancredi et al. 2005). $Aib^7$, $Aib^{11}$, and $Aib^{15}$ peptide derivatives were significantly more potent than the native ligand. On the other hand, $Pro^5$, $Pro^6$, $Pro^7$, and $Pro^{11}$ substitutions severely compromised the activity of the endogenous peptide.

Finally, $Thr^5$ or $Ser^{10}$ have been scrutinized as possible glycosylation sites of N/OFQ-related peptides (Biondi et al. 2006; Arsequell et al. 2011). These investigations agree in the identification of $Ser^{10}$ as preferred anchoring point for a monosaccharide unit. Of the reported compounds, [$Ser^{10}$-O-α-D-GalNAc]-N/OFQ exhibited a p$K_i$ value of 8.42 in competition binding experiments with similar affinity as N/OFQ (Arsequell et al. 2011). A NMR analysis performed in membrane mimicking environments indicated that, unlike for $Thr^5$ derivatives, $Ser^{10}$ glycosylated analogues exist prevalently as linear α-helix motifs that are supposed to interact in a more favorable binding pose with the NOP compared to folded structures.

Some of the chemical modifications of the N/OFQ sequence described above have been profitably combined in the search for potent NOP peptide ligands with different pharmacological activities spanning from full agonists (N/OFQ(1–13)-$NH_2$, [(pF)$Phe^4$]N/OFQ(1–13)-$NH_2$, [$Arg^{14}Lys^{15}$]N/OFQ, UFP-112) to partial agonists ([F/G]N/OFQ(1–13)-$NH_2$, UFP-113) and antagonists ([$Nphe^1$]N/OFQ (1–13)-$NH_2$, UFP-101). The structures and the in vitro pharmacological profile of these molecules at the human and murine NOP receptor are reported in Table 1.

## 2.2 Cyclic N/OFQ Derivatives

As mentioned above, different approaches including circular dichroism (CD) and NMR spectroscopy have been applied with the aim to elucidate the bioactive conformation of N/OFQ(1–17)-$NH_2$, N/OFQ(1–13)-$NH_2$, and related peptides (Lohman et al. 2015). These studies consistently suggest that the 7–17 address domain of N/OFQ would rather assume an amphipathic α-helical conformation in the binding pocket of NOP receptor thanks to a motif in which the positive Arg-Lys couples are regularly spaced by Ala residues. However, the α-helicity of linear N/OFQ analogues is irrelevant in aqueous medium making these compounds particularly subjected to the action of serum peptidases (Lohman et al. 2015). For these reasons, different efforts have been made in the design of proper C-terminal cyclization strategies that could promote water-stable α-helix motifs thus improving peptidase resistance and in vivo potency and duration of action of N/OFQ analogues.

As largely known, the addition of cysteine residues to peptides makes them prone to cyclization via the formation of intramolecular disulfide bridges. Following this approach, a small series of cyclic analogues of N/OFQ(1–13)-$NH_2$ has been reported in 2001 (Ambo et al. 2001). These compounds came from the cyclization of either the N-terminal or the C terminal region of N/OFQ thanks to the addition/replacement of Cys residues at key positions of the parent peptide sequence. Any cyclization involving the N-terminal part led to a complete loss of activity. On the other hand, the cyclization of the C-terminal portion led to potent derivatives especially when the disulfide linkage was introduced between the positions 10–14 where a serine and a leucine residue were replaced by two cysteines, respectively. Cyclo[$Cys^{10}$,$Cys^{14}$]N/OFQ(1–14)-$NH_2$ (compound **1**, Fig. 2) can be considered the first example of constrained N/OFQ-related peptide whose receptor affinity ($pIC_{50} = 9.91$), potency ($pEC_{50} = 8.36$), and efficacy were comparable to those of the native peptide (Ambo et al. 2001). In an attempt to identify a cyclic antagonist, Kitayama et al. reported in 2003 the synthesis of the $Nphe^1$ derivative of compound **1** (Kitayama et al. 2003) that however suffered from a severe reduction of NOP affinity.

As an alternative to the disulfide bridge strategy, a few N/OFQ cyclic analogues with side chain to side chain lactam linkage have been firstly reported by Charoenchai et al. (2008). Of this series, the cyclo-peptide **2** (cyclo[D-$Asp^7$,$Lys^{10}$]N/OFQ(1–13)$NH_2$, Fig. 2) showed subnanomolar binding affinity and high potency for the NOP receptor ($pK_i = 9.57$, $pEC_{50} = 8.80$). The usefulness of the

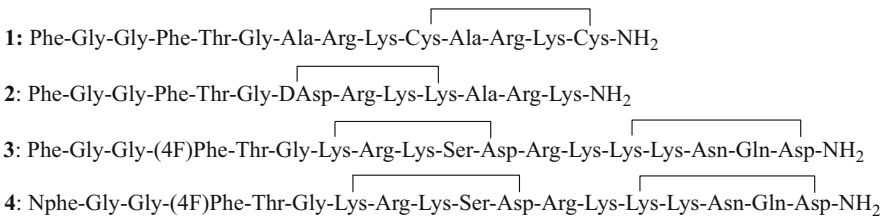



**Fig. 2** Structures of selected cyclic analogues of N/OFQ

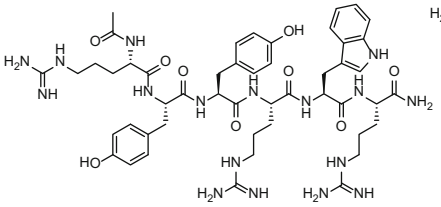
lactamization strategy was later confirmed by Harrison et al. who performed multiple cyclizations of the address domain of N/OFQ(1–17)-$NH_2$ (Harrison et al. 2010) each involving the minimum number of residues that span a canonical α-helix (five amino acids). In the latter case, cyclizations were combined with strategical substitution of the message sequence intended to enhance ligand potency (i.e., (pF)$Phe^4$) or induce antagonist activity (i.e., $Nphe^1$). The study resulted in the identification of the highly potent NOP agonist **3** (Fig. 2) with picomolar potency ($pEC_{50} = 10.40$) in vitro and significantly higher and longer lasting antinociceptive activity in vivo in comparison with unconstrained peptides (Harrison et al. 2010). With the same approach, the potent NOP antagonist **4** ($pIC_{50} = 8.12$) was discovered (Fig. 2). CD spectroscopy highlighted that constrained analogues of this series adopted α-helical conformation even in aqueous phosphate buffer where all the investigated unconstrained analogues exhibited minimal helicity. In addition, lactame derivatives showed longer half-life in human serum (Lohman et al. 2015).

## 3 Structure–Activity Relationship Studies on N/OFQ-Unrelated Peptides

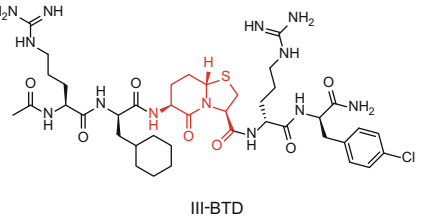
Dooley et al. have reported in 1997 the first examples of peptide ligands of the NOP receptor featuring an amino acid sequence totally unrelated to that of N/OFQ (Dooley et al. 1997). This study originated from the screening of a large peptide combinatorial library and resulted in the identification of a small series of positively charged hexapeptides reflecting the general structure Ac-RYY(R/K)(I/W)(R/K)-$NH_2$. The functional profile of such compounds evaluated in three different assays (stimulated [$^{35}$S]GTPγS binding and inhibition of forskolin-stimulated cAMP in cells expressing the recombinant human NOP, and the electrically stimulated mouse vas deferens bioassay) revealed a partial agonist behavior. One of the most representative member of this class namely Ac-RYYRWR-$NH_2$ (see Table 2) was later employed in photoaffinity labeling studies with the aim to determine its binding domain on the NOP receptor (Bes and Meunier 2003). Interestingly, these studies indicated that N/OFQ and hexapeptides interact with the NOP receptor in distinct, although partially overlapped, regions and this could be the reason of their diverse pharmacological activities. Of this series, Ac-RYYRWR-$NH_2$ showed the highest efficacy in inhibiting cAMP accumulation (75% versus 84% for N/OFQ), while the analogue Ac-RYYRWK-$NH_2$ (Table 2) exhibited lower maximal effect (58%). This suggests that the higher basicity of Arg compared to Lys at the 6-position could contribute to promote NOP activation. Of note, it was found that the side chain of $Arg/Lys^6$ can be replaced with shorter moieties (such as in Orn, Dab, or Dap) without significant loss of activity in vitro (Kasakov et al. 2010).

The negligible in vivo activity of Ac-RYYRWR-$NH_2$ prompted further efforts aimed at improving proteolytic resistance. The resulting SAR profile indicated the importance of each Arg residue to maintain high binding affinity while $Tyr^2$ and $Tyr^3$ seem to be less essential residues, although at least one of these must maintain its

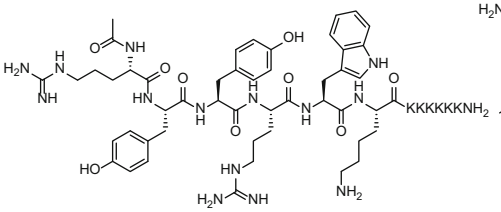
**Table 2** Structures and in vitro biological activity of N/OFQ-unrelated NOP peptide ligands



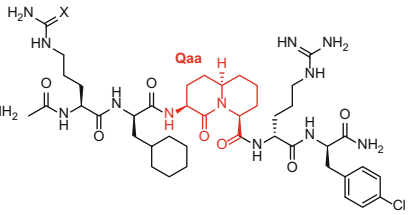
Ac-RYYRWR-NH$_2$



III-BTD



ZP120 (SER 100)



**5**: Ac-Arg-D-Cha-Qaa-D-Arg-D-pClPhe-NH$_2$ (X = NH)
**6**: Ac-Cit-D-Cha-Qaa-D-Arg-D-pClPhe-NH$_2$ (X = O)

| | Human NOP | | Mouse NOP | |
|---|---|---|---|---|
| | Binding affinity | Functional potency | Functional potency[a] | Reference |
| *NOP partial agonists* | p$K_i$ | pEC$_{50}$ | pEC$_{50}$ | |
| Ac-RYYRWR-NH$_2$ | 9.22 | 8.66[b]<br>9.28[c] | nd | Dooley et al. (1997) |
| Ac-RYYRWK-NH$_2$ | 9.15 | 8.68[b]<br>9.28[c] | 8.07 | Dooley et al. (1997) and Rizzi et al. (2002b) |
| Ac-R-(3Cl)Y-YRWR-NH$_2$ | 10.5 | 9.30[b] | nd | Judd et al. (2004) |
| ZP120 (SER100) | 9.6 | 9.30[c]<br>7.15[d] | 8.88 | Rizzi et al. (2002b), Kapusta et al. (2005), and Camarda et al. (2009) |
| *NOP antagonists* | p$K_i$ | pA$_2$/pK$_B$ | pA$_2$/pK$_B$ | |
| Isovaleryl-RYYRIK-NH$_2$ | 8.13 | Inactive[b] | 9.7 | Li et al. (2008) |
| III-BTD | 7.62 | 7.89[b]<br>7.49[c] | 6.57 | Becker et al. (1999), Hashiba et al. (2001), McDonald et al. (2002), and Bigoni et al. (2000) |
| **5** | 7.46 | 6.52[b] | nd | Halab et al. (2002) |
| **6** | 7.14 | 6.87[b] | nd | Van Cauwenberghe et al. (2004) |

pEC$_{50}$: agonist potency; pA$_2$/pK$_B$: antagonist potency
[a]mVD
[b][$^{35}$S]GTPγS
[c]cAMP
[d]$Ca^{2+}$

phenol group to sustain the agonist efficacy of the peptide (Kawano et al. 2002; Judd et al. 2004). Indeed, Ac-R-Phe(4-F)-Phe(4-F)-RWR-$NH_2$ ($pK_i = 8.82$) behaved as NOP antagonist while compound Ac-R-Tyr(3-Cl)-YRWR-$NH_2$ (Table 2) exhibited picomolar affinity for the NOP receptor with a functional profile of low efficacy partial agonist in different assays (Judd et al. 2004). Moreover, $Trp^5$ was substituted with a series of nonnatural aromatic amino acids and the following biological investigation suggested that the indole moiety of the side chain at this position is not mandatory for biological activity (Carra' et al. 2005). Nevertheless, it was found that the fifth amino acid residue plays a crucial role in the modulation of agonist/antagonist activity. Indeed an L-aliphatic/hydrophobic amino acid seems to favor antagonist activity, while a D-residue (especially D-Trp or a D-Arg) resulted in potent agonist activity (Ambo et al. 2007). More recently, Zamfirova et al. reported the synthesis and biological evaluation of a new series of congeners of Ac-RYYRWK-$NH_2$, modified at the 5-position with nonnatural tryptophan analogues (Zamfirova et al. 2013). This study highlighted the contribution of the 5-position to the modulation of selectivity over opioid receptors. Indeed, when a 5-methoxy $\beta^2$-tryptophan residue was incorporated at the 5-position of Ac-RYYRWK-$NH_2$ a compound with higher affinity for opioid receptors than for NOP was obtained.

Other modifications of the original hexapeptide scaffold include the substitution of the N-terminal acetyl group that led in some cases to reduced or abolished intrinsic activity. In particular, the peptide pentanoyl-RYYRWR-$NH_2$ displays high NOP affinity ($pK_i = 9.89$) with barely measurable agonist activity (Judd et al. 2003). Furthermore, the compounds Isovaleryl-RYYRIK-$NH_2$ (Table 2) (Li et al. 2008) and Ac-Diaminobutyl-YYRWR-$NH_2$ (Judd et al. 2004) are examples of pure NOP antagonists obtained with modifications of the N-terminal residue. $Arg^1$ was also substituted with aminophosphonate moieties with a severe loss of NOP affinity and selectivity (Naydenova et al. 2010). The effect of modifications of the C-terminal portion of the hexapeptide scaffold has been explored as well. A reduction of ligand efficacy has been observed when the C-terminal amide moiety has been replaced by a primary hydroxyl function like in the NOP antagonist Ac-RYYRIK-ol (Kocsis et al. 2004; Gündüz et al. 2006). Noteworthy, the parent hexapeptide structure has been extended at the C-terminal position with an oligo-lysine frame in compound ZP120 (Ac-RYYRWKKKKKKK-$NH_2$, Table 2) developed by Zealand Pharma (Rizzi et al. 2002b). The $(Lys)_6$ sequence of this derivative would likely induce an α-helix conformation with consequent reduction of enzymatic vulnerability (Larsen 1999). The pharmacological profile of ZP120 (more recently known as SER100) has been extensively investigated both in vitro and in vivo consistently demonstrating its action as a potent and selective NOP partial agonist (Toll et al. 2016). This compound is of particular interest since SER100 is now in clinical development as innovative treatment for systolic hypertension (Kantola et al. 2017).

A screening of a synthetic combinatorial library of β-turn-constrained peptides resulted in the identification of the pseudohexapeptide III-BTD (Ac-Arg-DCha-BTD-DArg-D(pCl)Phe-$NH_2$, Table 2) as NOP antagonist with low binding selectivity over opioid receptors (Becker et al. 1999). Interestingly, the compound behaves

as weak agonist toward all the three classical opioid receptors. The thiazolidinone bicycle inserted within the sequence of III-BTD as turn inducer has been later replaced with different azabicycloalkane amino acids in the search for more selective ligands (Halab et al. 2002). The introduction of a 6,6-bicyclic moiety (i.e., quinolizidine) in compound **5** determined the maintenance of NOP affinity with a significant improvement of selectivity especially over DOP receptors. However, at high concentration, **5** behaved as weak partial agonist at MOP and KOP receptors. Further SAR studies on this molecule focused on the importance of the Arg residues whose position, structure, and charge were modified (Van Cauwenberghe et al. 2004). This investigation resulted in the identification of the citrulline derivative **6** with slightly lower NOP affinity but improved potency and selectivity.

## 4 Bivalent NOP Peptide Ligands

With the aim to investigate the possible existence of NOP receptor homodimers, we recently reported a series of dimeric NOP ligands obtained by the linkage of two peptide or non-peptide pharmacophores with spacers of different length and chemical composition (Pacifico et al. 2017). A subset of homobivalent ligands have been obtained connecting the C-terminal portions of two N/OFQ(1–13) fragments chosen as pharmacophore units. The spacers varied from 18 to 32 atoms and were composed of Gly, Ala, β-Ala, Gaba, and Cys residues, in variable combinations. Neither ligand dimerization nor spacer length/composition seemed to affect agonist potency or efficacy. However, when low potency agonists, e.g., N/OFQ(1–12) and N/OFQ (1–11), were chosen as pharmacophores, dimerization resulted in total recovery of ligand potency. This effect of dimerization depends on the doubling of the C-terminal address sequence rather than the presence of an additional N-terminal message sequence or modifications of peptide conformation (Pacifico et al. 2017).

Together with homobivalent ligands, a few examples of peptide-based bifunctional MOP/NOP agonists have been recently examined. This research area is of particular interest since molecules that are able to modulate multiple opioid receptors may result into novel opioid analgesics possibly with reduced side effects (Günther et al. 2018). In particular several recent studies demonstrated that the mixed NOP/opioid receptor agonist cebranopadol displays analgesic effects similar to morphine associated with reduced side effects (reviewed in Calo' and Lambert 2018). Moreover, the small molecule AT-121, with a bifunctional NOP/MOP agonist profile, has been shown to promote nonaddictive analgesia in nonhuman primates with potential as treatment for opioid abuse disorders (Ding et al. 2018).

As far as heterobivalent peptides are concerned, Kawano et al. obtained a first hybrid template linking dermorphin, as mu receptor agonist, to the NOP peptide ligand Ac-RYYRIK-$NH_2$ (Kawano et al. 2006). A synergistic effect was observed on both MOP and NOP binding potency when a relatively long spacer −Gly-Gly-Gly-Lys(Gly-Gly-)-$NH_2$ was incorporated between the pharmacophores. In fact, compound **7** (Fig. 3) exhibited picomolar affinities for the investigated targets ($pK_i$ NOP = 10.33; $pK_i$ MOP = 11.63); despite this, the chimeric compound did not show

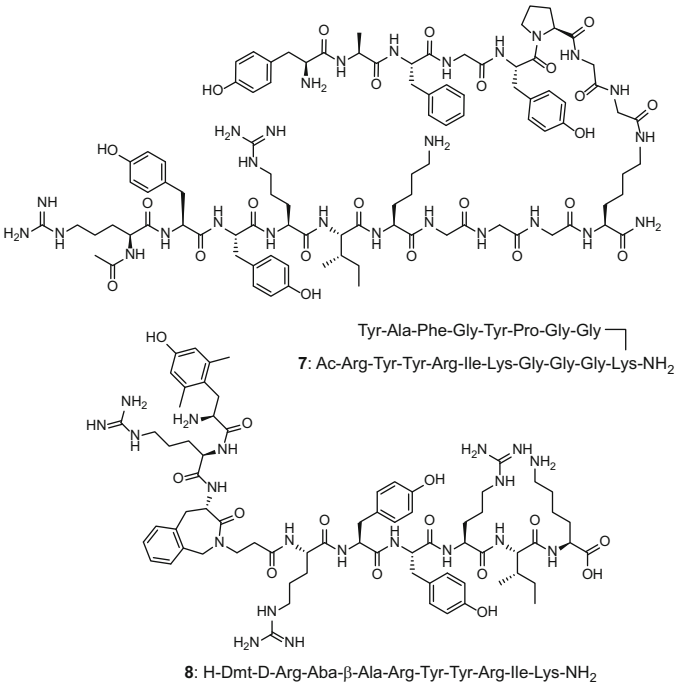



**Fig. 3** Structures of bifunctional NOP/opioid receptor peptide ligands

improved antinociceptive activity in vivo compared to the single peptides (Kawano et al. 2007). Dermorphin was also hetero-dimerized with the endogenous NOP agonist N/OFQ (Bird et al. 2016). The resulting compound showed in vitro the expected profile (i.e., the sum of that of the single monomeric components) but exhibited in vivo only weak antinociceptive properties.

More recently, Guillemyn et al. described chimeric compounds obtained through the linear combination of the N-terminal fragment H-Dmt-D-Arg-Aba-β-Ala-$NH_2$, as opioid pharmacophore, with different NOP peptide ligands at the C-terminal portion (Guillemyn et al. 2016; Lagard et al. 2017). Such hybrids were able to simultaneously activate opioid receptors and block NOP when evaluated in vitro. In this case, the merging strategy led to a slight loss of affinity toward both opioid and NOP receptors. Among the investigated compounds, the bifunctional peptide **8** ($pK_i$ NOP = 7.38; $pK_i$ MOP = 8.30, Fig. 3) elicited high and long lasting antinociceptive efficacy in vivo upon i.v. administration in mice. Of note, this molecule demonstrated higher analgesic efficacy in neuropathic pain models compared to morphine with limited effects on the respiratory function and reduced tolerance liability (Starnowska et al. 2017).

## 5 Tetrabranched NOP Peptide Ligands

Bracci et al. provided in 2003 the first evidences of the positive effect of N/OFQ multimerization on its stability in plasma and serum (Bracci et al. 2003). A high efficacy chemical strategy for the synthesis of multi-branched peptides, the peptide welding technology (PWT), has been developed more recently (Calo' et al. 2018). This approach showed to significantly extend the typical short half-life of a series of peptides of therapeutic interest possibly because of a reduced proteolytic metabolism. The methodology is based on the thiol-Michael conjugation of three different tetra-maleimide functionalized cores (PWT1, PWT2, and PWT3; see Fig. 4) with four linear peptide monomers strategically functionalized with a cysteine residue. N/OFQ was employed in early studies to produce the first examples of PTW homotetravalent peptides (Guerrini et al. 2014). PWT-N/OFQ derivatives displayed higher binding affinity (threefold) for NOP than N/OFQ and comparable selectivity over opioid receptors (Rizzi et al. 2014). Moreover, PWT-derivatization of N/OFQ preserved its behavior as NOP full agonist in vitro with even improved potency. Interestingly, N/OFQ clustering impacts also on the capability to discriminate between NOP/G protein and NOP/β-arrestin 2 interaction. In particular, PWT2-N/OFQ displayed a significant bias toward G protein (Malfacini et al. 2015). Remarkably, PWT-N/OFQ derivatives showed enhanced potency (40-fold) compared to

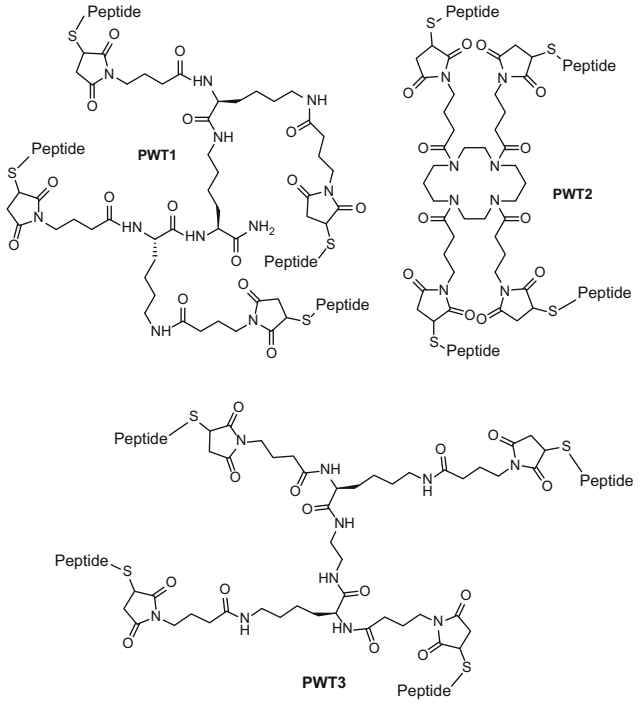



**Fig. 4** General structures of tetrabranched peptide ligands of the NOP receptor (PWT technology)

N/OFQ when evaluated in vivo for its inhibitory effects on mouse locomotor activity after supraspinal administration (Rizzi et al. 2014). Similar results, i.e., 40-fold higher potency than N/OFQ, were obtained investigating the spinal antinociceptive effects of PWT2-N/OFQ in neuropathic pain models in mice and in nonhuman primates (Rizzi et al. 2015).

[$Dmt^1$]N/OFQ(1–13)-$NH_2$ has been clustered into PWT cores with the aim to provide a potential example of a tetrameric ligand with mixed NOP and opioid receptor agonist properties (Cerlesi et al. 2017). PWT2-[$Dmt^1$]N/OFQ(1–13) displayed reduced NOP affinity if compared to the linear peptide monomer, with a similar selectivity profile (NOP = MOP = KOP > DOP). In functional assays, this compound behaved as a G protein biased NOP/MOP dual agonist. In addition, the compound prompted antinociceptive effects following spinal administration in monkeys, with tenfold higher potency than [$Dmt^1$]N/OFQ(1–13)-$NH_2$ and longer lasting effects (Cerlesi et al. 2017).

PWT2 technology was also employed to synthetize a tetrabranched derivative of the NOP antagonist UFP-101 (see Table 1). PWT2-UFP-101 preserved the NOP antagonist pharmacological activity displaying a value of potency ($pA_2 = 8.58$) comparable to that of UFP-101 ($pA_2 = 8.32$). Of note, tetramerization of UFP-101 determined a significant reduction of selectivity over opioid receptors, particularly for the DOP. When evaluated in vivo in the mouse forced swimming test, PWT2-UFP-101 exhibited antidepressant properties with higher potency (nearly tenfold) compared with UFP-101. In line with the PWT derivatives described above, the onset of the in vivo effects of PWT2-UFP-101 was significantly delayed. Nevertheless, unlike other tetrabranched derivatives, the duration of action of PWT2-UFP-101 was similar to that of UFP-101. It has been speculated that the latter finding may be due to the presence of the unnatural amino acid residue Nphe at N-terminal portion of UFP-101 resulting in an intrinsic proteolytic resistance that cannot be further improved through the PWT approach (Calo' et al. 2018).

## 6 Concluding Remarks

Collectively the diverse approaches to the obtainment of NOP peptide ligands resulted in the identification of important tools with pharmacological profiles spanning from full and partial agonists to pure antagonist. Some of these ligands largely contributed to the elucidation of the physiopathology of the N/OFQ-NOP system and its translational potential to the pharmacological treatment of different diseases. Of note, some of the design and synthetic efforts described above also contributed to reduce significantly the known pharmacokinetic weaknesses of peptide molecules paving the way to the concrete option of employing NOP-targeted peptide ligands for clinical purposes. In this regard, the extensive SAR studies performed on the endogenous sequence of N/OFQ drove to the identification of the NOP full agonist UFP-112 that is now under clinical development by Recordati under the name Rec 0438. This compound is currently in Phase II studies as innovative treatment for overactive

bladder patients (ClinicalTrials.gov Identifier: NCT03482037). Furthermore, SER 100 (alias ZP120) distinguishes as the most advanced N/OFQ-unrelated peptide that reached phase II clinical studies aimed at assessing its safety and efficacy after subcutaneous administration in patients with systolic hypertension (Kantola et al. 2017). Finally, preclinical studies performed in rodents and nonhuman primates suggested that the spinal administration of NOP selective as well as of mixed NOP/opioid peptides could be of value for the treatment of chronic pain (Toll et al. 2016). A relevant contribution in this area might come from N/OFQ-related PWT derivatives whose spinal analgesic action last for more than one day from injection (Cerlesi et al. 2017; Rizzi et al. 2015). These kind of compounds together with technological advances of targeted intrathecal drug delivery systems may hopefully provide in the near future interesting options for the management of chronic pain patients.

**Author Contributions** DP, GC, and RG wrote the chapter and approved its final version.

**Declaration of Interests** DP has nothing to declare. GC and RG are inventors of the patent applications WO2006/087340 and U.S. Serial No. 14/782,578 covering UFP-112 and PWT derivatives of N/OFQ, respectively, and are among the founders of the University of Ferrara spin-off company UFPeptides s.r.l., the assignee of these patents.

## References

Ambo A, Hamazaki N, Yamada Y, Nakata E, Sasaki Y (2001) Structure-activity studies on nociceptin analogues: ORL1 receptor binding and biological activity of cyclic disulfide-containing analogues of nociceptin peptides. J Med Chem 44(23):4015–4018. https://doi.org/10.1021/jm010092i

Ambo A, Kohara H, Kawano S, Sasaki Y (2007) Opioid receptor-like 1 (ORL1) receptor binding and the biological properties of Ac-Arg-Tyr-Tyr-Arg-Ile-Arg-$NH_2$ and its analogs. J Pept Sci 13 (10):672–678. https://doi.org/10.1002/psc.900

Arduin M, Spagnolo B, Calo' G, Guerrini R, Carra' G, Fischetti C, Trapella C, Marzola E, McDonald J, Lambert DG, Regoli D, Salvadori S (2007) Synthesis and biological activity of nociceptin/orphanin FQ analogues substituted in position 7 or 11 with Cα,α-dialkylated amino acids. Bioorg Med Chem 15(13):4434–4443. https://doi.org/10.1016/j.bmc.2007.04.026

Arsequell G, Rosa M, Mayato C, Dorta RL, Gonzalez-Nunez V, Barreto-Valer K, Marcelo F, Calle LP, Vázquez JT, Rodríguez RE, Jiménez-Barbero J, Valencia G (2011) Synthesis, biological evaluation and structural characterization of novel glycopeptide analogues of nociceptin N/OFQ. Org Biomol Chem 9(17):6133–6142. https://doi.org/10.1039/c1ob05197k

Becker JA, Wallace A, Garzon A, Ingallinella P, Bianchi E, Cortese R, Simonin F, Kieffer BL, Pessi A (1999) Ligands for kappa-opioid and ORL1 receptors identified from a conformationally constrained peptide combinatorial library. J Biol Chem 274(39):27513–27522. https://doi.org/10.1074/jbc.274.39.27513

Bes B, Meunier JC (2003) Identification of a hexapeptide binding region in the nociceptin (ORL1) receptor by photo-affinity labelling with Ac-Arg-Bpa-Tyr-Arg-Trp-Arg-$NH_2$. Biochem Biophys Res Commun 310(3):992–1001. https://doi.org/10.1016/j.bbrc.2003.09.113

Bigoni R, Rizzi A, Rizzi D, Becker JA, Kieffer BL, Simonin F, Regoli D, Calo' G (2000) In vitro pharmacological profile of peptide III-BTD: a novel ligand for nociceptin/orphanin FQ and opioid receptors. Life Sci 68(2):233–239. https://doi.org/10.1016/S0024-3205(00)00931-0

Biondi B, Goldin D, Giannini E, Lattanzi R, Negri L, Melchiorri P, Ciocca L, Rocchi R (2006) Novel nociceptin analogues: synthesis and biological activity. Int J Pept Res Ther 12 (2):139–144. https://doi.org/10.1007/s10989-006-9011-x

Bird MF, Cerlesi MC, Brown M, Malfacini D, Vezzi V, Molinari P, Micheli L, Di Cesare Mannelli L, Ghelardini C, Guerrini R, Calo' G, Lambert DG (2016) Characterisation of the novel mixed μ-NOP peptide ligand Dermorphin-N/OFQ (DeNo). PLoS One 11(6):e0156897. https://doi.org/10.1371/journal.pone.0156897

Bracci L, Falciani C, Lelli B, Lozzi L, Runci Y, Pini A, De Montis MG, Tagliamonte A, Neri P (2003) Synthetic peptides in the form of dendrimers become resistant to protease activity. J Biol Chem 278(47):46590–46595. https://doi.org/10.1074/jbc.M308615200

Calo' G, Guerrini R (2013) Medicinal chemistry, pharmacology, and biological actions of peptide ligands selective for the nociceptin/orphanin FQ receptor. In: Research and development of opioid-related ligands. ACS symposium series, vol 1131, pp 275–325. https://doi.org/10.1021/bk-2013-1131.ch015

Calo' G, Lambert DG (2018) Nociceptin/orphanin FQ receptor ligands and translational challenges: focus on cebranopadol as an innovative analgesic. Br J Anaesth 121(5):1105–1114. https://doi.org/10.1016/j.bja.2018.06.024

Calo' G, Rizzi A, Bogoni G, Neugebauer V, Salvadori S, Guerrini R, Bianchi C, Regoli D (1996) The mouse vas deferens: a pharmacological preparation sensitive to nociceptin. Eur J Pharmacol 311(1):R3–R5. https://doi.org/10.1016/0014-2999(96)00563-8

Calo' G, Guerrini R, Bigoni R, Rizzi A, Marzola G, Okawa H, Bianchi C, Lambert DG, Salvadori S, Regoli D (2000) Characterization of [Nphe$^{1}$]nociceptin(1-13)$NH_2$, a new selective nociceptin receptor antagonist. Br J Pharmacol 129(6):1183–1193. https://doi.org/10.1038/sj.bjp.0703169

Calo' G, Rizzi A, Rizzi D, Bigoni R, Guerrini R, Marzola G, Marti M, McDonald J, Morari M, Lambert DG, Salvadori S, Regoli D (2002) [Nphe$^{1}$,Arg$^{14}$,Lys$^{15}$]nociceptin-$NH_2$, a novel potent and selective antagonist of the nociceptin/orphanin FQ receptor. Br J Pharmacol 136 (2):303–311. https://doi.org/10.1038/sj.bjp.0704706

Calo' G, Lambert DG, Guerrini R (2013) Nociceptin/orphanin FQ. In: Handbook of biologically active peptides, 2nd edn, pp 1577–1585. https://doi.org/10.1016/B978-0-12-385095-9.00215-3

Calo' G, Rizzi A, Ruzza C, Ferrari F, Pacifico S, Gavioli EC, Salvadori S, Guerrini R (2018) Peptide welding technology – a simple strategy for generating innovative ligands for G protein coupled receptors. Peptides 99:195–204. https://doi.org/10.1016/j.peptides.2017.10.004

Camarda V, Fischetti C, Anzellotti N, Molinari P, Ambrosio C, Kostenis E, Regoli D, Trapella C, Guerrini R, Severo S, Calo' G (2009) Pharmacological profile of NOP receptors coupled with calcium signaling via the chimeric protein $G\alpha_{qi5}$. Naunyn Schmiedeberg's Arch Pharmacol 379 (6):599–607. https://doi.org/10.1007/s00210-009-0396-x

Carra' G, Calo' G, Spagnolo B, Guerrini R, Arduin M, Marzola E, Trapella C, Regoli D, Salvadori S (2005) Tryptophan replacement in the nociceptin/orphanin FQ receptor ligand Ac-RYYRWK-$NH_2$. J Pept Res 66(1):39–47. https://doi.org/10.1111/j.1399-3011.2005.00272.x

Cerlesi MC, Ding H, Bird MF, Kiguchi N, Ferrari F, Malfacini D, Rizzi A, Ruzza C, Lambert DG, Ko MC, Calo' G, Guerrini R (2017) Pharmacological studies on the NOP and opioid receptor agonist PWT2-[Dmt$^{1}$]N/OFQ(1-13). Eur J Pharmacol 794:115–126. https://doi.org/10.1016/j.ejphar.2016.11.026

Charoenchai L, Wang H, Wang JB, Aldrich JV (2008) High affinity conformationally constrained nociceptin/orphanin FQ(1-13) amide analogues. J Med Chem 51(15):4385–4387. https://doi.org/10.1021/jm800394v

Daga PR, Zaveri NT (2012) Homology modeling and molecular dynamics simulations of the active state of the nociceptin receptor reveal new insights into agonist binding and activation. Proteins 80(8):1948–1961. https://doi.org/10.1002/prot.24077

Ding H, Kiguchi N, Yasuda D, Daga PR, Polgar WE, Lu JJ, Czoty PW, Kishioka S, Zaveri NT, Ko MC (2018) A bifunctional nociceptin and mu opioid receptor agonist is analgesic without opioid

side effects in nonhuman primates. Sci Transl Med 10(456):eaar3483. https://doi.org/10.1126/scitranslmed.aar3483

Dooley CT, Houghten RA (1996) Orphanin FQ: receptor binding and analog structure activity relationships in rat brain. Life Sci 59(1):PL23–PL29. https://doi.org/10.1016/0024-3205(96)00261-5

Dooley CT, Spaeth CG, Berzetei-Gurske IP, Craymer K, Adapa ID, Brandt SR, Houghten RA, Toll L (1997) Binding and in vitro activities of peptides with high affinity for the nociceptin/orphanin FQ receptor, ORL1. J Pharmacol Exp Ther 283(2):735–741

Granier S, Manglik A, Kruse AC, Kobilka TS, Thian FS, Weis WI, Kobilka BK (2012) Structure of the δ-opioid receptor bound to naltrindole. Nature 485(7398):400–404. https://doi.org/10.1038/nature11111

Guerrini R, Calo' G, Rizzi A, Bianchi C, Lazarus LH, Salvadori S, Temussi PA, Regoli D (1997) Address and message sequences for the nociceptin receptor: a structure-activity study of nociceptin-(1-13)-peptide amide. J Med Chem 40(12):1789–1793. https://doi.org/10.1021/jm970011b

Guerrini R, Calo' G, Bigoni R, Rizzi D, Rizzi A, Zucchini M, Varani K, Hashiba E, Lambert DG, Toth G, Borea PA, Salvadori S, Regoli D (2001) Structure-activity studies of the $Phe^4$ residue of nociceptin(1-13)-$NH_2$: identification of highly potent agonists of the nociceptin/orphanin FQ receptor. J Med Chem 44(23):3956–3964. https://doi.org/10.1021/jm010221v

Guerrini R, Marzola E, Trapella C, Pela' M, Molinari S, Cerlesi MC, Malfacini D, Rizzi A, Salvadori S, Calo' G (2014) A novel and facile synthesis of tetra branched derivatives of nociceptin/orphanin FQ. Bioorg Med Chem 22(14):3703–3712. https://doi.org/10.1016/j.bmc.2014.05.005

Guerrini R, Marzola E, Trapella C, Pacifico S, Cerlesi MC, Malfacini D, Ferrari F, Bird MF, Lambert DG, Salvadori S, Calo' G (2015) Structure activity studies of nociceptin/orphanin FQ (1-13)-$NH_2$ derivatives modified in position 5. Bioorg Med Chem 23(7):1515–1520. https://doi.org/10.1016/j.bmc.2015.02.008

Guillemyn K, Starnowska J, Lagard C, Dyniewicz J, Rojewska E, Mika J, Chung NN, Utard V, Kosson P, Lipkowski AW, Chevillard L, Arranz-Gibert P, Teixidó M, Megarbane B, Tourwé D, Simonin F, Przewlocka B, Schiller PW, Ballet S (2016) Bifunctional peptide-based opioid agonist-nociceptin antagonist ligands for dual treatment of acute and neuropathic pain. J Med Chem 59(8):3777–3792. https://doi.org/10.1021/acs.jmedchem.5b01976

Gündüz O, Rizzi A, Baldisserotto A, Guerrini R, Spagnolo B, Gavioli EC, Kocsis L, Magyar A, Benyhe S, Borsodi A, Calo' G (2006) In vitro and in vivo pharmacological characterization of the nociceptin/orphanin FQ receptor ligand Ac-RYYRIK-ol. Eur J Pharmacol 539(1–2):39–48. https://doi.org/10.1016/j.ejphar.2006.03.075

Günther T, Dasgupta P, Mann A, Miess E, Kliewer A, Fritzwanker S, Steinborn R, Schulz S (2018) Targeting multiple opioid receptors-improved analgesics with reduced side effects? Br J Pharmacol 175(14):2857–2868. https://doi.org/10.1111/bph.13809

Halab L, Becker JA, Darula Z, Tourwe D, Kieffer BL, Simonin F, Lubell WD (2002) Probing opioid receptor interactions with azacycloalkane amino acids. Synthesis of a potent and selective ORL1 antagonist. J Med Chem 45(24):5353–5357. https://doi.org/10.1021/jm020078l

Harrison RS, Ruiz-Gómez G, Hill TA, Chow SY, Shepherd NE, Lohman RJ, Abbenante G, Hoang HN, Fairlie DP (2010) Novel helix-constrained nociceptin derivatives are potent agonists and antagonists of ERK phosphorylation and thermal analgesia in mice. J Med Chem 53(23):8400–8408. https://doi.org/10.1021/jm101139f

Hashiba E, Harrison C, Galo G, Guerrini R, Rowbotham DJ, Smith G, Lambert DG (2001) Characterisation and comparison of novel ligands for the nociceptin/orphanin FQ receptor. Naunyn Schmiedeberg's Arch Pharmacol 362:28–33. https://doi.org/10.1007/s002100000327

Judd AK, Kaushanskaya A, Tuttle DJ, Sanchez A, Khroyan T, Polgar W, Toll L (2003) N-terminal modifications leading to peptide ORL1 partial agonists and antagonists. J Pept Res 62(5):191–198. https://doi.org/10.1034/j.1399-3011.2003.00077.x

Judd AK, Tuttle DJ, Jones RW, Sanchez A, Polgar W, Berzetei-Gurske I, Toll L (2004) Structure-activity studies on high affinity NOP-active hexapeptides. J Pept Res 64(3):87–94. https://doi.org/10.1111/j.1399-3011.2004.00169.x

Kantola I, Scheinin M, Gulbrandsen T, Meland N, Smerud KT (2017) Safety, tolerability, and antihypertensive effect of SER100, an opiate receptor-like 1 (ORL-1) partial agonist, in patients with isolated systolic hypertension. Clin Pharmacol Drug Dev 6(6):584–591. https://doi.org/10.1002/cpdd.330

Kapusta DR, Thorkildsen C, Kenigs VA, Meier E, Vinge MM, Quist C, Petersen JS (2005) Pharmacodynamic characterization of ZP120 (Ac-RYYRWKKKKKKK-$NH_2$), a novel, functionally selective nociceptin/orphanin FQ peptide receptor partial agonist with sodium-potassium-sparing aquaretic activity. J Pharmacol Exp Ther 314(2):652–660. https://doi.org/10.1124/jpet.105.083436

Kasakov L, Nashar M, Naydenova E, Vezenkov L, Vlaskovska M (2010) In vitro studies of the activity of newly sinthesized nociceptin/orphanin FQ receptor ligand analogues. Protein Pept Lett 17(5):616–620. https://doi.org/10.2174/092986610791112747

Kawano C, Okada K, Honda T, Nose T, Sakaguchi K, Costa T, Shimohigashi Y (2002) Structural requirements of nociceptin antagonist Ac-RYYRIK-$NH_2$ for receptor binding. J Pept Sci 8 (10):561–569. https://doi.org/10.1002/psc.415

Kawano S, Ambo A, Sasaki Y (2006) Synthesis and receptor binding properties of chimeric peptides containing a mu-opioid receptor ligand and nociceptin/orphanin FQ receptor ligand Ac-RYYRIK-amide. Bioorg Med Chem Lett 16(18):4839–4841. https://doi.org/10.1016/j.bmcl.2006.06.060

Kawano S, Ito R, Nishiyama M, Kubo M, Matsushima T, Minamisawa M, Ambo A, Sasaki Y (2007) Receptor binding properties and antinociceptive effects of chimeric peptides consisting of a micro-opioid receptor agonist and an ORL1 receptor antagonist. Biol Pharm Bull 30 (7):1260–1264. https://doi.org/10.1248/bpb.30.1260

Kitayama M, Barnes TA, Carra' G, McDonald J, Calo' G, Guerrini R, Rowbotham DJ, Smith G, Lambert DG (2003) Pharmacological profile of the cyclic nociceptin/orphanin FQ analogues c[$Cys^{10,14}$]N/OFQ(1-14)$NH_2$ and c[$Nphe^{1}$,$Cys^{10,14}$]N/OFQ(1-14)$NH_2$. Naunyn Schmiedeberg's Arch Pharmacol 368(6):528–537. https://doi.org/10.1007/s00210-003-0821-5

Kocsis L, Orosz G, Magyar A, Al-Khrasani M, Kató E, Rónai AZ, Bes B, Meunier JC, Gündüz O, Tóth G, Borsodi A, Benyhe S (2004) Nociceptin antagonism: probing the receptor by N-acetyl oligopeptides. Regul Pept 122(3):199–207. https://doi.org/10.1016/j.regpep.2004.06.019

Lagard C, Chevillard L, Guillemyn K, Risède P, Laplanche JL, Spetea M, Ballet S, Mégarbane B (2017) Bifunctional peptide-based opioid agonist/nociceptin antagonist ligand for dual treatment of nociceptive and neuropathic pain. Pain 158(3):505–515. https://doi.org/10.1097/j.pain.0000000000000790

Larsen BD (1999) Pharmacologically active peptide conjugates having a reduced tendency towards enzymatic hydrolysis. WO 99/46283

Li J, Isozaki K, Okada K, Matsushima A, Nose T, Costa T, Shimohigashi Y (2008) Designed modification of partial agonist of ORL1 nociceptin receptor for conversion into highly potent antagonist. Bioorg Med Chem 16(5):2635–2644. https://doi.org/10.1016/j.bmc.2007.11.043

Lohman RJ, Harrison RS, Ruiz-Gómez G, Hoang HN, Shepherd NE, Chow S, Hill TA, Madala PK, Fairlie DP (2015) Helix-constrained nociceptin peptides are potent agonists and antagonists of ORL-1 and nociceptin. Vitam Horm 97:1–55. https://doi.org/10.1016/bs.vh.2014.10.001

Malfacini D, Ambrosio C, Gro' MC, Sbraccia M, Trapella C, Guerrini R, Bonora M, Pinton P, Costa T, Calo' G (2015) Pharmacological profile of nociceptin/orphanin FQ receptors interacting with G-proteins and beta-arrestins 2. PLoS One 10(8):e0132865. https://doi.org/10.1371/journal.pone.0132865

Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, Pardo L, Weis WI, Kobilka BK, Granier S (2012) Crystal structure of the μ-opioid receptor bound to a morphinan antagonist. Nature 485(7398):321–326. https://doi.org/10.1038/nature10954

McDonald J, Barnes TA, Calo' G, Guerrini R, Rowbotham DJ, Lambert DG (2002) Effects of [(pF)$Phe^{4}$]nociceptin/orphanin FQ-(1-13)$NH_2$ on GTPγ$^{35}$S binding and cAMP formation in Chinese

hamster ovary cells expressing the human nociceptin/orphanin FQ receptor. Eur J Pharmacol 443(1–3):7–12. https://doi.org/10.1016/S0014-2999(02)01577-7

McDonald J, Calo' G, Guerrini R, Lambert DG (2003) UFP-101, a high affinity antagonist for the nociceptin/orphanin FQ receptor: radioligand and GTPγ$^{35}$S binding studies. Naunyn Schmiedeberg's Arch Pharmacol 367(2):183–187. https://doi.org/10.1007/s00210-002-0661-8

Molinari S, Camarda V, Rizzi A, Marzola G, Salvadori S, Marzola E, Molinari P, McDonald J, Ko MC, Lambert DG, Calo' G, Guerrini R (2013) [Dmt$^{1}$]N/OFQ(1-13)-$NH_2$: a potent nociceptin/orphanin FQ and opioid receptor universal agonist. Br J Pharmacol 168(1):151–162. https://doi.org/10.1111/j.1476-5381.2012.02115.x

Mustazza C, Pieretti S, Marzoli F (2018) Nociceptin/orphanin FQ peptide (NOP) receptor modulators: an update in structure-activity relationships. Curr Med Chem 25(20):2353–2384. https://doi.org/10.2174/0929867325666180111095458

Naydenova ED, Todorov PT, Mateeva PI, Zamfirova RN, Pavlov ND, Todorov SB (2010) Synthesis and biological activity of novel small peptides with aminophosphonates moiety as NOP receptor ligands. Amino Acids 39(5):1537–1543. https://doi.org/10.1007/s00726-010-0624-1

Okada K, Sujaku T, Chuman Y, Nakashima R, Nose T, Costa T, Yamada Y, Yokoyama M, Nagahisa A, Shimohigashi Y (2000) Highly potent nociceptin analog containing the Arg-Lys triple repeat. Biochem Biophys Res Commun 278(2):493–498. https://doi.org/10.1006/bbrc.2000.3822

Okada K, Isozaki K, Li J, Matsushima A, Nose T, Costa T, Shimohigashi Y (2008) Synergistic effect of basic residues at positions 14-15 of nociceptin on binding affinity and receptor activation. Bioorg Med Chem 16(20):9261–9267. https://doi.org/10.1016/j.bmc.2008.09.014

Orsini MJ, Nesmelova I, Young HC, Hargittai B, Beavers MP, Liu J, Connolly PJ, Middleton SA, Mayo KH (2005) The nociceptin pharmacophore site for opioid receptor binding derived from the NMR structure and bioactivity relationships. J Biol Chem 280(9):8134–8142. https://doi.org/10.1074/jbc.M406405200

Pacifico S, Carotenuto A, Brancaccio D, Novellino E, Marzola E, Ferrari F, Cerlesi MC, Trapella C, Preti D, Salvadori S, Calo' G, Guerrini R (2017) Structure-and conformation-activity studies of nociceptin/orphanin FQ receptor dimeric ligands. Sci Rep 7:45817. https://doi.org/10.1038/srep45817

Rizzi D, Rizzi A, Bigoni R, Camarda V, Marzola G, Guerrini R, De Risi C, Regoli D, Calo' G (2002a) [Arg$^{14}$,Lys$^{15}$]nociceptin, a highly potent agonist of the nociceptin/orphanin FQ receptor: in vitro and in vivo studies. J Pharmacol Exp Ther 300(1):57–63. https://doi.org/10.1124/jpet.300.1.57

Rizzi A, Rizzi D, Marzola G, Regoli D, Larsen BD, Petersen JS, Calo' G (2002b) Pharmacological characterization of the novel nociceptin/orphanin FQ receptor ligand, ZP120: in vitro and in vivo studies in mice. Br J Pharmacol 137(3):369–374. https://doi.org/10.1038/sj.bjp.0704894

Rizzi A, Malfacini D, Cerlesi MC, Ruzza C, Marzola E, Bird MF, Rowbotham DJ, Salvadori S, Guerrini R, Lambert DG, Calo' G (2014) In vitro and in vivo pharmacological characterization of nociceptin/orphanin FQ tetrabranched derivatives. Br J Pharmacol 171(17):4138–4153. https://doi.org/10.1111/bph.12799

Rizzi A, Sukhtankar DD, Ding H, Hayashida K, Ruzza C, Guerrini R, Calo' G, Ko MC (2015) Spinal antinociceptive effects of the novel NOP receptor agonist PWT2-nociceptin/orphanin FQ in mice and monkeys. Br J Pharmacol 172(14):3661–3670. https://doi.org/10.1111/bph.13150

Starnowska J, Guillemyn K, Makuch W, Mika J, Ballet S, Przewlocka B (2017) Bifunctional opioid/nociceptin hybrid KGNOP1 effectively attenuates pain-related behaviour in a rat model of neuropathy. Eur J Pharm Sci 104:221–229. https://doi.org/10.1016/j.ejps.2017.03.029

Tancredi T, Carra' G, Guerrini R, Arduin M, Calo' G, Regoli D, Salvadori S, Temussi PA (2005) The interaction of highly helical structural mutants with the NOP receptor discloses the role of the address domain of nociceptin/orphanin FQ. Chemistry 11(7):2061–2070. https://doi.org/10.1002/chem.200401095

Thompson AA, Liu W, Chun E, Katritch V, Wu H, Vardy E, Huang XP, Trapella C, Guerrini R, Calo' G, Roth BL, Cherezov V, Stevens RC (2012) Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. Nature 485(7398):395–399. https://doi.org/10.1038/nature11085

Todorov PT, Mateeva PI, Zamfirova RN, Pavlov ND, Naydenova ED (2012) Synthesis and biological activity of new series of N-modified analogues of the N/OFQ(1-13)$NH_2$ with aminophosphonate moiety. Amino Acids 43(3):1217–1223. https://doi.org/10.1007/s00726-011-1177-7

Toll L, Bruchas MR, Calo' G, Cox BM, Zaveri NT (2016) Nociceptin/orphanin FQ receptor structure, signaling, ligands, functions, and interactions with opioid systems. Pharmacol Rev 68(2):419–457. https://doi.org/10.1124/pr.114.009209

Van Cauwenberghe S, Simonin F, Cluzeau J, Becker JA, Lubell WD, Tourwe D (2004) Structure-activity study of the ORL1 antagonist Ac-Arg-D-Cha-Qaa-D-Arg-D-p-ClPhe-$NH_2$. J Med Chem 47(7):1864–1867. https://doi.org/10.1021/jm031034v

Wright KE, McDonald J, Barnes TA, Rowbotham DJ, Guerrini R, Calo' G, Lambert DG (2003) Assessment of the activity of a novel nociceptin/orphanin FQ analogue at recombinant human nociceptin/orphanin FQ receptors expressed in Chinese hamster ovary cells. Neurosci Lett 346 (3):145–148. https://doi.org/10.1016/S0304-3940(03)00518-4

Wu H, Wacker D, Mileni M, Katritch V, Han GW, Vardy E, Liu W, Thompson AA, Huang XP, Carroll FI, Mascarella SW, Westkaemper RB, Mosier PD, Roth BL, Cherezov V, Stevens RC (2012) Structure of the human kappa-opioid receptor in complex with JDTic. Nature 485 (7398):327–332. https://doi.org/10.1038/nature10939

Zamfirova R, Pavlov N, Todorov P, Mateeva P, Martinez J, Calmes M, Naydenova E (2013) Synthesis and changes in affinity for NOP and opioid receptors of novel hexapeptides containing $\beta^2$-tryptophan analogues. Bioorg Med Chem Lett 23(14):4052–4055. https://doi.org/10.1016/j.bmcl.2013.05.064

Zaveri NT (2016) Nociceptin Opioid Receptor (NOP) as a therapeutic target: progress in translation from preclinical research to clinical utility. J Med Chem 59(15):7011–7028. https://doi.org/10.1021/acs.jmedchem.5b01499

Zhang C, Miller W, Valenzano KJ, Kyle DJ (2012) Novel, potent ORL-1 receptor agonist peptides containing alpha-Helix-promoting conformational constraints. J Med Chem 45(24):5280–5286. https://doi.org/10.1021/jm0202021